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Reference:
Ebo Didier, Rihs H.P., Mertens Christel, Van Gasse Athina, van der Poorten Marie-Line, Hagendorens Margo, Sabato Vito, Elst Jessy.- Exploring the thaumatin-like protein (TLP) as a candidate cannabis allergen in North-Western Europe
Allergy: European journal of allergy and clinical immunology - ISSN 1398-9995 - 79:1(2024), p. 257-259
Full text (Publisher's DOI): https://doi.org/10.1111/ALL.15953
To cite this reference: https://hdl.handle.net/10067/2009080151162165141

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Exploring the thaumatin-like protein (TLP) as a candidate cannabis allergen in North-western Europe.

To the editor,

Cannabis sativa (Can s) can elicit IgE-mediated allergy with a myriad of symptoms [1-3]. Most studies point to Can s 3, the nonspecific lipid transfer protein (nsLTP) as a major allergen [2]. However, Can s 3 does not cover the entire cannabis IgE-reactivity profile [1].

Earlier research [4] reported patients showing IgE-reactivity to a 38-kDa band, identified as the pathogenesis-related thaumatin-like protein (TLP). The TLP family has been identified as major allergens in several fruits such as kiwi, banana, peach, and apple and is considered a panallergenic family responsible for cross-reactivity between pollen and fruit. Moreover, some TLPs are glycoproteins which could explain their allergenic capacity [5]. However, in the absence of skin testing and functional cellular tests, the authors were unable to comment on the clinical relevance of their observation [4].

Here we aim to explore the TLP as a candidate *Cannabis sativa* allergen in CA in a North-western European region.

Patients with a history of immediate respiratory and/or cutaneous symptoms on cannabis exposure (CA), asymptomatic atopic cannabis users (henceforth designated as exposed atopic controls (EAC)) and asymptomatic exposed healthy controls (EHC) were included as described previously [1]. Total IgE and specific (s)IgE to hemp and recombinant (r) pollen components

were quantified by ImmunoCAP (Thermo Fisher Scientific) as described elsewhere [1]. Results were considered positive if ≥ 0.10 kU_A/L. To depict sensitization to cannabis TLP, sera were analyzed for IgE-reactivity towards rCan s-TLP (rCs-TLP) by using ELISA as described in the Online Repository. The recombinant protein synthesis is detailed in the Online Repository. Figure E1 of the online repository displays rCs-TLP by SDS-PAGE. Finally, rTLP sIgE effector cell activating capacity was evaluated by passive mast cell activation test (pMAT). As described in the Online Repository, in the pMAT, mast cells (MCs) were passively sensitized with serum from CA patients or controls (both EAC and EHC) and subsequently incubated with rCs-TLP.

All participants had skin prick tests (SPTs) with aeroallergens and cannabis extract as prepared in [1]. SPTs were read after 15 minutes and considered positive when the largest wheal diameter exceeded 3 mm. A positive control with histamine (10 mg/mL) and a negative saline control without allergen (ALK-Abello Ltd) were performed. A total of 75 individuals was included; 60 CA patients, 10 EAC, i.e. asymptomatic cannabis users with a documented birch and/or grass pollen sensitization and 5 EHCs. Demographics are shown in table E1 of the online repository.

In terms of cannabis diagnostics, 53/60 (88.3%) of CA patients demonstrate a positive sIgE hemp and 47 (78%) a positive SPT with the Can s extract. As shown in figure 1 and table 1, 16/60 (26.7%) of the CA patients demonstrate a positive sIgE result for rCs-TLP. rCs-TLP IgE reactivity was also demonstrable in 2/10 (20%) of the EAC but none of the EHC. As shown in figure 1, serum from 4 out of 5 randomly available selected sera from CA patients with sensitization to rCs-TLP triggered MC degranulation in response to rCs-TLP. No rCs-TLP-mediated degranulation was demonstrable with randomly available selected sera from 5 CA

patients and 5 EAC without demonstrable rCs-TLP sensitization. However, 1/2 sera of the EACwith rCan s-TLP IgE reactivity was available to evaluate the MC degranulation capacity and resulted in a clear degranulation of MCs. Note this patient experienced anaphylaxis to banana and demonstrated a positive sIgE and pMAT to purified Mus a 4, the TLP from banana (*Musa acuminata*) kindly provided by Araceli Diaz-Peralez. Sera of 4/5 EHCs was available to evaluate the MC degranulation capacity. Three of these did not trigger MC degranulation by rCs-TLP. A closer look to the EHC whose serum triggered MC degranulation revealed a sensitization to hemp as shown by a positive sIgE (1.09 kUA/L) and a positive SPT. Although uncertain, it cannot be excluded this apparent clinically irrelevant degranulation of MCs might be due to cross-reactivity to hemp, as shown by the positive sIgE hemp. A similar explanation could apply for the positive skin test with cannabis.

To summarize, as shown by IgE-binding and pMATs, about one-quarter of CA patients in a North-western European region demonstrate a potentially clinically relevant TLP sensitization. Meanwhile, the Can s-TLP has been indexed as Can s 7 by the WHO/IUIS Allergen Nomenclature Subcommittee.

Acknowledgements

The authors thank B. Van Camp, head of the Unit ‘drug production’, Central Drug Department, Directorate of organized crime, Belgian Federal Judicial police for his help with providing the necessary plant materials. We would also like to thank Mrs. K. Vandebos and N. Maes, our study nurses, for their help in the performance of the skin prick tests and blood sampling.

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Figure 1: sIgE binding and MC activation responses for rCs-TLP

Left: sIgE for rCs-TLP in Cannabis allergic patients (CA), exposed asymptomatic atopic individuals (EAC) and exposed asymptomatic healthy controls (EHC). Right: passive mast cell activation test: CD63 upregulation in CA with rCs-TLP-sensitization (red●) or without (blue■), EAC with rCs-TLP-sensitization (grey◆) or without (black▼) and EHC without sensitization (green▲).

Table 1: Demographics and allergy characteristics of cannabis allergic patients (CA) with positive sIgE rCan s TLP

PT.	Age (y)/Sex	Clinical characteristics	SPT cannabis	Total IgE (kU/L)	sIgE rCs-TLP (kUA/L)	sIgE hemp (kUA/L)	sIgE Bet v 1 (kUA/L)	sIgE Bet v 2 (kUA/L)	sIgE Phl p 1 (kUA/L)	sIgE Phl p 5 (kUA/L)
1	22/F	Rhinoconjunctivitis	+	165	2.7	3.72	8.77	<0.1	2.12	<0.1
2*	29/F	Anaphylaxis	+	328	0.80	10.68	0.05	<0.1	0.08	<0.1
3*	27/M	Anaphylaxis	+	5000	11.31	77.1	58.6	0.47	30.4	9.94
4	34/F	Pruritus	+	1188	8.17	15.7	100	<0.1	1.58	<0.1
5*	28/M	Rhinoconjunctivitis	+	250	59	4.27	0.11	ND	ND	ND
6*	26/F	Rhinoconjunctivitis Urticaria	+	553	1.51	11	6.67	0.68	ND	ND
7	39/M	Urticaria	+	227	12	5.95	13	0.1	ND	ND
8	27/F	Rhinoconjunctivitis	+	171	9.33	1.18	0.98	<0.1	1.45	ND
9	34/F	Rhinoconjunctivitis	-	1993	1.36	0.44	6.88	0.1	37.8	56.9
10	28/F	Urticaria and angioedema	+	5500	30	55.1	66.3	0.33	5.14	0.35
11*	45/M	Rhinoconjunctivitis	+	233	1.72	1.68	26.84	<0.1	<0.1	<0.1
12	35/F	Rhinoconjunctivitis	+	16.1	25	4.65	3.67	<0.1	<0.1	<0.1
13	24/F	Anaphylaxis	-	722	6.8	<0.1	<0.1	<0.1	13.4	14.4
14	24/F	Rhinoconjunctivitis	-	109	18	<0.1	1.89	0.45	13.56	8.54
15	30/F	Anaphylaxis	+	370	0.5	20	0.1	<0.1	0.03	<0.1
16	42/M	Rhinoconjunctivitis	+	235	7.6	7	4.21	ND	<0.10	ND

*** Sera used in passive mast cell activation test**

y, years; SPT, skin prick test; +, positive; -, negative; ND, not determined.

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Word count text: 600 – 1 figure and 1 table

Word count online repository: 878 – 1 figure and 1 table

Funding

This work was supported by the Agency for Innovation by Science and Technology (IWT) [grant number 140185]. Furthermore, V. Sabato is a Senior Clinical Researcher of the Research Foundation Flanders/Fonds Wetenschappelijk Onderzoek (FWO: 1804518N). D.G Ebo is a Senior Clinical Researcher of the Research Foundation Flanders/Fonds Wetenschappelijk Onderzoek (FWO: 1800614N).

Conflicts of interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.