

BP14, an allergen from *Cupressus sempervirens* pollen related to the family of snakin/gibberellin-regulated proteins

The missing link of the cross-reactivity between cypress pollen and peach or citrus

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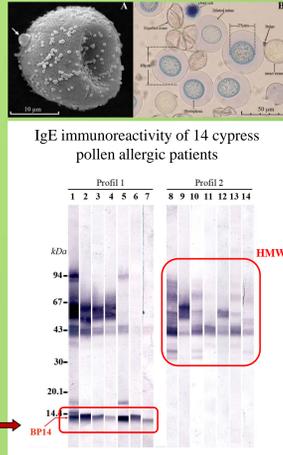
INTRODUCTION

Cypress pollinosis: 9 to 65 % of pollen sensitized patients
Cypress species: *Cupressus sempervirens*, *Hesperocyparis arizonica*, *Cryptomeria japonica*, *Juniperus ashei*, etc...

Cypress allergens: group 1: pectate lyase
 group 2: polygalacturonase
 group 3: thaumatin-like protein
 group 4: Ca-binding protein

and **BP14, a 14kDa basic allergen**

Worldwide cypress pollen allergy



Food associated syndromes



Peach

- Hugues et al. (2006) Allergy
- Delimi et al. (2007) Rev Fr Allergol Clin Immunol
- Caimmi et al. (2013) Allergy



Citrus

- Martinez et al. (2015) Rev Fr Allergol
- Martinez et al. (2015) CFA, EAACI

MASS SPECTROMETRY of BP14

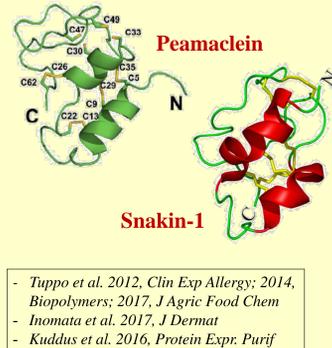
SEQUENCE ALIGNMENT

→ BP14 is a member of the snakin/gibberellin regulated protein (GRP)

PROTEIN		PEPTIDE			
Accession	Name	Species	MW [kDa]	pI	Sequence
P86888	Peamaclein	<i>Prunus persica</i>	6.9	8.6	K.YCGICCEK.C R.CLKYCGICCEK.C
A0A061FGF7	Gibberellin-regulated protein	<i>Theobroma cacao</i>	11.4	10.1	R.CLKYCGICCK.K
GRP (<i>Theobroma cacao</i>):	MKLILVTFLL VSLVLSSTFF EVSMAGSGFC DSKCKVRCSEK AGAKDRCLKY				50
Peamaclein (peach) :	-----GSSFC DSKCGVRCSEK AGYQERCLKY				25
Snakin-1 (potato) :	-----GSSFC DSKCKLRCSK AGLADRCLKY				25
GRP (<i>Theobroma cacao</i>):	CGICCKCKC VPSGTYGNKQ EPCPYRDMKN SKGQLALAGL NCTQR				95
Peamaclein (peach) :	CGICCEKCHC VPSGTYGNKD EPCPYRDLKN SKGNPKCP--				63
Snakin-1 (potato) :	CGICCEECKC VPSGTYGNKH EPCPYRDKKN SKGSKCP--				63

snakin/GRP protein family

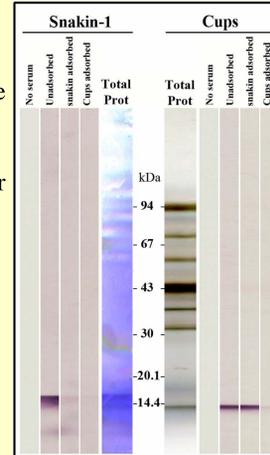
- Small cationic proteins with anti-microbial properties
- 12 cysteines, 6 disulfide bridges
- Resistant to trypsin, to heat
- Conserved C-terminal part, variable N-terminal
- Present in fruits and plants
- Involved in pollen maturation, plant responses to biotic and abiotic stress (ex: parasite or pollution), hormone crosstalk, redox homeostasis, ...
- peamaclein shares 82% identity with snakin-1 from potato
- **4 GRP = allergens in fruits: Pru p 7:** peach (*Prunus persica*), **Cit s 7:** orange (*Citrus sinensis*), **Pru m 7:** Japanese apricot (*Prunus mume*), **Pun g 7:** pomegranate (*Punica granatum*)



CROSSED INHIBITIONS

Inhibition protocole

- 1 - adsorption (depletion) of the patient's serum on *C. sempervirens* pollen extract (Cups) or snakin-1, dotted or blotted onto nitrocellulose membrane
- 2 - incubation of the adsorbed serum on blotted *C. sempervirens* pollen extract (Cups) or snakin-1



→ BP14 inhibits the IgE reactivity on snakin-1 but snakin-1 does not inhibit the IgE reactivity on BP14

Patient Nbr	IgE reactivity on:	Immunosorbent for depletion		
		snakin-1	Cypress pollen extract	
5	snakin-1	+	+	+
	BP14 in basic fraction	-	+	
12	snakin-1	+	+	-
	BP14 in basic fraction	-	+	
14	snakin-1	+*	+†	no inhibition
	BP14 in total extract	-	+	
16	snakin-1	ND	ND	-
	BP14 in basic fraction	-	+	
19	snakin-1	+	+	-
	BP14 in basic fraction	-	+	

The same results are obtained with the serum adsorbed either on snakin (*) or on BP14 electro-transferred on nitrocellulose membrane after migration in SDS-PAGE (†).

Patients' clinical data

Serum #	Gender	Age	Specific IgE (kU/L)				Other
			Cup s pollen	peach	citrus		
1	M	67	ND	ND	ND	(negative control, non atopic, non allergic)	
2	M	4	0.35	1.80	ND	potato: 1.22	
3	M	47	<0.10	ND	ND	nettle pollen: 9.84	
4	F	26	<0.10	ND	ND	pine pollen +	
5	M	19	10.2	2.62	1.09	mustard: 3.29; mite<0.10	
6	F	3	26.0	1.80	ND	grass: 87.5; weed: 28.6; mite: 0.23	
7	M	3	6.99	14.7	3.08	pea: 4.4; lentil: 4.2	
8	M	30	11.5	<0.10	0.14	Juniperus: 40.1; dander: 1.8; mite: 0.3	
9	M	14	2.27	0.29	<0.10	Juniperus: 13.6; pumpkin: 9.8	
10	F	17	4.89	0.6	0.55	weed: 3.2; olive: 11.4	
11	M	21	2.86	<0.10	<0.10	grass: 1.8; olive: 0.69; mite: 17.0	
12	M	44	4.33	0.11	<0.10	grass: <0.10; weed: 0.16	
13	F	31	5.82	ND	3.96		
14	F	17	8.39	0.41	0.43	grass: 0.26	
15	M	13	+	ND	+		
16	F	25	7.50	+	0.76		
17	F	31	10.9	ND	2.34		
18	F	39	59.8	<0.10	ND	<i>C. arizonica</i> : 36.7; <i>J. sabinoides</i> >100; mite 1.7	
19	F	48	4.66	0.98	1.44	curry: 1.19; coriandre: 0.74; gluten: 0.49	
20	M	42	23.1	21.7	15.6	grass: 26.7; mimosa: 1.44; dander: 5.3	
21	M	72	21.6	0.57	0.87	grass: 2.21; dander: 5.96	
22	M	10	8.15	0.18	0.29	grass: 24.4	
23	M	13	11.8	6.41	3.85	weed: 36.9; oak: 35.5	
24	M	19	8.15	<0.10	<0.10	grass: 0.11	
25	F	21	0.88	<0.10	<0.10	grass: <0.10; dander: <0.10; mite: 12.0	
26	F	11	12.1	5.2	4.57	Ara h 8: 9.6; Ara h 9: 4.7; grass: 8.2; olive: 75.5; dander: 3.3; mite	
27	M	5	3.35	<0.10	<0.10	weed: 0.1; mite: 0.3	
28	M	41	1.54	<0.10	<0.10	mimosa: <0.10	
29	M	11	8.8	4.69	8.15	pea, lentil, chickpea>100; grass: >100; olive: 18.0; dander: 7.7	
30	M	55	1.11	0.7	0.72	grass: 1.4; weed: 4.8; mite: 10.9	
31	F	48	0.47	0.35	0.35	grass: 0.48; mite: 0.11	
32	M	unknown	51.36	<0.10	ND		
33	F	37	3.62	0.12	0.16	grass: 0.18; weed: 0.16	
34	M	21	13.7	0.49	0.45	grass: 40.3; weed: 0.59	
35	F	44	13.1	ND	<0.10	weed: 0.17; dander: 13.0; mite: <0.10	

ND: non done ++: qualitative evaluation

IgE IMMUNO REACTIVITIES

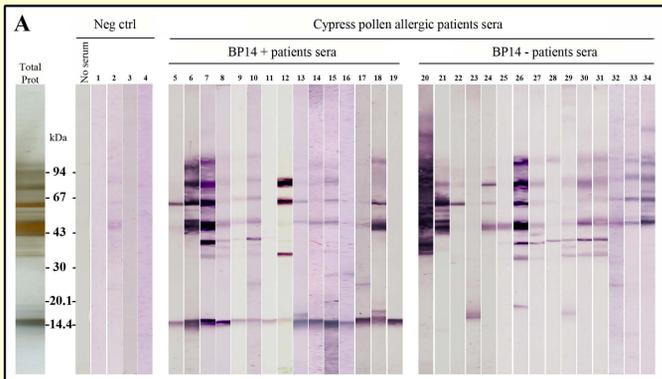
Western blots after SDS-PAGE 8-18%
 Revelation of IgE binding

30 sera from cypress pollen allergic patients are tested on blotted *C. sempervirens* pollen extract (A) or on blotted recombinant snakin-1 (B).

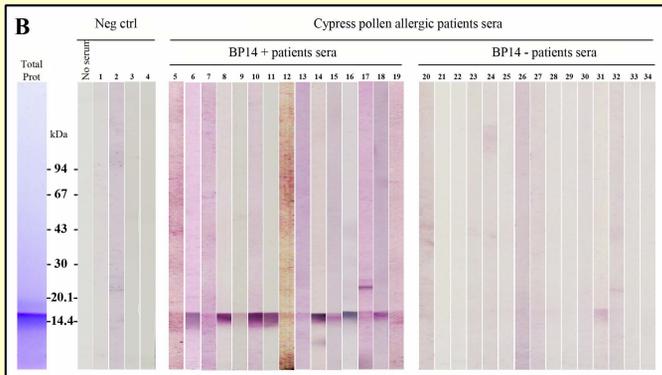
- 15 sera BP14+ (5 to 19)
 - 15 sera BP14- (20 to 34)

→ All BP14+ patients are snakin+

Cupressus sempervirens pollen extract



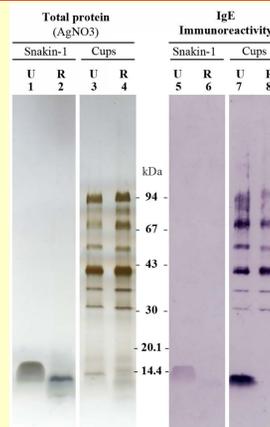
Snakin-1



Reduced snakin-1 (with β-mercapto éthanol) decreased its apparent mw and abolished the IgE reactivity... like BP14.

U : Unreduced,
 R : Reduced,
 Cups : *C. sempervirens* pollen extract

Serum N° 7



→ because of 6 disulfide bridges the protein is very folded favoring conformational IgE epitopes

CONCLUSION (1)

- 1 - A pollen allergen is a member of the snakin/GRP protein family
- 2 - It cross-reacts with other members of this protein family found in many fruits and plants.
- 3 - It may constitute the structural basis of the peach/cypress and citrus/cypress associated syndromes described, involving Pru p 7 and Cit s 7, two allergens, of the snakin/GRP protein family, described in peach and sweet orange.

CONCLUSION (2)

- 4 - Given the results of crossed inhibition it seems that the food sensitization is secondary to the pollen sensitization
- 5 - The sensitization to allergens from snakin/GRP protein family could be a new marker of the pollen food associated syndromes. (Charpin et al. 2017, Clin Rev Allergy Immunol).

(In relation to this presentation, we declare that there are no conflicts of interest)