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Brief Communication

Capsicum Allergy: Involvement of Cap a 7, a New Clinically Relevant Gibberellin-Regulated Protein Cross-Reactive With Cry j 7, the Gibberellin-Regulated Protein From Japanese Cedar Pollen

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ABSTRACT

The *Capsicum* genus belongs to the Solanaceae family. Bell or chili peppers are consumed worldwide, but allergy to *Capsicum* is rare. It is involved in the celery-birch-mugwort-spice syndrome and cross-reactivities were reported with latex. Several allergens have been described, but only 2 are referenced in the World Health Organization/International Union of Immunological Societies allergen data bank, a thaumatin-like protein and a profilin. A patient allergic to bell/chili pepper, peach, orange and Japanese cedar pollen was clinically and biologically analyzed including direct and competitive immunoblots and basophil activation tests (BATs) with allergenic source extracts and recombinant gibberellin-regulated proteins (GRPs). The patient was shown to be sensitized to Cap a 7, the GRP of *Capsicum annuum* newly described herein. Cross-reactivities were demonstrated between various GRPs from bell/chili pepper, peach, orange and Japanese cedar pollen either in native form in the different extracts or as recombinant allergens. A similar immunoglobulin E reactivity was found also in *Capsicum chinense* and against snakin-1, the GRP from potato. The patient showed a positive BAT with recombinant Cry j 7, Pru p 7 and Cap a 7, but not with recombinant snakin-1. Despite the ubiquitous nature of GRPs in plants and the immunochemical cross-reactivity observed between different GRPs, clinically relevant sensitization to this protein family seems restricted to some allergenic sources, often associated with *Cupressaceae* pollen allergy, and to some patients, therefore reflecting very specific and peculiar mechanisms of conditional sensitization.

Keywords: *Capsicum*; allergens; pollen; *Cryptomeria*; food hypersensitivity

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There are no financial or other issues that might lead to conflict of interest.

INTRODUCTION

Capsicum, originates from South America and encompasses 5 species: *annuum*, *chinense*, *baccatum*, *frutescens* and *pubescens*. They are commonly named bell (soft) or chili (spicy) pepper and can be red, orange, yellow or green variably tasting from sweet to very spicy on the Scoville scale. They are mainly eaten whole raw in salad composition or cooked, as in ratatouille and also as spices after drying and powdering like paprika. They belong to the Solanaceae family, which includes other genera such as tomato, potato, eggplant, or tobacco.

While allergy to *Capsicum* is considered rare, allergy symptoms were reported after consumption of bell or chili/cayenne pepper or paprika.[1-3] Solanaceae are involved in the celery-birch-mugwort-spice syndrome.[4] Nine allergens have been reported. Two of them are certified by World Health Organization/International Union of Immunological Societies (WHO/IUIS), Cap a 1, an osmotin, member of the thaumatin-like protein family (PR5) and Cap a 2, a profilin.[5,6] Seven additional allergens have been reported, a β-1,3-glucanase,[7,8] an ascorbate peroxidase,[9] a Bet v 1-like protein (PR10 family),[10] a defensin J1 and a vicilin,[2] a lipid transfer protein (LTP)[11] and a chitinase similar to the prohevein Hev b 6.[12] Cross-reactivities were reported between bell pepper and latex involving β-1,3-glucanase and profilin.[9]

We report a case of a 16-year-old Japanese patient allergic to Japanese cedar pollen and to fruits who suffered from an anaphylactic reaction after consuming chili pepper. The patient is shown to be sensitized to a clinically relevant allergen as yet undescribed in *Capsicum annuum* and *Capsicum chinense*, a member of the gibberellin-regulated protein (GRP) family, Cap a 7, thus extending the number of GRP allergen members characterized up to now.

MATERIALS AND METHODS

Protein extraction

Fresh bell (*C. annuum*) and chili peppers (*C. annuum* var. Serrano from Morocco and *C. chinense* var. Scotch bonnet from Ghana) and peaches were purchased from vegetable stores (Paris, France). The skins were removed and crushed in grinder. Solid pulp or crushed skin, free of juice, were extracted using a multidirectional grinder FastPrep 24 (MP-Biomedicals, Illkirch, France) in the presence of 500 glass beads (1-mm diameter) in phosphate-buffered saline. After filtrations and centrifugation, supernatants were lyophilized, dissolved in H_2O and stored at −20°C. Proteins from *Cryptomeria japonica* (Cryj) pollen grains (10%, w:v) (Allergon AB, Angelholm, Sweden) were extracted as previously described.[13]

For basophil activation test (BAT) chili pepper (*C. annuum*) extract was obtained after solubilisation of a commercially available chili pepper powder purchased at supermarket together with canned peach.

Recombinant proteins

The GRPs from Japanese cedar (Cry j 7), peach (Pru p 7), potato (snakin-1) and chili/bell pepper (Cap a GRP) were prepared as recombinant proteins in *Pichia pastoris.*[14] Seven sequences of Cap a GRPs were found as a result of a BLAST search using Pru p 7 sequence as query. XP_016553101.1 corresponding to A0A1U8F383 in the UniprotKB database was produced as recombinant protein because it showed the highest percentage of sequence identity with Pru p 7 (about 81%) (**Fig. 1**). It was named Cap a 7 by the WHO/IUIS Allergen

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Acession number	Source	Protein	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
XP_016553101.1	***Capsicum annuum***	**Cap a 7***	G	S	A	F	C	D	S	K	C	N	F	R	C	S	K	A	G	R	K	D	R	C	L	K	Y	C	G	I	C	C	A	D	C
XP_016568843.1			-	-	S	-	-	-	-	-	-	K	Q	-	-	-	-	-	-	L	A	-	-	-	-	-	-	-	-	-	-	-	E	Q	-
XP_016554341.1				-	D	-	-	-	-	-	-	-	V	-	-	-	-	-	S	A	H	-	-	-	-	-	-	-	-	-	-	-	-	E	-
PHT57516.1					-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AFQ40008.1			-	-	N	-	-	-	-	-	-	K	L	-	-	-	-	-	-	L	A	-	-	-	-	-	-	-	-	-	-	-	E	E	-
PHT81083.1				-	D	-	-	-	-	-	-	-	V	-	-	-	-	-	S	A	H	-	-	-	-	-	-	-	-	-	-	-	-	E	-
XP_016557751.1				-	S	Y	-	-	Q	-	-	T	A	-	-	W	-	-	-	L	-	K	-	-	-	-	-	-	-	-	-	-	G	L	-
A0A2G3CL03	***Capsicum chinense***		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A0A2G2WX49	***Capsicum baccatum***		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Q948Z4	Potato	Snakin-1	-	-	S	-	-	-	-	-	-	K	L	-	-	-	-	-	-	L	A	-	-	-	-	-	-	-	-	-	-	-	E	E	-
K4DF43	Tomato		-	-	D	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-	Q	-	-	-	-	-	-	-	-	-	-	-	E	E	-
P86888	Peach	Pru p 7*	-	-	S	-	-	-	-	-	-	G	V	-	-	-	-	-	-	L	Q	E	-	-	-	-	-	-	-	-	-	-	E	K	-
XP_016649029.1	Apricot	Pru m 7*	-	-	S	-	-	-	-	-	-	G	V	-	-	-	-	-	-	L	Q	E	-	-	-	-	-	-	-	-	-	-	E	K	-
A0A067D4T6	Orange	Cit s 7*	-	-	D	-	-	-	-	-	-	A	V	-	-	-	-	-	-	-	E	-	-	-	-	-	-	-	-	-	-	-	D	K	-
A0A218X6T8	Pomegranate	Pun g 7*	-	-	S	-	-	-	-	-	-	A	V	-	-	-	-	-	-	V	Q	-	-	-	-	-	-	-	-	-	-	-	E	K	-
A0A6P5SVH6	Sweet Cherry	Pru av 7*	-	-	S	-	-	-	-	-	-	G	V	-	-	-	-	-	-	Y	-	E	-	-	-	-	-	-	-	-	-	-	E	K	-
C0HLQ1	***Cryptomeria japonica***	Cry j 7*	A	H	I	D	-	-	K	E	-	-	R	-	-	-	-	-	S	A	H	-	-	-	-	-	-	-	-	-	-	-	E	K	-

Acession number	Source	Protein	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	Identity (%)
XP_016553101.1	***Capsicum annuum***	**Cap a 7**	N	C	V	P	S	G	T	F	G	N	K	D	E	C	P	C	Y	R	D	K	K	N	S	K	G	G	P	K	C	P	100.00
XP_016568843.1			K	-	-	-	-	-	-	Y	-	-	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	-	-	-	-	82.54
XP_016554341.1			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	88.89
PHT57516.1			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.00
AFQ40008.1			K	-	-	-	-	-	-	Y	-	-	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	K	S	-	-	-	80.95
PHT81083.1			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	90.48
XP_016557751.1			K	-	-	-	-	-	-	Y	-	-	-	S	-	-	-	-	-	-	-	M	L	-	-	-	-	K	-	-	-	-	-	74.19
A0A2G3CL03	***Capsicum chinense***		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.00
A0A2G2WX49	***Capsicum baccatum***		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100.00
Q948Z4	Potato	Snakin-1	K	-	-	-	-	-	-	Y	-	-	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-	K	S	-	-	-	-	80.95
K4DF43	Tomato		H	-	L	-	-	-	-	Y	-	H	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	85.71
P86888	Peach	Pru p 7*	H	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	N	-	-	-	-	-	80.95
XP_016649029.1	Apricot	Pru m 7*	H	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	N	-	-	-	-	-	80.95
A0A067D4T6	Orange	Cit s 7*	H	-	-	-	-	-	-	Y	-	H	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	K	-	-	-	-	-	82.54
A0A218X6T8	Pomegranate	Pun g 7*	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	M	-	-	-	-	-	K	-	-	-	-	-	84.13
A0A6P5SVH6	Sweet Cherry	Pru av 7*	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	-	-	-	L	-	-	-	-	-	N	-	-	-	-	-	84.13
C0HLQ1	***Cryptomeria japonica***	Cry j 7*	-	-	-	-	P	-	-	Y	-	-	E	-	S	-	-	-	-	A	N	L	-	-	-	-	-	-	H	-	-	-	-	68.25

Fig. 1. Sequence alignment of the 7 *Capsicum* candidates, plant and food GRPs. Asterisks indicate allergenic proteins. -: identical amino-acid with bell pepper GRP. Cysteines are in red. Percent sequence identity is calculated according to the first line bell pepper GRP.
GRP, gibberellin-regulated protein.

Nomenclature Sub-Committee. Recombinant Pun g 7 (pomegranate) and Cit s 7 (orange) were prepared as previously described.[13]

Patient data and sera

The patient's clinical data were recorded at the Department of Pediatrics, National Hospital Organization Sagamihara, Kanagawa, Japan.[13] Informed consent (validated by the Ethics Committee of the National Hospital Organization Sagamihara National Hospital) was obtained for each patient as well as for the approval from an ethical committee (#2013-201307916, #20141214 and #2019-038).

Japanese cedar pollinosis was defined by clinical symptoms during Japanese a cedar pollination period and specific immunoglobulin E (IgE) evaluation. Food allergies were defined by immediate allergy reactions after consuming causative food and positive results in

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skin prick and oral food challenge tests. The serum from a healthy individual and 3 sera from atopic patients with similar types of allergy (except bell/chili pepper) were used as negative controls and corresponded to residues from biological analysis laboratories. Clinical and biological parameters are presented in Table.

Protein analysis and immunoblotting

One-dimensional electrophoresis (1-DE) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described[13] and double 1-DE (D1-DE) according to Shahali *et al.*[15] Briefly, after a first dimension in isoelectric focusing, bands corresponding to selected pI ranges were cut on the whole width of the gel and co-axially run in SDS-PAGE.

After migration and electric protein transfer, the nitrocellulose membranes were processed as previously described.[13] Serum IgE binding was immuno enzymatically revealed using alkaline phosphatase-conjugated goat anti-human IgE (ε specific; Sigma-Aldrich, St. Louis, MO, USA). IgE binding competition with bromelain 0.1%[16] or rCry j 7, rPru p 7 or rCap a 7 at different concentrations were performed.

BAT

Chili pepper extract or canned peach as allergenic sources or recombinant GRPs (rCry j 7, rPru p 7, rCap a 7 or rsnakin-1) were used to activate patients' basophils. Activation was evaluated by the expression of CD203c by flow cytometry according to the supplier's recommendations (Allergenicity Kit; Beckman Coulter, Inc., Brea, CA, USA). For recombinant series 2 control patients, not allergic to fruit, were tested.

RESULTS

Clinical history of Capsicum-allergic P1

P1, a Japanese girl born in October 2000 and living in the Shizuoka prefecture, was diagnosed as having allergy to Japanese cedar pollen when she was 10 years old. Japanese cedar pollinosis was defined by the combination of clinical symptoms during a Japanese cedar pollination period and specific IgE evaluation. She has no history of asthma, atopic dermatitis or latex allergy. She suffered also from allergy to apple, peach and orange with symptoms of anaphylaxis exacerbated by physical exercise (or before menstruation) with an onset at the age of 12 after the consumption of canned peach. At the age of 14 years, consuming Korean cuisine containing beef, bean sprout, spinach, fiddlehead fern, chili pepper and rice, she developed anaphylaxis with symptoms of facial angioedema, systemic erythema, cough, dyspnea and cramp. At the age of 16, she again experienced a similar reaction after consuming Chinese cuisine containing tofu, ground meat and chili pepper. Chili pepper was suspected as a causative food and confirmed with specific IgE evaluation (**Table**) showing, in addition, a high titer to Japanese cedar pollen together with other pollen and food sensitizations to peach, apple, orange or potato. P1 has no IgE against LTPs and a low titer to PR-10. Oral food challenges to chili pepper (125 mg) or peach (30 g of canned peach) were positive inducing allergic symptoms that include anaphylaxis and required adrenaline and fluid supplement. In agreement, chili pepper extract and canned peach were able to *ex vivo* activate the patient's basophils as attested by the dose-dependent expression of CD203c (**Fig. 2A**).

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Table. Clinical and biological parameters of P1, the studied patient and control patients P2, P3 and P4

Patient			Total IgE (kIU/L)	Pollen sIgE (kUA/L)	Symptom	Food						
Nr	Sex	Age (yr)				Fruit & vegetable sIgE (kUA/L)	Type of FA symptom	Symptom				
								Skin	Oral cavity	Gastro intestinal	Respiratory	Anaphylaxis
P1	F	16	646	Japanese cedar: 220	R, C	Apple: 2.54	Immediate/ FEIAn	GU	LT	CR	CO	1
				Japanese cypress: 31.1		Mal d 1: 0.39						
				Alder: 2.99		Mal d 3: < 0.10						
				Ragweed: 1.34		Peach: 4.70		FL	LT	CR	CO	1
				Mugwort: 0.82		Pru p 1: 0.47						
				Orchard grass: 1.32		Pru p 3: < 0.10						
						Pru p 4: < 0.10						
						Orange: 4.55			POC	AP		1
						Potato: 1.08				AP		
						Chili pepper: 0.237		E, FA		CR	D	1
P2	M	15	378	Japanese cedar: 21.9	A, R, C	Cherry: N/A	Immediate/ FEIAn	GU	LT		W, CO, S	1
				Birch: 1.96		Peach: 2.59						
				Ragweed: 0.89		Apple: 1.3						
				Orchard grass: 3.58		Orange: 2.35						
				Mugwort: 1.47								1
P3	M	9	598	Japanese cedar: 222	N/A	Grapefruit: 2.29	Immediate/ FEIAn	I, SE	LT	AP	S, CAL	1
				Ragweed: 0.76		Peach: 6.45						1
				Mugwort: 0.45		Orange: 1.51						1
P4	F	8	923	Japanese cedar: 528	A, R	None	None					
				Birch: 16.1								
				Ragweed: 3.54								
				Orchard grass: 6.18								
				Mugwort: 2.47								

Specific IgE titers were evaluated using ImmunoCAP system (ThermoFisher Scientific, Uppsala, Sweden). Sensitization threshold for Japanese cedar was 0.35 and 0.1 kUA/L for other allergenic sources.
IgE, immunoglobulin E; N/A, not available; CO, cough; FEIAn, food-dependent exercise-induced anaphylaxis, POC, prurit oral cavity; A, asthma; CR, cramps; FL, flushing; R, rhinitis; AP, abdominal pain; D, dyspnea; GU, generalized urticaria; S, sneezing; C, conjunctivitis; E, systemic erythema; I, itching; SE, swollen eyelids; CAL, change in activity level; FA, facial angioedema; LT, laryngeal tightness; W, wheezing.

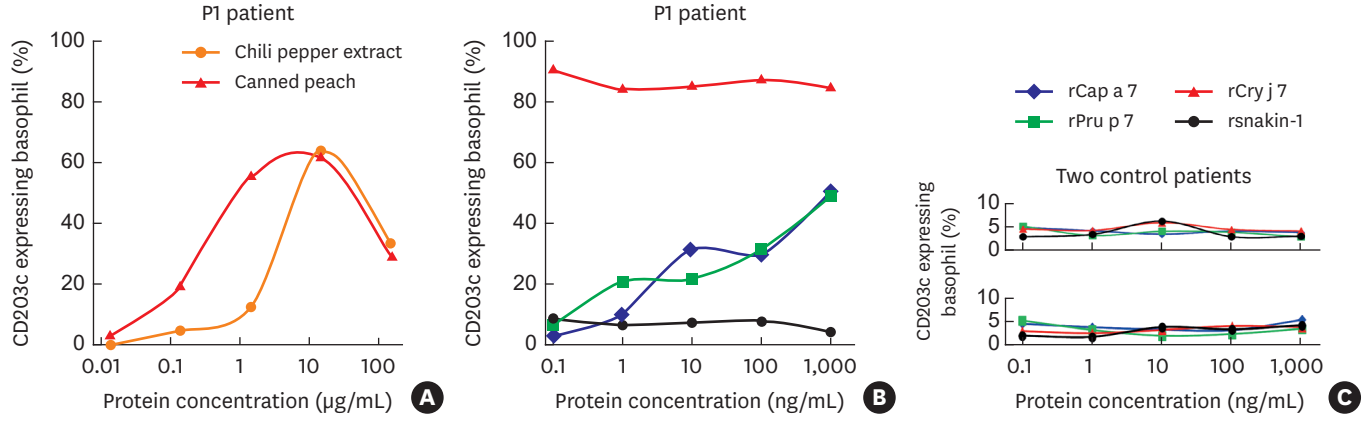

Fig. 2. BAT with P1's basophils tested against (A) chili pepper and canned peach extracts at different concentrations (0.01–100 µg/mL); (B) rCry j 7, rPrup 7, rCap a 7 and rsnakin-1 at different concentrations (0.1–1,000 ng/mL); and (C) BAT with 2 control patient's basophils tested against rCry j 7, rPrup 7, rCap a 7 and rsnakin-1 at different concentrations (0.1–1,000 ng/mL).
BAT, basophil activation test.

P1 sensitized to cationic low molecular weight (LMW) from Cryj pollen and *Capsicum* proteins

P1 showed IgE reactivities against 2 close LMW bands (< 14 kDa) in Cryj pollen (**Fig. 3A**). Similar to P1, control patients, P2 and P3 displayed LMW IgE reactivities corresponding to Cry j 7.[13]

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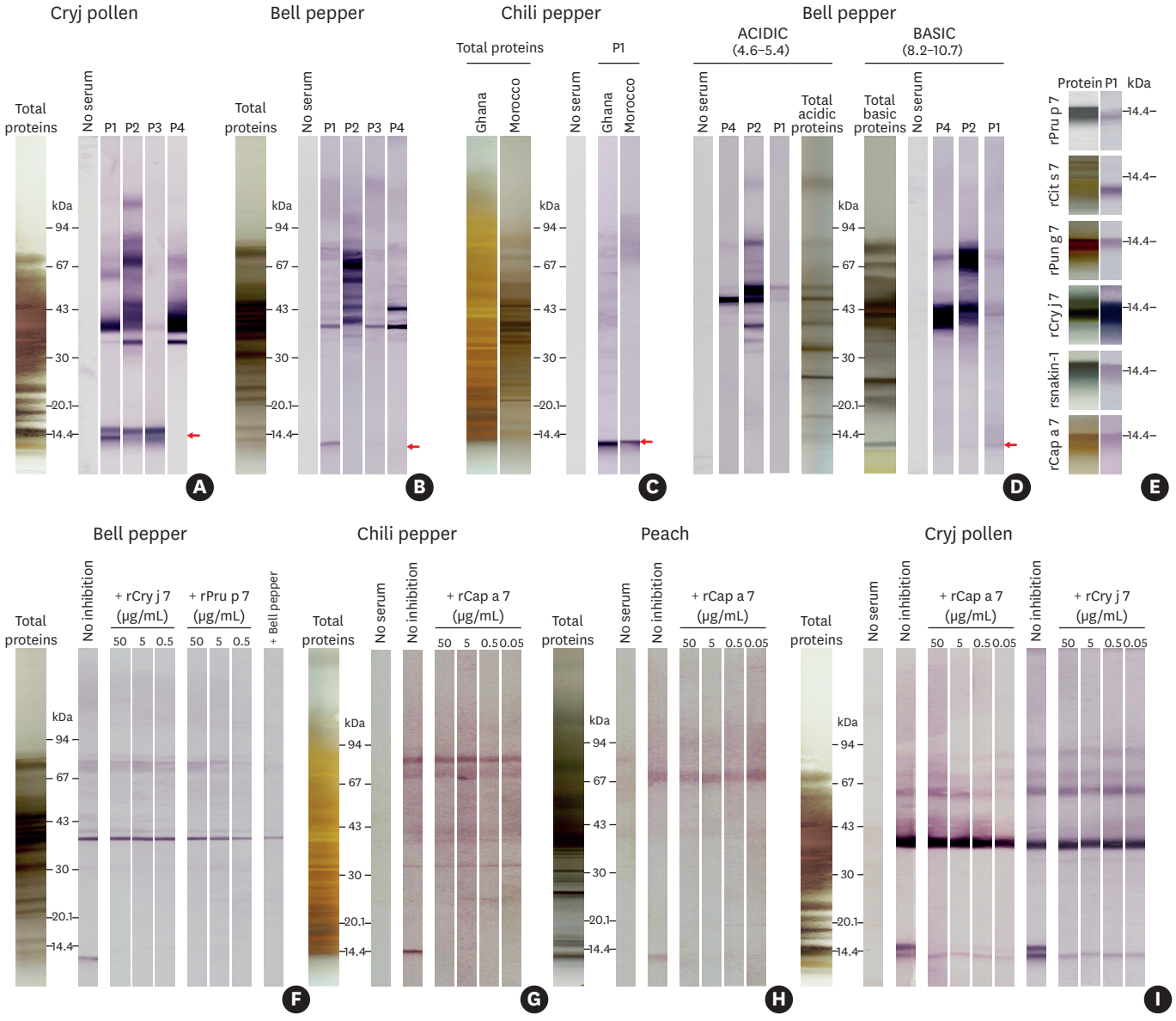

Fig. 3. IgE immunoreactivity of *Cupressaceae*-allergic patients. (A) Cryj pollen extract. (B) Bell pepper pulp protein extract. (C) Chili pepper pulp protein extracts from Morocco (*Capsicum annuum*) or Ghana (*C. chinense*). (D) Acidic (pH 4.6–5.4) or basic (pH 8.2–10.7) bell pepper proteins fractionated by in-gel isoelectric focusing were then separated by SDS-PAGE and blotted onto nitrocellulose membranes. (E) P1 tested against various recombinant GRPs as indicated using immunoblots. (F-I) Competitive inhibition experiments: bell pepper (F) or chili pepper from Ghana (*C. chinense*) (G) or peach (H) or Cryj pollen (I) extracts in absence or presence of different recombinant GRPs as competitive inhibitors as indicated. Arrows indicate the cationic LMW IgE reactivity for P1. Total proteins are silver-stained and molecular mass references are indicated in kDa.
IgE, immunoglobulin E; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; GRP, gibberellin-regulated protein; LMW, low molecular weight.

Only P1 showed an IgE reactivity in LMW proteins of bell pepper pulp extracts (**Fig. 3B**) barely observed in skin and juice (**Supplementary Data 1** and **Supplementary Fig. S1**). An intense reactivity band in LMW proteins extracted from the pulp of chili peppers originated from Morocco (*C. annuum*) or Ghana (*C. chinense*) was observed (**Fig. 3C**). This P1's LMW allergen is cationic (**Fig. 3D**) and IgE epitopes are not oligosaccharide related (**Supplementary Data S2** and **Supplementary Fig. S2**).

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P1 sensitized to a clinically relevant allergenic *Capsicum* GRPs

Since the patient was sensitized to a *Cupressaceae* pollen, Japanese cedar and to fruits such as peach and orange, and showed a reactivity in cationic LMW with no LTP sensitization, a GRP sensitization was suspected. Indeed, P1 was tested positive against 6 recombinant GRPs including rCap a 7, the GRP from *Capsicum* (**Fig. 3E**). The results are in agreement with the patient's symptoms when exposed to orange, peach, potato, bell/chili pepper or pollen and also with the immunochemical cross-reactivities described between the different GRPs.[13,17] No information is available about the ability of P1 to tolerate pomegranate because she has never consumed this fruit.

Competitive inhibitions with different concentrations of either rCry j 7 or rPru p 7 or rCap a 7 showed that rCry j 7 or rPru p 7, as low as 0.5 μg/mL, totally and specifically abolished the binding of P1 IgE against the LMW bell pepper allergen, while the reactivities at 35 kDa and higher MW were not affected (**Fig. 3F**). Homologous inhibition with bell pepper extract was nearly complete. Recombinant Cap a 7 was also able to compete with the LMW IgE reactivity found in *C. chinense* (**Fig. 3G**), therefore corresponding to the GRP of *C. chinense.* This result is in agreement with the 100% sequence identity observed between the different *Capsicum* genus GRPs (**Fig. 1**). Furthermore, rCap a 7 was shown to compete with native Pru p 7 in peach extract (**Fig. 3H**). Conversely, rCry j 7 and rCap a 7 were able to totally inhibit the upper LMW band and nearly totally the lower LMW one in Cryj pollen (**Fig. 3I**). These results confirmed the GRP nature of the allergen recognized in *Capsicum* extracts.

Using BAT, rCap a 7 was shown to be clinically relevant like rPru p 7, since about 50% of P1's basophils were activated at 1 μg/mL. The activation is higher with rCry j 7 (90% at 0.1 ng/mL) (**Fig. 2B**). Control patients did not show any basophil activation (**Fig. 2C**). Interestingly, rsnakin-1 did not induce any activation of P1's basophils, although P1 has unsystematic symptoms upon consumption of potatoes.

DISCUSSION

We report a new food allergen of the protein family GRP/gibberellic acid stimulated in *Arabidopsis* in the genus *Capsicum*, Cap a 7. The allergen is in *C. annuum* and *chinense*, more abundant in pulp than in skin and juice. The chili pepper-allergic P1 was also allergic to Japanese cedar pollen and to fruits such as peach, orange and apple, but not to latex.[9] Besides immunochemical characterization, a clinical relevance of Cap a 7 was shown, since not only the allergenic sources, chili pepper and peach extracts, but also rCap a 7 and rPru p 7 themselves were able to activate the patient's basophils.

Until now, 5 fruits have been reported to contain allergenic GRPs, peach (Pru p 7),[18] orange (Cit s 7),[19] pomegranate (Pun g 7),[20] the Japanese apricot (Pru m 7)[21] and sweet cherry (Pru av 7: www.allergen.org). Cross-reactive GRPs were found in *Cupressaceae* pollen, Mediterranean cypress (Cup s 7),[22] Japanese cedar (Cry j 7)[13] and mountain cedar (Jun a 7)[23] partly or entirely responsible for the cypress-peach[24] and cypress-citrus[25] pollen-food allergy syndrome. GRPs are 7 kDa proteins with 6 disulfide bonds generating very similar folded tertiary structures displaying a cleft in the molecule (**Supplementary Data S3** and **Supplementary Fig. S3**). Despite structural and sequence similarities among GRP, specific allergy profiles can be distinguished. The IgE reactivity of P1 to native bell pepper GRP is inhibited by rCry j 7 and also by rPru p 7 and, conversely, rCap a 7 significantly inhibited the binding of

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P1 IgE to nCry j 7. However, in a cohort of 45 Cup s 7/Cry j 7-positive patients, only P1 was positive on native *Capsicum* GRP in the whole extract (data not shown). Therefore, either the studied patient raised a specific IgE response to private epitope(s) on native *Capsicum* GRP or specific IgEs have a high affinity. Interestingly, fruit or vegetable GRP IgE responses often occur in association with Cupressaceae pollen sensitization. The reasons for this conditional sensitization remain unsolved and may be based on complexes and ligand complementarities occurring at molecular levels during the earlier steps of triggering of the immune system.

Unsystematically, P1 had allergic symptoms upon consumption of potatoes. Bell pepper and potato GRPs share 80% to 95% sequence identity with very similar surface characteristics. Despite an immunochemical IgE cross-reactivity, rsnakin-1 was unable to activate P1's basophils, reminiscent of similar results previously reported.[26] The explanation might be a low IgE affinity or the requirement for co-factors inducing symptoms such as physical exercise, non-steroidal anti-inflammatory drugs, proton pump inhibitors, alcohol or the rarely mentioned menstruations.

Our results extended the number of allergenic plant food GRPs to bell/chili pepper. The sensitization is associated with sensitizations to fruits and Cupressaceae pollen. Although GRPs are present in all plants, only a few of them have been shown to be allergenic up to now, reflecting very peculiar mechanisms of conditional sensitization.

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SUPPLEMENTARY MATERIALS

Supplementary Data S1

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Supplementary Data S2

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Supplementary Data S3

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Supplementary Reference

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Supplementary Fig. S1

IgE immunoreactivity of Cupressaceae allergic patients against bell pepper pulp protein extract or bell pepper skin protein extract or bell pepper juice as indicated. Arrows indicate the cationic LMW IgE reactivity for P1 patient. Total proteins are silver stained and molecular mass references are indicated in kDa.

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Supplementary Fig. S2

IgE immunoreactivity of Cupressaceae allergic patients against bell pepper protein extract in presence or absence of bromelain 0.1% to assess the oligosaccharide or peptidic nature of IgE epitopes. Arrow indicates the cationic LMW IgE reactivity for P1 patient. Total proteins are silver stained and molecular mass references are indicated in kDa.

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Supplementary Fig. S3

Three-dimensional modelling of 4 GRPs showing position of alpha helices: Cap a 7 from bell pepper, potato snakin-1, Cryj 7 from Japanese cedar pollen and Pru p 7 from peach. Comparative 3-D structure of GRPs was obtained by SWISS-MODEL using the structure of snakin-1 as a template (ID: 5e5q). The high reliabilities of the models were confirmed by their QMEAN z-scores (all above −4.0 and close to 1).

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