

Component-resolved diagnosis of kiwifruit allergy with purified natural and recombinant kiwifruit allergens

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Background: Kiwifruit is one of the most common causes of food allergic reactions. Component-resolved diagnostics may enable significantly improved detection of sensitization to kiwifruit.

Objective: To evaluate the use of individual allergens for component-resolved *in vitro* diagnosis of kiwifruit allergy.

Methods: Thirty patients with a positive double-blind placebo-controlled food challenge to kiwifruit, 10 atopic subjects with negative open provocation to kiwifruit, and 5 nonatopic subjects were enrolled in the study. Specific IgE to 7 individual allergens (nAct d 1-5 and rAct d 8-9) and allergen extracts was measured by ImmunoCAP.

Results: The diagnostic sensitivities of the commercial extract and of the sum of single allergens were 17% and 77%, respectively, whereas diagnostic specificities were 100% and 30%. A combination of the kiwi allergens Act d 1, Act d 2, Act d 4, and Act d 5 gave a diagnostic sensitivity of 40%, whereas diagnostic specificity remained high (90%). Exclusion of the Bet v 1 homolog recombinant (r) Act d 8 and profilin rAct d 9 from this allergen panel reduced sensitivity to 50% but increased specificity to 40%. Kiwifruit-mono-sensitized patients reacted more frequently ($P < .001$) with Act d 1 than polysensitized patients, whereas the latter group reacted more frequently with rAct d 8 ($P = .004$).

Conclusion: Use of single kiwifruit allergen ImmunoCAP increases the quantitative test performance and diagnostic sensitivity compared with the commercial extract. Bet v 1 homolog and profilin are important allergens in pollen-related kiwifruit allergy, whereas actinidin is important in monoallergy to kiwifruit, in which symptoms are often more severe. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Actinidin, allergens, diagnosis, ImmunoCAP, *in vitro* test, kiwifruit, food allergy

Allergy to kiwifruit is well documented in the literature and provides an illustrative example of the effect of introducing a novel food into the food chain.¹⁻³ Although regarded as an exotic fruit during the 1980s, kiwifruit has become one of the top 10 food allergy sources in recent studies from Finland,⁴ Sweden,⁵ and France.⁶

Kiwifruit allergy is most frequently associated with birch and grass pollinosis,^{5,7-9} but it is also involved in the latex-fruit syndrome, with 12% to 39% of individuals allergic to latex also allergic to this food.¹⁰⁻¹² In reported studies, the symptoms of 65% to 72% of patients with kiwifruit allergy were confined to the oral allergy syndrome (OAS), often seen in patients with pollen-fruit allergy,^{1,2,13} although in 18% to 28% of the cases, kiwifruit allergy was manifested by systemic reactions.^{1,2} Mono-sensitization to kiwifruit was observed in 21% to 50%. Many of these individuals reacted with severe symptoms such as urticaria, gastrointestinal symptoms, or anaphylactic shock on kiwifruit ingestion.^{1,2,12}

Because of the poor correlation between a suggestive case history and skin prick test (SPT) performed with commercial kiwifruit protein extracts,^{1,2,12} proper *in vivo* diagnosis of kiwifruit allergy requires the use of fresh fruit, such as in the prick-prick technique. However, 2 previous studies showed that prick-prick testing (PPT) resulted in 95%² or 100%¹ sensitivity but was accompanied by a low specificity (31%).² The reduced specificity of fresh kiwifruit for skin testing was observed in subjects with pollen allergy⁷ as well as latex allergy.^{14,15} A diagnostic sensitivity of *in vitro* IgE tests of 13%¹⁰ to 50%¹ and 60%² has been reported. The low diagnostic sensitivity of *in vitro* and *in vivo* tests is related to shortage or absence of kiwifruit proteins from commercially available kiwifruit extracts.^{1,2} The endogenous protease activity of actinidin, which makes up about 50% of the total fruit protein, may cause rapid protein degradation after tissue disruption.¹⁶

The aim of the current study was to validate the performance of purified kiwifruit allergens in ImmunoCAP (Phadia AB, Uppsala,

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Abbreviations used

DBPCFC: Double-blind, placebo-controlled food challenge

OAS: Oral allergy syndrome

PPT: Prick-prick testing

r: Recombinant

SPT: Skin prick test

Sweden) and to establish the allergen sensitization profiles of patients with kiwifruit allergy, confirmed by double-blind, placebo-controlled food challenge (DBPCFC). For this purpose, we produced a panel of 7 kiwifruit allergens either as natural or recombinant proteins.

METHODS**Patients and controls**

Thirty patients with a suspected kiwifruit allergy were recruited between August 2003 and January 2005 at the Allergy Unit of the University Hospital Zurich, Switzerland. The primary inclusion criterion was a positive case history of an allergic reaction to kiwifruit, whereas definitive inclusion into the study required either a positive food challenge with kiwifruit or a convincing case history of anaphylactic reactions to kiwifruit as recently defined by Sampson et al.¹⁷ Patients were divided according to clinical characteristics into 3 groups: patients monosensitized to kiwifruit (group A1, n = 8), patients with pollen and kiwifruit allergy (group A2, n = 17) and patients with a combined kiwifruit, pollen, and latex allergy (group A3, n = 5). Controls consisted of patients with a negative open food challenge with kiwifruit and (group B, n = 5) a case history of a birch pollen allergy and a positive skin test or ImmunoCAP to birch pollen extract, (group C, n = 5) a case history of latex allergy and a positive skin test or ImmunoCAP to latex extract, and (group D, n = 5) nonatopic subjects. Pregnancy, significant concurrent disease, or medication with glucocorticosteroids, H₁-receptor antagonists, angiotensin-converting enzyme inhibitor, or β -blocking agents were exclusion criteria.

Ethical considerations

The study was reviewed and approved by the local ethical committee. All subjects provided written informed consent before enrollment into the study.

Skin tests

Skin prick tests were performed with fresh kiwifruit by using the prick-prick-technique.¹⁸ Commercial extracts (Soluprick; ALK, Hørsholm, Denmark, and Allyostal, Stallergènes, France) from kiwifruit, latex, birch, mugwort, and grass pollen were tested (in duplicate), and results evaluated according to the recommendation of the European Academy of Allergy and Clinical Immunology.¹⁹

DBPCFC with kiwifruit

Double-blind placebo-controlled food challenge was performed as previously described for soy in a 2-step mucosal-systemic challenge.²⁰ Two different meals indistinguishable in color, texture, and taste were prepared. The active and placebo meals consisted of 400 g orange juice, 50 g peppermint syrup, 4 g agar agar, 60 g sugar, 10 g poppy seed, and 40 g banana flavor cooked to a pudding. To the active meal, 60 g kiwifruit and 10 g sugar were added. To the placebo meal, 60 g white grapes, 10 g sugar, and 0.2 g ascorbic acid were added.

Apart from kiwifruit, all ingredients were known to be tolerated by each patient. For the mucosal challenge, 4 doses containing 1.4 g, 2.8 g, 5.6 g, and 11.2 g kiwifruit were administered to the patients, whereas for the systemic challenge, 4 doses containing 2.8 g, 5.6 g, 11.2 g, and 22.4 g kiwifruit were

used. To prevent the effect of the hydrolytic activity of actinidin, the challenges were performed immediately after preparation of the meals.

Open food challenge with kiwifruit

Control subjects (groups B, C, D) underwent an open provocation with 1 fresh kiwifruit to exclude kiwifruit allergy.

Kiwifruit protein extract

Kiwifruit protein extract was prepared from *Actinidia deliciosa* cv Hayward (green kiwifruit) as described previously.¹³

Natural kiwifruit allergens

Act d 1 and Act d 2 were purified from kiwifruit extracts as described previously.^{16,21} For purification of Act d 3, extract was prepared by extraction in 0.5 mol/L NaCl. The sample was loaded onto a 1-mL SP Sepharose Fast Flow column (GE Healthcare Life Sciences, Uppsala, Sweden) and proteins eluted using a 0 to 500 mmol/L NaCl gradient. To purify Act d 4 and Act d 5, kiwifruit proteins were extracted in water containing 3% suspended solid polyvinylpyrrolidone. After ammonium sulfate precipitation and dialysis, the allergens were purified by Affi-Gel Blue column (Bio-Rad Laboratories, Hercules, Calif). Flow-through sample was collected and dialyzed against 20 mmol/L TRIS-HCl, pH 8.0, loaded onto a 1-mL Resource Q anion exchange column (GE Healthcare Life Sciences) and eluted with a 0 to 500 mmol/L NaCl gradient. Purity of the allergens was checked by SDS-PAGE and the identity confirmed by N-terminal sequencing.

Recombinant kiwifruit allergens

Bet v 1-related sequences and profilin were identified from EST cDNA libraries from *A. deliciosa* ripe fruit inner cortex.^{22,23} A full-length clone of Act d 8 was obtained by PCR using sequence-specific primers, cloned into a pET-23a(+) derived vector, and expressed as a C-terminally hexahistidine protein in *Escherichia coli* BL21. Recombinant (r) Act d 8 was purified by immobilized metal ion affinity chromatography on Ni²⁺-charged Chelating Sepharose FF (GE Healthcare Life Sciences) and size exclusion chromatography on Superdex 75 p.g. (GE Healthcare Life Sciences). Further, we cloned the kiwifruit profilin (EMBL/GenBank/DBJ accession no. FG438715), and the allergen was given the official designation Act d 9 by the International Union of Immunological Societies Allergen Nomenclature Subcommittee. The Act d 9 full length sequence was cloned into the (pCL476) plasmid, a heat-inducible expression vector.²⁴ The expression was performed in BL21-RIL *E. coli* cells (Novagen Merck KGaA, Darmstadt, Germany), and recombinant kiwifruit profilin was purified through a HiTrap HP nickel column (GE Healthcare Life Sciences) followed by a G75 Superdex column (GE Healthcare Life Sciences).

In vitro diagnosis

Specific IgE antibodies to kiwifruit extract, latex, birch pollen, rBet v 1 and rBet v 2 were measured by ImmunoCAP using a commercially available test. Experimental ImmunoCAP tests were prepared for all kiwifruit allergens and a self-made kiwifruit extract as previously described,²⁵ and specific IgE concentrations were determined in duplicate on an ImmunoCAP 250 instrument. Values ≥ 0.35 kU_A/L were regarded as positive. When appropriate, specific IgE antibody concentrations were expressed as classes by using the following intervals: class 1, 0.35 to 0.7 kU_A/L; class 2, 0.7 to 3.5 kU_A/L; class 3, 3.5 to 17.5 kU_A/L; class 4, 17.5 to 50 kU_A/L; class 5, 50 to 100 kU_A/L; and class 6, >100 kU_A/L.

Statistical analysis

Diagnostic sensitivity and specificity were calculated according to the method of Goldman²⁶ as described previously for carrot allergy.²⁷

TABLE I. Clinical characteristics of patients with kiwifruit allergy

Patients	Case history	DBPCFC	PPT	SPT	SPT grass	CAP kiwifruit	CAP latex	CAP birch	CAP rBet v 1	CAP rBet v 2
Group A1										
1	OAS	OAS	–	–	–	<0.35	<0.35	<0.35	<0.35	<0.35
2	N, E, Di, GIT, BP	OAS, N, F	+	+	–	<0.35	<0.35	<0.35	<0.35	<0.35
3	OAS	OAS	+	+	–	1.07	<0.35	<0.35	<0.35	<0.35
4	OAS, Dph	OAS	+	+	–	<0.35	<0.35	<0.35	<0.35	<0.35
5	OAS, N	OAS, N, C	+	+	–	0.4	<0.35	<0.35	<0.35	<0.35
6	OAS, F, T	OAS	+	+	–	<0.35	<0.35	<0.35	<0.35	<0.35
7	N, E, F, D, GIT, BP, A	ND	ND	ND	ND	<0.35	<0.35	<0.35	<0.35	<0.35
8	OAS	OAS	+	–	–	1.48	<0.35	<0.35	<0.35	<0.35
% Positive			86	71	0	38	0	0	0	0
Group A2										
1	OAS	OAS	–	–	+	<0.35	<0.35	<0.35	<0.35	<0.35
2	OAS	OAS	+	–	+	<0.35	0.46	1.15	<0.35	0.95
3	OAS	OAS	+	–	+	<0.35	0.71	18.6	47.1	2.12
4	OAS	OAS	–	–	+	<0.35	<0.35	<0.35	<0.35	<0.35
5	OAS,R, GIT	GIT	+	+	+	<0.35	0.44	0.63	0.59	<0.35
6	OAS	OAS	+	–	+	<0.35	<0.35	15.8	44.3	<0.35
7	OAS	OAS	–	–	+	<0.35	<0.35	<0.35	<0.35	<0.35
8	OAS	OAS	+	+	+	0.43	<0.35	11.1	16.5	<0.35
9	OAS, F, T, D	OAS, C, T, GIT	–	–	–	<0.35	<0.35	15.4	29.2	<0.35
10	OAS	OAS	+	–	+	<0.35	<0.35	6.05	21.1	<0.35
11	OAS	OAS	+	–	+	<0.35	1.67	1.24	<0.35	1.75
12	OAS, AE, Dph	OAS, Dph	+	+	+	<0.35	1.98	11.3	19.6	4.91
13	OAS, AE	OAS	+	–	–	<0.35	<0.35	19	36.1	<0.35
14	OAS	OAS	+	–	+	<0.35	<0.35	2.16	4.36	<0.35
15	OAS	OAS	+	–	+	<0.35	<0.35	2.37	6.18	<0.35
16	OAS, AE, C, D	OAS, D	+	–	–	<0.35	<0.35	36.6	51.5	<0.35
17	OAS, F, D	F	+	+	–	<0.35	1.21	1.46	<0.35	<0.35
% Positive			76	24	76	6	35	82	65	24
Group A3										
1	OAS, AE, F	AE, D	+	+	+	<0.35	31.6	<0.35	<0.35	<0.35
2	OAS, F	OAS, F	+	–	+	<0.35	1.82	12.5	28.1	4.14
3	OAS	OAS	+	+	+	<0.35	3.2	20.3	68.3	<0.35
4	OAS	OAS	+	+	+	<0.35	0.51	6.67	24.9	0.39
5	OAS,AE	OAS	+	+	+	0.38	2.84	54.4	89.2	<0.35
% Positive			100	80	100	20	100	80	80	40

A, anaphylactic shock; AE, angioedema; BP, drop of blood pressure of at least 20 mmHg; C, conjunctivitis; D, dyspnea; Di, diarrhea; Dph, dysphagia; E, emesis; F, flush; GIT, gastrointestinal cramps; N, nausea; ND, not done; PPT, PPT with native kiwi; R, rhinitis; SPT, kiwi extract (Allystal); SPT grass, SPT with grass pollen extract (Soluprick); T, tightness of the chest.

CAPs (ImmunoCAP) are reported as kU_A/L.

TABLE II. Kiwifruit allergens used in this study

Allergen	Accession number	Purity	Molecular weight, kD (SDS-PAGE)	Protein family/Biochemical designation	Cross-reactivity to birch and grass pollen and latex	References
nAct d 1	UniProt P00785	≥95%	27	Actinidin	Unknown	33
nAct d 2	UniProt Q5ND92	≥95%	24	Thaumatin-like protein	Unknown	34
nAct d 3	UniProt P85063	≥95%	42	Glycoprotein of unknown function	Birch and grass pollen, latex	12
nAct d 4	UniProt Q6TPK4	≥95%	11	Phytocystatin	Unknown	35
nAct d 5	UniProt P84527	≥95%	26	Kiwelin	Unknown	28
rAct d 8	EMBL/Genbank AM489568	≥99%	17	Pathogenesis-related proteins 10 family	Birch pollen	23
rAct d 9	EMBL/Genbank FG438715	≥99%	14	Profilin	Birch and grass pollen, latex	Unpublished

n, Protein purified from kiwifruit tissue; r, recombinant protein produced in *E. coli*.

The Fisher exact probability test was applied to assess statistically significant differences between the percentages of patients from group A1, A2, and A3 with individual kiwifruit allergens. The analysis of differences in

IgE levels and severity of symptoms between groups of patients was assessed using the Mann and Whitney *U* test. All statistical tests were performed using the SPSS software package (SPSS, Chicago, Ill).

Patients	Kiwi-CAP	Extract	Act d 1	Act d 2	Act d 3	Act d 4	Act d 5	Act d 8	Act d 9
Group A1									
1									
2		2	2	2					
3	1	2	2		2		2		
4		1	1						
5	1	2	2						
6									
7		1				1	1		
8	1	3	3		1		2		
% positive	38	75	63	13	25	13	38	0	0
Group A2									
1									
2									2
3		2			2			3	2
4									
5		2		2	2	1			
6		1			1			1	
7									
8	1	2	2					3	
9									
10								2	
11		2			2	2	1		3
12		1			1			2	3
13		1						3	
14								2	
15								2	
16		2						3	
17		2			3	2	1		
% positive	6	53	6	6	35	18	12	53	24
Group A3									
1									
2		2						3	2
3		1						4	
4		2				3	1	2	1
5	1	3		2	2	2	1	3	
% positive	20	80	0	20	40	40	20	80	40
Group B									
1					1			3	
2								1	
3					1			1	
4					2				2
5									
% positive	0	0	0	0	60	0	0	60	20
Group C									
1							1		
2									
3									
4					1			2	
5		2			1			2	3
% positive	0	20	0	0	40	0	20	40	20
Group D									
1									
2									
3									
4									
5									
% positive	0	0	0	0	0	0	0	0	0

FIG 1. IgE reactivity profiles to kiwifruit extracts and individual allergens in kiwifruit-mono sensitized subjects (A1), subjects with kiwifruit and pollen allergy (A2), subjects with kiwifruit, pollen, and latex allergy (A3), and controls with a birch pollen allergy (B), with a pollen and latex allergy (C), and nonatopics (D). Specific IgE concentrations are expressed as CAP classes as described in the Methods section. *Extract^a*, Commercial extract; *Extract^b*, self-produced extract.

RESULTS

Clinical allergy to kiwifruit shows a diverse sensitization pattern

Thirty patients (18 females and 12 males) with kiwifruit allergy were included in the study: 29 on the basis of a positive DBPCFC and 1 on the basis of a positive case history of an anaphylactic reaction to kiwifruit. The mean age $\pm$ SD was 33.1 ± 12.8 years (range, 16-62 years). Patients were divided according to clinical characteristics into 3 groups: 8 patients were monosensitized to

kiwifruit (A1), 17 patients had a pollen allergy in addition to a kiwifruit allergy (A2), and 5 patients had a combined kiwifruit, pollen, and latex allergy (A3). Eleven of the patients in group A2 had seasonal rhinitis during the birch pollen season and 9 during the grass pollen season. Four of the patients in group A3 had seasonal rhinitis during the birch pollen season and 4 during the grass pollen season.

Table I summarizes case histories with respect to kiwifruit allergy, symptoms observed during DBPCFC, skin test, and ImmunoCAP results. PPTs with fresh kiwifruit showed the highest diagnostic sensitivity with 83%, whereas diagnostic sensitivity of SPT with commercial kiwifruit extract was 45%, and for the *in vitro* determination of kiwifruit-specific IgE, 17%. Overall, the sensitivity of extract based diagnostic tests was lower in the group of patients with pollen-related kiwifruit allergy than in the other patients with kiwifruit allergy.

On challenge, 26 of 29 patients developed oropharyngeal symptoms such as itching and swelling. More severe symptoms were observed in 9 patients, including conjunctivitis ($n = 2$), angioedema ($n = 1$), flush ($n = 3$), nausea ($n = 2$), gastrointestinal pain ($n = 2$), dysphagia ($n = 1$), dyspnea ($n = 2$) and tightness of the chest ($n = 1$). Symptoms were elicited during mucosal challenge in 22 patients and only after systemic challenge in 7 patients.

Because the provocation was stopped in case of repetitive and intensive mucosal symptoms, recorded reactions during the challenge were in most cases milder than reactions to kiwifruit reported in the case history.

Fifteen subjects entered the control groups, consisting of 5 patients with a birch pollen allergy (group B; mean age $\pm$ SD, 27.4 ± 11.9 years), 5 patients with a pollen and latex allergy (group C; 32.8 ± 13.3 years) and 5 nonatopic subjects (group D; 29.6 ± 12.5 years), all with a negative open challenge to kiwifruit.

All patients with birch pollen allergy had seasonal rhinitis during the birch pollen season, and 60% also had seasonal asthma. All patients were SPT-positive and ImmunoCAP-positive to birch pollen extract. Likewise, all controls with pollen and latex allergy (group C) were affected by seasonal rhinitis to different pollens: 3 of them to birch pollen and 3 to grass pollen. Latex-induced symptoms consisted of contact urticaria in all patients and a bronchospasm induced by latex in 1 patient. All 5 patients with latex allergy had a positive ImmunoCAP result, and 4 had a positive skin test to latex extract.

Characterization of purified natural and recombinant kiwifruit allergens

Kiwifruit allergens Act d 1 to 5 were purified from kiwifruit tissue, whereas rAct d 8²³ and rAct d 9 were produced and purified from an *E coli* expression system. Biochemical characteristics, biological function, purity, and possible IgE cross-reactivity of the allergens are summarized in Table II. The identity of each of the purified proteins was confirmed by N-terminal amino acid sequence analysis. Circular dichroism spectroscopy of the purified proteins indicated appropriate folding patterns and secondary structures representative of their respective allergen families (data not shown). IgE binding properties tested by ELISA also confirmed that antibody binding properties were retained.

TABLE III. Diagnostic sensitivity and specificity determination of allergen specific IgE by ImmunoCAP using kiwifruit extracts and single allergens

	No. of patients tested positive (≥ 0.35 kU _A /L)														
	Extract n	Extract I*	Extract II†	All single allergens	nAct d 1	nAct d 2	nAct d 3	nAct d 4	nAct d 5	rAct d 8	rAct d 9	All w/o rAct d 8	All w/o rAct d 9	All w/o rAct d 8 and rAct d 9	nAct d 1, 2, 4 and 5 positive
Group A1	8	3	6	6	5	1	2	1	3	0	0	6	6	6	6
Group A2	17	1	9	13	1	1	6	3	2	9	4	8	12	7	4
Group A3	5	1	4	4	0	1	2	2	1	4	2	3	4	2	2
Group B	5	0	0	4	0	0	3	0	0	3	1	3	4	3	0
Group C	5	0	1	3	0	0	2	0	1	2	1	3	3	3	1
Group D	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kiwifruit allergy	30	5	19	23	6	3	10	6	6	13	6	17	22	15	12
Atopic controls	10	0	1	7	0	0	5	0	1	5	2	6	7	6	1
Sensitivity (%)		17	63	77	20	10	33	20	20	43	20	57	73	50	40
Specificity (control group, atopic controls; %)		100	90	30	100	100	50	100	90	50	80	40	30	40	90
Specificity (control group, nonatopic controls; %)		100	100	100	100	100	100	100	100	100	100	100	100	100	100

*Commercial extract.

†Self-produced kiwifruit extract.

w/o, Without.

A primary structure comparison of Act d 9 and profilins from other allergen sources revealed amino acid sequence identity with Cap a 2 from bell pepper (93%), Hev b 8 from latex (90%), and Bet v 2 from birch pollen (75%).

Using single allergen ImmunoCAP increases signal intensity and diagnostic sensitivity

Fig 1 represents the individual IgE reactivity profiles of patients with kiwifruit allergy and controls to 2 different kiwifruit extracts (commercial and self-prepared) and 7 individual natural and recombinant allergens on ImmunoCAP. In an initial screening, measurement of specific IgE levels to commercial kiwifruit ImmunoCAP, 5 of 30 patients (17%) were positive. Nineteen patients (63%) were positive when this extract was replaced by a self-produced kiwifruit extract, thus increasing the *in vitro* diagnostic sensitivity almost 4-fold (Table III). There was also a major difference in the ability of the 2 extracts to quantify specific IgE: median concentration values obtained among subjects who tested positive were 0.45 and 1.3 kU_A/L for the commercial and the self-prepared extract, respectively.

Furthermore, diagnostic sensitivity and specificity of the tests were calculated to evaluate the potential of the individual kiwifruit allergens as *in vitro* diagnostic reagents (Table III). In a first analysis, a serum was considered positive if at least 1 positive ImmunoCAP result was obtained. All sera from nonatopic controls reacted neither with the extract nor with individual kiwifruit allergens. The nonatopic control group diagnostic specificity was 100% for the both extracts and individual allergens. Using the range of allergens (at least 1 positive) proved superior (77% sensitivity) to both the commercial (17%) and self-produced kiwifruit extracts (63%), respectively. The evident rise of diagnostic sensitivity was accompanied by a decrease in diagnostic specificity from 100% of the commercial extract ImmunoCAP

to 30% for all single allergens. A diagnostic specificity of 100% was observed for the nonpollen or nonlatex-related kiwi allergens nAct d 1, nAct d 2, and nAct d 4 ImmunoCAP because no signals were obtained from the sera of control individuals (Table III), whereas the specificity was only 50% for the allergens nAct d 3 and rAct d 8 and 80% for rAct d 9. When the Bet v 1 homolog rAct d 8 was excluded, the diagnostic sensitivity was reduced from 77% to 57%, and the diagnostic specificity increased from 30 to 40%. Exclusion of kiwifruit profilin rAct d 9 led to a decrease in sensitivity from 77% to 73%, whereas specificity did not change. Just 1 patient with pollen and latex allergy without kiwifruit allergy showed IgE reactivity to nAct d 5. Thus, diagnostic specificity for nAct d 5 was 90%. Testing IgE binding to the 4 nonpollen or nonlatex-related kiwifruit allergens Act d 1, Act d 2, Act d 4, and Act d 5, the sensitivity increased to 40%, and the specificity remained high (90%).

Identification of Act d 1 as a marker molecule with diagnostic significance

Measurements of specific IgE to the individual kiwifruit allergens in 3 groups of subjects with kiwifruit allergy revealed clear differences in the sensitization patterns (Fig 2). Patients from group A1 (monosensitized patients) reacted significantly more frequently ($P < .001$) with Act d 1 than patients from group A2 (patients with kiwifruit and pollen allergy) and group A3 (patients with kiwifruit, pollen, and latex allergy). None of the patients from group A1 reacted to rAct d 8 or rAct d 9. In contrast, patients from groups A2 and A3 reacted frequently with rAct d 8 (13/23) and rAct d 9 (6/23).

No significant correlation between IgE reactivity to kiwifruit allergens and the severity of symptoms could be observed (see this article's Fig E1 in the Online Repository at www.jacionline.org).

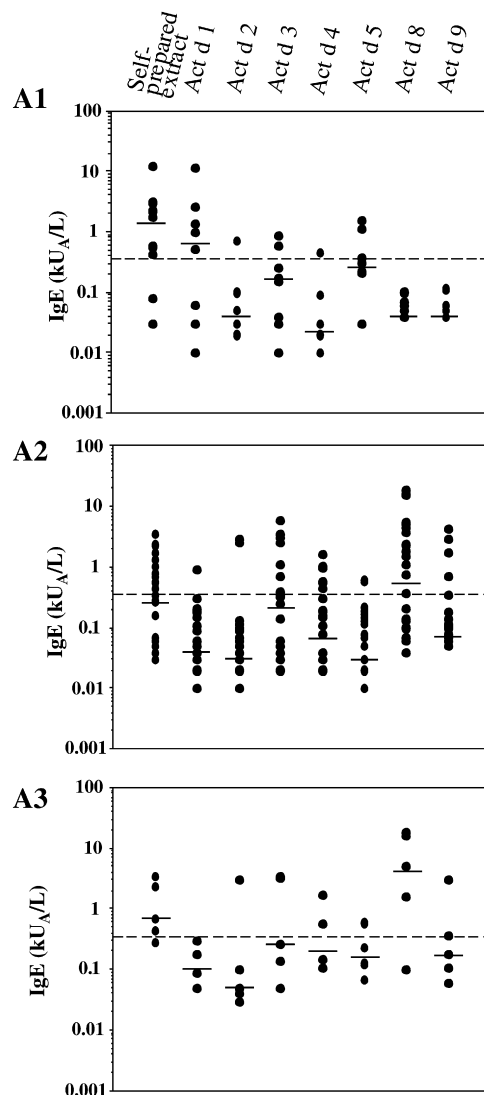


FIG 2. Sensitization profiles to kiwifruit allergen components in kiwifruit-monosensitized subjects (A1), subjects with kiwifruit and pollen allergy (A2), and subjects with kiwifruit, pollen, and latex allergy (A3). Dotted lines indicate the 0.35 kU_A/L cutoff level. Median values are indicated.

DISCUSSION

Currently, diagnosis of kiwifruit allergy relies mainly on the PPT with fresh kiwifruits or on the determination of specific IgE with commercial kiwifruit extracts.

Biochemical variability in allergen source and relatively high false-positive rates represent limitations of PPT for sensitization assessment.^{2,7,14,15} Most importantly, degradation of allergens by actinidin and other proteases heavily affects the diagnostic sensitivity of extract-based and commercially available diagnostic tests.^{1,2} Degradation of proteins starts with the enzymes being released during extraction procedure and continues during storage, thus heavily affecting the quality of the extract. Our data clearly showed that the commercial extract ImmunoCAP had a low diagnostic sensitivity. This was particularly true in the 2 groups of patients with kiwifruit/pollen and kiwifruit/pollen/latex allergy, in which it failed to detect specific IgE in 91% of the subjects. A slightly higher sensitivity of commercial extract ImmunoCAP and SPT was obtained in the group of

monosensitized patients with kiwifruit allergy, presumably because of the high content of actinidin in the extract in combination with a predominant actinidin sensitization among these subjects. Actinidin makes up about 50% of the total kiwifruit protein, and the proportion is even higher in commercial extracts.²

In the current study, individual kiwifruit allergens applied as *in vitro* reagents for ImmunoCAP analysis proved to be a sensitive tool to diagnose IgE-mediated kiwifruit allergy. The allergen panel included natural purified kiwifruit allergens nAct d 1, nAct d 2, nAct d 3, nAct d 4, and nAct d 5. In addition, we have cloned and expressed the Bet v 1-related allergen, Act d 8, and the kiwifruit profilin, Act d 9. The diagnostic sensitivity of a positive ImmunoCAP result to at least 1 of these 7 kiwifruit allergens was 77%, comparable to that of PPTs with fresh kiwifruit and more than 4-fold higher than that of the commercial extract ImmunoCAP. The sensitivity of the self-prepared kiwifruit extract ImmunoCAP (63%) approached that of the single allergens (77%), suggesting that an improved production of kiwifruit extracts would also improve *in vitro* allergy diagnosis. However, preparation of reproducible and high-quality allergen extracts has been limited by the natural biochemical variability observed in disparate sources of kiwifruit. For example, it has been shown that both ripening stage and postharvest handling of kiwifruit can influence the relative amount of many protein compounds as well as the profile of IgE-binding components.²⁸ Furthermore, it has also been suggested that the profile of the IgE-binding components in the kiwifruit protein extract is significantly affected by the proteolytic activity of actinidin, which is present in high amounts.²⁹ To overcome the drawbacks of the kiwifruit extract, a single ImmunoCAP test for diagnosis of kiwifruit allergy could be manufactured by coupling an adequately balanced mixture of purified kiwifruit allergens. This approach has been shown to be highly suitable to demonstrate sensitization in patients with suspected cherry allergy.³⁰ Still, if a mix of purified allergens is used in a single test, the information about the individual reactivity pattern of a patient potentially linked with mild or severe symptoms will be missed.

To compare the sensitization pattern to the individual kiwifruit allergens (component-resolved diagnostics) with the clinical response, we used sera from well characterized patients with positive DBPCFC or anaphylactic reactions to kiwifruit and an appropriate control population. However, a total of 7 DBPCFC-positive patients were still negative in all ImmunoCAP tests used in this study. Two of these were also PPT-positive. We therefore assume that 1 or more relevant kiwifruit allergens are missing in the extracts used and remain to be identified. Our results indicate that the Bet v 1 homolog, Act d 8, and profilin, Act d 9, are important allergens in pollen-related kiwifruit allergy. Fifty-nine percent of the 22 patients with combined kiwifruit/pollen or kiwifruit/pollen/latex allergy were sensitized to rAct d 8, 27% to rAct d 9, and 36% to nAct d 3. Because the Bet v 1 homolog and profilin are not present in sufficient quantities in natural kiwifruit extract,²³ rAct d 8 and rAct d 9 are suitable tools to detect a sensitization to the kiwifruit in patients with pollen allergy with suspected kiwifruit allergy.

Fifty percent of the atopic control subjects, however, were also sensitized to rAct d 8 and to nAct d 3, and 20% to rAct d 9, indicating that IgE from control subjects with pollen or pollen/latex allergy recognizes Bet v 1 or pollen profilin-related epitopes on rAct d 8 or rAct d 9 and/or cross-reactive carbohydrate determinants on nAct d 3. Thus, IgE binding to highly cross-reactive

rAct d 8, rAct d 9, or nAct d 3 is not a clinically specific marker for kiwifruit allergy in the presence of pollen or pollen/latex sensitization. This observation is common in birch pollen-related food allergy and has been reported from other studies using purified Bet v 1 homologs and profilins.^{31,32} In contrast, it is noteworthy that none of the kiwifruit-mono-sensitized patients had rAct d 8 or rAct d 9-specific IgE.

Only 1 of 22 patients with combined kiwifruit/pollen or kiwifruit/pollen/latex allergy had a positive ImmunoCAP to actinidin (Act d 1), which has been reported in many studies to be a major kiwifruit allergen.^{1,12,13,33} However, actinidin was recognized by 5 of 8 kiwifruit-mono-sensitized patients, which may therefore qualify it as a specific biomarker for isolated allergy to kiwifruit, which tends to be clinically more severe than cross-reactive intolerance to the same food. Most importantly, sensitization to actinidin proved to be highly specific, because none of the atopic controls tested positive with nAct d 1. Apart from actinidin, the other kiwifruit specific allergens such as kiwellin and cystatin also seem to be relevant for the *in vitro* diagnosis of patients with a latex or pollen independent kiwifruit allergy. According to other studies, 21%¹ to 50%^{2,12} of kiwifruit allergy occurs in patients not allergic to pollen or latex.

No specific allergen recognition pattern could be attributed to the patients with combined allergy to kiwifruit, pollen, and latex (A3). These results are in agreement with those reported in 2 former studies in which the allergen recognition pattern of patients with kiwifruit and latex allergy has been assessed.^{1,12} Consequently, further research is necessary to identify a molecular basis of IgE cross-reactivity between latex and kiwifruit to account for the pattern of allergic responses observed in our subject pool.

In conclusion, we found that both an improved kiwifruit extract and the use of a panel of individual kiwifruit allergens had a major impact on the sensitivity of *in vitro* diagnosis of kiwifruit allergy, increasing the sensitivity from 17% of a commercial extract to 63% and 77%, respectively. Furthermore, the application of component-resolved diagnostic reagents enables the classification of patients into different reactor groups. Whereas actinidin may serve as a marker for isolated kiwifruit allergy, Act d 8 and Act d 9 are indicative for typical cross-reactivity patterns. Even though not statistically significant, there was a trend toward more severe reactions among patients with monoallergy. Furthermore, a component resolved diagnostic approach may facilitate precise selection of patients for kiwifruit immunotherapy and contributes to improve patient-tailored immunotherapy.

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Clinical implications: Measuring IgE specific to single kiwifruit allergens results in a high diagnostic sensitivity and allows the differentiation of subjects with primary and secondary kiwifruit allergy.

REFERENCES

- Aleman A, Sastre J, Quirce S, de las Heras M, Carnes J, Fernandez-Caldas E, et al. Allergy to kiwi: a double-blind, placebo-controlled food challenge study in patients from a birch-free area. *J Allergy Clin Immunol* 2004;113:543-50.
- Lucas JS, Grimshaw KE, Collins K, Warner JO, Hourihane JO. Kiwi fruit is a significant allergen and is associated with differing patterns of reactivity in children and adults. *Clin Exp Allergy* 2004;34:1115-21.
- Lucas JSA. Kiwi fruit allergy: a review. *Pediatr Allergy Immunol* 2003;14:420-8.
- Mattila L, Kilpelainen M, Terho EO, Koskenvuo M, Helenius H, Kalimo K. Food hypersensitivity among Finnish university students: association with atopic diseases. *Clin Exp Allergy* 2003;33:600-6.
- Eriksson NE, Werner S, Foucard T, Möller C, Berg T, Kiviloog J, et al. Self-reported hypersensitivity to exotic fruit in birch pollen-allergic patients. *Allergy International* 2003;52:199-206.
- Rance F, Grandmottet X, Grandjean H. Prevalence and main characteristics of schoolchildren diagnosed with food allergies in France. *Clinical and Experimental Allergy* 2005;35:167-72.
- Gall H, Kalveram KJ, Forck G, Sterry W. Kiwi fruit allergy: a new birch pollen-associated food allergy. *J Allergy Clin Immunol* 1994;94:70-6.
- Gavrovic-Jankulovic M, Cirkovic T, Burazer L, Vuckovic O, Jankov RM. IgE cross-reactivity between meadow fescue pollen and kiwi fruit in patients' sera with sensitivity to both extracts. *J Investig Allergol Clin Immunol* 2002;12:279-86.
- Pastorello EA, Pravettoni V, Ispano M, Farioli L, Ansaloni R, Rotondo F, et al. Identification of the allergenic components of kiwi fruit and evaluation of their cross-reactivity with timothy and birch pollens. *J Allergy Clin Immunol* 1996;98:601-10.
- Brehler R, Theissen U, Mohr C, Luger T. "Latex-fruit syndrome": frequency of cross-reacting IgE antibodies. *Allergy* 1997;52:404-10.
- Kim KT, Hussain H. Prevalence of food allergy in 137 latex-allergic patients. *Allergy Asthma Proc* 1999;20:95-7.
- Palacin A, Rodriguez J, Blanco C, Lopez-Torres G, Sanchez-Monge R, Varela J, et al. Immunoglobulin E recognition patterns to purified Kiwifruit (*Actinidia deliciosa*) allergens in patients sensitized to Kiwi with different clinical symptoms. *Clin Exp Allergy* 2008;38:1220-8.
- Bublin M, Mari A, Ebner C, Knulst A, Scheiner O, Hoffmann-Sommergruber K, et al. IgE sensitization profiles toward green and gold kiwifruits differ among patients allergic to kiwifruit from 3 European countries. *J Allergy Clin Immunol* 2004;114:1169-75.
- Latasa M, Dieguez I, Sanz ML, Parra A, Pajaron MJ, Oehling A. Fruit sensitization in patients with allergy to latex. *J Investig Allergol Clin Immunol* 1995;5:97-102.
- Beezhold DH, Sussman GL, Liss GM, Chang NS. Latex allergy can induce clinical reactions to specific foods. *Clin Exp Allergy* 1996;26:416-22.
- Bublin M, Radauer C, Knulst A, Wagner S, Scheiner O, Mackie AR, et al. Effects of gastrointestinal digestion and heating on the allergenicity of the kiwi allergens Act d 1, actinidin, and Act d 2, a thaumatin-like protein. *Mol Nutr Food Res* 2008;52:1130-9.
- Sampson HA, Munoz-Furlong A, Campbell RL, Adkinson NF Jr, Bock SA, Branum A, et al. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J Allergy Clin Immunol* 2006;117:391-7.
- Dreborg S, Foucard T. Allergy to apple, carrot and potato in children with birch pollen allergy. *Allergy* 1983;38:167-72.
- Dreborg S, Frew A. Position paper—allergen standardization and skin-tests. *Allergy* 1993;48:49-82.
- 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.
- Brocklehurst K, Baines BS, Malthouse JP. Differences in the interaction of the catalytic groups of the active centres of actinidin and papain: rapid purification of fully active actinidin by covalent chromatography and characterization of its active centre by use of two-protonic-state reactivity probes. *Biochem J* 1981;197:739-46.
- Crowhurst RN, Gleave AP, MacRae EA, Ampomah-Dwamena C, Atkinson RG, Beuning LL, et al. Analysis of expressed sequence tags from Actinidia: applications of a cross species EST database for gene discovery in the areas of flavor, health, color and ripening. *BMC Genomics* 2008;9:351.
- Oberhuber C, Bulley SM, Ballmer-Weber BK, Bublin M, Gaier S, DeWitt AM, et al. Characterization of Bet v 1-related allergens from kiwifruit relevant for patients with combined kiwifruit and birch pollen allergy. *Mol Nutr Food Res* 2008;52(suppl 2):S230-40.
- Love CA, Lilley PE, Dixon NE. Stable high-copy-number bacteriophage lambda promoter vectors for overproduction of proteins in *Escherichia coli*. *Gene* 1996;176:49-53.
- 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.
- Goldman L. Quantitative aspects of clinical reasoning. In: Principles of internal medicine. New York: McGraw-Hill; 1987.

27. Ballmer-Weber BK, Wuthrich B, Wangorsch A, Fotisch K, Altmann F, Vieths S. Carrot allergy: double-blinded, placebo-controlled food challenge and identification of allergens. *J Allergy Clin Immunol* 2001;108:301-7.
28. Ciardiello MA, Giangrieco I, Tuppo L, Tamburrini M, Buccheri M, Palazzo P, et al. Influence of the natural ripening stage, cold storage, and ethylene treatment on the protein and IgE-binding profiles of green and gold kiwi fruit extracts. *J Agric Food Chem* 2009;57:1565-71.
29. Tuppo L, Giangrieco I, Palazzo P, Bernardi ML, Scala E, Carratore V, et al. Kiwellin, a modular protein from green and gold kiwi fruits: evidence of in vivo and in vitro processing and IgE binding. *J Agric Food Chem* 2008;56:3812-7.
30. Ballmer-Weber BK, Scheurer S, Fritsche P, Enrique E, Cistero-Bahima A, Haase T, et al. Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol* 2002;110:167-73.
31. Hansen KS, Ballmer-Weber BK, Sastre J, Lidholm J, Andersson K, Oberhofer H, et al. Component-resolved in vitro diagnosis of hazelnut allergy in Europe. *J Allergy Clin Immunol* 2009;123:1134-41.
32. Reuter A, Lidholm J, Andersson K, Ostling 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.
33. Pastorello EA, Conti A, Pravettoni V, Farioli L, Rivolta F, Ansaloni R, et al. Identification of actinidin as the major allergen of kiwi fruit. *J Allergy Clin Immunol* 1998;101:531-7.
34. Gavrovic-Jankulovic M, Cirkovic T, Vuckovic O, Atanaskovic-Markovic M, Petersen A, Gojic G, et al. Isolation and biochemical characterization of a thaumatin-like kiwi allergen. *J Allergy Clin Immunol* 2002;110:805-10.
35. Rassam M, Laing WA. Purification and characterization of phytocystatins from kiwifruit cortex and seeds. *Phytochemistry* 2004;65:19-30.

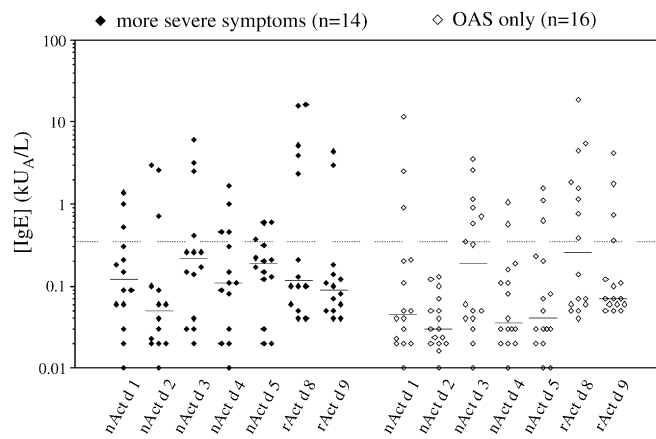


FIG E1. Specific serum IgE levels to the individual allergens of patients responding with more severe or systemic symptoms (see Table I, symptoms by case history) to ingestion of kiwifruit were compared with those with a pure OAS. Dotted lines indicate the 0.35 kU_A/L cutoff level. Median values are indicated.