

New Food Allergies in a European Non-Mediterranean Region: Is *Cannabis sativa* to Blame?

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Key Words

Allergy · Basophil · Cannabis · Lipid transfer protein · Food · Specific IgE

Abstract

Background: Allergy to fruit and vegetables exhibit geographic variation regarding the severity of symptoms and depending on the sensitization profile of the patient. These sensitization profiles and routes remain incompletely understood. Cannabis is a very popular drug and derived from *Cannabis sativa*, a plant containing lipid transfer proteins (LTP) also known as important allergens in plant and fruit allergies. In this study we sought to elucidate a potential connection between *C. sativa* allergy and plant food allergies. **Methods:** A case-control study involving 21 patients consulting for plant food allergies. Twelve patients were cannabis allergic and 9 had a pollen or latex allergy without cannabis allergy. Testing for cannabis IgE implied measurement of specific IgE, skin testing and basophil activation tests. Allergen component analysis was performed with a microarray technique. **Results:** Plant food allergy in patients with documented cannabis allergy had more severe reactions than patients without cannabis allergy and frequently implied fruits and vegetables that are not observed in a (birch) pollen-related food

syndrome. With the exception of 1 patient with cannabis allergy, all were sensitized to nonspecific (ns)-LTP. **Conclusion:** Our data suggest that illicit cannabis abuse can result in cannabis allergy with sensitization to ns-LTP. This sensitization might result in various plant-food allergies. Additional collaborative studies in different geographical areas are needed to further elucidate on this hypothesis.

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Introduction

Allergy to fruits and vegetables is an important and evolving health issue [1] that is most frequently acquired via a direct sensitization over the gastrointestinal tract or a primary sensitization to pollen or to a lesser extent *Hevea latex* [2]. Today, it appears that fruit and vegetable allergies exhibit geographic and age-related variation regarding the severity of symptoms and depending on the sensitization profile of the patient. In Belgium, as in most other north European regions, plant food allergy in adults is generally characterized by an oral allergy syndrome (OAS) due to sensitization to the labile homologues of Bet v 1, the major allergen from birch (*Betula verrucosa*) pollen [3–8]. In contrast, children frequently demonstrate

more severe and generalized reactions that are generally not related to pollen allergy but are frequently due to sensitization to different seed-storage proteins (e.g. vicillins and legumines from the Cupin superfamily) and 2S albumins that belong to the superfamily of prolamins [2, 6, 8, 9].

In contrast, in the Mediterranean non-birch endemic region plant food allergy originates frequently from sensitization to nonspecific lipid transfer proteins (ns-LTPs), another member of the prolamins superfamily [4, 7, 8, 10]. Different ns-LTPs have been identified in various foods including Rosaceae, nuts, peanuts and cereals [11, 12]. Among these food ns-LTPs, Pru p 3 from peach (*Prunus persica*) [13] appears to dominate the ns-LTP-related plant food allergies, resulting from a direct sensitization via the gastrointestinal tract [14–18]. However, alternative sensitization routes via the respiratory tract or even skin have been suggested to clarify the observed geographical differences in ns-LTP sensitization, and several studies have shown the presence of ns-LTP in various pollen species [12, 19–21]. In our regions, sensitization towards ns-LTP is present in less than 5% of pollen allergic patients [5, 12] and it remains obscure whether pollen ns-LTP might be an important cause of food allergy.

Cannabis is a generic term for different preparations (marijuana or weed, hashish, hashish oil) derived from *Cannabis sativa* (order: Rosales, family: Cannabaceae) that contain high levels of psychoactive substances like cannabinoids, particularly δ^9 -tetrahydrocannabinol. Although cannabis use is illegal in most countries, it is ubiquitously used for its relaxing or euphoric effects, especially by inhalation/smoking, mostly by adolescents and young adults. The global number of illicit cannabis users was (under)estimated at 125–203 million people (2.8–4.5% of the global population aged 15–64 years in 2009) [22, 23]. Medical cannabis has been suggested to exhibit antispasmodic and muscle-relaxant properties as well as stimulating appetite. Its effectiveness as an analgesic agent has been proposed and disputed as well. Recently, an ns-LTP has been purified from *C. sativa* and designated as Can s 3 [24], and it has been speculated that Can s 3 constitutes a primary source of ns-LTP sensitization that might explain particular plant food allergies in Southern Europe [24–26].

For some time, we have been observing an increasing number of patients with marijuana or hashish allergy presenting with plant food allergies difficult to explain by a cross-reactivity with pollen. These patients reported generalized reactions to fruit and vegetables rarely observed in our regions. Therefore, we sought to further elucidate

on a potential connection between use of *C. sativa* and plant food allergies, and to assess whether this ‘*cannabis plant-food syndrome*’ could originate from cross-reacting ns-LTPs or involves other plant allergen components.

Materials and Methods

Patients and Control Subjects

Twenty-one patients were included reporting an OAS and/or systemic reactions to fruits, vegetables, nuts or cereals as detailed elsewhere [5, 6]. Twelve patients (median age 29 years, range 5–41) were selected because of reporting compelling symptoms towards *C. sativa* with immediate respiratory (rhinitis, conjunctivitis or asthma) and/or cutaneous (itching, urticaria) symptoms upon active and/or passive (e.g. smoking, cutaneous transmission by others) exposure to marijuana or hashish. The remaining 9 patients (median age 33 years, range 21–51) did not use cannabis. All patients completed a standardized questionnaire about inhalant allergy and latex allergy. Confirmatory testing for pollen and latex allergy comprised measurement of specific IgE (sIgE) and skin-prick tests (SPT) as described elsewhere [27]. Testing for cannabis involved quantification of sIgE antibodies, SPT and basophil activation tests (BATs) that were developed as described below. Therefore, 5 healthy control individuals (median age 49, range 24–59) were also included. All patients and control individuals gave informed consent approved by the local ethics committee (study reference No. B300201213041).

Extract Preparation

Preparations of cannabis ns-LTP extracts were performed as described before [28]. Before use, the lyophilisate was resolubilized in PBS and the total amount of protein was quantified by the Micro BCA Protein Assay (Pierce, Rockford, Ill., USA). Protein concentration of the cannabis extract used varied between 0.01 and 100 μ g/ml. PAGE characterization of the extract showed a clear band with a molecular weight of about 9 kDa, corresponding to the ns-LTPs (not shown).

Total and sIgE

Total and sIgE for rBet v 1 (as a marker for birch pollen allergy), Timothy grass, *Hevea* latex, rPru p 3 and *C. sativa* (industrial hemp) were quantified by Immuno-CAP (Thermo Fisher Scientific, Uppsala, Sweden). All these sIgE assays are readily available, except for the *C. sativa*, from which a prototype was kindly provided by Thermo Fisher Scientific. Assays were performed according to the manufacturer’s instructions and results ≥ 0.35 kUA/l were considered positive.

Multiplexed Component-Resolved Diagnosis

In order to establish the sensitization profile of our patients we employed the allergen microarray immunoassay (ISACTM 112, Thermo-Fisher Scientific) as recommended by the manufacturer. Briefly, microarray reaction sites were incubated with 30 μ l 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

Table 1. Demographics, clinics and relevant laboratory findings of the 21 food-allergic patients and 5 control individuals

Patient No.	Sex/age	<i>C. sativa</i>				Food allergy	Total IgE	Specific IgE		
		cutaneous symptoms	respiratory symptoms	SPT	sIgE			rPru p 3	rBet v 1	GP
1	M/24 ¹	U, A	B	+	1.85	Orange, banana, beer	121	2.73	1.94	3.16
2	F/31 ¹	–	R, C, B*	+	4.65	Hazelnut, almond, tomato, celery, cherry	151	6.61	4.60	<0.35
3	F/34 ¹	U	R, C	+	3.49	Apple, grape, tomato, banana, walnut	752	6.18	9.04	<0.35
4	F/27 ¹	–	R, C, A	NA	5.67	Peanut, hazelnut, cashew, apple, plum, peach	192	8.54	3.44	1.88
5	F/24	U, A	R, C, B	+	11.0	Apple, cherry, hazelnut, walnut, peanut	166	20	<0.35	<0.35
6	M/28 ¹	–	A	–	31.40	Hazelnut, peach	1,050	40	14.40	11.4
7	M/29 ¹	–	R	+	6.21	Apple, hazelnut, banana	81	31.6	0.64	<0.35
8	M/40 ¹	–	A	NA	1.48	Peach, peanut	208	0.71	14.10	<0.35
9	F/24 ¹	I, U*	R, C*	+	3.10	Apple, peach, tomato, nuts	43	2.62	0.36	<0.35
10	F/31 ¹	U	R, C	+	1.82	Apple, pear, kiwi, soy, pineapple	447	<0.35	38.80	24.3
11	M/30	–	R, C	+	0.54	Banana, apple, grape, orange, pine, kiwi	48	<0.35	<0.35	<0.35
12	M/5 ^{1, 3}	–	R, C*	+	7.48	Apple	423	42.4	44.8	6.54
13	F/25 ¹	–	–	–	<0.35	Apple, peanut	86	<0.35	6.79	NA
14	F/25 ¹	–	–	–	1.02	Apple, pear, melon, peanut	510	<0.35	40.8	NA
15	F/36 ¹	–	–	–	0.35	Apple, pear, kiwi, peanut, hazelnut, banana, melon	554	<0.35	7.55	100
16	M/39 ¹	–	–	–	<0.35	Hazelnut	82	<0.35	4.14	8.51
17	M/25 ¹	–	–	–	0.87	Apple, pear, kiwi, salad, spinach, peanut, carrot	454	<0.35	21.2	8.51
18	F/21 ¹	–	–	–	<0.35	Apple, peach, cherry	10	<0.35	2.06	3.56
19	M/33 ¹	–	–	–	<0.35	Apple	21	<0.35	8.35	6.12
20	F/21 ¹	–	–	–	<0.35	Melon	87	<0.35	<0.35	22.6
21	F/43 ²	–	–	–	<0.35	Pineapple	39	<0.35	<0.35	<0.35
HC1–5	3 M/2 F (24–59 years)	–	–	–	<0.35	–	<120	<0.35	<0.35	<0.35

Patients 1–12 are CSA⁺, patients 13–21 are CSA[–]. HC1–5 represents data from 5 healthy control individuals. Words in bold indicate systemic reactions toward the indicated food. * CSA reaction (also) due to passive exposure. I = Itching; U = urticaria; A = angioedema; R = rhinitis; C = conjunctivitis; B = bronchospasm; NA = not available; GP = grass pollen.

¹ Denotes presence of pollen allergy. ² Denotes latex allergy. ³ Exposure by passive smoking.

of allergen-bound sIgE were stained with a secondary, fluorescence-labeled anti-human IgE for 30 min at room temperature, protected from light. After a second rinsing and washing procedure, the obtained fluorescence signals 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 known IgE content. Data were expressed as ISAC standardized units (ISU/l), with measuring range 0.30–100 ISU/l.

Skin-Prick Tests

SPT included the afore-mentioned inhalant allergens (HAL, Haarlem, The Netherlands) and *C. sativa* (for extract preparation see above). SPT responses were considered positive only when the wheal reaction exceeded 3 mm (longest diameter).

Basophil Activation Tests

BATs were performed as described elsewhere [29]. Briefly, whole blood samples were stimulated with cannabis extract (0.01, 0.1, 1, 10 and 100 µg/ml), anti-IgE (BD Biosciences, Erembodegem, Belgium) as a positive control, or buffer to measure spontaneous CD63 expression. To quantify activated basophils, cells were

stained with anti-human IgE (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) conjugated with AlexaFluor 488 (Life Technologies, Gent, Belgium), CD203c-APC (BioLegend, San Diego, Calif., USA) and phycoerythrin-conjugated CD63 (Pharmingen, BD Biosciences). Red blood cells were lysed and white blood cells fixed (FACS Lysing solution, BD Biosciences) for 20 min at room temperature. After centrifugation, cells were resuspended in washing solution and flow cytometric analysis of basophil activation was performed on a FACSCalibur flow cytometer (BD Immunocytometry Systems, San Jose, Calif., USA). IgE staining, CD203c and side scatter were employed to gate on at least 500 basophils that expressed CD203c and a high density of surface IgE. Within this gate the percentage of activated basophils (coexpressing CD63) was measured. Percentages of activated basophils were corrected by subtracting spontaneous CD63 expression from the value obtained with allergen stimulation. The threshold for positivity of BAT was calculated using ROC analysis.

Statistics

Results are reported as median (range) where appropriate. Nonparametric statistical analysis was conducted using IBM SPSS Statistics version 20.0 (IBM, Chicago, Ill., USA). A *p* value >0.05 was considered significant.

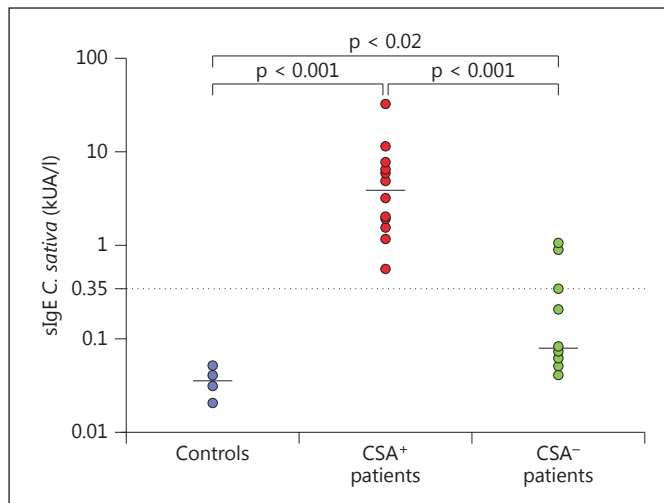


Fig. 1. sIgE to cannabis is higher in CSA⁺ food-allergic patients as compared to CSA⁻ food-allergic patients and to healthy control individuals. Note that 3 CSA⁻ patients display a false-positive sIgE to cannabis.

Results

Demographics, Clinics and Skin Tests

Table 1 displays the demographics, clinics and relevant laboratory findings of the 21 food-allergic patients and 5 control individuals. Symptoms of *C. sativa* allergy (CSA) included local itching and contact urticaria from cannabis leaves and rhinitis, conjunctivitis and/or asthma whilst smoking joints or evaporating the drug. Some patients presented their symptoms when entering places where cannabis allergen had become airborne or via indirect exposure through skin-skin contact. Ten out of 12 of the CSA patients (CSA⁺) and 8 out of the 9 patients without CSA (CSA⁻) allergy suffered from seasonal rhinoconjunctivitis and/or asthma suggestive for a tree and/or grass pollen allergy. One CSA⁻ patient allergy presented contact urticaria and rhinoconjunctivitis upon exposure to powdered latex gloves.

In both patient groups, food allergy was heterogeneous and involved taxonomically more or less related fruits, vegetables and nuts. However, in CSA⁺ patients it cannot be excluded that some food allergies result from cross-reactivity with pollen allergens. Ten out of the 12 CSA⁺ patients presented systemic reactions that are only rarely observed in pollen-related food allergy. Furthermore, in CSA⁺ patients, plant food allergy frequently involved fruits and vegetables such as banana, tomato, citrus fruits and grape. Allergy to peach was not predominant in our

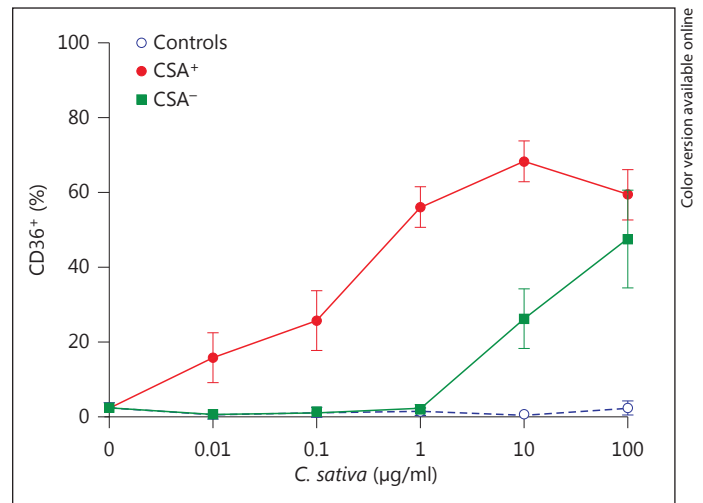


Fig. 2. Cannabis-induced basophil activation in CSA⁺ food-allergic patients (closed red circles, $n = 12$), CSA⁻ patients (closed green squares, $n = 9$) and healthy control individuals (open blue circles, $n = 4$). Results are expressed as percentages CD63⁺ basophils (mean $\pm$ SEM). Stimulation with 1 μ g/ml yielded the best discriminative capacity between CSA⁺ patients and CSA⁻ patients or healthy control individuals without CSA allergy. Colors refer to the online version only.

series and some patients reacted to banana, previously described to be safe in peach-allergic patients. Particular interest was paid to the moment of initial appearance of cannabis and food-related symptoms. However, the majority of the patients were unable to recollect the time relationship between CSA and food allergy. One patient demonstrated generalized urticaria within 30 min after drinking beer. Furthermore, table 1 shows that 9 out of 10 CSA⁺ patients demonstrated a positive SPT for cannabis, whereas atopic patients and healthy control CSA⁻ individuals showed negative SPT.

sIgE to *C. Sativa*

Figure 1 shows that sIgE to *C. sativa* (industrial hemp) in CSA is significantly higher as compared to food-allergic CSA⁻ patients ($p < 0.001$, Mann-Whitney U test). However, a positive sIgE cannabis result cannot be considered predictive for the clinical outcome, as it was also found in 3 out of 9 (33%) CSA⁻ patients. Two out of these 3 CSA⁻ patients had a positive sIgE to profilins. In healthy control individuals no sIgE to cannabis was demonstrable.

Basophil Activation to *C. Sativa*

Figure 2 summarizes the dose-finding experiments of the BAT with *C. sativa* extract. It appears that *C. sativa*

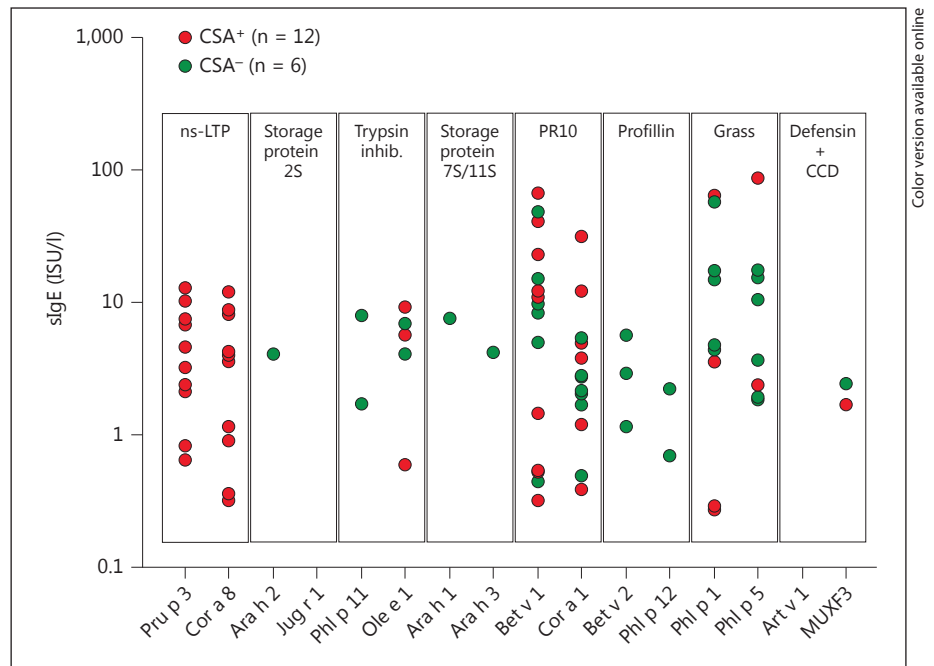


Fig. 3. sIgE quantifications by ISAC 112 ImmunoCAP microarray for the plant food and pollen components in CSA⁺ and CSA⁻ patients. Note the frequent sensitization towards the ns-LTP rPru p 3 from peach (*P. persica*) and rCor a 8 from hazelnut (*C. avellana*) in CSA⁺ but not in CSA⁻ patients with food allergy. Sensitization to 2S albumins (rAra h 2 from peanut and nJug r 1 from walnut) and trypsin inhibitors (rPhl p 11 from Timothy grass and nOle e 1 from olive tree), vicillins (rAra h 1 from peanut) and legumines (rAra h 3 from peanut) was rarely observed. Eleven CSA patients

demonstrated a positive sIgE to the major birch pollen allergen rBet v 1. rPhl p 1 and rPhl p 5 from Timothy grass (*Phleum pratense*) are markers for genuine grass pollen allergy. Sensitization to the defensin nArt v 1 from mugwort was rare. Three out of 9 food-allergic CSA⁻ patients demonstrated a sensitization to profilin (rBet v 2 from birch and rPhl p 12 from Timothy grass). One food-allergic CSA⁻ patient was sensitized to MUXF-type cross-reactive carbohydrates (CCD). Sensitization to these components was not demonstrable in any of the healthy control individuals.

elicits a dose-dependent activation above spontaneous expression of the cells in food-allergic patients, irrespective of the presence or absence of CSA. Nevertheless, BAT with cannabis proved to be absolutely discriminative between CSA⁺ patients and food-allergic CSA⁻ patients, provided a correct stimulation concentration was applied (e.g. 1 µg/ml). Stimulation at a high concentration of 100 µg/ml failed to discriminate between CSA⁺ and CSA⁻ patients. In healthy control individuals, cannabis did not trigger basophil activation and CD63 expression remained unaltered as compared to buffer stimulation.

Component Resolved Diagnosis

Figures 3 and 4 display the individual ISAC results for relevant pollen and food allergen components in 18 patients (12 CSA⁺, 6 CSA⁻). Most interestingly, sensitization towards ns-LTP was observed in all CSA⁺ patients but none of the CSA⁻ individuals. Generally, patients demonstrated a positive sIgE result for various ns-LTP. Specific

IgE was positive for rPru p 3 from peach (*P. persica*), rPla a 3 from plane tree (*Plantanus acerifolia*) and nJug r 3 from walnut (*Juglans regia*) in 10 patients, rCor a 8 from hazelnut (*Corylus avellana*) in 9 patients, and rAra h 9 from peanut (*Arachis hypogaeae*) and nArt v 3 from mugwort (*Artemisia vulgaris*) in 8 CSA⁺ patients, respectively. sIgE to rTri a 14 from wheat (*Triticum aestivum*) was positive in 3 patients, and to nOle e 7 from olive tree (*Olea europaea*) in 2 patients. No sensitization to rPar j 2 was demonstrable. Note that sensitization towards ns-LTP was frequently not predictive for overt food allergy. For example, only 4 out of 10 CSA⁺ patients sensitized to Pru p 3 reported an allergic reaction to peach. Sensitization to other prolamins (2S albumins and trypsin inhibitors) and vicillins and legumines from the Cupin superfamily was only rarely observed. Alternatively, 10 out of the 12 CSA⁺ patients also demonstrated a documented birch pollen allergy with positive sIgE to Bet v 1, and 5 of them were also allergic to grass pollen with a sensitization to Phl p 1 and/

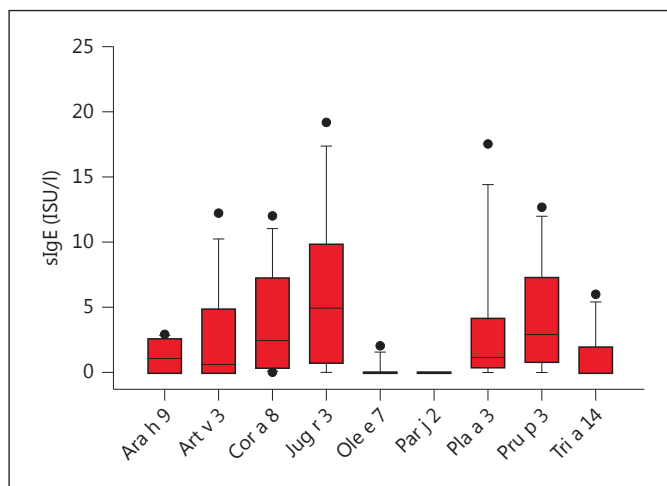


Fig. 4. sIgE to the native and recombinant ns-LTP panel spotted on the microarray rPru p 3 from peach (*P. persica*), rPla 3 from plane tree (*P. acerifolia*), nJug r 3 from walnut (*J. regia*), rCor a 8 from hazelnut (*C. avellana*), rAra h 9 from peanut (*A. hypogaeae*), nArt v 3 from mugwort (*A. vulgaris*), rTri a 14 from wheat (*T. aestivum*) and nOle e 7 from olive tree (*O. europaea*). Note that there was no sensitization to rPar j 2, the ns-LTP from wall pellitory (*Parietaria judaica*).

or Phl p 5 (fig. 3). Three out of 9 food allergic CSA⁻ patients demonstrated a sensitization to profilin. None of 5 healthy control individuals demonstrated sensitization to the components spotted on the microarray.

Discussion

Although there have been several studies on occupational respiratory and cutaneous allergy to plants of the *Cannabaceae* family, including Cannabis (hemp) and Humulus (hops) [30–32], descriptions of CSA in illicit drug abusers remain rare. The first report dates from 1971 and describes a patient who, after smoking a marijuana cigarette, suffered from an allergic reaction [33]. Since then, various cases of CSA after exposure through inhalation, cutaneous contact, ingestion and even intravenous use have been published [24, 25, 34–38].

This is the first case series of patients from a European non-Mediterranean region presenting with various fruit, vegetable and nut allergies from which some might result from a CSA. Our data reveal that CSA⁺ patients can exhibit various clinical and biological particularities.

First, patients generally present respiratory and/or cutaneous symptoms upon exposure to cannabis. However,

these symptoms are easily overlooked as they cannot always be discriminated from symptoms related to their pollen allergy, could be masked by intake of drugs (e.g. antihistamines, nasal corticosteroids) or are not always recognized. In addition, patients might not be eager to disclose their illicit drug abuse.

Second, patients frequently display different food allergies. Although it cannot be excluded that some of these food allergies result from cross-reactivity with pollen allergens (which was clearly present in 10 out of the 12 CSA⁺ patients), it is noteworthy that these reactions frequently were more severe than an OAS, as generally observed in pollen-related food allergy. Furthermore, several patients demonstrated allergic reactions towards fruits and vegetables such as banana, tomato, citrus and grape fruits that are rarely involved in a well-known Bet v 1-associated food syndrome [2, 39]. Alternatively, unlike in southern European surveys [14, 15, 40, 41], allergy to peach was not predominant and some patients reacted to banana, which was previously described to be safe in peach-allergic patients [42]. Finally, most patients did not easily recognize their drug allergy and also suffered from a birch pollen allergy, they were generally unable to recollect the exact sequence of appearance of cannabis and food-related symptoms.

Third, it is demonstrated that the BAT constitutes a reliable instrument to document CSA. Comparative analysis between patients and healthy control individuals revealed the technique to be absolutely discriminative. On the other hand, it is confirmed that the validation of a new diagnostic instrument cannot be considered as appropriate when it failed to identify factors that could have confounded the outcome. Like Herzinger et al. [32], we show that correct diagnosis of CSA might not be straightforward, as patients with pollen allergy might demonstrate positive test results without overt CSA. The most likely explanation for these false-positive results should probably be sought in terms of stimulation concentrations. This phenomenon has already been described for other allergens such as *Hevea* latex [43, 44]. Moreover, it seems justified to state that sensitization to profilin could also account for the false-positive sIgE cannabis results observed in 1 out of 3 plant food-allergic CSA⁻ patients.

Fourth, multiplexed component-resolved diagnosis (CRD) with purified and recombinant proteins confirms the ns-LTP Can s 3 could be the candidate major allergen for CSA [24–26]. ns-LTP are highly stable structures that resist thermal processing [45–47] and gastroduodenal proteolysis [48, 49]. Thus, both sensitization and triggering of reactions towards *C. sativa* might result from vari-

ous routes of exposure such as inhalation, transdermal and also ingestion (e.g. from cannabis tea, space cake). In addition, it cannot be excluded that sensitization might occur indirectly via passive exposure to airborne allergen as demonstrated in our youngest patient. This respiratory sensitization route could be particularly relevant in children exposed to cannabis allergens through parental drug abuse (e.g. cannabis smoking) and could parallel an observation in occupational asthma to asparagus LTP [50]. Nonetheless, according to clinics and CRD it seems unlikely that sensitization towards ns-LTP in our series results from allergy to food (particularly peach) [14–18] or pollen from *Artemisia* [51] or *Parietaria* [52]. Alternatively, our data reveal that not all patients are sensitized to the ns-LTP spotted on the microarray and that there is currently no ns-LTP that captures all CSA patients.

Fifth, as ns-LTP are highly cross-reactive and ubiquitously present allergenic proteins, sensitization to cannabis ns-LTP could explain the various fruit, vegetable and nut allergies in our patients. Moreover, as observed in 1 of our patients, sensitization towards ns-LTP from hop and grape could explain allergic reactions to alcoholic beverages such as beer [53, 54] and wine [55–58]. To our knowledge, this series is the first description of sensitization towards ns-LTP from cannabis as a possible explanation for plant food allergy in a non-Mediterranean country. Moreover, sensitization to Cas s 3 and its homologues could also explain (severe) plant food allergy in other central European series [59]. Alternatively, it is again evi-

denced that a positive sIgE result is not per se predictive for the clinical outcome, as sensitization towards ns-LTP as disclosed by CRD was frequently not associated with overt food allergy.

In conclusion, we report on a series of patients with food allergies with and without IgE-mediated cannabis allergy. Our data indicate that diagnosis of CSA is best documented by skin tests and BATs that more closely mirror the in vivo situation than conventional IgE technique. Future larger surveys are needed to determine the potential existence and prevalence of a 'cannabis plant-food syndrome' and elucidate on the sensitization routes and implicated allergenic components. These studies should further investigate the clinical relevance of the cross-reactivity or cosensitization due to ns-LTP. Ideally, such collaborative studies should involve centers from different geographical regions in order to assess whether these observations also apply to Mediterranean countries and other regions.

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