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T Guiddir, H Sénéchal, M A Selva, R Couderc, I Swoboda, C Hilger, A Kuehn, Y Chantran, S Yen-nicolaÿ, T Vrai, et al.

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LETTER

Chicken meat allergy in children: Complex sensitization profiles with newly described allergen candidates

To the Editor,

Chicken meat allergy (ChMA) has an estimated incidence of 0.6%–5%.¹ Symptoms range from mild oral syndrome to systemic reactions. Individual sensitization profiles remain poorly understood. The objective was to exhaustively and individually explore the clinical parameters (Table 1) and the IgE repertoire of five children (P1 to P5, 6–12 years old) with primary ChMA, without egg allergy, in order to compare clinical and IgE sensitization profiles. IgE repertoires were evaluated using multi-allergen chip (ALEX2©), dotline immunoassay on selected purified proteins, allergomic analysis, that is, two-dimensional (2D) gel electrophoresis of cooked chicken breast meat extract followed by IgE immunoblot (Figure 1A; Figure S1) and identification of allergens by liquid chromatography (LC) and mass spectrometry (MS) (see Methods in Data S1). Patients developed ChMA as the result of a break in tolerance. The skin prick test (SPT) and oral food challenge (OFC) were positive using chicken breast meat, poached and grilled. As depicted in 1D and 2D western blots (WB) (Figure 1D; Figure S1), profile complexity was low for P1 and P2, high for P4 and P5, and intermediate for P3. Proteins present in 30 excised gel spots matching 2D IgE-reactivities were identified by LC/MS/MS analysis (Figure S1). Out of 95 different proteins identified, 25 were considered to be the most relevant allergen candidates (Figure 1B) (see explanations in Data S1). Nine proteins corresponded to known allergens. Gal d 3, an allergen of mainly, but not only, egg white, is restricted to P2 and P3 and hemoglobin to P4 and P5. Gal d 7, 9, 10 and triose phosphate isomerase are potential allergens for P2, P3, P4 and P5. Gal d 5, also described in egg and chicken meat, is recognized by P5 only and Gal d troponin by P4 only. Gal d 7 was found for all patients in WB and also in a dot/line immunoassay (Figure 1C), in agreement with its major characteristic.² IgE reactivity to parvalbumin, not explored in 2D WB because the protein is not present in chicken breast,³ was

shown to be recognized by five patients by the dot/line immunoassay. Ten chicken candidate allergens already described as allergens in other animal sources (fish, crustaceans, cattle, worms, mites, and insects) were identified, such as troponin and cofilin-2, candidate allergens for all five patients. Patients P3 and P4 are allergic to other fowl (turkey and duck) and P3 is allergic to tuna fish, likely because of reported IgE cross-reactivities between known allergen families such as parvalbumin or myosin light chain,^{2–5} but also other uncharacterized allergens. Some of these, mainly muscle proteins, are proposed as candidate allergens. As these proteins are part of large protein complexes, they might be processed together leading to a joint/synergistic Th2 sensitization. The ALEX2© multiplex assay (Table 1) detected reactivities to fish parvalbumin, probably as a consequence of IgE cross-reactivity not systematically related to allergy-induced symptoms. Finally, six additional putative IgE reactive proteins, not reported in animal food allergies, were found and some of them, like ATP synthase, malate dehydrogenase, and phosphoglycerate kinase, include allergens in plants and molds exhibiting more than 50% sequence identity with chicken orthologs. Chicken allergens with potential cross-reactivity with fish or crustacean are mentioned in Figure 1B either because they were reported cross-reactive in literature^{1,6} or because they share more than 60% sequence identity (Uniprot Blast and Align). Allergens and patient overlaps are represented in a Venn diagram (Figure S2) and location of candidate allergens is shown on 1D WB (Figure 1D).

In conclusion, the allergomic study, performed with the sera from five ChMA children, showed highly diversified sensitization profiles with no correlation with clinical severity evaluated by symptoms, SPT, sIgE, OFC results, and potential cross-reactivities. The 25 candidate allergens pointed out pave the way for the identification of additional clinically relevant allergens as an approach to patient endotyping and stratification.

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TABLE 1 Clinical and sIgE reactivity (animal food) data on patients allergic to chicken.

Clinical data		P1	P2	P3	P4	P5					
Gender		M	M	M	M	F					
Age (years)		6	12	10	16	15					
Age at allergic reaction (years)		5	11	5	15	10					
Personal atopy symptom		No atopy	MA, R	AD	AD	MA					
Consumed chicken		In sauce	In sauce	Roast & grilled	Nuggets	Nuggets					
SPT (mm) with cooked chicken breast		6	6	10	14	6					
Symptoms with chicken meat (oFASS-5)		FU, PE, RC (3)	LA, LE, Ana (4)	FE, FE (2)	GU, LA, Ana (4)	TI, LI (1)					
Chicken OFC failure reactogen dose		5g	N/A	2.5g	N/A	2g					
Symptoms during OFC (oFASS-5)		Co, VM (4)		FE, PI, CB (5)		TI (1)					
Other allergies inducing symptoms (oFASS-5)		None	None	Guinea fowl, duck, turkey	Tuna: FE, E (1)	Green pea (7.18 ^a): TI (1) Birch and grass pollen MA					
sigE reactivities											
Proteins	Homologous chicken allergen	Species	kUA/L	Species	kUA/L	Species	kUA/L				
Poultry meat		Chicken	1.17 ^a , 0.81 ^b	Chicken	0.62 ^a , <0.1 ^b	Chicken	8.61 ^a , 3.49 ^b	Chicken	12.1 ^a , 3.04 ^b	Chicken	44.4 ^a , 26.65 ^b
Mammalian meat				Turkey		Turkey	1.55 ^a , 0.93 ^b	Turkey	3.39 ^a , 1.57 ^b	Turkey	10.47 ^b
Fish meat		Tuna	0.91 ^b	Tuna		Tuna	7.86 ^a , 1.19 ^b	Rabbit		Rabbit	1.58 ^b
								Tuna		Tuna	2.45 ^b
								Herring		Herring	0.85 ^b
								Mackerel		Mackerel	0.65 ^b
		Cod	1.72 ^b	Cod		Cod	0.78 ^b	Cod		Cod	4.12 ^b
								Curly ray		Curly ray	1.11 ^b
Crustacean meat				Crab		Crab	21.41 ^b	Crab		Crab	0.84 ^b
				Northern red shrimp		Northern red shrimp	9.52 ^b	Northern red shrimp		Northern red shrimp	0.33 ^b
				White shrimp		White shrimp	31.27 ^b				
				Lobster		Lobster	20.59 ^b				
				Squid		Squid	5.24 ^b				
				Mussel		Mussel	0.50 ^b				
				Oyster		Oyster	2.82 ^b				
				Scallop		Scallop	0.67 ^b				
				Clam		Clam	7.00 ^b				
				Tenebrio		Tenebrio	16.97 ^b				
Mollusk meat				Migratory locust		Migratory locust	8.67 ^b				
				Cricket		Cricket	9.50 ^b				
Insect meat											
											1.14 ^b
											0.42 ^b
											0.49 ^b

TABLE 1 (Continued)

sIgE reactivities		Homologous chicken allergen		Species		kUA/L		Species		kUA/L		Species		kUA/L	
Proteins	Gal d 5	Gal d 8	Gal d 9 + Gal d 10	Gal d 5	Gal d 8	Gal d 9 + Gal d 10	Gal d 5	Gal d 8	Gal d 9 + Gal d 10	Gal d 5	Gal d 8	Gal d 9 + Gal d 10	Gal d 5	Gal d 8	Gal d 9 + Gal d 10
Serum albumin	Beef			0.38 ^b			Herring			0.64 ^b			Tuna		
	Pork			0.63 ^b			Salmon			0.57 ^b			Herring		
Parvalbumin	Herring			0.90 ^b			Atlantic cod			0.37 ^b			Mackerel		
	Mackerel			0.99 ^b			Carp			0.35 ^b			Salmon		
Tropomyosin	Tuna			1.73 ^b									Carp		
													Tuna		
Sarcoplasmic calcium-binding protein							Anisakis			24.6 ^b			Anisakis		
							Black tiger prawn			39.2 ^b			Black tiger prawn		
							Dermatoph. Pt.			34 ^b			Dermatoph. Pt.		
							Blomia tropicalis			31.7 ^b			Blomia tropicalis		
							American cockroach			27.8 ^b			American cockroach		
Enolase+aldolase							Black tiger prawn			0.41 ^b			Atlantic cod		
													Chicken		
Conalbumin/ovotransferrin															
Gal d 3															

Note: Results obtained using: ^aImmunoCap, ^bALEX microchip. Only positive results for animal food are noted in the table. For plant sensitization see Table S1 in supplementary data. SPT: skin prick test. N/A: not available. Patients P2 and P4 did not wish to do an OFC because of the severity of the first allergic reaction with chicken meat.

Abbreviations: AD, atopic dermatitis; CB, change behavior; Co, cough; E, edema; FE, facial edema; FER, facial erythema; FU, facial urticaria, GU, generalized urticaria; LA, laryngeal angioedema; LE, lip edema; LI, lip itching; MA, mild asthma; OFC, oral food challenge; PE, palpebral edema; PI, palpebral itching; R, rhinitis; PSP, palm & sole pruritus; RC, rhinoconjunctivitis; TI, throat itching; VM, voice modification.

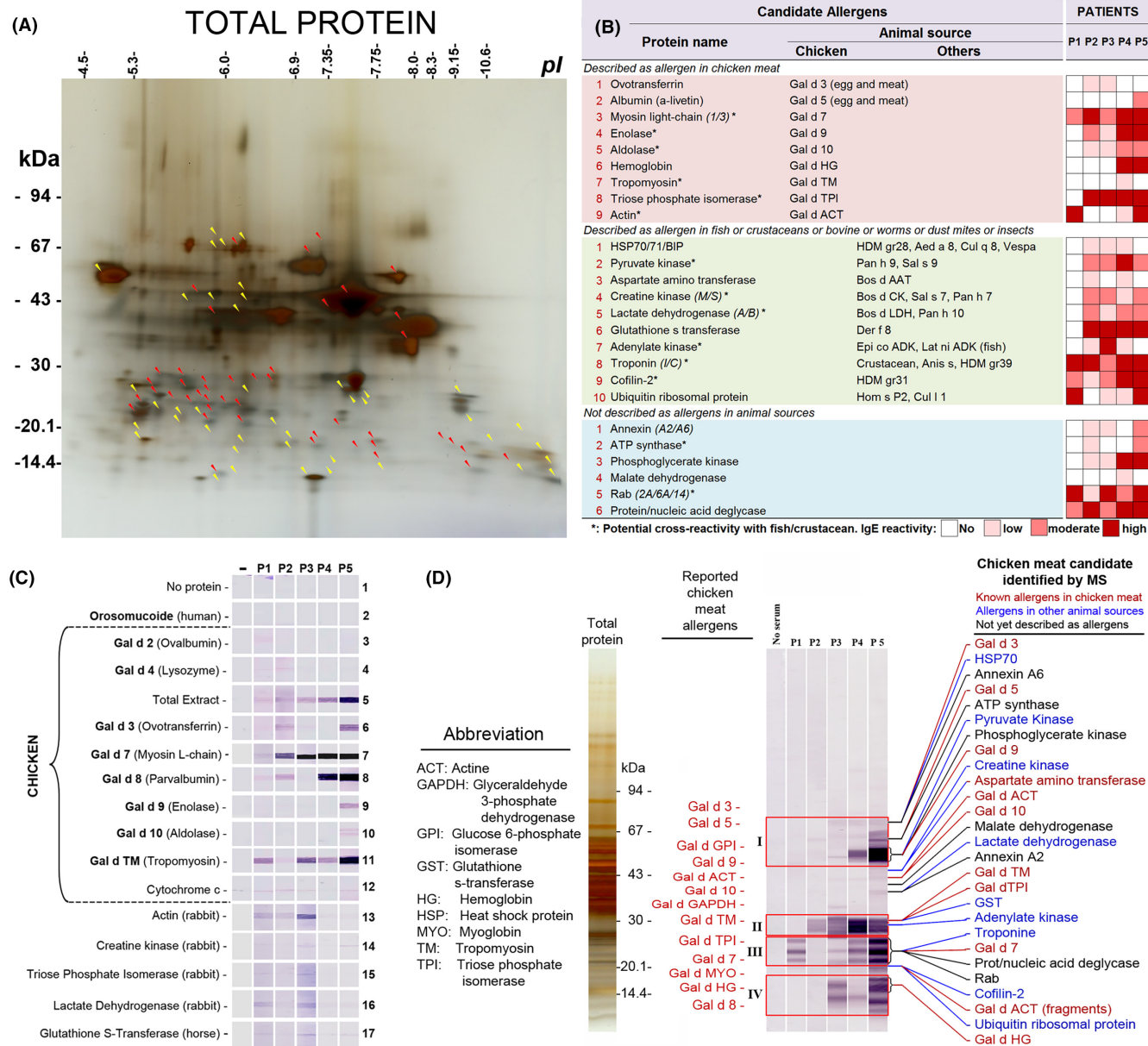


FIGURE 1 (A) Silver-stained 2D gel electrophoresis of chicken meat extract. IgE immunoreactivity is represented by ellipses. Red ellipses: proteins recognized by at least two patients. Yellow ellipses: proteins recognized by one patient. (B) Candidate allergens. IgE reactivity was semi-quantitatively evaluated according to the intensity of the immunoenzymatic signal. Reactivity: White: no; Yellow: poor; Orange: moderate; Red: high. (C) Dot/line immunoassay against purified proteins (D) Location of described and potential allergens in 1D analysis. Chicken meat allergens: red: known; blue: other animal sources, black: not yet described. Four red boxes (I, II, III, and IV) delineate 4 IgE reactivity areas.

AUTHOR CONTRIBUTIONS

Conceptualization, writing original draft, review and editing: T. Guiddir, A. Nemni; 2D electrophoresis, western blot, protein spotting and data interpretation, writing, review and editing: H. Sénéchal, P. Poncet, M-A. Selva; Data processing, review and editing: R. Couderc, B. Baudin; Production of reagents, review and editing: I. Swoboda, C. Hilger, A. Kuehn; Serum laboratory analysis: Y. Chantran; Supervision of proteomic analysis from experiment design to data interpretation, review

and editing: S. Yen-Nicolaÿ; Tryptic digestion, LC/MS analysis and data processing: G. Ruellou and T. Vrai.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article.

T. Guiddir¹ 
 H. Sénéchal² 
 M. A. Selva³
 R. Couderc²
 I. Swoboda⁴ 
 C. Hilger⁵ 
 A. Kuehn⁵
 Y. Chantran³
 S. Yen-Nicolay⁶
 T. Vrai⁶
 G. Ruellou⁶
 B. Baudin²
 P. Poncet^{2,7} 
 A. Nemni⁸ 

¹Paris-Saclay University, AP-HP, Bicêtre Hospital, Pediatric Pulmonology and Allergology Unit, Le Kremlin Bicêtre, France

²Sorbonne University, AP-HP, Armand Trousseau Children Hospital, Biochemistry Department, Allergy & Environment Team, Paris, France

³Sorbonne University, AP-HP, Armand Trousseau Children Hospital, Immunology Department, Allergology Sector, Paris, France

⁴The Molecular Biotechnology Section, FH Campus Wien, University of Applied Sciences, Vienna, Austria

⁵Department of Infection and Immunity, Luxembourg Institute of Health, Esch-sur-Alzette, Luxembourg

⁶Proteomic Facility-UMS IPSIT, Paris-Saclay University, Orsay, France

⁷Immunology Department, Institut Pasteur, Paris, France

⁸Children-Adult Allergology Department, Robert Ballanger Hospital, Aulnay-sous-Bois, France

Correspondence

T. Guiddir, Paris-Saclay University, AP-HP, Bicêtre Hospital, Pediatric Pulmonology and Allergology Unit, Le Kremlin Bicêtre, France.

Email: tamazoust.guiddir@aphp.fr

A. Nemni and P. Poncet equally contributed to this work.

ORCID

T. Guiddir  <https://orcid.org/0000-0002-8452-9918>
 H. Sénéchal  <https://orcid.org/0000-0002-8415-8803>
 I. Swoboda  <https://orcid.org/0000-0002-9164-1721>
 C. Hilger  <https://orcid.org/0000-0001-9455-5442>
 P. Poncet  <https://orcid.org/0000-0003-0757-5424>
 A. Nemni  <https://orcid.org/0000-0003-1240-2739>

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.