

## R E V I E W

# Component resolved diagnosis and risk assessment in food allergy

*Alberto Martelli<sup>1</sup>, Mauro Calvani<sup>2</sup>, Thomas Foiadelli<sup>3,4</sup>, Mariangela Tosca<sup>5</sup>, Giuseppe Pingitore<sup>6</sup>, Amelia Licari<sup>3,4</sup>, Alessia Marseglia<sup>3,4</sup>, Giorgio Ciprandi<sup>7</sup>, Carlo Caffarelli<sup>8</sup>*

<sup>1</sup> Pediatrician at the University of Pavia, Pavia, Italy; <sup>2</sup> UOC di Pediatria. Azienda ospedaliera S. Camillo Forlanini, Rome, Italy; <sup>3</sup> Pediatric Unit, Department of Clinical, Surgical, Diagnostic, and Pediatric Sciences, University of Pavia, Pavia, Italy; <sup>4</sup> Pediatric Clinic, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; <sup>5</sup> Allergy Centre, IRCCS G. Gaslini Pediatric Hospital, Genova, Italy; <sup>6</sup> Pediatrician and Allergist, Rome, Italy; <sup>7</sup> Allergy Clinic, Casa di Cura Villa Montallegro, Genoa, Italy; <sup>8</sup> Pediatric Clinic, Department of Medicine and Surgery, University of Parma, Parma, Italy

**Abstract.** Allergy testing should only be performed in the context of the clinical history as history provides the cornerstone of diagnosis. In food allergy, some allergy tests often give rise to false positive results and thus can lead to unnecessary avoidance or delay on foods introduction. The use of Component Resolved Diagnosis in combination with conventional sensitization testing improves analytical and diagnostic performance and can lead to the reduction of diagnostic oral food challenges. Component Resolved Diagnosis can be helpful in identifying some risks for the allergic child. Molecular diagnosis can help also in predicting the development of the allergy march, in severe reactions (lipid transfer protein, seed storage proteins, etc.) in food allergy and for potential clinical cross-reactivity. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** Component resolved diagnosis, IgE, food allergy, primary care, children

## Introduction

False positive food allergy diagnosis can lead to unnecessary food avoidance and potential impact on nutrition and growth, heightened anxiety and inappropriate recommendation of emergency medication (1). Molecular diagnosis provides a major step in improving the accuracy of diagnosing IgE-mediated food allergy.

In the clinical practice, component resolved diagnosis (CRD) can improve diagnostic clinical efficiency and assist the physician in many aspects of the allergy work-up. CRD allows for discriminatory co-sensitization versus cross-sensitization phenomena and can be

useful to stratify the clinical risk associated with a specific sensitization pattern, in addition to the oral food challenge (OFC).

CRD is a diagnostic approach that utilizes purified native or recombinant allergens to detect monomolecular sIgE antibodies response against the individual allergenic components (2).

Thanks to the capacity to exactly establish the sIgE molecular profile the CRD can allow for the discrimination of genuine sensitization from sensitization due to cross-reactivity (3). Moreover, it can be useful to assess and stratify the clinical risk associated with a specific sensitization pattern and predict the outcome of the OFC (4).

## Risk for late tolerance and prognosis of food allergy

The presence of specific IgE to some food proteins can be a predictive factor for a longer duration of food allergy. We can have new information in this prognostic direction specially for cow's milk, egg and peanuts. All of the proteins present in cow's milk are also present in human breast milk, with an exception of beta-lactoglobulin, present in negligible quantities in breast milk. Caseins, beta-lactoglobulin and alpha-lactalbumin are considered major allergens. More than 50% of the individuals with cow's milk allergy are sensitized to these proteins and most of the patients are polysensitized to several proteins (5). The allergenicity of cow's milk protein is modified by extensive heating e.g., baking. Caseins are more resistant to heating compared to whey proteins. Casein fraction is very resistant to high temperatures, retaining strong IgE binding after 90 minutes of boiling at  $> 90^{\circ}\text{C}$  (6). The presence of IgE directed against caseins determines both a longer duration of cow's milk allergy and a lower probability that cow's milk can be tolerated baked. The majority (70-80%) of the cow's milk allergic children tolerate cow's milk as an ingredient in the baked products (7). Reactivity to baked milk is a marker for more severe and more persistent cow's milk allergy. Inclusion of the baked products containing cow's milk into the diet of children with cow's milk allergy appears to accelerate development of tolerance to unheated cow's milk (8). Thus, in the diagnostic work-up for cow's milk allergic children, the study of sIgE to Bos d 6 could be relevant to identify patients at risk of beef-induced reactions (9). The high specific IgE against casein play a risk for reaction for baked milk (10). With CRD it is possible also to identify IgE directed towards an oligosaccharide of red meat, the galactosyl- $\alpha$ -(1,3)-galactose ( $\alpha$ -Gal) (11). These reactions can also be severe and induce anaphylaxis.

The egg proteins have completely different properties. The main proteins of the egg are differently represented in the yolk and in the albumen and the sensitization to ovomucoid, an egg white protein, has about the same meaning of the casein. The ovomucoid (Gal d 1) has been shown to be the immunodominant component (12) also if represent the 11%

of egg white protein. Children with persistent egg allergy have significantly higher ovomucoid-specific serum IgE levels than children who have outgrown their egg allergy. Other egg white proteins, including ovotransferrin, ovoalbumin and lysozyme, appear to be less important in the pathogenesis of egg allergy. Specific IgE against lysozyme can cause an allergic reaction in adolescents, after taking a species of white wine (13). Another study demonstrated that children with persistent egg allergy had significantly higher levels of serum IgE to ovomucoid and ovalbumin than those with transient egg allergy. Ovomucoid is resistant to heat, acid and proteolytic enzymes. Usually, cooked egg is slightly less allergenic than raw. It has been known also that Ig E against ovomucoid increased risk to late progress to multiple environmental allergen sensitization (14). Within allergenic sources causing symptoms by inhalation, the patients with sensitization to ovomucoid, had the highest prevalence of inhalant allergenic molecule sensitization rate, being grass allergens the most frequent sensitizers followed by olive pollen, mites, cypress pollen, and mammal epithelia.

Peanuts are a common trigger of food induced anaphylaxis also in Italy. In many parts of the world such as US or the Europe, even if less, peanuts are primarily consumed in roasted form. Peanuts have a high protein content of 24%–29% and contain various allergens. The processing of peanuts seems to be important in relation to their allergenicity as roasting at high temperatures likely promotes the formation of compact globular protein aggregates that can increase the allergenicity of Ara h 1 and 2 (15). Ara h 6 and Ara h 2 are the best predictors of peanut allergy at diagnosis in Mediterranean pediatric patients. Ara h 1, Ara h 8, are associated with peanut allergy persistence (16). For the diagnosis of hazelnut allergy, Cor a 14 is an extremely predictive marker of allergy to this type of tree nut allergy (17) and Ara h 2- and Cor a 14-specific IgE are useful to estimate the probability for a positive challenge outcome in the diagnostic work-up of peanut or hazelnut allergy making some food challenges superfluous (18) even if diagnostic accuracy of hazelnut components is low (19).

## Risk for severe reactions

Identifying patients at high risk of a severe reaction to foods is important for the management of patients diagnosed with adverse reaction to food. In past, there are contradictory results about the utility of food-specific IgE levels in assessing severity of food allergy (19,20). The severity of the reaction depends also on the type of protein involved and the IgE directed towards this protein (Table 1). But the risk assessment of allergic patients depends on factors other than mere individual players of IgE-mediated food induced allergic reactions (such as single allergens or epitopes, IgE or basophils) and requires a holistic clinical evaluation of the patient.

So the mechanisms that determine the severity of an allergic reaction are regulated by multiple factors. In addition to the type of food and how it is prepared, the other considerations should be given to the release capacity of mast cells and the number of receptors on target organs (vessels, bronchial tubes, etc.) (21,22).

The molecular diagnostics have allowed to identify the IgE directed against the single proteins of a food and not more, as it happened in the past, against the mixture of proteins of that same food.

Serum specific IgE to certain allergen components, such as Ara h 2 in peanut, has been associated with more severe reactions than sIgE to whole peanut or other single allergens, which is corroborated by *in vitro* studies of basophil activation and mediator release assays where Ara h 2 and Ara h 6 have been shown to be the most potent elicitors of effector cell response (23).

In southern Europe, the Lipid Transfer Protein (LTP) (Ara h 9) may act as a marker of severity, as it is associated with systemic and more severe reactions (24). Moreover, clinical reactions to nuts may reflect sensitization to non-specific LTP (e.g., Ara h 9, Cor a 8). Severe reactions in walnut-allergic patients are associated with Storage proteins (Jug r 1, Jug r 2) or LTP (Jug r 3) sensitization (25). For the nut-induced anaphylaxis, there are a lot of potential clinical cross-reactivity (26).

Tropomyosin is also considered to be a major allergen of shrimps and crustaceans and represents a marker of food allergy: 72–98% of the subjects allergic

to shrimps has IgE specific for tropomyosin. Sensitization towards tropomyosin increases the risk of reaction to oral food challenge in subjects with suspected shellfish allergy (27).

The implicated allergen for peach allergy in countries like, Spain, Italy and Greece is the non-specific LTP, i.e. Pru p 3. IgE antibodies against Pru p 3 can cross-react quite broadly to other fruits, as well as to tree nuts, legumes and some vegetables (28,29). They are associated with an increased risk for severe systemic reactions (30). This more “dangerous” profile of LTPs has been attributed to their high degree of protease (and food-processing) resistance (31).








Gibberellin-regulated proteins are members of cysteine-rich antimicrobial peptide families and are conserved in a broad range of plants. Some Gibberellin-regulated proteins in fruits and pollens have been identified as allergens including peach Pru p 7, Japanese apricot Pru m 7, orange Cit s 7, pomegranate Pun g 7, and cypress pollen Gibberellin-regulated proteins (32). The clinical implications of fruit-derived Gibberellin-regulated proteins allergies frequently include systemic reactions, multiple allergies regardless of plant kingdom classifications and, less frequently, cofactor-dependence.

The non-specific LTP Tri a 14 is a relevant food allergen in Italian wheat allergic patients (33). The allergy work-up in patients with suspected wheat allergies always includes an accurate history, investigating the tolerance to other cereals and the evaluation also of sensitization to wheat proteins.

## Conclusion

Progress in molecular biology and recent development on genetic technologies over the last 3 decades has allowed us to identify and characterize single allergens in detail at a molecular level. For this reason, large allergen data banks have been recently prepared. More of these allergens have already and will become available for *in vitro* allergy diagnostics, either as highly purified native or recombinant proteins. The use of molecular diagnostics can be of great help to the allergist. Daily routine molecular allergy diagnostics offers many benefits that give us a higher diagnostic accuracy and

Table 1. Risk assessment in food allergy.

Allergen	Food	Protein family	Risk for late tolerance	Risk for severe reaction
(Bos d 8) Casein		Phosphoprotein	x	
(Gal d 1) Ovomucoid		serine protease inhibitor	x	
Cor a 8		LTP		x
Cor a 9		11S globulin (storage protein)		x
Cor a 14		2S albumin (storage protein)		x
Ara h 1		Storage protein	x	x
Ara h 2		Storage protein	x	x
Ara h 3		Storage protein		x
Ara h 8		PR -10	x	
Ara h 9		LTP		x
Jug r 1		Storage protein		x
Jug r 2		Storage protein		x
Jug r 3		LTP		x
Tri a 14		Non – specific LTP 1		x (WDEIA)*
Pru p 3		Non – specific LTP 1		x
Pru p 7		Gibberellin		x

\* Wheat dependent exercise induced anaphylaxis

allow for better patient management. IgE reactivity to members of the same allergen family reflect the degree

of protein homology and IgE cross-reactivity level. If the level is high, the relevance needs to be sorted out clinically. If the cross-reactivity level is low, selected IgE testing of other family members can provide additional information. Positive sensitizations to allergen extracts or molecules are only clinically relevant in case of corresponding symptoms. In conclusion, molecular allergens for IgE testing can lead not only diagnostic definitions that relate to predictions of severe reactions but can also drive the study of cross reactions and the prognosis of food allergy.

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## References

- Flammarion S, Santos C, Guimber D, Jouannic L, et al. Diet and nutritional status of children with food allergies. *Pediatr Allergy Immunol* 2011;22:161-165.
- Treudler, R, Simon, JC. Overview of component resolved diagnostics. *Curr Allergy Asthma Rep* 2013;13:110-117.
- Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. EAACI food allergy and anaphylaxis guidelines: diagnosis and management of food allergy. *Allergy* 2014;69:1008-1025.
- Matricardi PM, Kleine-Tebbe J, Homann, HJ, et al. EAACI molecular allergology user's guide. *Pediatr Allergy Immunol* 2016;27:1-250.
- Fiocchi A, Dahdah L, Albarini M, Martelli A. Cow's milk allergy in children and adults. *Chem Immunol Allergy* 2015;101:114-123.
- Bloom KA, Huang FR, Bencharitiwong R, et al. Effect of heat treatment on milk and egg proteins allergenicity. *Pediatr Allergy Immunol* 2014;25:740-746.
- Nowak-Węgrzyn A, Bloom KA, Sicherer SH, et al. Tolerance to extensively heated milk in children with cow's milk allergy. *J Allergy Clin Immunol* 2008;122:342-347.
- Kim JS, Nowak-Węgrzyn A, Sicherer SH, Noone S, Moshier EL, Sampson HA. Dietary baked milk accelerates the resolution of cow's milk allergy in children. *J Allergy Clin Immunol* 2011;128:125-131.
- Martelli A, De Chiara A, Corvo M, Restani P, Fiocchi A. Beef allergy in children with cow's milk allergy; cow's milk allergy in children with beef allergy. *Ann Allergy Asthma Immunol* 2002;89(Suppl 1):38-43.
- Pouessel G, Jean-Bart C, Deschildre A, et al. Food-induced anaphylaxis in infancy compared to preschool age: a retrospective analysis. *Clin Exp Allergy* 2020;50:74-81.
- Jappe U, Minge S, Kreft B, et al. Meat allergy associated with galactosyl- $\alpha$ -(1,3)-galactose ( $\alpha$ -Gal)-Closing diagnostic gaps by anti- $\alpha$ -Gal IgE immune profiling. *Allergy* 2018;73:93-105.
- Dhanapala P, De Silva C, Doran T, Suphioglu C. Cracking the egg: an insight into egg hypersensitivity. *Mol Immunol* 2015;66:375-83.
- Weber P, Kratzin H, Brockow K, Ring J, Steinhart H, Paschke A. Lysozyme in wine: a risk evaluation for consumers allergic to hen's egg. *Mol Nutr Food Res* 2009;53:1469-77.
- Alessandri C, Zennaro D, Scala E, et al. Ovomuroid (Gal d 1) specific IgE detected by microarray system predict tolerability to boiled hen's egg and an increased risk to progress to multiple environmental allergen sensitisation. *Clin Exp Allergy* 2012;42:441-50.
- Visser YM, Blanc F, Skov PS, et al. Effect of heating and glycation on the allergenicity of 2S albumins (Ara h 2/6) from peanut. *PLoS One* 2011;6:e23998.
- Agabriel C, Ghazouani O, Birnbaum J, et al. Ara h 2 and Ara h 6 sensitization predicts peanut allergy in Mediterranean pediatric patients. *Pediatr Allergy Immunol* 2014;25:662-7.
- Eller E, Mortz CG, Bindslev-Jensen C. Cor a 14 is the superior serological marker for hazelnut allergy in children, independent of concomitant peanut allergy. *Allergy* 2016;71:556-62.
- Beyer K, Grabenhenrich L, Härtl M, et al. Predictive values of component-specific IgE for the outcome of peanut and hazelnut food challenges in children. *Allergy* 2015;70:90-8.
- Caffarelli C, Mastroianni C, Santoro A, Criscione M, Proccaccianti M. Component-Resolved Diagnosis of hazelnut allergy in children. *Nutrients* 2021;13:640.
- Wainstein BK, Studdert J, Ziegler M, Ziegler JB. Prediction of anaphylaxis during peanut food challenge: usefulness of the peanut skin prick test (SPT) and specific IgE level. *Pediatr Allergy Immunol* 2010;21:603-11.
- Hourihane JO, Grimshaw KE, Lewis SA, et al. Does severity of low-dose, double-blind, placebo-controlled food challenges reflect severity of allergic reactions to peanut in the community? *Clin Exp Allergy* 2005;35:1227-33.
- Wernersson S, Pejler G. Mast cell secretory granules: armed for battle. *Nat Rev Immunol* 2014;14:478-94.
- Brochetta C, Suzuki R, Vita F, et al. Munc18-2 and syntaxin 3 control distinct essential steps in mast cell degranulation. *J Immunol* 2014;192:41-51.
- Kukkonen AK, Pelkonen AS, Mäkinen-Kiljunen S, Voutilainen H, Mäkelä MJ. Ara h 2 and Ara 6 are the best predictors of severe peanut allergy: a double-blind placebo-controlled study. *Allergy* 2015;70:1239-1245.
- Krause S, Reese G, Randow S, et al. Lipid transfer protein (Ara h 9) as a new peanut allergen relevant for a Mediterranean allergic population. *J Allergy Clin Immunol* 2009;124:771-778.
- Costa J, Carrapatoso I, Oliveira MBPP, Mafra I. Walnut allergens: molecular characterization, detection and clinical relevance. *Clin. Exp. Allergy* 2014;44:319-341.
- Lomas JM, Järvinen KM. Managing nut-induced anaphylaxis: challenges and solutions. *J Asthma Allergy* 2015;8:115-23.

28. Farioli L, Losappio LM, Giurida, MG, et al. Mite-induced asthma and IgE levels to shrimp, mite, tropomyosin, arginine kinase and Der p 10 are the most relevant risk factors for challenge-proven shrimp allergy. *Int. Arch. Allergy Immunol* 2017;174:133–143.
29. Mastrorilli C, Cardinale F, Giannetti A, Caffarelli C. Pollen-food allergy syndrome: A not so rare disease in childhood. *Medicina (Kaunas)* 2019;55:641.
30. Asero R. In patients with LTP syndrome food-specific IgE show a predictable hierarchical order. *Eur Ann Allergy Clin Immunol* 2014;46:142-146.
31. Fernández-Rivas M, Bolhaar S, González-Mancebo E et al. Apple allergy across Europe: how allergen sensitization profiles determine the clinical expression of allergies to plant foods. *J Allergy Clin Immunol* 2006;118:481-488.
32. Asero R, Mistrello G, Roncarolo D et al. Lipid transfer protein: a pan-allergen in plant-derived foods that is highly resistant to pepsin digestion. *Int Arch Allergy Immunol* 2000;122:20-32.
33. Inomata N. Gibberellin-regulated protein allergy: clinical features and cross-reactivity. *Allergol Int* 2020;69:11-18.

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**Correspondence:**

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Alberto Martelli,

University of Pavia, Viale Golgi 19, Pavia, 27100 Italy

Phone: +39 333-4274390

E-mail: albmartelli@tiscali.it