REVIEW ARTICLE

# Determination of the clinical egg allergy phenotypes using component-resolved diagnostics

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**Abstract**

IgE-mediated egg allergy presents as one of the most common food allergies in children and is a food which is widely consumed all over the world. Measurement of egg white-specific IgE levels has been shown to be a poor predictor of clinical phenotypes of egg allergy, including to raw egg white, but particularly to baked or cooked egg. Egg white and yolk contain more than 20 different glycoproteins, including ovomucoid, ovalbumin, ovotransferrin, alpha-livetin, and the newly identified Gal d 6. Recent developments in component-resolved diagnostic technology, including microarrays, have enabled us to improve the way in which we diagnose food allergy. This technology allows us to measure specific IgE antibodies to individual egg allergens which have been highly purified. Characterization of the major egg allergens could help profile the relevant binding epitopes to each region and may also help diagnose the different clinical phenotypes of egg allergy.

IgE-mediated raw egg allergy presents as one of the most common food allergies in young infants, with a recent meta-analysis on the prevalence of food allergy estimating that egg allergy affects 0.5–2.5% of young children in the western world (1). However, the exact prevalence is difficult to ascertain, largely due to the differences in the cohort and definition of egg allergy. In a recent population study of age-matched 1-yr old infants with challenge proven outcomes, the prevalence of raw egg allergy was as high as 8.9% (2). Despite previous claims that egg allergic infants generally have a good prognosis, recent reports indicate that the age of resolution appears to be increasingly delayed (3–5). Symptoms usually present rapidly and can manifest as mild urticarial, nausea, abdominal pain, and/or vomiting, to more severe respiratory distress, hypotension, cardiovascular collage, and/or death (6). The current therapy for egg allergy is strict avoidance (7) in all dietary forms, although recent developments suggest this does not apply to all phenotypes of egg allergy. It's been reported that up to 70% of infants and children with raw egg allergy are tolerant to the baked form of egg (8, 9). Children who were tolerant to baked egg were 12.2 times more likely to develop tolerance to raw egg compared to children who were allergic to baked egg (10), of which only about half (43–56%) of the baked egg allergic infants will develop tolerance at the age of two (11, 12). While egg skin prick test (SPT) and specific IgE (sIgE) can be used for diagnosis of raw egg allergy (with low sensitivity), they are unable to diagnose baked egg allergy (9, 13). Furthermore, the use of these current tests as a clear prognostic indicator of resolution is limited (11, 14, 15).

As hen's egg is a versatile ingredient used for cooking in many cultures, as well as a wide range of manufactured food products, dietary avoidance of egg can be difficult (16, 17). In patients who have had a clinical reaction to egg current tools for diagnosing egg allergy include clinical history, skin prick test, and measurement of IgE specific for egg white protein (9, 18). Oral food challenge (OFC) is required when the history or IgE antibody levels are equivocal. However, history is not usually able to predict with precision which egg allergy phenotype any one individual belongs to because most egg allergy presents early in life and exposure to the full range of egg products (raw, cooked, or baked) may not have occurred. So, to clearly define an infant's egg allergy phenotype and their

related prognosis based on whether or not they are tolerant to baked egg an oral food challenge may be required.

Therefore, new methodologies are needed to define egg allergy phenotypes and to assist in determining the likelihood of resolution of egg allergy. Further understanding of the molecular basis of the different egg allergy phenotypes may provide valuable opportunities to better differentiate prognosis for egg allergic infants. The identification of major allergenic proteins in egg has been difficult due to the number of proteins in egg white and yolk, which is further complicated by the complexities surrounding the effect of heat treatment on many of the proteins (19).

## Major egg allergens and its immunodominance

It is recognized that egg white contains >20 proteins, a mixture of both allergenic and non-allergenic proteins, and may be one of the reasons why this egg white sIgE testing fails to differentiate between the clinical phenotypes of egg allergy (19). New molecular diagnostic technologies have been recently introduced into allergological research, and testing for individual egg allergen specific IgE may provide increased accuracy in diagnosis although further research is required before its role in clinical practice is delineated (20). Despite ovalbumin (Gal d 2, OVA) being the most abundant protein in egg white (54% by weight of protein), ovomucoid (Gal d 1, OVM) makes up 11% and is the predominant egg allergen (Table 1) (21–23). There has been much debate over whether OVA as the major egg allergen is immune dominant, responsible for causing the majority of clinically adverse reactions following egg ingestion. It has recently been shown that commercial preparations of OVA contain a considerable amount of OVM as well as some contamination with transferrin (20), raising questions about the previous estimates of sensitization rates to OVA (24).

In two different studies, children with persistent egg allergy had significantly higher titers of specific IgE (sIgE) to OVM compared to OVA than children who outgrew their egg allergy (24, 25). Those with an absent or declining titer of OVM sIgE had a favorable prognosis (11, 24). Furthermore, OVM, a highly glycosylated protein, containing as much as 25% (w/w) carbohydrate, is considered the dominant allergen in heated egg products due to its ability to remain stable after heat treatment through cooking, and its resistance to digestion with proteinases compared to other egg allergens (26, 27). In contrast to OVM, OVA is a heat labile protein that undergoes conformational changes following heat treatment, and it is postulated to be less allergenic making it unlikely to be the major allergen (28, 29). However, it has recently been shown that OVA that is transformed through certain heating conditions into a thermostable form (S-ovalbumin), although the allergenicity in this form is still currently unclear (30, 31).

Other egg white allergens such as ovotransferrin and lysozyme, like OVA, are sensitive to heat treatment. Their contribution to allergic response to egg is largely unknown, as they have not been well characterized. There are two main egg yolk allergens, Gal d 5, and the recently discovered Gal d 6. Little is known about Gal d 6, with only one small study to characterize the reactivity in patients' serum, showing that 5 of 27 patients produced IgE to Gal d 6 (32). The main allergen in egg yolk is alpha-livetin (chicken serum albumin, Gal d 5), and patients sensitized to Gal d 5 commonly experience respiratory symptoms such as rhinitis and/or asthma with egg ingestion (33, 34). It is postulated that primary sensitization usually occurs through aeroallergens, which commonly leads to cross-sensitization to serum albumins present in food later in life (35). Therefore, testing for Gal d 5 sIgE early in life might help predict sensitization to aeroallergens (34) later in life as food sensitization appears to precede aeroallergen sensitization (36), however, further work is needed to confirm this.

## Matrix modification of egg allergens and impact on allergenicity

Egg is a complex allergenic source, the allergenicity of which may be modified by mixing and baking with other food ingredients such as wheat (and other grains containing gluten) in addition to the effect of various forms of heating during cooking (38). The conformation of most of these allergenic proteins changes during the cooking process with evidence of variation depending on whether the egg is boiled alone or baked in a food matrix (39). Kato et al. (38) previously showed that a decreased in solubility of Gal d 1 when egg was mixed with wheat flour and wheat gluten, suggesting that Gal d 1 forms insoluble complexes with gluten, potentially leading to decreased digestibility. Rendering the protein insoluble could explain why such high levels of sIgE to Gal d 1 are needed to predict reactivity to baked egg in baked egg allergic children compared to children tolerating baked and lightly cooked egg (8).

## Component-resolved diagnostics to predict allergy

Currently, the use of the ImmunoCAP–fluoro-enzyme immunoassay (FEIA) technology to measure sIgE to total extracts,

**Table 1** Major egg allergens (32, 37)

| Allergen | Name | Source | Constitute (%) | Mw (kDa) | Carbohydrate (%) | Heat treatment | Digestive enzyme treatment |
|---|---|---|---|---|---|---|---|
| Gal d 1 | Ovomucoid | Egg white | 11 | 28 | 25 | Stable | Stable |
| Gal d 2 | Ovalbumin | Egg white | 54 | 44 | 3 | Partially | Unstable |
| Gal d 3 | Ovotransferrin | Egg white | 12 | 66–78 | 2.6 | Unstable | Unstable |
| Gal d 4 | Lysozyme | Egg white | 3.4 | 14.3 | 0 | Unstable | Unstable |
| Gal d 5 | Alpha-livetin | Egg yolk | Unknown | 66 | Unknown | Partially | Unknown |
| Gal d 6 | N/A | Egg yolk | Unknown | 35 | Unknown | Stable | Stable |

or a single allergen, has been adopted in improving the way we diagnose food allergy, such as Ara h 2 in peanut allergy (40). However, the protein microarray has recently become available for measuring sIgE in the form of ImmunoCAP–ISAC (Immuno Solid phase Allergen Chip) (41). This chip currently has 112 native/recombinant component allergens from 43 different allergen sources, including nGal d 1, nGal d 2, nGal d 3, and nGal d 5. It assesses simultaneously sIgE to different components and requires small amounts of serum (20 μl).

### Use of microarray-based CRD to predict allergy

One cross-sectional study has determined the sensitization status of a large cohort of Italian patients using ISAC, but the allergic outcomes of these patients and their sIgE were not investigated (42). Only two studies with small clinical longitudinal cohorts have used the CRD–ImmunoISAC platform to predict the raw egg OFC outcome of egg allergic children and found it performed more accurately than whole egg sIgE (43, 44). They report that it could be used as a second step assay, if the ImmunoCAP–sIgE to egg white is less than the 95% clinical threshold as was carried out in the Dang et al. 2012 study (40) which used peanut IgE followed by Ara h 2 as a 2-step diagnostic algorithm. D'Urbano et al. studied 104 patients (median age of 4.9 yr) with a history of cow's milk or hen's egg consumption and performed an OFC to confirm current allergy status. They found the microarray useful in predicting the OFC outcome compared to the current diagnostic tests (44), but used the test to predict concurrent egg allergy rather than predicting development of resolution as a way to avoid OFC at the time of diagnosis. In contrast, Ott et al. (43) used a retrospective cohort of cow's milk or hen's egg allergy in 130 infants (median age of 14 months) and failed to find any advantage of this microarray platform over current diagnostic tests. However, the majority of their cohort had a clinical manifestation of atopic eczema (>95%), with exacerbation of atopic eczema indicative of a positive food challenge, and this difference in patient selection could explain the difference in results between the two studies. Both studies did not assess the predictive ability of this platform to determine baked egg allergy. Further studies with larger cohorts are needed to determine the efficacy of this microarray platform in diagnosing and distinguishing the clinical phenotypes of egg allergy.

### Use of immunocap–CRD to predict egg allergy

Three small studies have found that Gal d 1 levels were more accurate in predicting raw egg allergy compared to egg white sIgE and that higher levels of Gal d 1 are also associated with persistent egg white allergy (8, 27, 45). While it was suggested that the lower the Gal d 1-sIgE concentration, the higher the probability of tolerance to cooked eggs, contradictory results were found from these studies.

Lemon-Mule et al. performed baked egg challenges in 117 subjects and found that Gal d 1 was no better than egg white sIgE in predicting outcome of baked egg challenge[8]. Similarly, Bartnikas et al. (46) found that Gal d 1 was not superior to egg white sIgE in predicting baked egg tolerance in a cohort of 169 children. However, Ando et al. and Haneda et al. found that Gal d 1 sIgE was more accurate in predicting the challenge outcomes of extensively heated egg in 108 and 100 subjects, respectively (27, 45). Comparisons between these should be drawn with caution; however, because the preparation of egg challenge material was different and not standardized (47). The two studies by Ando et al., and Haneda et al. challenged their subjects to liquid egg white heated at 95°C and boiled egg, respectively (27, 45), without the addition of wheat; whereas Lemon-Mulé et al., and Bartnikas et al. challenged their subjects to egg cooked in the form of a cupcake or muffin (8, 46), with the addition of wheat. Cooking egg together with wheat flour leads to aggregation and insolubility of OVM (38). Thus, concentrations and conformations of egg proteins may vary depending on the baked egg protocol used, leading to differences in food challenge outcomes and predictive values of immunologic parameters.

### Predicting the severity of egg allergic reactions

Despite the ability of the egg allergen component sIgE tests to provide an indication of likelihood of clinical reactivity to raw and baked egg, none of the studies were able to predict the severity of allergic reaction to eggs. Although they were unable to determine the natural history of egg allergy, these studies listed above saw that the specific IgE levels declined over time and may still be used a prognostic indicator for the development of tolerance (8, 27, 43–45).

### Varying levels of allergen in different regions

Another factor which may influence the ability to predict allergic outcomes with CRD is the varying levels of the major allergens in different regions. Gal d 1 has been identified as the predominant egg allergen in a number of countries including North America, Japan, and Australia and may be considered as a utility in the diagnosis of egg allergy (8, 27, 48). However, the use of Gal d 2 sIgE testing may not by applicable to all populations – for example Gal d 2 is reported to be the dominant allergen in Spain and Taiwan (49, 50). Testing of egg allergens to identify the dominant allergen in each region will be required before implementing the CRD method to diagnose egg allergy.

### Epitope discrimination using CRD

CRD is not limited to whole protein allergens and is capable of being adapted to capture more specific regions of an allergen, in the form of linear peptide epitopes. Few studies have looked at IgE-binding linear epitopes in OVM, but have reported that all the binding sites resemble each other (21, 51). Identification of linear epitopes is important for determining the specific region of an allergen which remains immunogenic and causes an allergic reaction, even after digestion and absorption from the gastrointestinal tract (52). It has been shown that egg allergic patients with IgE antibodies reacting against sequential

epitopes tended to have persistent allergy, whereas those with IgE antibodies primarily reacting against conformational epitopes tended to have transient allergy (21, 25). Further characterization of IgE epitopes of egg allergens will not only assist in the diagnosis of egg allergy, but may be important for the design of a therapeutic target.

## Conclusion

Molecular diagnosis technologies are likely improve diagnosis of IgE-mediated egg allergy although currently OFCs remain the gold standard for diagnosing egg allergy due to the poor predictive abilities of skin prick test and sIgE test to egg white (9, 18). Measurement of sIgE antibodies to individual egg white subcomponents is considered as a new method for diagnosis, with promising evidence of their ability to predict different clinical phenotypes of egg allergy. CRD based on a microarray platform is especially promising, with the ability to measure a number of allergens at the same time using minimal amounts of serum. The use of CRD may extend beyond diagnosis, by providing a tool to improve immunotherapy treatment by improving patient selection with a more targeted approach. With further characterization of the individual egg white allergens in larger cohorts, we could validate the accuracy of these tests for diagnosis of egg allergy and its various clinical phenotypes (raw versus cooked) which may reduce the need for an OFC. Furthermore, it may also enable us to better predict the likelihood of resolution later in life.

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