

ORIGINAL ARTICLE

Sensitization profiles in birch pollen-allergic patients with and without oral allergy syndrome to apple: lessons from multiplexed component-resolved allergy diagnosis

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Clinical & Experimental Allergy

Summary

Background Component-resolved diagnosis (CRD) using microarray technology has recently been introduced into the field of clinical allergology.

Objective To further validate the use of CRD by microarray technology in allergy diagnosis.

Methods Thirty-seven patients allergic to birch pollen were included. The discriminative value of apple-specific IgE (sIgE), recombinant Mal d 1 (rMal d 1) sIgE, apple skin prick test and rMal d 1 on the microarray was assessed between patients with a birch-related oral allergy syndrome to apple (OAS⁺, *n* = 20) and healthy control individuals (HC, *n* = 8) without a history of inhalant allergies or apple-induced OAS. An additional comparative analysis was carried out with individuals allergic to birch pollen allergy without OAS (OAS[−]; *n* = 17).

Results rMal d 1 coupled to the microarray constitutes a discriminative marker between OAS⁺ and HC with a sensitivity 95% and a specificity of 100%. However, in parallel with the traditional sIgE assay, 15 out of 17 OAS[−] individuals (88%) also displayed IgE reactivity to rMal d 1 coupled to the microarray. OAS[−] individuals are more frequently sensitized to mite (about three to four times), cat and dog dander (about two to three times) and grass pollen (about 1.5 times) as compared with OAS⁺ patients.

Conclusion At first glance, CRD by microarray seems to be a reliable instrument in the diagnosis of apple-mediated OAS in birch pollen allergy. However, for discriminating between sensitization and a real allergy, micro-arrayed rMal d 1 offers no advantage over conventional quantification of rMal d 1 sIgE. Most interestingly, within a single run, birch pollen-allergic patients without OAS to apple were shown to display a broader sensitization to classical inhalant allergens than birch pollen-allergic patients with an apple-related OAS.

Keywords apple, component-resolved diagnosis, IgE, microarray, oral allergy syndrome

Submitted 30 April 2009; revised 4 July 2009; accepted 7 July 2009

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Introduction

The cloning of the major allergen Der p 1 from house dust mite (HDM) (*Dermatophagoides pteronyssinus*) in the late 1980s [1] constituted the birth of component-resolved diagnosis (CRD), a new instrument to manage IgE-mediated allergy. In contrast to the traditional sIgE assays, CRD does not rely on whole extract preparations (potentially ill-defined mixtures that contain relevant and irrelevant components) from native allergens but specific IgE antibodies are quantified to single purified or recombinant components. The principles and potential of CRD have been detailed extensively elsewhere [2–5]. Briefly,

CRD includes marker allergens to investigate the genuine sensitization of patients towards a given allergen source as well as cross-reactive molecules that point to cross-sensitization to several allergen sources. These ‘gate-keeper’ tests not only create the opportunity to establish individual sensitization profiles but also help in discriminating between sensitization and real allergy, enable an analysis of the individual risk and might facilitate the selection of patients for specific immunotherapy and to monitor the immunological effects of treatment.

However, the number of different purified and recombinant allergen components has steadily increased, and, for several reasons, it has become virtually impossible to

analyse all the single allergen components in a single patient by traditional singleplexed sIgE tests. Recently, the CRD concept has been extended to multiplex testing with several (>100) components on microarrays [6–9]. This multiplexing allows, with a minute amount of serum and in a single measurement, the identification of diagnostic patterns that facilitate the formulation of diagnostic algorithms.

Although the technique seems highly promising, additional comprehensive studies are necessary before it can enter mainstream clinical application [10].

In our regions, pollen-associated food allergy is primarily caused by apple and is almost exclusively related to birch pollen allergy [11]. Symptoms are generally associated with the consumption of raw apples and include rapid-onset pruritus and/or angio-oedema of the oropharynx and occasionally pruritus in the ears, tightness of the larynx or both [oral allergy syndrome (OAS)]. The prevailing hypothesis is that sensitization to Bet v 1, the major birch pollen allergen (birch is *Betula verrucosa*), triggers the synthesis of specific sIgE antibodies that are capable of cross-reacting with its homologue in apple Mal d 1 (*Malus domestica*). It was anticipated that the OAS to apple in birch pollen-allergic patients could serve as a suitable model for further clinical validation of CRD by microarray technology, as this condition can be diagnosed easily on the grounds of history and a standardized questionnaire [12]. Moreover, we hypothesized that the OAS model could enable us to determine whether multiplexed CRD can discriminate between sensitization to apple and genuine apple allergy in patients suffering from birch pollen allergy. Actually, it has been speculated that microarrays could be better in discriminating between sensitization and allergy based on the stoichiometry of the assay. In contrast to traditional sIgE assays that are characterized by an excess of allergen coupled to the solid phase, only a minor amount of allergen material is spotted on the microarray.

Materials and methods

Subjects

Thirty-seven patients allergic to birch pollen already partially characterized elsewhere [13] were included. Briefly, the diagnosis of birch pollen allergy was established by an evocative history in combination with a positive skin prick test (SPT) and sIgE for birch pollen. All patients were free of medication and specific immunotherapy. All investigations were performed out of the pollen season.

The discriminative value of apple sIgE, recombinant Mal d 1 (rMal d 1) sIgE, apple SPT and rMal d 1 on the microarray was assessed in 20 patients with a birch-related OAS to apple [OAS⁺, M: 5, F: 15; age 38 years

(11–65)] and eight healthy control individuals [HC, M: 2, F: 6; age 36 years (21–52)] without a history of inhalant allergy or apple-induced OAS. The diagnosis of OAS was based on a compelling history of repetitive pruritus and/or angio-oedema of the lips, tongue, throat and/or palate due to consumption of raw apples. In HC, inhalation allergy was ruled out by a negative sIgE and SPT for common inhalant allergens. An additional comparative analysis was carried out in 17 birch pollen-allergic patients with regular and recent uneventful apple consumption [OAS⁺; M: 6, F: 11; age 28 years (14–41)]. The local ethics committee approved the study and patients and controls gave informed consent.

Specific immunoglobulin E

Quantification of total IgE and sIgE for HDM, birch, timothy grass, mugwort, cat, dog, *Cladosporium herbarum*, the recombinant major apple allergen rMal d 1 and bromelain [as an indicator for sensitization to highly cross-reactive carbohydrate determinants (CCD)] was performed with ImmunoCAP FEIA (Phadia, Brussels Belgium) according to the manufacturer's instructions. Values of allergen-specific IgE <0.35 kU/L were considered negative.

Skin prick tests

SPTs included the above-mentioned inhalant allergens (HAL, Haarlem, the Netherlands) and Jonagold apple (for extract preparation see [13]). Skin test responses were considered positive only when the weal reaction exceeded 3.

Basophil activation test

The principles of the basophil activation test (BAT) were detailed elsewhere [13]. Briefly, within 3 h of sampling, aliquots of endotoxin-free heparinized (Vacuette, Greiner Labortechnik GmbH, Kremsmünster, Austria) whole blood were pre-incubated with an IL-3-containing stimulation buffer. Pre-activated blood samples were stimulated with different concentrations of dialysed Jonagold apple extract applied in the skin tests, anti-IgE (BD Pharmingen, Biosciences, Erembodegem, Belgium) as a positive control or washing solution to measure spontaneous CD63 expression (negative control). To estimate activated basophils, cells were stained with monoclonal biotinylated anti-human IgE (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and monoclonal phycoerythrin-conjugated anti-human CD63 (BD, Biosciences, San José, CA, USA) or phycoerythrin-conjugated irrelevant control antibody of identical isotype (BD Biosciences). After washing, streptavidin conjugated with Alexa Fluor 488 (Molecular Probes, Leiden, the Netherlands) was added.

Red blood cells were lysed and white blood cells were fixed (FACS lysing solution, BD Immunocytometry Systems, San José, CA, USA). After centrifugation, washing solution was added to the cell pellets. Flow cytometric analysis of basophil activation was performed on a FACScan flow cytometer (BD, Immunocytometry Systems). Green fluorescence and side scatter were used to gate out the basophils that expressed a high density of surface IgE. Subsequently, within this gate, the percentage of activated basophils, i.e., co-expressing CD63, was measured. For this purpose, a histogram marker was set on the 99th percentile value obtained with an irrelevant isotype control antibody. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression from the value obtained with allergen stimulation. According to dose-response curves and ROC curve analysis, the cut-off point was set at 17% [13].

Component resolved diagnosis microarray

We used a commercially available allergen microarray immunoassay (ISACTM, VBC Genomics Bioscience Research, Vienna, Austria) as recommended by the manufacturers and recently described elsewhere [8, 14]. Briefly, microarray reaction sites were incubated with 20 µL of undiluted patients' serum for 2 h in order to capture allergen-specific IgE antibodies by their corresponding allergen. Subsequently, the microarray slides were rinsed

and washed with buffer solution in order to remove unbound serum sIgE. After drying, complexes of allergen-bound specific IgE were stained with a secondary, fluorescence-labelled anti-human IgE for 1 h at room temperature protected from light. After a second rinsing and washing procedure, the fluorescence signals obtained were scanned using a laser scanner (LuxScan 10K, CapitalBio, Beijing, China). Analysis of the corresponding digitized microarray image data was performed with ImmunoCAP ISAC software and image information was transformed into numerical data according to a reference serum with a known IgE content. Data were expressed as ISAC standardized units (ISU/L).

Statistical analysis

Results were expressed as median (range). Non-parametric tests were used where appropriate. SPSS 16 software was applied for data analyses.

Results

Skin tests

The Jonagold SPT was positive in 19 out of the 20 OAS⁺ patients, and in none of the HC and 2/17 (11.8%) OAS⁻ individuals (Fig. 1).

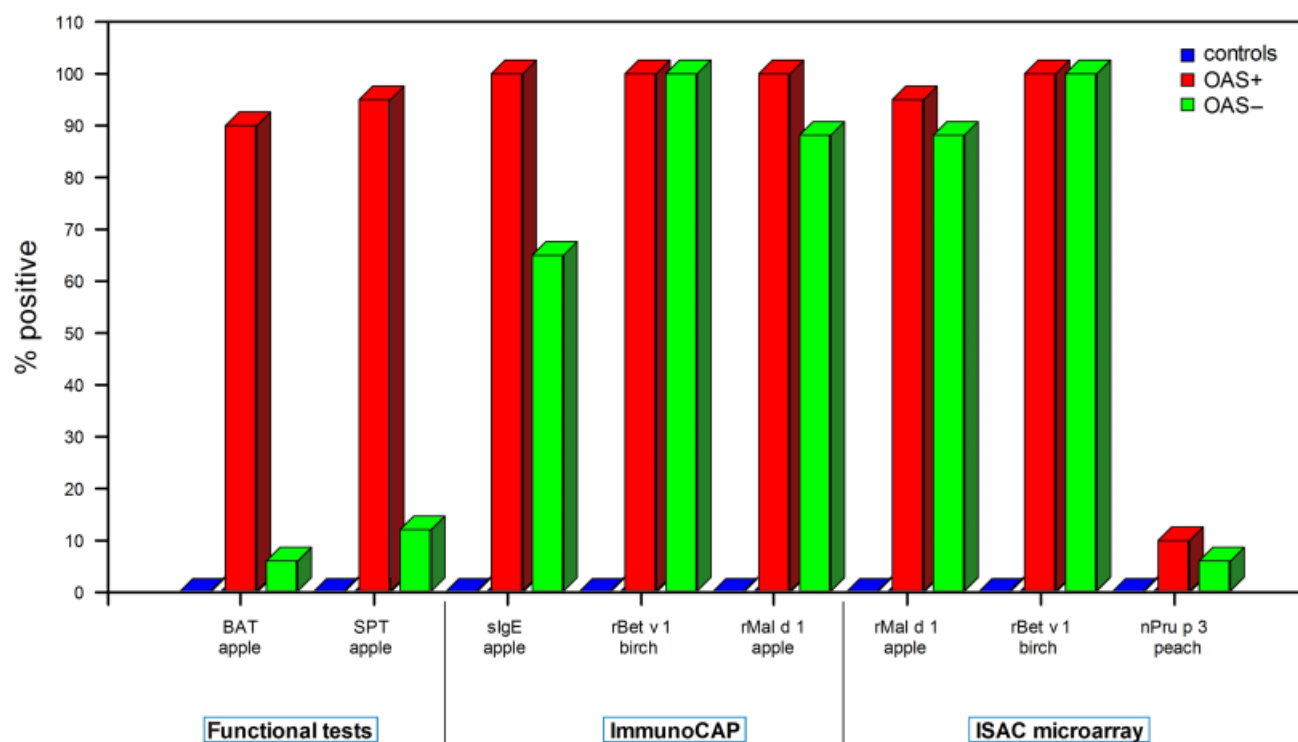


Fig. 1. Percentages of positive basophil activation tests (BAT), skin prick tests (SPT) and sIgE results by ImmunoCAP and ISAC microarray in control individuals (CO), birch pollen-allergic patients with an oral allergy syndrome (OAS) to apple (OAS⁺) and without apple allergy (OAS⁻).

Table 1. OAS⁺: birch pollen allergy with an oral allergy syndrome (OAS) to apple, OAS⁻: birch pollen allergy without apple-mediated OAS

	Controls (<i>n</i> = 8)			Patients OAS ⁺ (<i>n</i> = 20)			Patients OAS ⁻ (<i>n</i> = 17)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
Plants									
Primarily genuine markers									
nAct d 1	-	-	-	-	-	-	1	0.16	-
nAct d 2	-	-	-	-	-	-	1	7.39	-
nAct d 5	-	-	-	1	0.33	-	-	-	-
rBer e 1	-	-	-	-	-	-	1	0.62	-
rAna o 2	-	-	-	-	-	-	-	-	-
nCor a 9	-	-	-	-	-	-	-	-	-
nSes i 1	-	-	-	2	1.00	(0.74–1.25)	-	-	-
nAra h 1	-	-	-	1	0.4	-	1	1.4	-
nAra h 2	-	-	-	-	-	-	1	0.93	-
nAra h 3	-	-	-	-	-	-	-	-	-
nGly m β-conglycinin	-	-	-	7	3.25	(0.38–7.21)	6	1.01	(0.34–4.27)
nGly m glycinin	-	-	-	-	-	-	-	-	-
nTri a 18	-	-	-	-	-	-	-	-	-
rTri a 19.0101	-	-	-	-	-	-	-	-	-
nTri a aA_TI	-	-	-	-	-	-	-	-	-
nTri a Gliadin	-	-	-	-	-	-	-	-	-
nCyn d 1	-	-	-	9	6.76	(0.77–15.81)	8	1.71	(0.42–9.07)
rPhl p 1	-	-	-	14	7.09	(0.28–49.63)	16	4.37	(0.39–22.81)
rPhl p 2	-	-	-	7	1.21	(0.4–48.67)	11	5.12	(0.37–26.46)
rPhl p 4	-	-	-	6	8.68	(1.4–30.19)	9	2.09	(0.96–11.95)
rPhl p 5	-	-	-	7	12.23	(1.38–61.81)	7	9.71	(0.52–35.45)
r'Phl p 6	-	-	-	5	2.68	(2.13–24.01)	5	1.68	(0.58–8.83)
rPhl p 11	-	-	-	3	1.26	(0.4–12.13)	3	4.07	(3.61–6.48)
nOle e 1	1	0.79	-	3	3.32	(0.28–14.39)	2	1.02	(1–1.04)
rPla a 1	-	-	-	-	-	-	-	-	-
nPla a 2	-	-	-	5	0.61	(0.24–1.23)	2	2.09	(0.64–3.54)
nCry j 1	-	-	-	4	0.68	(0.57–0.87)	-	-	-
nCup a 1	-	-	-	4	0.38	(0.33–0.64)	-	-	-
nAmb a 1	-	-	-	-	-	-	-	-	-
nArt v 1	-	-	-	1	6.72	-	1	6.73	-
nSal k 1	-	-	-	-	-	-	1	0.32	-
rHev b 1	-	-	-	-	-	-	1	0.26	-
r'Hev b 3	-	-	-	-	-	-	-	-	-
rHev b 5	-	-	-	-	-	-	1	1.86	-
rHev b 6	-	-	-	1	0.73	-	1	0.19	-
Cross-reactive markers									
PR-10/Bet v 1 homologues									
rBet v 1	-	-	-	20	34.66	(5.32–71.91)	17	12.18	(1.12–55.82)
rAln g 1	-	-	-	18	7.09	(0.58–56.42)	15	2.83	(0.46–22.1)
rCor a 10101	-	-	-	19	6.15	(0.68–50.49)	14	2.75	(0.38–18.22)
rCor a 10401	-	-	-	16	11.63	(2.53–61.45)	14	2.70	(0.41–26.82)
rMal d 1	-	-	-	19	6.07	(0.02–57.87)	15	1.48	(0.3–15.53)
r'Pru p 1	-	-	-	18	5.65	(0.43–57.75)	14	1.63	(0.3–22.97)
rGly m 4	-	-	-	-	-	-	-	-	-
rAra h 8	-	-	-	14	2.83	(0.49–34.25)	10	0.89	(0.27–7.9)
rApi g 1	-	-	-	8	4.15	(0.51–9.96)	2	4.09	(1.42–6.76)
rDau c 1	-	-	-	4	1.15	(0.28–3.27)	1	1.94	-
nAct d 8	-	-	-	7	3.13	(0.52–7.61)	1	0.48	-
Lipid transfer proteins									
nPru p 3	-	-	-	2	1.17	(0.07–2.26)	1	3.39	-
rCor a 8	-	-	-	1	0.63	-	1	0.89	-
nArt v 3	-	-	-	2	1.20	(0.37–2.03)	2	1.48	(1.07–1.88)
rPar j 2	-	-	-	-	-	-	-	-	-

Table 1. continued

	Controls (<i>n</i> = 8)			Patients OAS ⁺ (<i>n</i> = 20)			Patients OAS ⁻ (<i>n</i> = 17)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
Profilins									
rBet v 2	–	–	–	4	2.05	(0.54–16.83)	2	2.94	(1.64–4.24)
nOle e 2	–	–	–	3	3.39	(0.4–14.03)	2	2.56	(1.28–3.84)
rHev b 8	–	–	–	5	3.21	(0.57–19.54)	2	3.54	(1.58–5.49)
rMer a 1	–	–	–	4	2.01	(0.01–17.89)	2	5.60	(2.08–9.11)
rPhl p 12	–	–	–	3	1.51	(0.33–14.87)	2	3.74	(1.44–6.03)
Calcium-binding 2-EF-hand proteins									
rBet v 4	–	–	–	–	–	–	2	5.92	(4.53–7.31)
rPhl p 7	–	–	–	1	0.29	–	2	7.17	(5.1–9.24)
CCD marker									
nAna c 2	–	–	–	1	0.71	–	1	0.59	–
Non-plants									
Primarily genuine markers									
nGal d 1	–	–	–	–	–	–	–	–	–
nGal d 2	–	–	–	–	–	–	–	–	–
nGal d 3	–	–	–	–	–	–	–	–	–
nBos d 4	–	–	–	–	–	–	–	–	–
nBos d 5	–	–	–	–	–	–	–	–	–
nBos d 8	–	–	–	1	0.57	–	–	–	–
nBos d lactoferrin	–	–	–	–	–	–	–	–	–
rBla g 1	–	–	–	–	–	–	1	0.35	–
rBla g 2	–	–	–	–	–	–	–	–	–
rBla g 4	–	–	–	–	–	–	–	–	–
rBla g 5	–	–	–	–	–	–	–	–	–
rFel d 1	–	–	–	8	5.18	(0.26–14.9)	14	4.70	(0.99–22.52)
rFel d 4	–	–	–	–	–	–	3	1.55	(0.38–1.57)
rCan f 1	–	–	–	3	0.76	(0.25–6.84)	6	7.38	(0.25–27.99)
rCan f 2	–	–	–	1	0.66	–	1	2.84	–
nMus m 1	–	–	–	–	–	–	1	0.40	–
rAlt a 1	–	–	–	2	12.22	(0.7–23.73)	1	10.68	–
rAlt a 6	–	–	–	–	–	–	1	4.14	–
rAsp f 1	–	–	–	3	0.39	(0.29–0.44)	–	–	–
rAsp f 2	–	–	–	–	–	–	–	–	–
rAsp f 3	–	–	–	–	–	–	–	–	–
rAsp f 4	–	–	–	–	–	–	–	–	–
rAsp f 6	–	–	–	–	–	–	1	5.35	–
rCla h 8	–	–	–	–	–	–	–	–	–
rAni s 1	1	0.8	–	–	–	–	–	–	–
nApi m 1	–	–	–	2	2.14	(0.94–3.34)	2	0.86	(0.52–1.2)
nApi m 4	–	–	–	1	0.54	–	–	–	–
Cross-reactive markers									
Cysteine protease									
nDer p 1	–	–	–	4	7.33	(0.84–24.9)	13	15.58	(1.99–59.62)
nDer f 1	–	–	–	4	5.92	(0.54–28.96)	13	13.43	(0.5–56.07)
NPC2 family									
rDer f 2	–	–	–	6	7.44	(1.22–56.26)	12	29.18	(2.47–61.96)
nDer p 2	–	–	–	6	6.49	(0.88–46.72)	12	26.45	(3.36–61.76)
rEur m 2	–	–	–	5	2.64	(0.52–13.2)	12	4.17	(0.29–31.87)
Parvalbumin									
rCyp c 1	–	–	–	–	–	–	–	–	–
rGad c 1	–	–	–	–	–	–	–	–	–
Tropomyosin									
rPen a 1	–	–	–	–	–	–	–	–	–
nPen i 1	–	–	–	–	–	–	–	–	–

Table 1. continued

	Controls (<i>n</i> = 8)			Patients OAS ⁺ (<i>n</i> = 20)			Patients OAS ⁻ (<i>n</i> = 17)		
	<i>N</i>	Median	Range	<i>N</i>	Median	Range	<i>N</i>	Median	Range
nPen m 1	-	-	-	-	-	-	-	-	-
rDer p 10	-	-	-	-	-	-	-	-	-
nBla g 7	-	-	-	1	0.26	-	-	-	-
rAni s 3	-	-	-	-	-	-	-	-	-
Serum albumin									
nBos d 6	-	-	-	-	-	-	-	-	-
nFel d 2	-	-	-	1	0.73	-	1	3.22	-
nCan f 3	-	-	-	1	0.8	-	1	2.09	-
nEqu c 3	1	0.24	-	1	0.25	-	2	1.83	(0.43–3.22)
nGal d 5	-	-	-	-	-	-	-	-	-

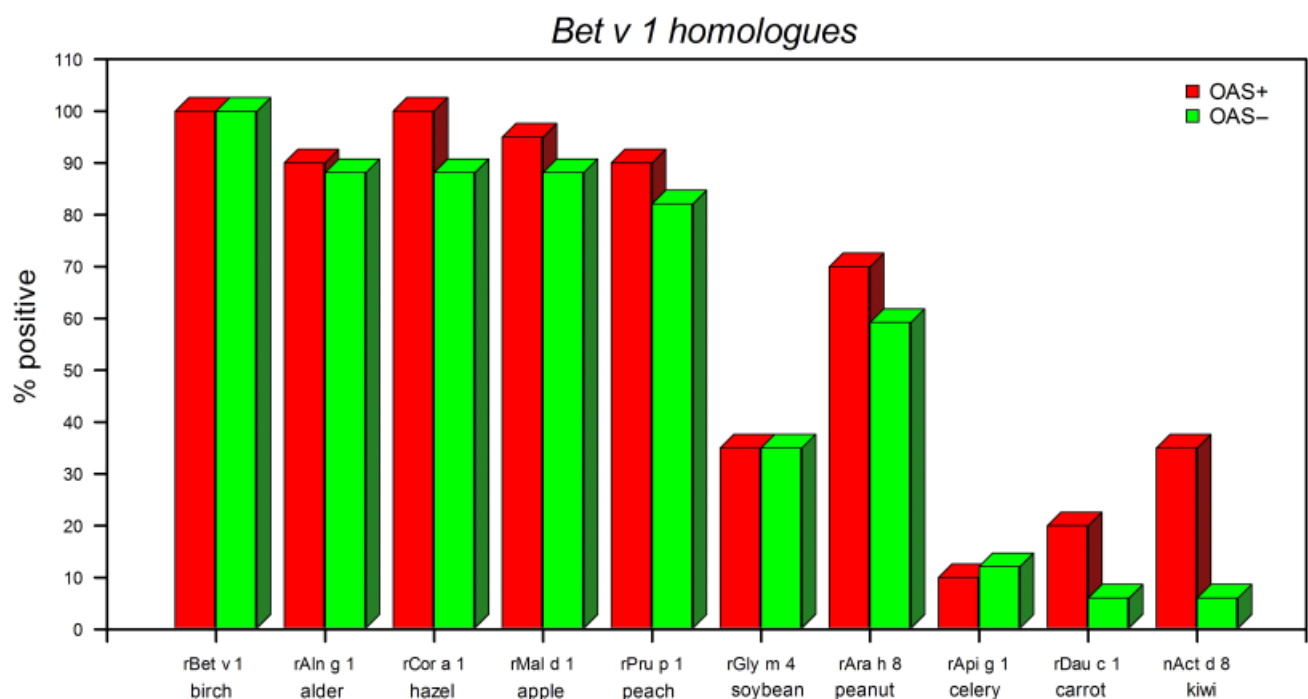


Fig. 2. Prevalence of sensitization to the different recombinant and purified Bet v 1 homologues. Apart from rDau c 1 from carrot (*Daucus carota*) and nAct d 8 from kiwi (*Actinidia deliciosa*), which were found to be more frequently positive in OAS⁺, OAS⁺ patients and OAS⁻ individuals demonstrated a comparable prevalence of sensitization for rBet v 1 from birch (*Betula verrucosa*), Aln g 1 from alder (*Alnus glutinosa*), Cor a 1 from hazel (*Corylus avellana*), Pru p 1 from peach (*Prunus persica*), Gly m 4 from soy (*Glycine max*), Ara h 8 from peanut (*Arachis hypogaea*) and Api g 1 from celery (*Apium graveolens*). OAS, oral allergy syndrome.

Total and specific immunoglobulin E

Total IgE in OAS⁺ patients, OAS⁻ individuals and HC was 124 kU/L (13–2886), 259 kU/L (38–7600) and 31 kU/L (5–92), respectively (Kruskal–Wallis test $P = 0.001$).

Apple sIgE in the OAS⁺ patients was 2.8 kUA/L (0.4–41.8) and 1.1 kUA/L (1.3–4.5) in the OAS⁻ group (Mann–Whitney U -test $P = 0.01$). IgE for the recombinant major apple allergen (rMal d 1) was 8.3 kUA/L (1 to > 100) in the OAS⁺ patients and 1.9 kUA/L (< 0.35–26.0) in the OAS⁻ individuals, respectively (Mann–Whitney U -test

$P = 0.002$). The percentages of positive sIgE results for apple and rMal d 1 are shown in Fig. 1.

Healthy control individuals displayed negative sIgE results for the above-mentioned classical inhalant allergens, apple and rMal d 1.

Basophil activation test

The BAT for apple was positive in 18 out of 20 OAS⁺, 1/17 OAS⁻ and none of the eight HC. The percentages

of positive BATs in OAS⁺, OAS⁻ and HC are displayed in Fig. 1.

Microarray

Table 1 displays the results of all 103 purified and recombinant components tested in the microarray. From these data, it is clear that the technique constitutes a specific tool with almost no false quantifications in healthy control children and adults without classical inhalant and/or food allergy. Actually, only three positive results were observed out of a total of 824 quantifications (0.004%) performed in HC individuals.

Figure 1 summarizes the most relevant results of the apple-related OAS in birch pollen allergy. These data show that rMal d 1 coupled to the microarray constitutes a highly discriminative marker between OAS⁺ and HC with a sensitivity 95% and a specificity of 100%. However, in parallel with the traditional singleplexed sIgE assay, 15/17 OAS⁻ individuals (88%) also displayed IgE reactivity for rMal d 1 coupled to the microarray. Moreover, both techniques were highly related (Spearman $r=0.801$, $P<0.001$). Receiver-operating curve (ROC) analysis between OAS⁺ patients and OAS⁻ individuals generated an rMal d 1-specific threshold of 4.4 kUA/L for the traditional sIgE and 2.4 ISU/L for the microarray assay. However,

these calculated decision thresholds did not improve the discriminative capacity of the rMal d 1-based tests between OAS⁺ and OAS⁻ (data not shown). Three patients also demonstrated sensitization to Pru p 3, a lipid transfer protein from peach (*Prunus persica*). The two OAS⁺ patients demonstrated more systemic reactions associated with their OAS, whereas the OAS⁻ individual was asymptomatic.

Figure 2 summarizes the sensitization to different recombinant and purified Bet v 1 homologues. Except for rDau c 1 from carrot (*Daucus carota*) and nAct d 8 from kiwi (*Actinidia deliciosa*), which were found to be more frequently positive in OAS⁺, OAS⁺ patients and OAS⁻ individuals demonstrated a comparable prevalence of sensitization for rBet v 1 from birch (*B. verrucosa*), Aln g 1 from alder (*Alnus glutinosa*), Cor a 1 from hazel (*Corylus avellana*), Pru p 1 from peach (*P. persica*), Gly m 4 from soy (*Glycine max*), Ara h 8 from peanut (*Arachis hypogaea*) and Api g 1 from celery (*Apium graveolens*).

Figure 3 summarizes the sensitization profiles for traditional inhalant allergens. It emerges that OAS⁻ individuals are more frequently sensitized to common aeroallergens such as mite (about three to four times), cat and dog dander (about two to three times) and grass pollen (about 1.5 times) than OAS⁺ patients. Figure 3 also displays the percentages of positive results of CCD and

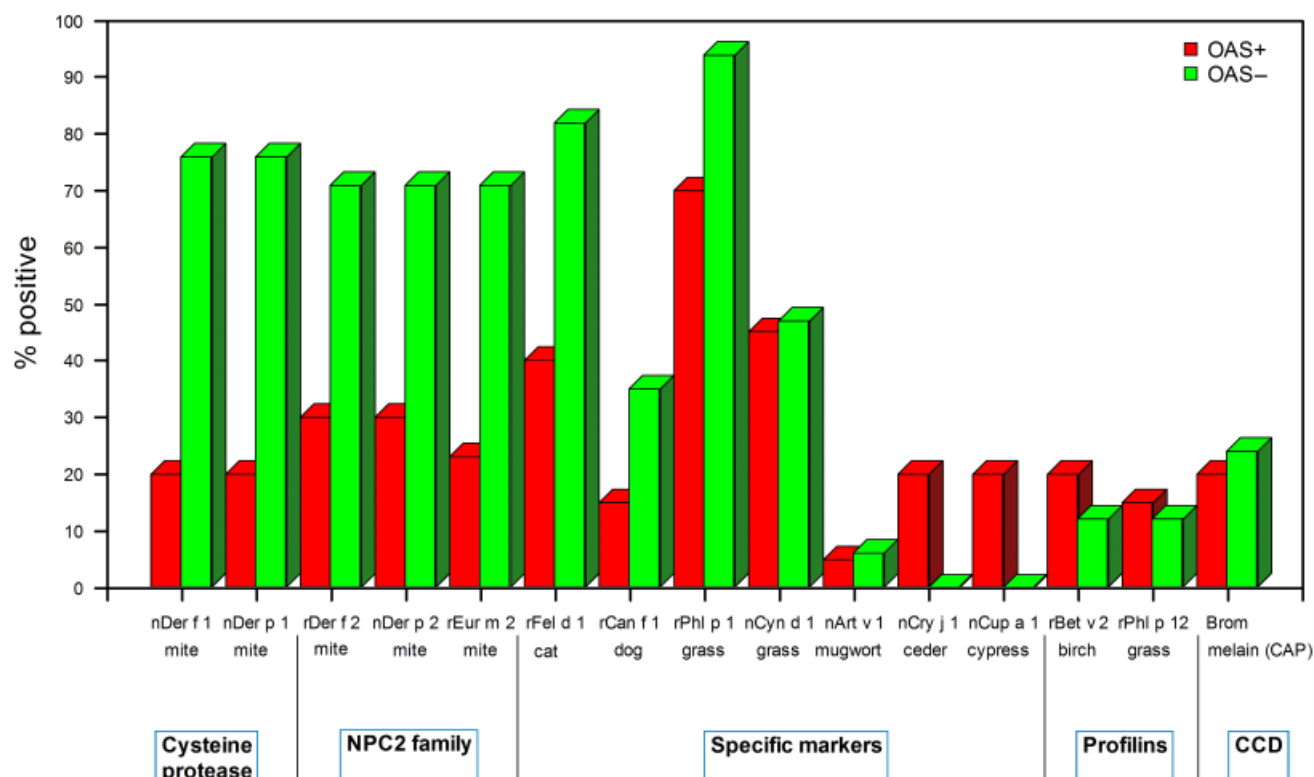


Fig. 3. Sensitization profiles for genuine markers of classical inhalant allergens in birch pollen-allergic patients with apple-related OAS (OAS⁺) and without OAS to apple (OAS⁻). Der f, *Dermatophagoides farinae*; Der p, *Dermatophagoides pteronyssinus*; Eur m, *Euroglyphus maynei*; Fel d, *Felis domesticus*; Can f, *Canis familiaris*; Phl p, *Phleum pratense*; Cyn d, *Cynodon dactylon*; Art v, *Artemisia vulgaris*; Cry j, *Cryptomeria japonica*; Cup a, *Cupressus arizonica*; Bet v, *Betula verrucosa*; OAS, oral allergy syndrome.

profilins, both structures well known to mimic allergy to apple [15]. No difference in sensitization to CCD and profilin between OAS⁺ patients and OAS⁻ individuals is demonstrable. Finally, Fig. 4 shows the box plots of microarray values of the most relevant genuine and cross-reactive plant and non-plant components in OAS⁺ and OAS⁻. In most cases, the OAS⁺ group shows higher sIgE antibody levels to Bet v 1 homologues (mid panel), whereas the OAS⁻ group shows generally higher levels to genuine allergens from mites and animal dander (bottom).

Discussion

At first glance, our data suggest that quantification of sIgE antibodies against the recombinant major apple allergen (rMal d 1) by microarray technology and ImmunoCAP constitute reliable instruments in the diagnosis of OAS to apple in birch pollen allergy. However, the most critical need for *in vitro* testing for food allergy generally, and especially for pollen-related food allergy, are tests that discriminate between cross-reactivity and true allergy [16]. As already exemplified in the introductory paragraph, in our regions, apple-mediated OAS almost invariably results from a primary sensitization to the major birch pollen allergen Bet v 1 [11, 12]. Consequently, an additional comparative analysis in non-apple-allergic individuals with birch pollen allergy (OAS⁻) was performed. As our results indicate, quantification of sIgE for the recombinant major apple allergen fails to discriminate between birch pollen-allergic patients with and without an OAS to apple, irrespective of the method applied. Thus, the OAS model does not seem to endorse the presumption that a smaller amount of allergen on the microarray might better discriminate between sensitization and real allergy than a traditional solid-phase assay that is characterized by an excess of allergen. Alternatively, our data confirm that functional tests such as skin tests and basophil activation assays can discriminate between sensitization and genuine birch-related apple allergy.

One of the major advantages of CRD by microarray is that the technique offers the opportunity to establish and compare individual sensitization profiles in one single measurement. Our data suggest that birch-allergic patients with an OAS to apple exhibit a clearly different overall sensitization profile as compared with birch pollen-allergic patients tolerant to apple. Actually, birch pollen-allergic patients without apple allergy are more frequently sensitized to other common inhalant allergens such as mite, animal dander from cats and dogs and grass pollen. In contrast, no significant difference in sensitization to ubiquitous structures such as β -1,2 xylose and core α -1,3 fucose-containing CCD, both structures well known to mimic allergy for apple [15], was demonstrated.

In conclusion, this study re-emphasizes that the validation of a diagnostic test cannot be considered appropriate

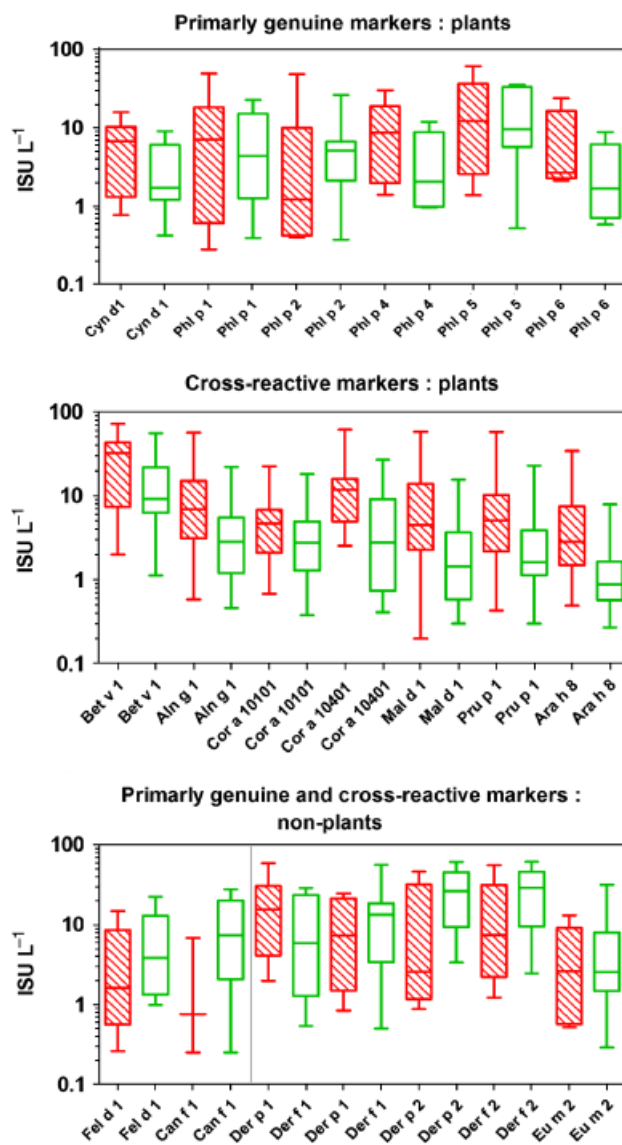


Fig. 4. Box plot and whiskers representing the minimum and maximum values of microarray results of genuine and cross-reactive plant components in OAS⁺ (red hatched) and OAS⁻ (green open). In most cases, the OAS⁺ group shows higher sIgE antibody levels to Bet v 1 homologues (mid panel), whereas the OAS⁻ group shows generally higher levels to genuine allergens from mites and animal dander (bottom). Cyn d, *Cynodon dactylon*; Phl p, *Phleum pratense*; Bet v, *Betula verrucosa*; Aln g, *Alnus glutinosa*; Cor a, *Corylus avellana*; Pru p, *Prunus persica*; Ara h, *Arachis hypogea*; Fel d, *Felis domesticus*; Can f, *Canis familiaris*; Der p, *Dermatophagoides pteronyssinus*; Der f, *Dermatophagoides farinae*; Eur m, *Euroglyphus maynei*; OAS, oral allergy syndrome.

when the assessment is restricted to a comparison between patients and healthy controls without taking into account conditions that might confound the outcome. Once again, it is confirmed that cross-reactivity has a pronounced effect on the specificity of *in vitro* sIgE quantification that fails to discriminate between sensitization and real allergy. Most intriguingly, this study reveals that birch pollen-allergic patients with and without OAS to apple display

different sensitization profiles to traditional indoor and outdoor aeroallergens, particularly HDM and animal dander. However, additional studies are essential to improve the confidence of the observation that birch pollen-allergic patients with and without associated food allergy present with different overall sensitization profiles.

Acknowledgements

The authors would like to thank Mrs Christel Mertens, Mr Paul van Endert and Mr Nicky Dillis for their technical skills and Phadia for providing the microarrays and ImmunoCAP rMal d 1.

This study was supported by the 'Instituut voor de Aanmoediging van Innovatie door Wetenschap en Technologie van Vlaanderen': basophil activation new applications in nutritional allergy (BANANA) project (IWT 060521).

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