

Clinical Communications

Molecular diagnosis in cannabis allergy

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Clinical Implications

- *Cannabis* may be an important allergen in young people.
- The most important allergens implicated were lipid transfer proteins, which may be responsible for primary sensitization and might induce further lipid transfer protein food allergies.
- Patients sensitized to tobacco or tomatoes are at risk.

TO THE EDITOR:

Studies have described isolated cases of *Cannabis sativa* sensitization as unusual cases of lipid transfer protein (LTP) allergy.^{1,2} Serologic investigations that used ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) and ISAC (ThermoFisher Scientific, Uppsala, Sweden) microarray—confirmed IgE positivity for peach LTP (nPrup3), hazelnut lipid transfer protein (rCor a 8), and mugwort LTP (nArt v3). Reports suggest that *C sativa* sensitization may be mediated by 2 mechanisms, cross-reactivity (mainly with LTPs and thaumatin-like protein), and exposure-related *de novo* sensitization.³ LTPs sensitize primarily through the airways.⁴ We characterized the sensitization profile of patients diagnosed with primary cannabis allergy who experienced new-onset asthma after cannabis handling or smoking.⁵ The protocol was approved by the clinical research ethics committees, and all participants gave written informed consent.

We included 130 patients (mean age 26 ± 9 years; 97 men) diagnosed with primary cannabis allergy (positive skin prick test, specific IgE, and bronchial challenge) (Table I).⁴ Fifty patients with asthma who were sensitized to pollen and 50 healthy blood

donors who were not atopic (mean age 24 ± 12 years) were included as controls. Microarrays (ThermoFisher Scientific, Uppsala, Sweden), including native cannabis LTP (nCan s 3), were performed in all patients and controls.

Extracts used for *in vivo* tests were the following: Fresh *C sativa* cut top leaves cold-milled and defatted with acetone (2×1 ; 1:5 wt/vol for 1 hour at 4°C) and, after drying, extracted with 0.1 mol/L Tris-HCl pH 7.5, 10 mM EDTA (1:5 wt/vol for 1 hour at 4°C). After centrifuging at $9000 g$ for 30 minutes at 4°C , the supernatant was dialyzed against H_2O (cutoff point, 3.5 kDa) and lyophilized. A wheal $\geq 19.62 \text{ mm}^2$ was considered clearly positive, which corresponded to a diameter of 5 mm, as in a previous study.⁵

Native LTPs from cannabis were obtained from *L. ssp. Sativa* cv. Kompolti by following the method reported by Rihs et al (written communication, ISMA meeting 2013: IgE-binding properties in patients with symptoms to cannabis).

In patients sensitized to cannabis, a response to cannabis LTP was found in 95.3%, to LTPs from tomato (Lyc e 3) in 89%, to mugwort (nArt v3) in 61.5%, to tobacco allergens in 56.9%, to cross-reactivity carbohydrate determinants (native ananas bromelain carbohydrate determinants) in 33.8%, and to group 1 grass allergens (recombinant *Lolium perenne* group 1 in 32% and group 1 allergen of *Cynodon dactylon* (Bermuda grass pollen) (nCyn d 1) in 33.8%), and to nPrup3, the major sensitizer in LTP allergy in the Mediterranean area, in 47% of patients. Cross inhibition experiments by using nCan s3 and nPrup3 were made to demonstrate that nCan s3 was the primary sensitizer.

Analysis of our results suggests that smokers and patients with difficulties ingesting tomato also are at risk, but cannabis-related symptoms appeared a mean of 4 years after starting smoking and symptoms after eating tomato also appeared later. Although tomatoes possess allergens that cross-react with *C sativa* allergens,⁶ 72% of patients with tomato allergy admitted previous cannabis consumption. Smokers sensitized to tobacco also responded to challenge with pure extract of *C sativa* leaf, which showed that not only tobacco provoked their symptoms.

Sensitization to cross-reactivity carbohydrate determinants may be explained by the Maillard reaction⁷ after burning cannabis in

TABLE I. Response to main allergens by study group

	Cannabis allergy		Controls		Pollen allergy	
	Array, n/%	IgE/CAP, n/%	Array, n/%	IgE/CAP	Array, n/%	IgE/CAP, n/%
r Lol p1 Lolium	42/32%	42/32%	2/4%	0	49/98%	50/100%
Cyn d1/Cynodon	44/33.8%	44/38.8%	2/4%	0	35/70%	50/100%
nPrup3 Peach	62/47%	60/46.15%	0	0	11/22%	14%
rCor a 8 Hazelnut	42/32.3%	32/41.6%	1/2%	0	2/4%	8%
r Hev b8 latex	26/20%	12/15.6%	0	0	2/4%	0%
LTP tomato	104/80%	nd	0	0	2/4%	nd
LTP cannabis	124/95.3%	nd	0	0	1/2%	nd
LTP tobacco	74/56.9%	nd	0	0	2/4%	nd
nArt v3 Artemisia	80/61.5%	66/85.8%	0	0	6/12%	22%
nAna c2 bromelina	44/33.8%	nd	0	0	1/2%	nd

r Lol p1, recombinant *Lolium perenne* group 1; nCyn d 1, *Cynodon dactylon*; nPrup3, peach LTP; rCor a 8, hazelnut lipid transfer protein; LTP, lipid transfer protein; nd, Not done; nArt v3, mugwort; nAna c2, native ananas bromelain carbohydrate determinants.

cigarettes. Deglycosylation studies helped refine protein allergen identification and demonstrated significant IgE antibodies against plant oligosaccharides that could help explain cross-reactivity.⁸ Sensitization to group 1 grass antigens is highly prevalent in our area. *Cynodon nCyn d 1* has antifungal function against *Claviceps* spores, a source of alkaloids (ergonovine and ergonovine), which causes tremorgenic syndrome in cattle due to *Cynodon* consumption.⁹

In summary, increasing consumption of marijuana has resulted in a rise in sensitized individuals, but little is known about the causal allergens associated with *C sativa* allergy. Molecular diagnosis showed that the major allergens implicated are LTPs, which may be responsible for primary sensitization and might induce further LTP food allergies.

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