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Egg allergen specific IgE diversity predicts resolution of egg allergy in the population cohort HealthNuts

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ABSTRACT

Background: IgE-mediated egg allergy presents as one of the most common food allergies in children. Measurement of egg white-specific IgE levels in serum or skin prick test has been shown to be a poor predictor of clinical allergy to raw egg white, and also to baked or cooked egg. Recent developments in component resolved diagnostic (CRD) technology have enabled us to improve the way in which we diagnose and predict peanut allergy through examining IgE specificity to individual peptides.

Objectives: We aimed to investigate whether egg CRD could improve current methods to diagnose various egg allergy phenotypes as well as predict the development of tolerance to egg.

Methods: Using the HealthNuts cohort of food challenge-proven egg allergic and egg sensitised tolerant age matched 12 month infants with longitudinal follow-up at 2 and 4 years (n=451), we measured serum egg white, Gal d 1, 2, 3, and 5 specific IgE using ImmunoCAP.

Results: Gal d 1 sensitisation increased the risk of persistent egg allergy by 2.5-fold. The production of specific IgE to all four egg allergens (Gal d 1, 2, 3 or 5) increased the risk of having persistent raw egg allergy 4-fold (OR 4.19 (95% CI: 1.25-14.07). We did not find any improvements of using Gal d 1, 2, 3 or 5 to diagnose current egg allergy compared to egg white sIgE.

Conclusion: Sensitization to multiple egg allergens Gal d 1, 2, 3 or 5 may be a prognostic marker that could be useful for patient management and identifying individuals at risk of developing persistent egg allergy

Key words: Egg allergy, baked egg allergy, diagnosis, prognosis, tolerance, HealthNuts, IgE, component resolved diagnostics, ovomucoid, ovalbumin

Abbreviations:

OFC: Oral Food challenge

ImmunoCAP FEIA: ImmunoCAP fluorescence enzyme immunoassay

CRD: Component Resolved Diagnostics

SPT: Skin prick test

sIgE: Specific immunoglobulin E

PPV: Positive predictive value

ROC: Receiver operating characteristic

AUC: Area under curve

Introduction

IgE-mediated raw egg allergy presents as one of the most common food allergies in young infants in the developed world, affecting up to 9% of infants, compared to other major food allergies such as peanut (3%), and sesame (0.8%)^{1, 2}. While egg allergy in infancy often resolves, approximately 20% of egg allergy persists into later childhood and these individuals are at continuing risk of potentially severe reactions³. Diagnosis of egg allergy is relatively straightforward when there is an unequivocal history of clinical reaction following egg ingestion, however, can be more complicated when the clinical history is not clearly defined or in children who are yet to be exposed to egg. While egg skin prick test (SPT) and specific IgE (sIgE) can be used for diagnosis, the accuracy of these tests are low^{4, 5}, and an oral food challenge remains the gold standard. Furthermore, the use of these current tests as a prognostic indicator of resolution is limited^{2, 6, 7}.

We previously showed that component resolved diagnostics (CRD) for peanut allergy using Ara h 2 specific IgE (sIgE) in a 2 step algorithm significantly reduced the need for oral food challenges for the diagnosis of peanut allergy⁸. We have also shown that the sensitivity of egg white sIgE and SPT is low³. By contrast, ability to tolerate baked egg offers a potential predictor of transient egg allergy, with infants who are unable to tolerate baked egg being five times less likely to develop tolerance³. This is important considering that the current

management for egg allergy is strict avoidance⁹. However, recent developments suggest this does not apply to all phenotypes of egg allergy. It has been reported that up to 80% of infants and children with raw egg allergy are tolerant to the baked form of egg^{3, 10}, however egg white SPT and sIgE thresholds are poor predictors of baked egg allergy as we have not been able to identify thresholds with 95% PPV to baked egg allergy. Prediction of egg allergy phenotypes (baked egg allergic versus tolerant) would be useful for providing prognostic information to the clinician. Thus accurate diagnosis and prognosis of all forms of egg allergy is important for correct patient management and avoiding time consuming and costly oral food challenges.

Several recent clinical cohort studies have suggested that the use of CRD may be useful in both diagnosis and prognosis of egg allergy, however, it is still unclear whether it is effective due to conflicting results from these small clinical studies which did not consistently use the diagnostic gold standard of an oral food challenge to define egg allergy¹⁰⁻¹⁴.

Ovalbumin (Gal d 2) is the most abundant protein in egg white (54%), while ovomucoid (Gal d 1) makes up 11% and is hypothesised to be the predominant egg allergen due to its heat resistant properties compared to ovalbumin which is heat labile¹⁵. The role of sensitization to Gal d 3 or the egg yolk allergen Gal d 5 has not yet been well characterised in egg allergic individuals. To determine whether egg CRD could i) improve the diagnosis of raw and baked egg allergy and ii) predict the prognosis of egg allergy later in life, we measured specific IgE levels to each of these egg allergens in the HealthNuts cohort of food challenge-proven egg allergic and sensitised tolerant age matched children.

Methods

Selection of subjects for component resolved diagnostic testing

A subset of 451 subjects were selected from the HealthNuts cohort for this study (Figure 1)¹⁶ based on the availability of sufficient volumes of plasma. Median age was 12 and 14 months at the time of SPT and OFC respectively [ranges 11-15 and 11.5-20 months]). Subjects selected for this study were challenged to egg at the Royal Children's Hospital, Melbourne. OFC was performed according to standardised protocols¹⁶ in all subjects to confirm egg allergy status. Egg allergic infants (n=297) had a positive SPT $\geq$ 2mm to egg and an unequivocal objective allergic reaction during OFC. Egg sensitised tolerant infants (n=97) had a positive SPT $\geq$ 2mm to egg and a negative OFC. Healthy control infants were non

sensitised, and non allergic (n=57) with a negative SPT (wheal 0mm) to peanut, egg, sesame and cow's milk together with a negative egg challenge outcome (Figure 1). A total of 451 plasma samples from age matched participants of the HealthNuts study were analysed.

Recruitment and follow up at age 2 years and 4 years

All infants with challenge-confirmed egg allergy at 12 months of age from the HealthNuts study were invited to participate in an additional baked egg OFC. The study methods have been previously described³. A frequent reason for declining the baked egg OFC was because parents reported that infants were already consuming and tolerating foods containing baked egg. Therefore tolerance to baked egg at 1 year of age was defined as a negative baked egg OFC result or parental report of tolerance to baked egg in those who declined the baked egg OFC.

At 2 years of age, all children with egg allergy who had been offered a baked egg OFC, even if they did not participate in the baked egg OFC, were invited to return for follow-up. At follow-up, participants undertook repeat raw egg OFCs, SPTs, and sIgE tests. From 297 egg allergics at age 1, we were able to establish the age 2 raw egg allergy outcomes for 125 of the infants, of which 60 had resolved their egg allergy and 65 persisting with their egg allergy. Of the 297 egg allergic infants we were able to determine that 246 were baked egg tolerant and 18 were baked egg allergic based on parent report of baked egg ingestion in 106, while the remaining 158 received a baked egg OFC to determine their outcomes.

At 4 years of age, all children with egg allergy undertook repeat raw egg OFCs, SPTs, and sIgE tests to confirm their current egg allergy status. From 297 egg allergics at age 1, we were able to establish the age 4 outcomes for 248 of the infants, of which 208 had resolved their egg allergy and 40 persisting with their egg allergy. All follow-up OFCs were conducted irrespective of previous or current SPT and sIgE results and severity of previous OFC reactions. All clinical staff performing OFCs were blinded to previous history of reaction and sIgE and SPT results. Furthermore, we have previously reported that there are few demographic differences between food allergic children who did and did not attend the study clinic at age 4 years¹⁷. Attendees were more likely to have Australian-born parents, eczema at age 1 year, and other allergies at age 4 years. We also determined that there are no demographic differences between those lost at follow-up and those included in this study.

Predefined criteria for a positive oral food challenge

Subjects received increasing doses of the suspected food allergen at 15 minute intervals until they received the equivalent of 60g of egg. Baked egg challenges were in the form of a muffin equivalent to a cumulative dose of 10 g of whole egg. The food challenge was stopped if an objective allergic reaction was observed that fulfilled the predetermined stopping criteria from the study protocol i.e. at least one of the following: three or more concurrent non-contact hives (urticarial lesion) lasting for more than 5 min, perioral or periorbital angioedema, vomiting (excluding immediate post-ingestion gag/vomits), or evidence of anaphylaxis as defined by the Australian Society of Clinical Allergy and Immunology (evidence of circulatory or respiratory compromise) within 2 h of the last dose within the food challenge¹⁸.

Plasma collection and allergen-specific IgE analysis

Blood was collected into a sodium heparin tube (Sarstedt) 1-2 hours after the last dose of the OFC. The blood was centrifugated off at 700g for 10 minutes within 2 hours after the blood was taken and the plasma was collected and frozen at -20°C until use.

Allergen-sIgE was measured with the ImmunoCAP System FEIA (Phadia AB, Uppsala, Sweden). Plasma samples from our age 1 cohort was analysed for IgE to whole egg, and major egg allergens Gal d 1, Gal d 2, Gal d 3, and Gal d 5.

Statistical Analysis

Population prevalences were estimated as observed proportions that were compared between groups using the two-sample z-test for proportions. Egg white sIgE, Gal d 1, and Gal d 2 sIgE had skewed distributions so we report summaries of the distribution of all sIgE measurements as the median and interquartile range. We studied the association between egg allergen component sIgE and the risk of raw, baked and persistent egg allergy by generating the receiver operating characteristic (ROC) curve based on empirical sensitivities and specificities for a range of cut-offs values for sIgE measurements. We report the area under the curve (AUC) along with 95% confidence intervals for the corresponding population quantity based on the asymptotic standard error (ASE). We also quote the estimated positive and negative likelihood ratios, as their interpretation is not dependent on the underlying disease prevalence or, equivalently, the pre-test probability that an individual is allergic, which potentially permits the reader to transfer results to their own patients and the corresponding population - a full discussion of the role of the likelihood ratio and interpretation of thresholds is given by Roberts and Lack¹¹. Positive predictive values (PPVs)

were calculated for a series of component IgE thresholds based on the estimated positive likelihood ratio (sensitivity / (1 – specificity)) using previously published estimates of the population prevalence of egg allergy from the same (HealthNuts) study. Confidence intervals for the estimated PPV's were generated by applying Fieller's Theorem to the positive likelihood ratio. Logistic regression was used to model the association between the risk of having persistent raw egg allergy and the measure of sensitization to each of the allergen components by assuming a linear relationship between the log odds persistent egg allergy and the magnitude of sensitization. All statistical analyses were performed using Stata release 13.0 (StataCorp, College Station, Texas).

Ethics

Ethics approval was obtained for the HealthNuts study from the Victorian State Government Office for Children (reference no. CDF/07/492), the Victorian State Government Department of Human Services (reference no. 10/07), and the Royal Children's Hospital Human Research Ethics Committee (reference no. 27047).

Results

Clinical features of the study cohort

Egg allergic infants were more likely to have current eczema (52%) compared to non-egg sensitized infants (15%), $p < 0.05$ (Table 1). Egg allergic infants were more likely to have co-existent sensitisation to other foods (47%), and were also more likely to be allergic to other foods (20%) compared to egg sensitised tolerant infants (10%). There were no differences observed in the rates of family history of eczema or food allergies between the clinical phenotypes. No differences in demographics data between the subjects for whom blood samples were not available (Supplementary Table 1).

We report that 97.3% ($n=289$) of egg allergic infants had detectable egg white sIgE (0.1 kUA/l), compared to 83.5% ($n=81$) of egg sensitised tolerant infants. Egg allergic infants were more likely to be sensitised to Gal d 1 and Gal d 2 than sensitised-tolerant infants (67.9% vs 32.1% and 88.6% versus 48.1% (Figure 2). Less than 8% of all egg sensitised infants (irrespective of allergy status) were sensitised to Gal d 3 or Gal d 5.

Using component testing to predict long-term outcomes of egg allergy

To determine if egg CRD at age 1 year was able to predict later resolution of egg allergy, we plotted a ROC of each allergen component level for raw egg allergic infants at 12 months against outcomes of egg allergy at 4 years of age (n=208 resolved egg allergy and n=40 persistent egg allergic). Gal d 1 and Gal d 2 had moderate ability to predict persistence of egg allergy at 4 years with an area under the curve (AUC) of 0.73 [95% CI: 0.63-0.83] and 0.75 [95% 0.66-0.85] respectively (Figure 3a, Table 2). Egg white sIgE remained the best predictor of persistent egg allergy, with an AUC of 0.83 [95% CI: 0.75-0.91]. An egg white sIgE cut off of 11.0 kUA/l had a specificity of 95% and a sensitivity of 45%, accurately predicting the persistence of almost half the number of raw egg allergies at 4 years old. At the same specificity, a Gal d 1 cut off of 3.45 kUA/l and Gal d 2 cut off of 6.11 kUA/l provided a sensitivity of 35% [95% CI: 27-59%] and 43% [95% CI: 21-52%] respectively, and did not increase the performance compared to egg white sIgE.

Prediction of persistent versus transient egg allergy

Plots of the predicted probability of persistent raw egg allergy based on sIgE at age 1 are displayed in Figure 3b. We found that the Gal d 1 curve is “tighter” (shifted left of egg white curve) compared to Gal d 2, and suggests an association with persistent egg allergy. A logistic regression found that infants sensitised to either Gal d 1 or Gal d 2 were more likely to have persistent raw egg allergy at 2 years (**OR 2.3**, p=0.02; and **OR 3.4**, p=0.038 respectively), however, only Gal d 1 was associated with long term persistence of raw egg allergy at 4 years (**OR 2.5**, p=0.02) compared to Gal d 2 (**OR 0.67**, p=0.50) (Table 3). Furthermore, we observed that Gal d 5 was strongly associated with persistent egg allergy (**OR 6.89**, p<0.01), however was only 9 subjects in this group for comparison.

Sensitisation to multiple egg allergen components predicts natural history of egg allergy

Sensitisation to all four egg allergen components increased the risk of persistent egg allergy (**OR 4.19**, p=0.007). The rate of resolution of egg allergy in infants who were sensitised to only one egg allergen was 60% at 2 years of age, with that number increasing to 93% by 4 years (Figure 4). The rate of resolution was significantly lower in infants sensitised to three egg allergens, with resolution of egg allergy in only 27% at 2 years, and 65% by 4 years. All egg allergic infants who were sensitised to all four egg components had persistent egg allergy, with no infants having resolution of egg allergies by 2 years, or 4 years.

Egg component ImmunoCAP testing to diagnose current phenotypes

For raw egg allergy, measurement of Gal d 1, and Gal d 2 sIgE provided receiver operator curves (ROC) with an area under the curve (AUC) of 0.83 [95% CI: 0.81-0.87] and 0.63 [95% CI: 0.60-0.67] respectively, however, the AUC of egg white sIgE was 0.89 [95% CI: 0.85-0.92] indicating that it remains the most accurate way to diagnose raw egg allergy at 1 year of age (Supplementary Figure 1a and Supplementary Table 2).

For baked egg allergy, Gal d 1 and Gal d 2 provided ROCs with an AUC of 0.65 [95% CI: 0.61-0.70] and 0.77 [95% CI: 0.73-0.81] respectively for diagnosis of baked egg allergy (Supplementary Figure 1b), however, egg white sIgE generated the best ROC with an AUC of 0.80 [95% CI: 0.76-0.84].

Discussion

This is the first study to assess the utility sIgE against egg allergen components to predict both egg allergy phenotype (baked allergic vs. tolerant) and persistent vs. transient raw egg allergy in a population setting. Using the gold standard of oral food challenges to confirm egg allergy, we found that sensitisation to egg allergen components early in life can aid in predicting the outcomes of egg allergy in childhood. The production of specific IgE to all four egg allergen components (Gal d 1, 2, 3 or 5) increased the risk of having persistent raw egg allergy by 4-fold. Of the individual allergen components, Gal d 1 sensitisation was the best predictor of persistent egg allergy with a 2.5-fold increase in risk of persistent egg allergy compared to no Gal d 1 sIgE. These findings may have important clinical implications as they allow identification of those likely to have resolution of egg allergy by 4 years. Furthermore, they may provide help inform on the individual's ability to tolerate baked egg, and not unnecessarily avoid all forms of eggs.

Our findings demonstrate that a large proportion of infants allergic to raw egg are sensitised to egg white allergen components but not egg yolk allergen (Gal d 5). As expected, Gal d 2 was the predominant egg allergen, with 88.6% cohort sensitised compared to only 48.1% sensitised to Gal d 1. However, most infants sensitised to Gal d 2 alone had transient egg allergy, with 90% becoming tolerant by age 4 years. Gal d 1, unlike Gal d 2, is a fairly heat stable molecule with relative resistance to digestion by proteinases and it is postulated to be the dominant egg allergen^{19, 20}. We found that there was a left shift in the Gal d 1 probability curve from the baseline of whole egg white in predicting persistent egg allergy. Furthermore, sensitisation to Gal d 1 alone increased the risk of persistent egg allergy by 2.5 times in our challenge confirmed cohort. The strongest risk factor for persistent egg allergy was

sensitisation to all 4 egg allergen components, with an OR of 4.19. Evaluating the diversity of IgE binding to egg allergen components allowed us to identify for persistent egg allergy, and may prove to be a useful tool for prognostic prediction of egg allergy.

Component testing for the diagnosis of current egg allergy in our population cohort remained inferior to whole egg sIgE testing. Although smaller studies with clinical cohorts have evaluated the utility of Gal d 1 sIgE levels for egg allergy diagnosis and found it to be slightly superior to egg white in predicting tolerance to raw and heated egg^{11, 12, 21}, we found that the best ROC with an AUC of 0.89 was whole egg white sIgE, compared to Gal d 1 and Gal d 2 (AUC of 0.65 and 0.83) sIgE respectively in our population cohort. Egg sIgE also provided the best sensitivity in our cohort, as previously described. Compared to an egg white sIgE 95% PPV level of 1.70kUA/l, a cut off of 1.97kUA/l Gal d 2 sIgE was the next best predictor of current raw egg allergy, with an equivalent specificity of 98% and a sensitivity of 31%, while a Gal d 1 cut-off of 1.39kUA/l only provided a sensitivity of 21%. Taken as a whole, in line with previously published data^{10, 13, 22}, Gal d 1 and Gal d 2 sIgE levels offer no additional value above standard raw egg white sIgE testing in distinguishing patients with or without raw egg allergy.

Ingestion of baked egg products is well tolerated and safe in most patients with egg allergy^{3, 10}. We found that sIgE to egg allergen components Gal d 1-3, and 5, did not improve the ability to predict baked egg challenge outcomes and that egg sIgE remained the best predictor of ability to tolerate baked egg challenge, as our group has previously published³. The aforementioned studies by Ando *et al*, Haneda *et al*, and Benhomau *et al* also examined the utility of Gal d 1 sIgE levels to diagnose baked egg allergy, and found that it was superior to egg white sIgE. However, our study did not find that Gal d 1 or Gal d 2 sIgE were superior to egg white sIgE, and the sensitivity of these components was less than half of egg sIgE. Although previous studies suggest that Gal d 1 sIgE levels may be superior to egg sIgE levels to predict baked egg tolerance, it is important to note that these studies used heated egg in a form without a wheat matrix, which could explain the different findings compared with our study. This is important as the wheat matrix may help reduce allergenicity of the egg²³ and thus alter the predictive values of Gal d 1 sIgE. Lemon-mule *et al* looked at the utility of Gal d 1 and Gal d 2 sIgE levels in a cohort challenged to baked egg in a wheat matrix and found that these components were also poor predictors of baked egg allergy¹⁰. Immunological predictors of baked egg challenge outcomes remain elusive, and although the use of CRD

may aid in the prediction of egg allergy phenotypes, oral food challenge remains the only means of conclusively establishing baked egg tolerance.

The strengths of this study include the large cohort of challenge proven egg allergic and tolerant children. True population negative controls (sensitised tolerant and non sensitised non allergic infants) provided better evaluation of the performance of these tests as a screening tool for diagnosis of egg allergy and prediction of natural history. This is also the first study to present data on egg white sIgE in combination with egg allergen components on all subjects. A limitation of this study is that we have only examined outcomes between 1 and 4 years of age, so may not be generalised to diagnosis or natural history of egg allergy at other ages. Our data are population-based and may not reflect specialised clinical practice. However, our results are likely to be useful in a community setting where most children with food allergy will present in early life (up to 8.9% of Australian 12 month old infants have egg allergy)¹², and testing for egg allergy by age 1 year is common in clinical practice. Although current standard sIgE as well as component resolved sIgE diagnostics evaluate responses to proteins, epitope mapping evaluates IgE binding to specific segments of proteins, and offers a more precise method for determining the egg allergy phenotypes. If it is possible to identify which subset of linear epitopes results in more persistent or severe allergy, it would provide a potential screening tool to identify subgroups of egg allergy.

In conclusion, although we found that component resolved diagnostics remains inferior to current testing methods in the diagnosis of egg allergy, its use in the aid of determining prognostic outcomes could be important for patient management. By providing a method for screening those at risk of developing persistent egg allergy and those likely to develop tolerance. These patients would then be targeted for treatment interventions as they become available.

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Contributions

TD and KA were involved in the conception and design of the study, TD performed the experiments, TD, RP, JK, SD, LG, ALP, DM, MN, MLKT, and KA analysed and interpreted the results; TD, RP, JK, SD, LG, ALP, DM, MN, MLKT, and KA drafted and provided intellectual input into the manuscript.

Conflict of Interests

There is no conflict of interest from any of the authors.

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425

	Egg Allergic (n=297)			Egg Tolerant (n=152)		
	All Egg Allergic (n=297)	Resolved by 4 yo (n=208)	Persistent at 4 yo (n=40)	All Egg Tolerant (n=152)	Egg Sensitized (n=97)	Non-Egg sensitized (n=55)
Males (%)	54%	54%	45%	49%	53%	44%
Co-existent Sensitization to egg and one or more other foods at	79%	78%	82%	13%**	19%**	0%**

1yo [^] (%)						
Co-existent Food Allergy to egg and one or more other foods at 1yo [^] (%)	21%	16%	21%	7%**	10%**	0%**
Current Eczema at 1yo (%)	52%	38%	23%	22%*	46%	15%**
Family History of Eczema (%)	43%	42%	46%	36%	39%	41%
Family History of Food Allergy (%)	13%	14%	12%	10%	11%	9%
Gal d 1 sIgE, med (IQR, kUA/l)	0.06 (0-0.95)	0.03 (0-0.43)	1.93 (0.03-5.56)	0 (0-0.03)	0.00 (0,0.13)	0.00 (0-0)
Gal d 2 sIgE, med (IQR, kUA/l)	0.66 (0.22-2.72)	0.50 (0.17-1.52)	3.99 (0.76-16.4)	0.03 (0-0.2)	0.14 (0.03-0.35)	0.00 (0-0)
Gal d 3 sIgE, med (IQR, kUA/l)	0 (0-0.02)	0.00 (0-0)	0.00 (0-0.02)	0.00 (0-0)	0.00 (0-0)	0.00 (0-0)
Gal d 5 sIgE, med (IQR, kUA/l)	0 (0-0.04)	0.00 (0-0)	0.00 (0-0.04)	0.00 (0-0)	0.00 (0-0)	0.00 (0-0)

Table 1. Clinical features of the study group

* p < 0.05, ** p < 0.001 compared to egg allergic group

[^] Other foods tested were peanut, sesame, shellfish and cow's milk

Table 2. Sensitivity and specificity of various cut-offs for the prediction of egg allergy resolution at 4yo comparing persistent egg allergics (n=40) versus resolved egg allergics (n=208)

<u>Cut-off</u> (KUA/l)	<u>PPV (95% CI)</u>	<u>Sensitivity %</u> (95% CI)	<u>Specificity %</u> (95% CI)	<u>PLR*</u>	<u>NLR[^]</u>
<u>Egg White sIgE</u>					
> 0.35	19 (17-20)	95 (83-99)	21 (15-27)	1.20	0.24
> 1.17	31 (26-36)	90 (76-97)	61 (54-68)	2.31	0.16
> 1.70	33 (27-40)	85 (70-94)	67 (61-74)	2.53	0.19
> 11.0	64 (43-81)	45 (29-62)	95 (91-98)	9.36	0.53
> 20.0	82 (48-96)	33 (19-50)	99 (95-100)	22.5	0.68
<u>Gal d 2 sIgE (Ovalbumin)</u>					
> 0.35	21 (18-23)	83 (67-93)	39 (32-46)	1.34	0.46
> 1.97	35 (26-43)	63 (46-77)	78 (71-83)	2.73	0.647
> 6.11	61 (40-78)	43 (27-59)	95 (91-97)	8.04	0.61
> 22	82 (37-97)	23 (11-38)	99 (95-99)	23.4	0.78

Gal d 1 sIgE (Ovomucoid)					
> 0.35	29 (23-36)	65 (48-79)	92 (64-76)	2.18	0.50
> 1.39	42 (30-55)	55 (36-69)	86 (81-91)	3.95	0.53
> 5.95	72 (37-91)	25 (13-41)	98 (95-100)	13.0	0.76
> 12.7	86 (26-99)	10 (3-24)	100 (98-100)	31.2	0.85

- * PLR is the positive likelihood ratio calculated by (sensitivity/(1-specificity)) and indicates the likelihood of having raw egg allergy the sensitivity of the changes
- ^ NLR is the negative likelihood ratio calculated by ((1-sensitivity)/specificity)) and indicates the likelihood of not having raw egg allergy

Table 3. Sensitisation to egg allergens at 1 year as risk factors for persistent egg allergy.

Early sensitisation associated with risk of persistent egg allergy at 2yo	<u>Adjusted OR[^]</u>	<u>95% CI</u>	<u>p-value</u>
Resolved egg allergy by 2yo (n=60)	1		
Sensitisation to all components (n=65)	2.30*	1.31-4.03	0.004
Gal d 1 sensitisation (n=39)	2.31*	1.11-4.84	0.020
Gal d 2 sensitisation (n=65)	3.48	1.07-11.29	0.038
Gal d 3 sensitisation (n=5)	0.68	0.15-3.05	0.617

Gal d 5 sensitisation (n=8)	5.41	0.62-47.77	0.128
Early sensitisation associated with risk of persistent egg allergy at 4yo	<u>Adjusted OR[^]</u>	<u>95% CI</u>	<u>p-value</u>
Resolved egg allergy by 4yo (n=208)	1		
Sensitisation to all components (n=39)	4.19*	1.25-14.04	0.007
Gal d 1 sensitisation (n=29)	2.50*	1.15-5.17	0.020
Gal d 2 sensitisation (n=39)	0.67	0.20-2.19	0.504
Gal d 3 sensitisation (n=6)	1.89	0.58-6.11	0.289
Gal d 5 sensitisation (n=9)	6.39	2.03-20.09	<0.001

[^]One adjusted model included all four components therefore OR are adjusted for other component sensitisation. Sensitisation to all components versus no sensitisation were examined separately.

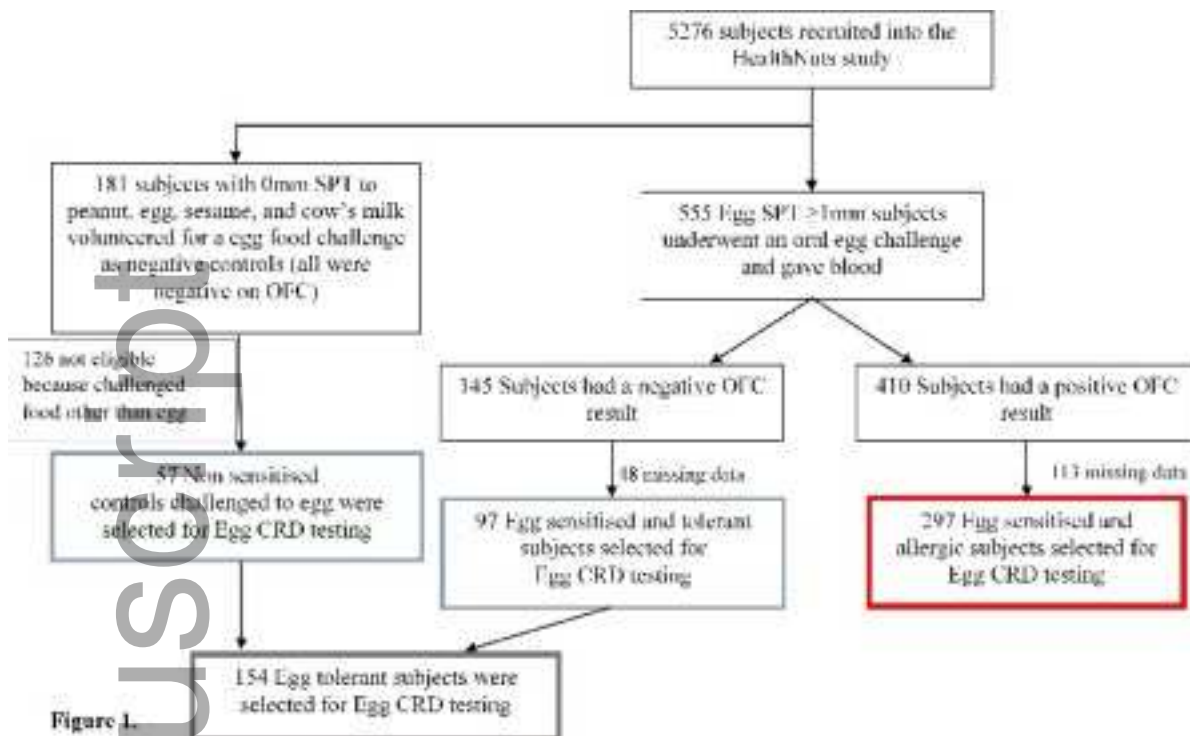
Figure Legends

Figure 1. Selection of subjects for component resolved diagnostic testing

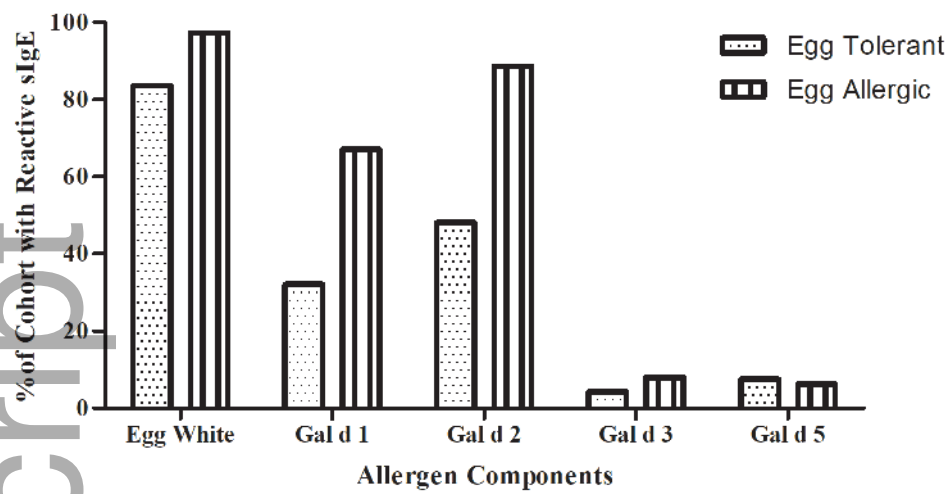
Figure 2. Proportion of 1 year old infants sensitised to egg allergen components. Egg allergic (vertical stripe bars) and egg sensitised tolerant infants (dotted bars) defined as a SPT $\geq$ 2mm. Egg white RAST $\geq$ 0.1kUA/l is used as a reference range to calculate the proportion of infants sensitised to each of the components ($\geq$ 0.1kUA/l).

Figure 3a-b. (a) ROC of Egg Allergen Components testing for persistent egg allergy at 4 years old; **(b)** Predictive probability curve of Gal d 1 (red triangles), Gal d 2 (green squares), and Egg White sIgE (blue circles) in determining persistent egg allergy.

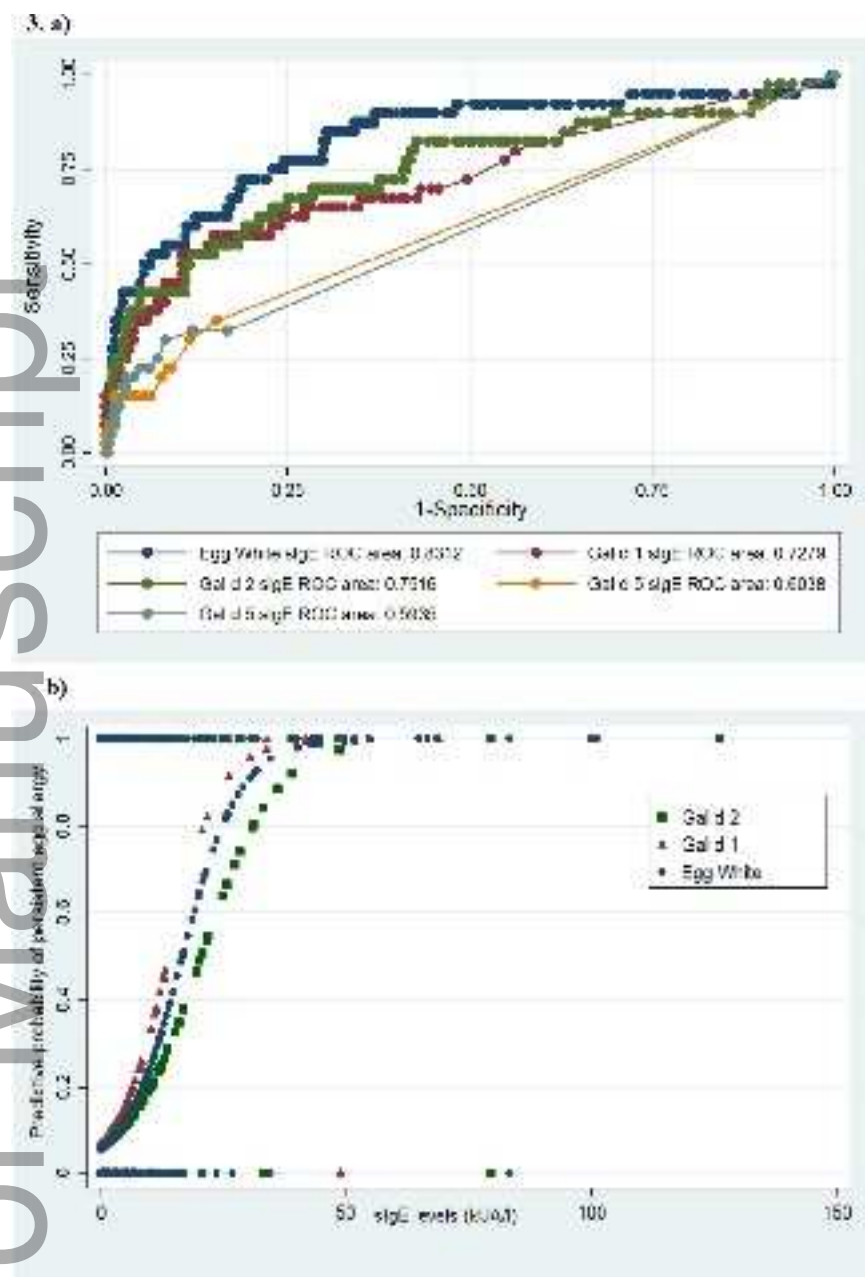
Figure 4. The rate of tolerance development to raw egg at 2 years of age (round dots) and 4 years of age (squares) depending on the number of egg allergens sensitised at age 1.



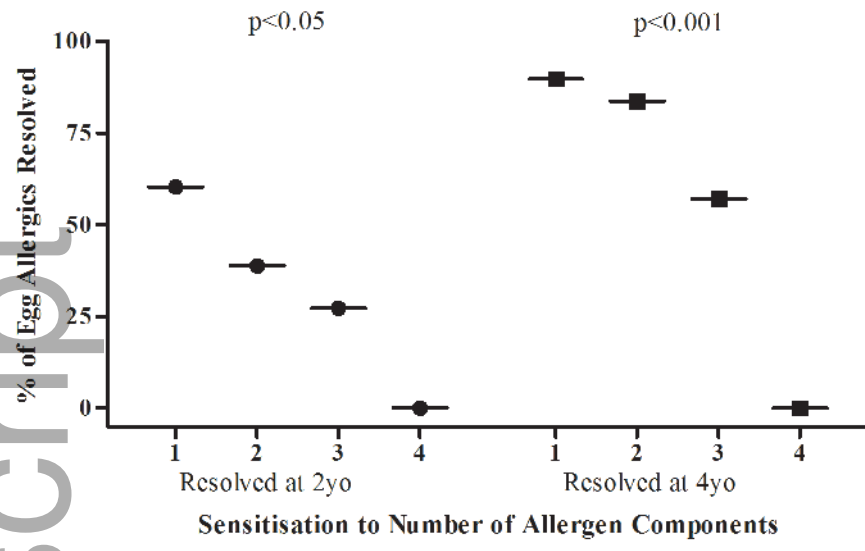
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