

**Pru p 7 sensitization and fruit allergy associate with cypress allergen immunotherapy**

**Running title: Cypress allergen immunotherapy and Pru p 7 sensitization**

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**Conflict of interest :**

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## Abstract

Up to 60% of food allergies in school children, adolescent and adults are related to an inhalant allergy. Sensitization to Pru p 7, the first identified gibberellin-regulated protein, is associated with cypress pollinosis and appears to be driven by cypress pollen exposure. It is currently unknown whether cypress allergen immunotherapy is a risk factor for Pru p 7 sensitization and fruit allergy.

Monocentric retrospective (January 2014 – December 2019) study of three hundred and ninety-one patients aged 15 years or older, residing in southern France and attending allergy work-up in one of the allergy departments of the University Hospitals of Marseille, France. Collected data included clinical history of immediate reaction to fruit, cypress pollinosis, cypress allergen immunotherapy, skin and biological sensitization.

Among 267 cypress-allergic patients, 39 had received cypress allergen immunotherapy. Fruit allergy was found in 77% of cypress-allergic patients who had received cypress allergen immunotherapy and in 55% of those who did not ( $p = 0.006$ ), while the prevalence of Pru p 7 sensitization was 69% and 39%, respectively ( $p < 0.0001$ ). Cypress pollen immunotherapy was also associated with higher levels of IgE to Pru p 7. The difference was not imputable to patients' age, cypress pollinosis duration, or time elapsed since the initiation of cypress allergen immunotherapy. These results suggest that cypress allergen immunotherapy might favor Pru p 7 sensitization and its clinical expression as fruit allergy.

**Keywords (up to 10):** allergen immunotherapy; cypress pollinosis; food allergy; gibberellin-regulated protein; IgE; peamaclein; Pru p 7

## Abbreviations

CUP: cypress pollinosis

CUP AIT : Cypress allergen immunotherapy

CUP+AIT + : patients had received cypress allergen immunotherapy

CUP+AIT - : patients had not received cypress allergen immunotherapy

GRP : Gibberellin-regulated proteins

FA: fruit allergy

PFAS : pollen-food allergy syndrome

To the Editor,

Up to 60% of food allergies in school children, adolescents and adults are related to an inhalant allergy (1). Food allergy related to pollen cross-reactivity is termed pollen-food allergy syndrome (PFAS). Gibberellin-regulated proteins (GRP) emerged in recent years as a novel family of pollen-food cross-reactive allergens causative of severe reactions, with peach Pru p 7 (peamaclein) first identified and characterized in 2013 (2-4). The number of established allergenic GRP is rapidly increasing, as four fruit GRPs have been registered by the World Health Organization (WHO)-International Union of Immunology Societies (IUIS). Pru p 7 sensitization is associated with cypress pollinosis (CUP) and appears to be driven by cypress pollen exposure, with GRP from Cupressaceae pollen as a primary sensitizer (3-6). Cupressaceae pollen is highly allergenic, massively released during the pollen season (up to 40% of total pollen counts in Mediterranean regions such as Marseille, France), and efficiently spread over long distances (3). Cypress allergen immunotherapy (CUP AIT) is effective and safe for rhinoconjunctivitis related to cypress exposure (5). However, allergen immunotherapy might elicit *de novo* sensitization to allergenic molecules. In this study,

A monocentric retrospective study of medical records and laboratory results was performed. Data were collected for patients aged 15 years or older, residing in southern France and attending allergy work-up for a suspicion of CUP or FA in one of the participating allergy departments of the University Hospitals of Marseille, France, from January 2014 through December 2019.

Data from 391 patients were analyzed (**Supplementary Table1**). CUP was defined as winter (January to April) pollinosis with positive skin prick tests (SPT) or specific IgE to Cupressaceae pollens. Fruit (Rosaceae, citrus, or exotic fruit: kiwi, banana, mango, lychee, guava, papaya, pineapple) allergy was established according to ICON guidelines for food allergy (7). Briefly, FA was defined as the combination of a convincing clinical history of immediate reaction to culprit fruit in the past year, positive SPT (wheal >50% positive control) to culprit fruit extract (Stallergènes-Greer, Antony, France, and ALK-Abelló, Varennes en Argonne, France) or fresh culprit fruit, and/or specific IgE to culprit fruit (3). CUP AIT initiated prior to the index consultation was considered for association analysis. Specific IgE (Cupressaceae pollen, fruit extracts, and corresponding molecular allergens) were measured with the ImmunoCAP™ autoanalyzer (Thermo Fisher Scientific, Uppsala, Sweden). The positivity threshold was set at the assay's limit of quantitation of 0.10 kU<sub>A</sub>/L. Results of 0.10 kUA/L or lower were expressed as 0.05 kUA/L.

Statistical analysis was performed with GraphPad Prism (version 8, La Jolla, CA, USA). IgE levels were expressed as median and interquartile range (IQR). The  $X^2$  test with Yate's correction, Fisher's exact test, t test, Mann Whitney test and Wilcoxon test were used to compare clinical and laboratory variables between groups as appropriate. Statistical significance was defined as  $p < 0.05$ .

CUP diagnosis was established in 267 patients (CUP+), of whom 39 had received CUP AIT (CUP+ AIT+) and 228 had not (CUP+AIT-). FA was confirmed in 30 (77%) CUP+AIT+, in 126 (55%) CUP+AIT- patients, and in 59 (48%) patients free of cypress pollinosis (CUP-), multivariate  $p < 0.0001$ . FA was more prevalent in CUP+AIT+ patients than in CUP+AIT- ( $p = 0.006$ ) and in CUP- ( $p = 0.0003$ ) counterparts (**Supplementary Figure 1**).

The prevalence of Pru p 7 sensitization followed a similar pattern, with the highest figures in CUP+AIT+ (27/39, 69%), followed by CUP+AIT- (90/228, 39%) and CUP- (20/126, 16%) ( $p < 0.0001$ ). Among CUP+ patients, CUP AIT was associated with higher levels of Pru p 7 sensitization,  $p < 0.0001$  (**Figure 1**).

Considering CUP AIT, FA, and Pru p 7 sensitization, higher prevalence and levels of Pru p 7 sensitization were strongly associated with confirmed FA in CUP+ patients, and among these, with CUP AIT+ ( $p < 0.0001$ ) (**Figure 2**). Pru p 7 sensitization was also found, albeit at lower prevalence and IgE levels, in patients without CUP or FA.

These results were not influenced by the patient's age, duration of cypress pollinosis or time since CUP AIT initiation, which were similar across patient groups (**Supplementary Table 1**).

Taken together, these results show that CUP AIT is associated with increased prevalence and levels of Pru p 7 sensitization and fruit allergy. In fact, cypress pollinosis by itself was associated with a higher prevalence of Pru p 7 sensitization and fruit allergy, but a history of CUP AIT further increased the probability of fruit allergy and Pru p 7 sensitization. We also report the occurrence of apparently asymptomatic Pru p 7 sensitization in patients free not only of fruit allergy, but also of cypress pollinosis. However, in this study all Pru p 7-sensitized subjects were also skin or IgE sensitized to Cupressaceae pollen extracts or molecules, even if patients did not complain of cypress pollen-related symptoms. This retrospective, cross-sectional study did not allow to infer a causal effect of CUP AIT. The main limitations of the present study are its monocentric and retrospective design and the possible bias due to patient recruitment to a tertiary allergy care structure. Its major strength is the analysis of a large, clinically and biologically well-characterized cohort of 391 patients.

Given the potential severity of Pru p 7 sensitization and fruit allergy (3,8-9), future longitudinal studies should address Pru p 7 sensitization prior to the initiation of, during, and following the completion of CUP AIT, its relationship with the clinical expression and severity of fruit allergy, and with cypress pollinosis symptoms and severity.

#### **Ethics statement**

This retrospective, non-interventional study was based on the review of clinical and laboratory medical records. Under the French law, ethics committee approval and patient consent were not required for this type of study, provided the patients had received information about the potential use of anonymized medical data for research purposes, and retained the right to oppose it. The study was approved by the local ethics committee (APHM 2020-86).

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## Figure Legends

### **Figure 1. Cypress pollinosis and cypress allergen immunotherapy are associated with more prevalent and higher-level Pru p 7 sensitization.**

Pru p 7 sensitization was more prevalent and displayed higher levels in patients with cypress pollinosis having received cypress allergen immunotherapy (CUP+AIT+) than in patients with cypress pollinosis not treated with cypress allergen immunotherapy (CUP+AIT-), 27/39 (69%) versus 89/228 (55%),  $p < 0.0001$ , and 2.02 (CI95% 0.16-5.89, IQR 0.05-8.31) versus 0.05 (CI95% 0.05-0.05, IQR 0.05-1.31),  $p < 0.0001$ . Data for patients not allergic to cypress pollen are shown for comparison. CI95%, 95% confidence interval; IQR, interquartile range. Statistical significance flag for p-value level: \*\*\*\*  $< 0.0001$ .

### **Figure 2. Cypress pollinosis and cypress allergen immunotherapy are associated with more prevalent and higher-level Pru p 7 sensitization in fruit-allergic patients.**

Pru p 7 sensitization was more prevalent and displayed higher levels in patients with fruit allergy and cypress pollinosis (CUP+) than in those without cypress pollinosis (CUP-). Previous cypress allergen immunotherapy (AIT+) was associated with a further increase in the prevalence and levels of Pru p 7 sensitization. IQR, interquartile range. Statistical significance flag for p-value level: \*\*  $< 0.01$ ; \*\*\*\*  $< 0.0001$ .

**Supplementary Figure 1. Cypress pollinosis and cypress allergen immunotherapy are associated with an increased prevalence of fruit allergy.** Fruit allergy was present in 30/39 (77%) patients with cypress pollinosis having received cypress allergen immunotherapy (CUP+AIT+), in 126/228 (55%) patients with cypress pollinosis not treated with cypress allergen immunotherapy (CUP+AIT-), and in 59/124 (48%) of patients without cypress pollinosis (CUP-). Statistical significance flags for p-value levels: \*  $< 0.05$ ; \*\*  $< 0.01$ ; \*\*\*  $< 0.001$ .



Figure 1. Cypress AIT is associated with more prevalent and higher-level Pru p 7 sensitization.

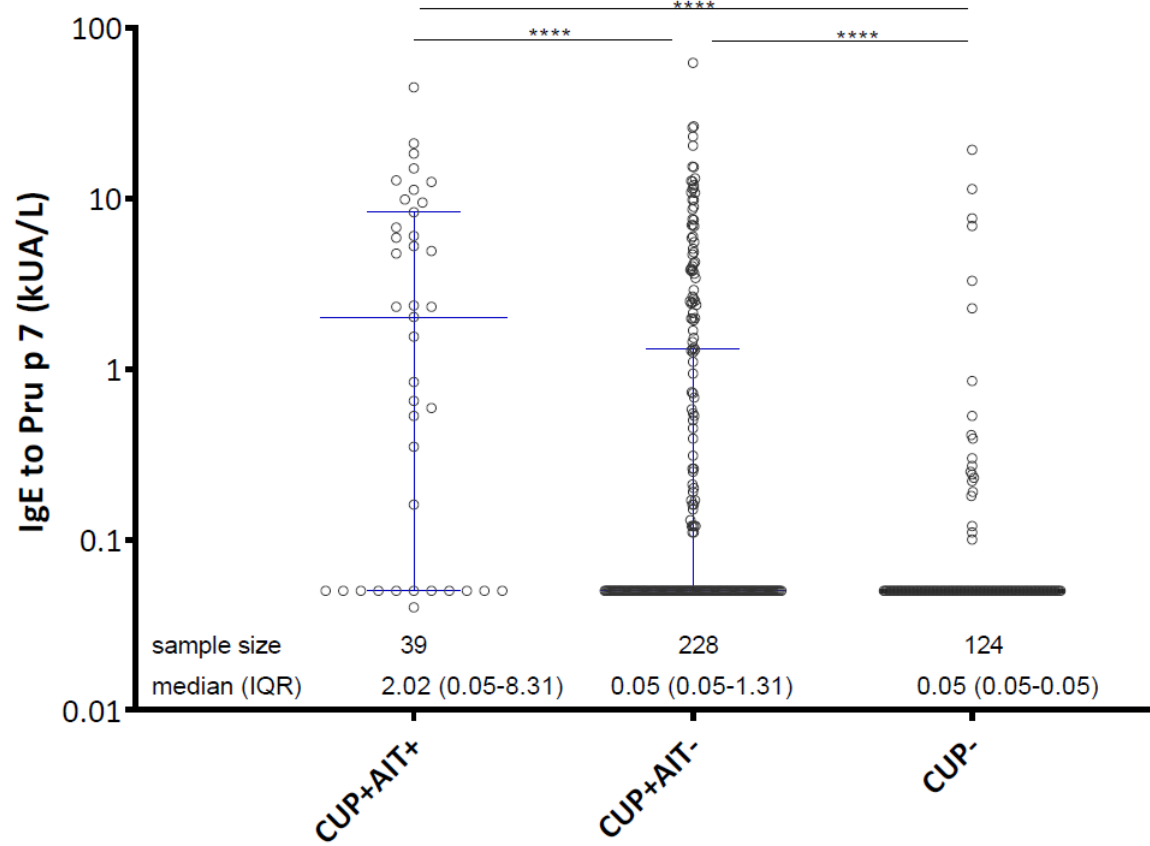
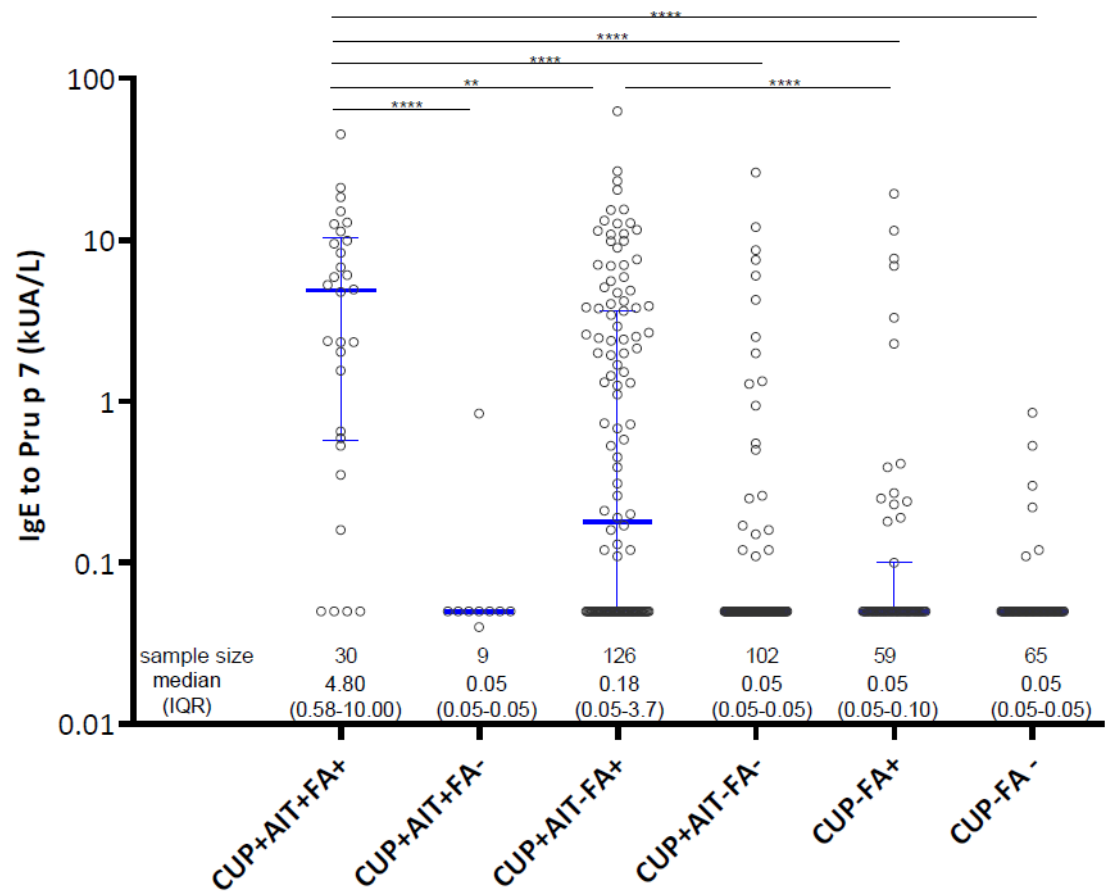


Figure 2. Cypress pollinosis and cypress AIT are associated with more prevalent and higher-level Pru p 7 sensitization in fruit-allergic patients.



	Sample size	Age (years) (median, IQR)	Sex ratio (M/F)	CUP duration (years) (median, IQR)	Time since CUP AIT initiation (median, IQR)
<b>CUP+</b>	<b>267</b>	<b>28 (19-44)</b>	<b>0.6</b>	<b>11 (5-19)</b>	
<b>CUP+AIT+</b>	<b>39</b>	<b>25 (19-45)</b>	<b>0.4</b>	<b>12 (9-16)</b>	<b>8 (4-11)</b>
CUP+AIT+FA+	30	24 (18-42)	0.4	11 (8-14)	7 (3-10)
CUP+AIT+FA-	9	44 (23-61)	0.5	19 (11-40)	11 (5-20)
<b>CUP+AIT-</b>	<b>228</b>	<b>29 (19-44)</b>	<b>0.6</b>	<b>11 (5-20)</b>	<b>NA</b>
CUP+AIT-FA+	126	27 (17-41)	0.5	12 (5-20)	NA
CUP+AIT-FA-	102	31 (24-44)	0.9	8 (5-19)	NA
<b>CUP-</b>	<b>124</b>	<b>33 (17-43)</b>	<b>0.5</b>	<b>NA</b>	<b>NA</b>
CUP-FA+	59	33 (18-43)	0.3	NA	NA
CUP-FA-	65	38 (22-50)	0.9	NA	NA
p (multivariate)		0.98	0.9	0.15	0.15

**Supplementary Table 1. Demographic characteristics of the study population.** CUP, cypress pollinosis; AIT, cypress allergen immunotherapy; FA, fruit allergy; IQR, interquartile range.

Supplementary Figure 1. Cypress pollinosis and cypress allergen immunotherapy are associated with an increased prevalence of fruit allergy.

