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A critical assessment of allergen component-based *in vitro* diagnosis in cherry allergy across Europe

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Clinical and Experimental Allergy

Summary

Background Food allergy to cherry occurs throughout Europe, typically with restricted oral reactions in the central and northern parts but with frequent systemic reactions in the Mediterranean region. Previous studies have demonstrated insufficient sensitivity of commercially available cherry extract reagents in the diagnosis of cherry allergy.

Objective To assess the diagnostic performance of specific IgE tests based on recombinant cherry allergens in comparison with an extract-based assay and to skin prick test (SPT). A secondary objective was to analyse the frequency of systemic reactions in cherry-allergic subjects across Europe, including the largest population of LTP-sensitized subjects from central Europe studied to date.

Methods A total of 186 subjects from central Europe and Spain were studied. Serum IgE was analysed with ImmunoCAP tests carrying rPru av 1, 3 and 4, combined and separately, and cherry extract.

Results Among the central European cherry allergics, the mix of rPru av 1, 3 and 4 had a sensitivity of 95%, compared with 65% for cherry extract, and the IgE binding capacity of the recombinant mix was considerably higher. The sensitivity of the two tests was more comparable in the Spanish population, 95% and 86%, respectively. The recombinant allergen ImmunoCAP equalled SPT in terms of sensitivity and specificity. Consistent with previous reports, major geographic differences in sensitization pattern and prevalence of systemic reactions were found. A significantly higher rate of systemic reactions was found in Spanish patients sensitized to Pru av 3 whereas German patients sensitized to LTP only had oral allergy syndrome.

Conclusions The recombinant cherry allergen ImmunoCAP is a highly sensitive diagnostic tool, clearly superior to any diagnostic method based on cherry extract. Three cherry allergens are sufficient for detecting sensitization in 95% of cherry-allergic subjects. Systemic reactions are common in LTP-sensitized individuals but seem to require at least one additional causative factor.

Keywords cherry allergy, diagnostic sensitivity and specificity, ImmunoCAP, lipid transfer protein, recombinant allergen

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Introduction

The reliability of *in vitro* or *in vivo* diagnostic tests for food allergies depends strongly on the quality of the allergen reagents applied. Protein extracts derived from fruits often show an unbalanced allergen content or even a lack of allergenic activity caused by inherent enzyme

activities or other factors, as recently reviewed [1, 2]. Especially, diagnosis of birch pollen-related food allergy, the most common type of food allergy in northern and central Europe, can be affected as their major food allergens, belonging to the Bet v 1 homology group, are labile and are often degraded or otherwise inactivated during extraction.

Reagents currently applied in the diagnosis of birch pollen-related food allergies are based on protein extracts and frequently fail to detect specific IgE [3, 4]. For cherry in particular, a sensitivity as low as 20% in the ImmunoCAP assay [1] and the skin prick test (SPT) [5] has been reported. Prick-to-prick testing with fresh food is often applied, but cannot be standardized and depends on seasonal food availability.

The major cherry allergen Pru av 1 was recently shown to be particularly susceptible to degradation and to be largely missing in commercial cherry extracts [6, 7]. Such problems of allergen deficiency in natural extracts may be overcome by the application of recombinant allergens, produced at high purity and with batch-to-batch consistency, as diagnostic tools.

Recombinant allergens from several foods serologically related to pollens, such as hazelnut [8], celery [9, 10], apple [11], carrot [12, 13] and cherry [14] are now available. The recombinant cherry allergens rPru av 1, rPru av 3 and rPru av 4 have been found suitable in experimental measurement of specific IgE antibodies and as SPT reagents [5, 6]. Although recombinant food allergens have previously been shown to enable higher sensitivity in the detection of specific IgE than natural extracts of the corresponding food source [8, 15, 16], no diagnostic assay has been described so far that combines all relevant allergens of one food, thereby mimicking the allergenic complexity of a natural extract.

Characteristic differences in the sensitization pattern and clinical food reactions to cherry between Swiss/German and Italian/Spanish patients have been described, showing that systemic reactions are more common in patients from the Mediterranean region, who are predominantly sensitized to the lipid transfer protein Pru av 3 [5]. Similar north-south differences have also been reported for hazelnut [17] and unpublished data from the EU funded project SAFE [18] indicate a similar geographic pattern in apple allergy. In addition, studies performed in Spanish and Italian patients suggest that LTPs have the potential to elicit systemic reactions. This has been shown for peach [19], apple [20, 21] as well as other foods rarely reported to cause allergy, such as asparagus [22], lettuce [23] or maize [24]. A comprehensive review on the clinical relevance of LTPs has been published recently [25]. However, LTP-sensitized patients from central or northern Europe have never been studied before in a number that allowed the comparison with a population from southern Europe on a statistical basis.

The aim of this study was to investigate the suitability of the recombinant cherry allergens rPru av 1, rPru av 3 and rPru av 4, in particular a mix of the three recombinant allergens, as *in vitro* diagnostic tools in the ImmunoCAP system. For this purpose, the performance of recombinant allergen ImmunoCAP was compared with the performance of a commercially available cherry extract Immu-

noCAP and with previously published results of SPT that had been performed with the same recombinant cherry allergens in a subgroup of our study population [5].

The study was further aimed at identifying LTP-sensitized subjects in central Europe in order to assess the generality of the association between LTP sensitization and systemic reactions to fruits, which has been previously reported to exist in Mediterranean populations [19, 20, 23, 25, 26].

Methods

Subjects

A total of 143 cherry-allergic subjects, 20 atopic controls and 23 non-atopic controls from central Europe (Switzerland and Germany) and Spain were studied. Twenty subjects from Switzerland and two from Germany were included on the basis of a positive double-blind placebo-controlled food challenge (DBPCFC) result to cherry [5] and 97 subjects from Germany, 22 from Spain and two from Switzerland were included on a clinical history of cherry allergy. Symptoms after ingestion of cherry were either observed during oral provocation in patients who underwent DBPCFC or were reported by patients with a history of cherry allergy in the course of a detailed interview. The control populations comprised 20 (including subjects No. 29–47 of Ballmer-Weber *et al.* [5]) birch pollen-allergic subjects and 23 non-atopic individuals without cherry allergy, in all cases confirmed by a negative result in an open challenge with fresh cherries. All controls were included in Switzerland and Germany. Sixty-five percent of the atopic controls were SPT-positive to rPru av 1 and 30% to Pru av 4 whereas no positive result was observed with rPru av 3. All but one of the control subjects of this group had serum IgE specific for Bet v 1 [5]. The number of females/males, median age and age range of the study groups were as follows: 96/47, 30 years, 8–58 years (cherry-allergic subjects); 13/7, 27.5 years, 19–48 years (atopic controls); 8/15, 31 years, 24–39 years (non-atopic controls). Seventy-four of the subjects were previously part of another study on cherry allergy [5]. Detailed information about allergy symptoms and sensitization to birch, weed, grass, olive and plane pollen are summarized in Tables 1 and 2. All study participants provided written informed consent. Ethical approval was obtained from the local ethical committee.

Skin prick tests and double-blind placebo-controlled food challenge

SPT and DBPCFC with cherry were performed in the course of a previously published study [5]. Briefly, DBPCFC was performed by means of a two-step spit-and-

Table 1. Frequency of specific symptoms in cherry allergic subjects (%)

Subject category	Oral allergy a.s. only		Larynx-										Drop of blood pressure		
	n	Oral allergy a.s. only	Urticaria	Angiodema	Flush	Rhinitis	Conjunctivitis	edema	Cough	Dyspnea	Dysphagia	Nausea	Emesis	Diarrhoea	Shock
DBPCFC-positive, Switzerland and Germany	22	45	100	0	0	5	0	0	0	41	18	0	0	0	0
	99	73	92	2	1	1	5	6	1	5	11	0	0	4	0
History-positive, Germany and Switzerland	121	68	93	1.7	0.8	0.8	5.0	5.0	0.8	4.1	17	3.3	0.0	0.0	3.3
	22	50	64	27	27	0	9	0	0	0	18	0	0	0	9
Entire study population	143	50	68	4.3	3.8	0.5	4.3	3.2	0.5	2.7	13	2.2	0.0	0.0	2.2
															1.1

*Comprises DBPCFC-positive subjects from Switzerland and history positive subjects from Germany and Switzerland. DBPCFC, double-blind placebo-controlled food challenge.

swallow procedure. Placebo and active drinks were prepared according to the recipe suggested by the interest group of food allergy of the European Academy of Allergy and Clinical Immunology on their website (www.ig-food.org). SPT test was performed on the flexor aspect of the forearm, applying 25 µL of rPru av 1, rPru av 3 and rPru av 4 at 100, 50 and 10 µg/mL each. Areas of weal-and-flare reactions were recorded after 15 min. A weal size of 7 mm² was regarded as positive.

Specific immunoglobulin E measurements

Specific IgE levels were determined using the Pharmacia CAP System (ImmunoCAP FEIA) (Phadia AB, Uppsala, Sweden). A commercially available cherry ImmunoCAP was used to measure IgE to natural cherry extract. The cherry extract was prepared under conditions designed to preserve allergen activity [27]. Recombinant cherry allergens rPru av 1.0101, rPru av 3 and rPru av 4 were expressed in *Escherichia coli* and purified as previously described [6, 9, 14]. Experimental ImmunoCAP tests were manufactured by coupling the recombinant allergens combined in equimolar concentrations (rMIX) or individually to the solid phase, as described elsewhere [28, 29]. Bromelain ImmunoCAP was used to determine IgE reactivity to cross-reactive carbohydrate determinants (CCD) [30, 31] by ImmunoCAP tests.

Statistical analysis

Statistical data analysis was performed using SAS/STAT software (SAS Institute Inc., Cary, NC, USA). The χ^2 -test and Mann-Whitney rank sum test were applied where appropriate and are noted in the Results section. Statistical significance was accepted at a probability value of $P < 0.05$.

Results

Clinical observations

Allergy to cherry was accompanied by sensitization to birch pollen in 98% of the subjects from central Europe. Sensitization to grass and weed pollens was present in 74% and 54%, respectively. The remarkable difference regarding sensitization to weed pollen between the DBPCFC-positive and the history-positive group (Table 2) reflects very likely a geographic difference between both groups. The DBPCFC-positive subjects were exclusively Swiss, whereas the history-positive group was German, except one Swiss subject. No birch pollen sensitization was found among the Spanish cherry-allergic subjects, whereas grass and weed pollen sensitization was present in 36% and 55%, respectively. More characteristic for the Spanish subjects was sensitization to plane or olive tree

Table 2. Frequency of sensitization to different pollens and allergy to different foods (%)

Subject category	<i>n</i>	Birch pollen	Grass pollen	Weed pollen	Plane pollen	Olive pollen	Wall pellitory pollen	Apple	Peach	Hazelnut	Carrot	Celery
DBPCFC-positive, Switzerland	22	100	77	18	ND	ND	ND	100	82	91	41	32
History-positive, Germany and Switzerland	99	97	73	62	ND	ND	ND	95	61	79	53	28
Central Europe, total*	121	98	74	54	ND	ND	ND	96	64	81	50	29
History-positive, Spain	22	0	36	55	64	32	27	45 [†]	90 [†]	60 [†]	0 [‡]	9 [‡]
Entire cherry allergic subjects	143	83	68	54	–	–	–	89 [§]	68 [§]	78 [§]	46 [§]	27 [§]

*Comprises DBPCFC-positive subjects from Switzerland and history-positive subjects from Germany and Switzerland. [†]Data were not available for two subjects. [‡]Data were not available for 11 subjects. [§]Calculated for 141 or 132 subjects, not including the two or 11 patients for whom this information was not available.

DBPCFC, double-blind placebo-controlled food challenge; ND, not done.

pollen, which was found in 64% and 32% of the subjects, respectively. In addition to reactions to cherry, most subjects had experienced allergic reactions to other foods such as apple, peach, hazelnut carrot and celery. Allergy to apple and hazelnut was found to be most relevant in patients from central Europe, whereas allergic reactions to peach were characteristic of the Spanish population. Detailed information about co-sensitization to pollen and allergy to other foods is summarized in Table 2.

The most frequently reported symptoms to cherry were oral allergy syndrome (OAS) and dyspnea. OAS was found in 68% and dyspnea in 13% of the entire cherry-allergic population. None of the other recorded symptoms (angio-oedema, urticaria, rhinitis, flush, conjunctivitis, larynx oedema, cough, dysphagia, nausea, emesis, diarrhea and cardiovascular reactions) were reported in more than 5% of the subjects.

However, the subjects from central Europe and Spain showed marked differences regarding the frequency of specific symptoms. Ninety-three per cent (113/121) of the central European study population reported OAS and other mild symptoms, 1.7% (2/121) urticaria and 0.8% (1/121) angio-oedema, after ingestion of cherry. The same symptoms were reported by 64%, 27% and 27% of the Spanish subjects, respectively. These regional differences were found to be statistically significant ($P < 0.0001$, χ^2 -test). Further, episodes of anaphylactic reactions upon consumption of cherry were reported by three of the Spanish but none of the central European subjects. A peculiar observation made was that one Swiss subject (No. 143), who displayed symptoms upon cherry ingestion that were unusually severe (urticaria, severe angio-oedema, cough, dyspnea and gastrointestinal symptoms) in relation to other central European subjects, in fact originated from the Mediterranean area. Interestingly, allergy to cherry occurred the first time when the subject had already been living in Switzerland for a couple of years and was working as a fruit seller. Detailed information on symptoms to cherry among the subjects studied are shown in Table 1.

Recombinant allergens enable superior diagnostic sensitivity

Two different cherry allergen reagents, natural cherry extract and a mix of rPru av 1, 3 and 4 (rMIX), were evaluated in parallel with respect to their capacity to detect specific IgE in the central European and Spanish cherry-allergic subjects studied and in the atopic controls.

From the results of the testing (Fig. 1), three main observations were made: (i) the two tests displayed a significant difference in sensitivity and quantitative performance, (ii) the differences between them were more pronounced in the central European than in the Spanish population and (iii) the higher sensitivity of the rMIX tests were accompanied by more frequent detection of specific IgE in the birch pollen-allergic control population.

Of the 121 central European cherry-allergic subjects' sera, five were not detected with the rMIX test, as compared to 42 with the extract test. Equally striking were the differences in the ability of the two tests to quantify specific IgE: median concentration values obtained among subjects who tested positive were 7.7 and 1.3 kU_A/L, respectively, i.e. a sixfold difference between the rMIX and the extract.

Of the 22 Spanish subjects, only one tested negative with the rMIX ImmunoCAP and three with the extract. In this population, the median IgE values obtained with the two reagents were 5.6 and 3.6 kU_A/L, respectively, among subjects who tested positive.

Whereas four (20%) of the atopic controls tested positive with cherry extract ImmunoCAP, 16 (80%) tested positive with the rMIX, resulting in a lower clinical specificity of the rMIX test when this birch pollen-allergic control population was used as comparator. The median IgE value obtained with the rMIX for those 16 subjects was 3.2 kU_A/L, i.e. significantly ($P = 0.002$; Mann-Whitney rank sum test) lower than that for the cherry-allergic population from the same region (7.7 kU_A/L). However, as the range of IgE concentrations to the rMIX overlapped between the central European cherry-allergic subjects and the birch pollen-sensitized controls, the quantitative test

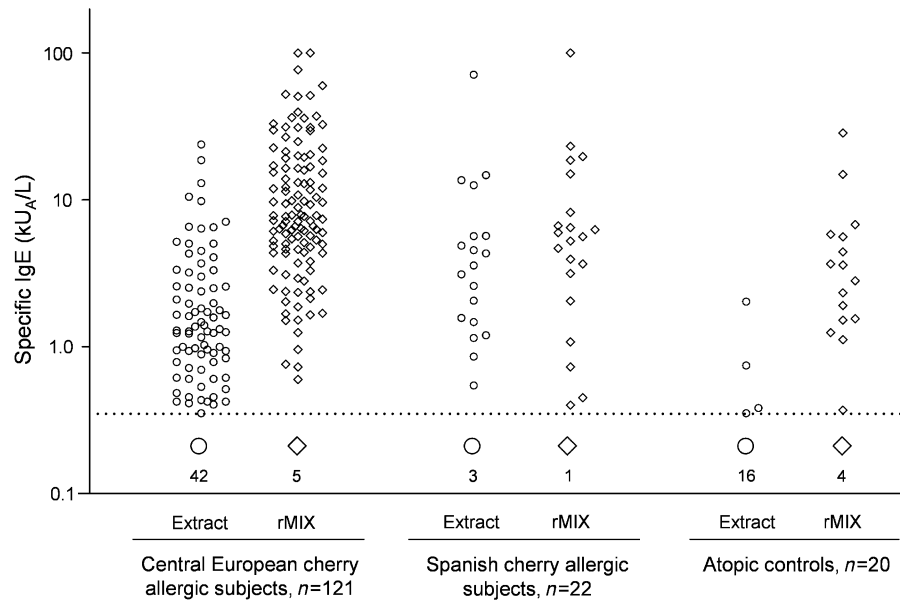


Fig. 1. Measurement of specific IgE to cherry extract and a mix of rPru av 1, rPru av 3 and rPru av 4 (rMIX) in the sera of cherry-allergic subjects from central Europe and Spain and birch pollen-sensitized control subjects without cherry allergy. The number of negative test results in each set of measurements is indicated below the dotted cut-off line (0.35 kU_A/L).

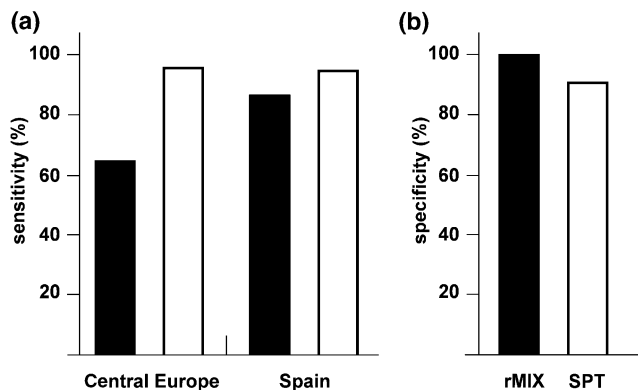


Fig. 2. (a) Sensitivity of extract ImmunoCAP (black) and rMIX ImmunoCAP (white) in subjects from central Europe and Spain. (b) Specificity of rMIX ImmunoCAP (black) and skin prick test (SPT) with recombinant allergens (white) in non-atopic individuals.

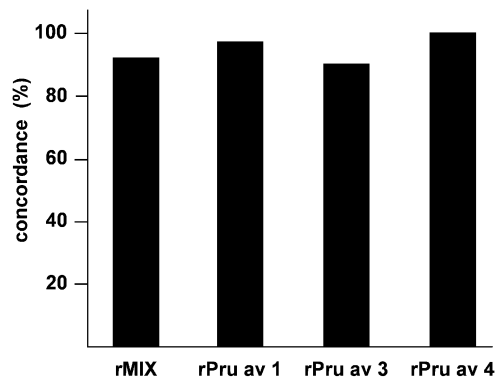


Fig. 3. Concordance between rMIX ImmunoCAP and skin prick test (SPT) with any of the three recombinant cherry allergens (rMIX) and between ImmunoCAP with single recombinant allergens and SPT with single recombinant cherry allergens (rPru av 1, 3 and 4).

result could not be used as a discriminating parameter at the level of individual subjects.

The sensitivity and clinical specificity of the tests, depicted in Figs 2a and b, were calculated to evaluate the potential of the rMIX ImmunoCAP as an *in vitro* diagnostic tool. The sensitivity of the rMIX was clearly superior to that of the extract-based ImmunoCAP in both the central European and the Spanish population, 96% and 95% as compared with 65% and 86%, respectively (Fig. 2a). An assay specificity of 100% was observed with the rMIX ImmunoCAP as no signals were obtained from the sera of non-atopic individuals (Fig. 2b).

Specific immunoglobulin E testing equals or exceeds skin prick test in diagnostic performance

Previously reported skin testing with a low-temperature cherry extract in the same non-atopic control population as the one used in this study demonstrated a specificity slightly lower (91%) [5] than that we observed with the rMIX ImmunoCAP. Concordance between IgE measurement and SPT results was assessed in a subset of subjects for which matching data were obtained: the 20 DBPCFC-positive patients, nine subjects from Spain, the birch pollen-allergic subjects without cherry allergy and the non-atopic controls. When the recombinant cherry

allergens Pru av 1, 3 and 4 were applied individually in both tests, concordant results were found in 90–100% of the cases (Fig. 3). Further, when the rMIX ImmunoCAP results were matched with SPT results to any of the individually applied recombinant cherry allergens, concordance with the SPT was found in 92% of the paired observations. It is interesting to note that SPT and ImmunoCAP showed similar performance in the birch pollen-allergic subjects with and without cherry allergy. Nine of the atopic controls had a positive SPT with cherry extract and 14 with any of the recombinant allergens, whereas 15 showed IgE binding to the rMIX ImmunoCAP.

Component-resolved ImmunoCAP measurements identify regional sensitization patterns

Measurements of specific IgE to the individual recombinant allergens in the central European and Spanish cherry-allergic study populations (Fig. 4) revealed dramatic regional differences in the sensitization pattern, which is consistent with previously reported observations [5, 6]. In the central European population, all but five subjects (96%) showed IgE reactivity to rPru av 1 whereas only one Spanish subject (5%) was sensitized to this allergen. In contrast, 20 of the 22 Spanish subjects (91%) were sensitized to rPru av 3, as compared with 13 of the 121 central European subjects (11%).

More similar proportions of the subjects showed IgE reactivity to rPru av 4, 22/121 (18%) in the central European population and 2/22 (9%) in the Spanish group. IgE antibody binding to bromelain, used here as an indicator of anti-CCD reactivity, was somewhat more common among the central European subjects (26%) than

in the Spanish population (9%). No relevant difference in the sensitization pattern was found between the DBPCFC- and case history-positive groups.

Interestingly, the vast majority of subjects showed IgE reactivity to only one of the recombinant allergens tested. Mono-sensitization to Pru av 1 was found in 75% of the subjects from central Europe whereas more than 80% of the Spanish individuals showed IgE binding only to rPru av 3. Predominant IgE reactivity to rPru av 4 occurred in six cherry-allergic subjects of whom one was sensitized to this allergen alone. Qualitative results of the ImmunoCAP analyses are summarized in Table 3.

Conditional association between Pru av 3 and systemic reactions?

A correlation between the sensitization pattern and clinical symptoms was observed for more severe reactions, namely urticaria, angio-oedema, drop of blood pressure and shock. Twelve subjects in this study reported one or more of these symptoms and 10 of those subjects (all but No. 27 and 52) were sensitized to Pru av 3. Interestingly, all but two of the subjects (No. 52 and 143) who had experienced systemic symptoms belonged to the Spain population whereas only one of the 13 central European subjects sensitized to Pru av 3 (No. 143, who originated from the Mediterranean area) had a history of such reactions. No statistical significant difference in the level of specific IgE to rPru av 3 was found between the central European and Spanish LTP-sensitized subjects ($P=0.306$; Mann-Whitney rank sum test). It is noteworthy that all subjects who had experienced systemic reactions were mono-sensitized to the LTP whereas all LTP-reactive

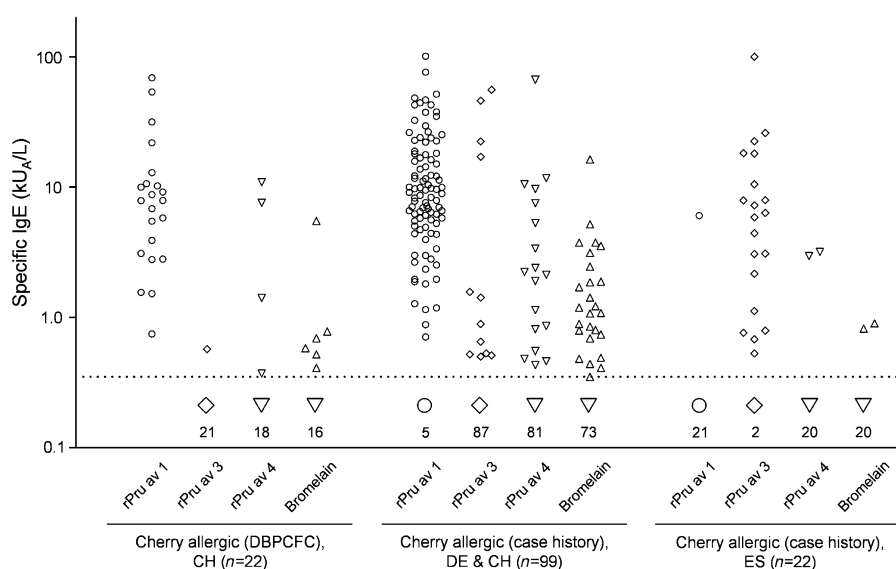


Fig 4. Component-resolved analysis of specific IgE in cherry-allergic subjects from central Europe and Spain, using ImmunoCAP tests carrying cherry allergens rPru av 1, rPru av 3, rPru av 4. Bromelain was included to determine IgE reactivity to cross-reactive carbohydrate determinants (CCD). The number of negative test results in each set of measurements is indicated below the dotted cut-off line.

Table 3. Prevalence* of specific IgE to Pru av 1, 3 and 4, rMIX and bromelain

Subject category	n	rPru av 1		rPru av 3		rPru av 4		rMIX	Extract	Bromelain
		Overall	Mono [†]	Overall	Mono [†]	Overall	Mono [†]			
All cherry allergic	143	82	64	23	14	17	0	96	68	23
Central Europe, total [‡]	121	96	75	11	1	18	0	96	64	26
Germany/Switzerland, DBPCFC-positive	22	100	77	5	0	18	0	95	59	27
Germany/Switzerland, history-positive	99	95	75	12	1	18	0	96	66	25
Spain, history-positive	22	5	5	91	86	9	0	95	86	9
Atopic control [§]	20	ND	ND	ND	ND	ND	ND	75	20	ND
Non-atopic control	23	ND	ND	ND	ND	ND	ND	0	0	ND

*Percentage of subjects with a specific IgE value ≥ 0.35 kUA/L. [†]Mono-sensitized. [‡]Germany/Switzerland DBPCFC- and history-positive. [§]Birch pollen allergic without cherry allergy.

DBPCFC, double-blind placebo-controlled food challenge; ND, not done.

subjects from central Europe, except patient No. 143, were co-sensitized to other cherry-allergens (Pru av 1 and/or Pru av 4). In LTP-sensitized subjects, regardless of origin, neither co-sensitization to plane or olive pollen, nor quantitative level of IgE to Pru av 3, correlated with type or severity of symptoms.

Discussion

Diagnostic applications of recombinant allergens have been intensely studied during recent years. The majority of studies have been performed on allergy to mites, moulds or pollen, especially birch pollen. However, even though a number of allergens relevant in allergy to plant-derived foods have been cloned and produced as recombinant proteins, few studies have been undertaken to evaluate their potential as tools for routine diagnosis, despite the well-known difficulties in diagnosis of allergy to many fruits and vegetables [32].

Ballmer-Weber *et al.* [5] demonstrated in a recent study the utility of recombinant cherry allergens Pru av 1, Pru av 3 and Pru av 4 as *in vivo* reagents in the diagnosis of cherry allergy. In the present study, the same allergens were evaluated with respect to their performance as *in vitro* reagents in a professional assay system used in clinical diagnosis of IgE-mediated allergy.

The recombinant allergens were used both combined in a single assay (rMIX) and individually, to establish sensitization profiles of the subjects studied.

The rMIX ImmunoCAP proved to be highly sensitive when used to detect specific IgE in the present study population comprising more than 140 cherry-allergic subjects. The sensitivity was $\geq 95\%$ in both the central European and Spanish populations investigated, which is at par with results typically obtained for respiratory allergies and remarkably high for food allergy. The natural extract ImmunoCAP displayed significantly lower sensitivity than the rMIX in the central European population, in which it failed to detect specific IgE in 35% of the

subjects. Higher sensitivity, 86%, was obtained in the Spanish population, presumably because of a more adequate content of Pru av 3 than Pru av 1 in the extract, in combination with a predominant LTP sensitization among these subjects.

The difference between the recombinant cherry allergens and the natural extract became even more evident when IgE quantitation results were compared. Among paired observations from the entire study population, the median factorial difference between the rMIX and extract test results was 6.3, suggesting that only about 15% of the IgE quantified with the rMIX test was typically bound by the natural extract reagent.

The findings reported here demonstrate that the rMIX ImmunoCAP is an excellent diagnostic reagent, displaying a sensitivity and quantitative capacity clearly exceeding what seems achievable with natural cherry extract.

Another observation made was a more frequent detection of specific IgE among the birch pollen-allergic controls with this test than with the extract-based test, presumably as a consequence of the higher antibody binding capacity of the rMIX ImmunoCAP. This phenomenon would particularly be related to Pru av 1, which is highly cross-reactive with birch pollen allergen Bet v 1, to which the majority of the atopic control subjects were sensitized. Thus, it is evident that specific IgE binding to rPru av 1 is not a clinically specific marker for cherry allergy in the presence of birch pollen sensitization. However, the rMIX ImmunoCAP would be highly suitable to confirm a sensitization to cherry allergens in patients with suspected cherry allergy.

It is noteworthy that such pollen-food cross-reactivity, which acts to hamper the conclusive interpretation of a positive test result, also affects SPT results to a similar extent when the two tests are performed with the same reagents. The SPT was positive in 14 (70%) of the birch pollen-allergic subjects when the recombinant allergens were applied and 15 (75%) showed IgE binding to the rMIX ImmunoCAP. These findings demonstrate that an allergen reagent with a

potency sufficient to reliably confirm sensitization in cherry-allergic patients will also detect cross-reactive sensitization in birch pollen allergics without cherry allergy more frequently, both in *in vitro* and in *in vivo* test systems. Food challenges are required to resolve cases when a positive test result cannot be conclusively interpreted.

The IgE antibody reactivity profiles established in the study populations were consistent with and corroborated previously reported findings on geographic differences in sensitization pattern to cherry allergens [5]. In the central European population, sensitization to Pru av 1 was vastly dominant and little sensitization to Pru av 3 was present, whereas the opposite was true for the Spanish population. Sensitization to Pru av 4 was of minor importance in both populations.

An almost equally salient north-south asymmetry as that for Pru av 1 and Pru av 3 sensitization existed for severity of symptoms upon cherry ingestion. All subjects but one experiencing systemic reactions were from the Mediterranean region and all but two were sensitized to LTP. This observation would suggest that Pru av 3 is able to induce severe and generalized reactions more frequently than Pru av 1 in sensitized individuals, a property that has been previously attributed to LTPs from several plant-derived foods [5, 7, 17, 19, 21–23, 25, 33]. However, the present study for the first time revealed LTP sensitization in 13 cherry-allergic subjects from central Europe (11%) but this group was found to be clinically different from Spanish LTP-sensitized subjects, as no clear correlation with symptom severity was found. Only one of these 13 subjects sensitized to Pru av 3 had systemic reactions and this patient originated from the Mediterranean area. Analysis of specific IgE levels to Pru av 3 did not provide an explanation for this phenomenon. As 10 of the 20 Spanish cherry allergics reported only OAS even though they were mono-sensitized to the LTP, we analysed sensitization to pollens characteristic of the Mediterranean region, such as *Parietaria*, olive and plane tree pollen, as possible co-factors. Again, no correlation was found with the severity of reactions in Spanish subjects. Furthermore, the severity of symptoms to cherry could not be correlated to allergic reactions to other foods such as peach, which is a particularly relevant allergenic food in Spain, apple, hazelnut, celery or carrot. Neither a history of allergic reactions to these foods in general, nor a history of particularly severe reactions to one or more of these foods indicated an increased probability to experience severe reactions to cherry. Consequently, our data suggest that even if rPru av 3 may carry diagnostic significance over rPru av 1 in relation to severe food reactions, the association appears moderately strong and seems to exist mostly in southern Europe. Level and composition of pollen exposure, amount of fruits consumed and other dietary habits, duration of relevant allergen exposure per year, climate, etc. may be taken into consideration as causative

factors enhancing clinical reactivity. It is also tempting to speculate that avidity of the antibody-allergen interaction may be of importance in this respect and that exposure and co-sensitization to LTPs from other sources, such as peach, could play a role in severe LTP-mediated food allergy. Whatever the nature may be of a factor that acts to potentiate the clinical reaction to LTPs, a recent report by Schad *et al.* [33], of anaphylactic reactions to grapes, raisins and wine in a German LTP-sensitized patient, suggests that it is not completely confined to the Mediterranean area. Further studies on this issue are clearly required before definitive conclusions can be drawn regarding the role of LTP sensitization in severe reactions to fruits.

We conclude that the experimental rMIX allergen ImmunoCAP carrying the recombinant cherry allergens rPru av 1, 3 and 4 is an excellent diagnostic tool that equals or exceeds SPT in terms of sensitivity as well as specificity. Even if other allergens, such as Pru av 2 [34] (thaumatin-like protein) or allergen isoforms (e.g. Pru av 1.0201 [35]), may be relevant in cherry allergy, the results indicate that they are not of critical importance for diagnostic purposes. Thus, for the first time in food allergy diagnosis, a mixture of recombinant allergens successfully mimicked a complex natural allergen extract.

Our data further indicate that the association between symptoms and sensitization profile, especially that between LTP and more severe reactions, seems to be complex and not restricted to the molecular properties of the offending allergen.

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References

- Bohle B, Vieths S. Improving diagnostic tests for food allergy with recombinant allergens. *Methods* 2004; 32:292–9.
- Vieths S, Scheurer S, Reindl J *et al.* Optimized allergen extracts and recombinant allergens in diagnostic applications. *Allergy* 2001; 56 (Suppl. 67):78–82.
- Vieths S, Hoffmann A, Holzhauser T, Muller U, Reindl J, Hausteine D. Factors influencing the quality of food extracts for *in vitro* and *in vivo* diagnosis. *Allergy* 1998; 53:65–71.
- Bruijnzeel-Koomen CA, Ortolani C, Aas K *et al.* Position paper: adverse reactions to food. *Allergy* 1995; 50:623–35.
- Ballmer-Weber BK, Scheurer S, Fritsche P *et al.* Component-resolved diagnosis with recombinant allergens in patients with cherry allergy. *J Allergy Clin Immunol* 2002; 110:167–73.
- Scheurer S, Pastorello EA, Wangorsch A, Kastner M, Hausteine D, Vieths S. Recombinant allergens Pru av 1 and Pru av 4 and a

- newly identified lipid transfer protein in the *in vitro* diagnosis of cherry allergy. *J Allergy Clin Immunol* 2001; 107:724–31.
- 7 Scheurer S, Lauer I, Foetisch K *et al.* Strong allergenicity of Pru av 3, the lipid transfer protein from cherry, is related to high stability against thermal processing and digestion. *J Allergy Clin Immunol* 2004; 114:900–7.
 - 8 Luttkopf D, Muller U, Skov PS *et al.* Comparison of four variants of a major allergen in hazelnut (*Corylus avellana*) Cor a 1.04 with the major hazel pollen allergen Cor a 1.01. *Mol Immunol* 2002; 38:515–25.
 - 9 Scheurer S, Wangorsch A, Nerkamp J *et al.* Cross-reactivity within the profilin panallergen family investigated by comparison of recombinant profilins from pear (Pyr c 4), cherry (Pru av 4) and celery (Api g 4) with birch pollen profilin Bet v 2. *J Chromatogr B Biomed Sci Appl* 2001; 756:315–25.
 - 10 Scheurer S, Wangorsch A, Hausteiner 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.
 - 11 Vanek-Krebitz M, Hoffmann-Sommergruber K, Laimer da Camara MM *et al.* Cloning and sequencing of Mal d 1, the major allergen from apple (*Malus domestica*), and its immunological relationship to Bet v 1, the major birch pollen allergen. *Biochem Biophys Res Commun* 1995; 214:538–51.
 - 12 Hoffmann-Sommergruber K, O'Riordain G, Ahorn H *et al.* Molecular characterization of Dau c 1, the Bet v 1 homologous protein from carrot and its cross-reactivity with Bet v 1 and Api g 1. *Clin Exp Allergy* 1999; 29:840–7.
 - 13 Ballmer-Weber B, Wangorsch A, Bohle B *et al.* Component-resolved *in vitro* diagnosis of carrot allergy: does the use of recombinant carrot allergens improve the reliability of the diagnostic procedure? *Clin Exp Allergy* 2005; 35:970–8.
 - 14 Scheurer S, Metzner K, Hausteiner D, Vieths S. Molecular cloning, expression and characterization of Pru a 1, the major cherry allergen. *Mol Immunol* 1997; 34:619–29.
 - 15 Schocker F, Luttkopf D, Scheurer S *et al.* Recombinant lipid transfer protein Cor a 8 from hazelnut: a new tool for *in vitro* diagnosis of potentially severe hazelnut allergy. *J Allergy Clin Immunol* 2004; 113:141–7.
 - 16 Mittag D, Vieths S, Vogel L *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.
 - 17 Pastorello EA, Vieths S, Pravettoni V *et al.* Identification of hazelnut major allergens in sensitive patients with positive double-blind, placebo-controlled food challenge results. *J Allergy Clin Immunol* 2002; 109:563–70.
 - 18 Hoffmann-Sommergruber K. The SAFE project: 'plant food allergies: field to table strategies for reducing their incidence in Europe' an EC-funded study. *Allergy* 2005; 60:436–42.
 - 19 Fernandez-Rivas M, Gonzalez-Mancebo E, Rodriguez-Perez R *et al.* Clinically relevant peach allergy is related to peach lipid transfer protein, Pru p 3, in the Spanish population. *J Allergy Clin Immunol* 2003; 112:789–95.
 - 20 Sanchez-Monge R, Lombardero M, Garcia-Selles FJ, Barber D, Salcedo G. Lipid-transfer proteins are relevant allergens in fruit allergy. *J Allergy Clin Immunol* 1999; 103:514–9.
 - 21 Pastorello EA, Pravettoni V, Farioli L *et al.* Clinical role of a lipid transfer protein that acts as a new apple-specific allergen. *J Allergy Clin Immunol* 1999; 104:1099–106.
 - 22 Diaz-Perales A, Tabar AI, Sanchez-Monge R *et al.* Characterization of asparagus allergens: a relevant role of lipid transfer proteins. *J Allergy Clin Immunol* 2002; 110:790–6.
 - 23 San Miguel-Moncin M, Krail M, Scheurer S *et al.* Lettuce anaphylaxis: identification of a lipid transfer protein as the major allergen. *Allergy* 2003; 58:511–7.
 - 24 Pastorello EA, Farioli L, Pravettoni V *et al.* The maize major allergen, which is responsible for food-induced allergic reactions, is a lipid transfer protein. *J Allergy Clin Immunol* 2000; 106:744–51.
 - 25 Pastorello EA, Robino AM. Clinical role of lipid transfer proteins in food allergy. *Mol Nutr Food Res* 2004; 48:356–62.
 - 26 Asero R, Mistrello G, Roncarolo D *et al.* Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000; 122:20–32.
 - 27 Björkstén F, Halmeupuro L, Hannuksela M, Lahti A. Extraction and properties of apple allergens. *Allergy* 1980; 35:671–7.
 - 28 Axen R, Drevin H, Kober A, Yman L. A new laboratory system applied to allergy testing. *N Engl J Allergy Proc* 1988; 9:503.
 - 29 Marknell DeWitt A, Niederberger V, Lehtonen 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.
 - 30 Mari A. IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the *in vivo* and *in vitro* reactivity. *Int Arch Allergy Immunol* 2002; 129:286–95.
 - 31 Mari A. Skin test with a timothy grass (*Phleum pratense*) pollen extract vs. IgE to a timothy extract vs. IgE to rPhl p 1, rPhl p 2, nPhl p 4, rPhl p 5, rPhl p 6, rPhl p 7, rPhl p 11, and rPhl p 12: epidemiological and diagnostic data. *Clin Exp Allergy* 2003; 33:43–51.
 - 32 Ortolani C, Ispano M, Pastorello EA, Ansaloni R, Magri GC. Comparison of results of skin prick tests (with fresh foods and commercial food extracts) and RAST in 100 patients with oral allergy syndrome. *J Allergy Clin Immunol* 1989; 83:683–90.
 - 33 Schad SG, Trcka J, Vieths S *et al.* Wine anaphylaxis in a German patient: IgE-mediated allergy against a lipid transfer protein of grapes. *Int Arch Allergy Immunol* 2005; 136:159–64.
 - 34 Inschlag C, Hoffmann-Sommergruber K, O'Riordain G *et al.* Biochemical characterization of Pru a 2, a 23-kD thaumatin-like protein representing a potential major allergen in cherry (*Prunus avium*). *Int Arch Allergy Immunol* 1998; 116:22–8.
 - 35 Reuter A, Fortunato D, Garoffo LP *et al.* Novel isoforms of Pru av 1 with diverging immunoglobulin E binding properties identified by a synergistic combination of molecular biology and proteomics. *Proteomics* 2005; 5:282–9.