

Clinical Communications

Relevance of lipid transfer protein to *Cannabis* sensitization in North America

Henry P. Morelli, BS^a, Cathy Thorpe, BS^b,
Didier G. Ebo, MD^c, Martin D. Chapman, PhD^b,
Khaldon Abbas, BSc^d, Gordon L. Sussman, MD^d, and
Ajay P. Nayak, MSc, PhD, FAAAAI^a

Clinical Implications

The clinical determination of allergic sensitization to cannabis is difficult owing to the lack of objective diagnostic measures. Our studies demonstrate that cannabis nonspecific lipid transfer protein (Can s 3) is a clinically relevant allergen in North American cohorts.

Allergic reactions to cannabis, although sporadic, have been reported over the past 5 decades.¹ With increased access and decriminalization of cannabis, cases of symptomatic exposures have become increasingly frequent.² Clinical investigations into allergic reactions to cannabis are challenging owing to the broad symptomatology that makes it difficult to distinguish cannabis allergy from general physiologic reactions to the distinct plant components. More recently, investigations from our laboratory and our collaborators have resulted in the identification and subsequent validation of four putative allergens of cannabis,²⁻⁵ thus paving the way for the development of novel diagnostic platforms.

One major cannabis allergen deemed critical for developing allergic sensitization is the nonspecific lipid transfer protein (nsLTP), also known as Can s 3.⁵ In Europe, Can s 3 was identified as a critical driver of cross-reactivities to homologous allergens commonly found in other plants and foods.⁶ Furthermore, Can s 3 was reported in cannabis pollen, which extends the scope of its allergenic potential.⁷ However, in our previous study,³ while examining type I hypersensitivity to cannabis among symptomatic recreational users in North America, we did not identify IgE binding to nsLTP using protein extracts. Although this observation raised questions about the relevance of Can s 3 in North America, concerns existed regarding the methodologic limitations presented at the time. Specifically, drawbacks included the relatively crude nature of the cannabis protein extracts, intrinsic limitations of the immunoproteomics approach, and the lack of complimentary analytical platforms required for validation.

In this report, we detail methodologic advances that have aided in establishing the relevance of Can s 3 to cannabis-linked allergic reactions in North America. First, we collected serum samples from 14 symptomatic recreational users of cannabis from Canada. Patients with suspected allergic sensitization to cannabis demonstrated typically diverse symptoms including urticaria, angioedema, rhinitis, chest tightness, and exacerbation of asthma. Importantly, all patients tested positive on skin prick test with

cannabis leaf and bud extract when tested according to methods described elsewhere.⁸

To overcome the limitations presented by crude cannabis protein extracts employed in our prior study,³ we developed a purified Can s 3 expressed in *Escherichia coli* using recombinant protein expression approaches (RE-CS3-1, InBio, Charlottesville, Va). Recombinant Can s 3 (rCan s 3) contains a 6xHis-tag on the C-terminus of the protein. Purity of the rCan s 3 was resolved using a standard SDS-PAGE approach and subsequent protein staining with Imperial protein stain (Thermo Fisher, Waltham, MA), which revealed a protein band at approximately 12 kDa (Figure 1, left). Western blot approach (15% SDS-PAGE) was used to probe rCan s 3 (5 µg) with serum (40 µL each, diluted 1:5 v/v in PBS containing BSA from 14 patients with cannabis allergy. Patient serum IgE binding to rCan s 3 was established using an anti-human IgE mouse monoclonal antibody (1:2,000 v/v in PBS containing BSA) and subsequent incubation with an anti-mouse antibody coupled to infrared dye (IRDye, LiCOR, Lincoln, NE) according to methods described elsewhere.³ Immunoreactive signals were detected using a LiCOR instrument. Sera from five patients (ID nos. 1, 2, 3, 9, and 14) revealed IgE binding to rCan s 3 indicating positive reactivity (Figure 1, right). We did not observe IgE binding to rCan s 3 in the remaining serum samples.

To investigate Can s 3-specific IgE further in symptomatic cannabis users, serum samples were compared by chimeric ELISA using either polyclonal or monoclonal anti-IgE for detection. In addition to the samples indicating positive immunoreactivity (IgE binding to rCan s 3) in western blot analysis (Figure 1, right panel), the polyclonal anti-IgE ELISA detected positive reactivity in three additional serum samples (Figure 2). In comparison, the monoclonal anti-IgE ELISA was mostly consistent with western blot analysis; only one additional sample demonstrated positive IgE immunoreactivity. These studies suggest that chimeric ELISA methods provided better resolution of serum IgE binding to Can s 3 protein than did immunoblotting.

Nonspecific lipid transfer proteins from cannabis and other food plant sources have been suggested to be critical molecular drivers of cross-reactive allergies in Europe.⁵ However, reports on cannabis allergies in North America have been limited, and the relevance of Can s 3 in North American cohorts has been inconclusive to date. Our studies demonstrate that cannabis nsLTP (Can s 3) is a relevant allergen for symptomatic exposure to cannabis. Furthermore, patients with symptomatic exposure to cannabis in Europe and North America alike exhibit IgE antibodies to Can s 3 at comparable rates. As accessibility to cannabis increases for recreational and medicinal use, allergic reactions to cannabis may emerge as a public health issue. In addition, a significant workforce is now engaged in the cannabis industry. The latest estimates identified over 420,000 workers employed in growing and processing cannabis.⁹ Finally, our report is one of the few to demonstrate the relevance of the nsLTP class of allergens in North American cohorts, which underscores the need to examine nsLTP sensitization, particularly in the context of food allergy. This is a clarion call for the

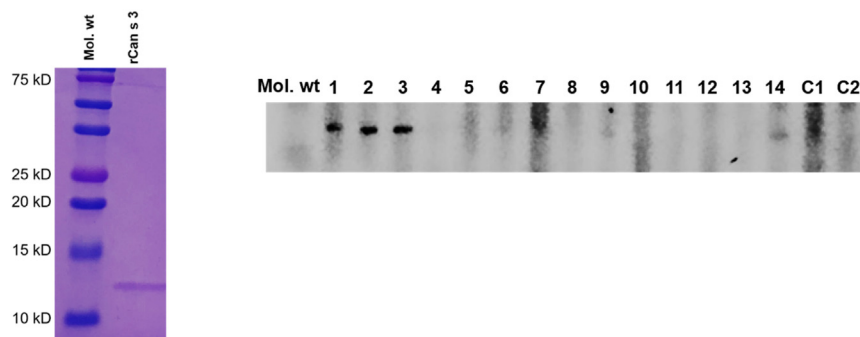


FIGURE 1. Recombinant Can s 3 and IgE immunoreactivity in western blot. Recombinant Can s 3 (500 ng) subjected to an SDS-PAGE approach migrates as an approximately 12-kDa protein (*left*). Western blot analysis of serum IgE reactivity to rCan s 3 (*right*).

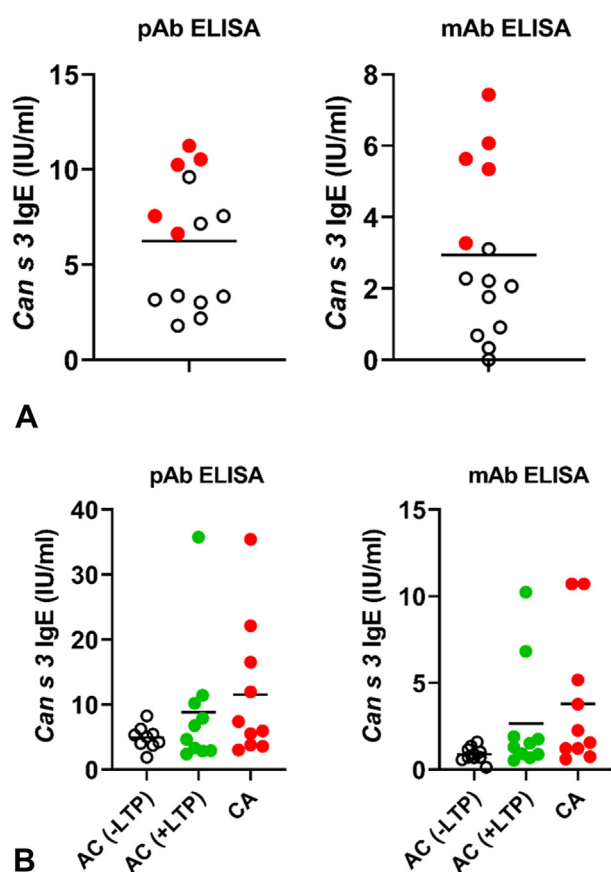


FIGURE 2. Chimeric ELISA approaches for detecting Can s 3-specific IgE. Can s 3 IgE (IU/mL) levels were examined by chimeric ELISA using either polyclonal or monoclonal anti-IgE. *Red circles* indicate patient samples with a positive result; *open circles* indicate patient samples with a negative result in the western blot approach; *black line* defines the threshold for positive immunoreactivity. *AC*, atopic; *CA*, cannabis atopic; *LTP*, lipid transfer protein; *pAb*, polyclonal antibody.

continued development of component-resolved diagnostics approaches and increased participation by allergists to address an emerging public health issue.

^aDepartment of Medicine, Division of Pulmonary, Allergy, and Critical Care Medicine and Center for Translational Medicine, Jane and Leonard Korman Lung Center, Thomas Jefferson University, Philadelphia, Pa

^bDepartment of Immunology, University of Antwerp, Antwerp, Belgium

^cInBio, Charlottesville, Va

^dDepartment of Medicine, University of Toronto, Toronto, Ontario, Canada

Funding was provided to A.P. Nayak by Grant R21AI140411 from the National Institutes of Health/National Institute of Allergy and Infectious Diseases.

Conflicts of interest: The authors declare that they have no relevant conflicts of interest.

Received for publication May 16, 2023; revised June 13, 2023; accepted for publication June 14, 2023.

Available online ■■

Corresponding author: Ajay P. Nayak, MSc, PhD, FAAAAI, Center for Translational Medicine, Thomas Jefferson University, JAH Ste 543B, 1020 Locust St, Philadelphia, PA 19107. E-mail: ajay.nayak@jefferson.edu.

2213-2198

© 2023 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2023.06.039>

REFERENCES

- Nayak AP, Green BJ, Sussman G, Beezhold DH. Allergenicity to Cannabis sativa L. and methods to assess personal exposure. In: Chandra S, Lata H, ElSohly M, editors. *Cannabis sativa L. - Botany and Biotechnology*. Cham: Springer; 2017.
- Skypala IJ, Jeimy S, Brucker H, Nayak AP, Decuyper II, Bernstein JA, et al. Cannabis-related allergies: an international overview and consensus recommendations. *Allergy* 2022;77:2038-52.
- Nayak AP, Green BJ, Sussman G, Berlin N, Lata H, Chandra S, et al. Characterization of Cannabis sativa allergens. *Ann Allergy Asthma Immunol* 2013;111:32-7.
- Faber MA, Van Gasse AL, Decuyper II, Uytendaele A, Sabato V, Hagendorens MM, et al. IgE-reactivity profiles to nonspecific lipid transfer proteins in a northwestern European country. *J Allergy Clin Immunol* 2017;139:679-682.e5.
- Skypala IJ, Asero R, Barber D, Cecchi L, Diaz Perales A, Hoffmann-Sommergruber K, et al. Non-specific lipid-transfer proteins: allergen structure and function, cross-reactivity, sensitization, and epidemiology. *Clin Transl Allergy* 2021;11:e12010.
- Ebo DG, Swerts S, Sabato V, Hagendorens MM, Bridts CH, Jorens PG, et al. New food allergies in a European non-Mediterranean region: is Cannabis sativa to blame? *Int Arch Allergy Immunol* 2013;161:220-8.
- Nayak AP, Lobundo C, Bielory L. Immunomodulatory actions of cannabinoids: clinical correlates and therapeutic opportunities for allergic inflammation. *J Allergy Clin Immunol Pract* 2023;11:449-57.
- Tessmer A, Berlin N, Sussman G, Leader N, Chung EC, Beezhold D. Hypersensitivity reactions to marijuana. *Ann Allergy Asthma Immunol* 2012;108:282-4.
- Leafly. Find, order, and learn about weed. Accessed July 31, 2023. <https://www.leafly.com/>.