HHS Public Access

Author manuscript

Allergy. Author manuscript; available in PMC 2021 October 01.

Published in final edited form as:

Allergy. 2020 October ; 75(10): 2633–2643. doi:10.1111/all.14357.

Ovomucoid epitope-specific repertoire of IgE, IgG_4, IgG_1, IgA_1, and IgD antibodies in egg-allergic children

Maria Suprun, MPH[1], **Robert Getts, PhD**[2], **Galina Grishina, MS**[1], **Angela Tsuang, MD**[1], **Mayte Suárez-Fariñas, PhD**[1], **Hugh A. Sampson, MD**[1]

[1]Icahn School of Medicine at Mount Sinai, New Yok, NY, USA

[2]AllerGenis LLC, Hatfield, PA, USA

Abstract

Background: Egg-white ovomucoid, that is, Gal d 1, is associated with IgE-mediated allergic reactions in most egg-allergic children. Epitope-specific IgE levels have been correlated with the severity of egg allergy, while emerging evidence suggests that other antibody isotypes (IgG_1, IgG_4, IgA, and IgD) may have a protective function; yet, their epitope-specific repertoires and associations with atopic comorbidities have not been studied.

Methods: Bead-based epitope assay (BBEA) was used to quantitate the levels of epitope-specific (*es*)IgA, *es*IgE, *es*IgD, *es*IgG_1, and *es*IgG_4 antibodies directed at 58 (15-mer) overlapping peptides, covering the entire sequence of ovomucoid, in plasma of 38 egg-allergic and 6 atopic children. Intraclass correlation (ICC) and coefficient of variation (CV) were used for the reliability assessment. The relationships across *es*Igs were evaluated using network analysis; linear and logistic regressions were used to compare groups based on egg allergy status and comorbidities.

Results: BBEA had high reliability (ICC >0.75) and low variability (CV <20%) and could detect known IgE-binding epitopes. Egg-allergic children had lower *es*IgA_1 ($P = .010$) and *es*IgG_1 ($P = .016$) and higher *es*IgE ($P < .001$) and *es*IgD ($P = .015$) levels compared to the atopic controls. Interestingly, within the allergic group, children with higher *es*IgD had decreased odds of anaphylactic reactions (OR =0.48, $P = .038$). Network analysis identified most associations between *es*IgE with either *es*IgG_4 or *es*IgD; indicating that IgE-secreting plasma cells could

Correspondence Hugh A. Sampson, Kurt Hirschhorn Professor of Pediatrics, Department of Pediatrics; Box 1089, Icahn School of Medicine at Mount Sinai, New York, 10029-6574 NY, USA. hugh.sampson@mssm.edu.

AUTHOR CONTRIBUTIONS

The experiments were conceived and designed by MS, M.SF., and HS Sample acquisition and experimental procedures were performed by MS, GG, and AT; BG provided technical information and advice on the components of the BBEA. Data were analyzed by MS and M.SF; MS, M.SF, and H.S drafted the paper. All authors reviewed and approved the manuscript.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

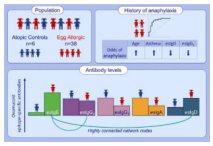
CONFLICT OF INTEREST

MS has nothing to disclose. RG is an employee of Genisphere LLC and scientific consultant of AllerGenis LLC; in addition, RG has a patent PCT/US15/020715 (WO) pending. GG has nothing to disclose. AT has nothing to disclose. M.SF. received research funding to Mount Sinai by a grant from AllerGenis LLC. HS reports nonfinancial support from AllerGenis LLC during the conduct of the study; grants from Immune Tolerance Network; NIAID/NIH, personal fees from N-Fold Therapeutics, other from DBV Technologies, outside the submitted work; and Mount Sinai has licensed the technology for a bead-based epitope assay for food-allergen epitope analyses to AllerGenis LLC. HS serves as an unpaid Board of Directors member and advisor to AllerGenis LLC.

originate from either sequential isotype switch from antigen-experienced intermediate isotypes or directly from the IgD^+ B cells.

Conclusions: Collectively, these data point toward a contribution of epitope-specific antibody repertoires to the pathogenesis of egg allergy.

Graphical Abstract



Egg-allergic children have lower levels of epitope-specific *(es)*IgA_1 and *es*IgG_1 but higher *es*IgE and *es*IgD compared to the atopic controls. Within the allergic group, children with higher *es*IgD antibodies have decreased odds of anaphylactic reactions. Network analysis identifies most associations between *es*IgE with either *es*IgG_4 or *es*IgD, indicating potentially different origins of IgE.

Keywords

anaphylaxis; antibody repertoire; egg allergy; epitopes; ovomucoid

1 | INTRODUC TION

Hen's egg allergy is one of the most common pediatric food allergies affecting 0.5%−10% of infants and children.[1–9] Allergic reactions occur when antigen-specific immunoglobulin E (IgE) binds to its high-affinity receptors on granulocytes, activating their effector functions and release of inflammatory mediators.[10,11] Several egg-white proteins contribute to IgE sensitization, of which ovomucoid (OVM) is considered a major allergen, designated as *Gal d 1* (WHO-IUIS).[12–16] Ovomucoid is resistant to heat and digestive enzymes, and IgE recognition of the OVM protein is indicative of a more persistent allergic phenotype.[15,17–21] Additionally, IgE recognition of a greater number of sequential epitopes is shown to be associated with greater severity of allergic reactions.[22–24]

Over the past few years, different groups demonstrated that induction of other antibody isotypes, such as IgA, IgG_1, IgG_4, and IgD, can ameliorate IgE-mediated reactions.[15,25–40] Specifically, several clinical trials of egg oral immunotherapy reported increases of serum antigen-specific IgA, IgG_1, and IgG_4 in patients that responded to treatment.[32,33] Interestingly, the ability of the antibodies to block IgE-mediated responses could be largely dependent on their antigen specificity rather than isotype.[31] However, recent research in food allergy has been mostly focused on understanding epitope-specific repertoire of IgE and IgG_4, with only a limited number of studies addressing epitope specificity of IgG1, IgA, and IgD.[41,42]

We have previously presented a bead-based epitope assay that is highly reliable and sensitive for detecting milk[43,44] and peanut[45] epitope-specific IgE and IgG4. In this work, we developed an assay to measure levels of five antibody classes, that is, IgE, IgG4, IgG1,

IgA1, and IgD, directed at the peptides covering the entire sequence of the OVM protein. We then investigated how epitope-specific antibody repertoires are associated with egg allergy and anaphylaxis, as well as atopic comorbidities, such as asthma, rhinitis, and eczema. To our knowledge, this is the first report investigating the relationships among epitope-specific adaptive humoral responses in pediatric food allergy.

2 | MATERIALS AND METHODS

2.1 | Study participants

Plasma from children with specific IgE (sIgE) to the egg-white extract >0.35 kU_A/L (ImmunoCAP) was obtained from the Food Allergy Research Initiative (FARI) biorepository at Mount Sinai. Allergy status was determined by sIgE or skin prick testing and/or a convincing history, with moderate-to-severe, clear-cut allergic reactions following the ingestion of egg, as documented by a patient's local physician. A negative control pool (a mix of sera from four adults with no history of any food or environmental allergies), a positive control pool (plasma from three children with egg sIgE >100 kU_A/L), and atopic controls with food allergies other than egg were also included. The study was approved by the Institutional Review Board at the Icahn School of Medicine at Mount Sinai. All the study subjects provided informed consent.

2.2 | Measurement and quantification of epitope-specific (es) Ig levels

A peptide library consisting of 58 15-mer biotinylated peptides (12-mer overlap; CS Bio) was generated covering the entire sequence of hen's egg white OVM (UniProt ID P01005). The bead-based epitope assay (BBEA) was carried out as described previously,[45] with a few modifications. A master mix of biotinylated peptides, conjugated to LumAvidin beads (Luminex Corporation), was prepared in PBS-TBN buffer (1xPBS, 0.02% Tween-20, 0.1% BSA), and 100 μL/well of mix was added to 96-well filter plates. Plates were washed twice with PBS-TBN, and 100 μL/well of 1:10 plasma dilution was added in duplicates and incubated on a shaker for 2 hours at room temperature. To account for potential well position and plate effect, samples were randomized to wells using *PlateDesigner*.[46] For the nonspecific signal detection, each plate included two wells with only PBS-TBN. Plates were washed and incubated for 30 minutes with 50 μL/well of mouse anti-human PE-labeled secondary antibody (2 μg/mL of IgE, clone BE5, Cat. MA1–10375, Thermo Fisher Scientific; or 0.25 μg/mL of IgG_4, clone HP6025, Cat. 9200–09, Southern Biotech; or 62.5 ng/mL of IgG_1, clone HP6001, Cat. 9054–28, Southern Biotech; or 0.2 μg/mL of IgA_1, clone IS11–8E10, Miltenyi Biotec; or 0.17 μg/mL of IgD, clone IADB6, Cat. OB903009, Southern Biotech). Plates were washed three times, and beads were resuspended in 100 μL of PBS-TBN. The signal was quantified as a median fluorescence intensity (MFI) on the Luminex200 instrument (Luminex Corporation). For each peptide, MFIs were log_2-normalized and converted to nMFI values by subtracting the average of the non-specific binding wells, as previously described.[45] Since MFI is representative of antibody concentrations,[47] these *epitope-specific immunoglobulin (esIg)* nMFI levels represent a relative quantity of an antibody in plasma directed at a particular peptide.

2.3 | Statistical analyses

The plate effect for the IgG_4 experiment was quantified using the principal variance component analysis (PVCA),[48] with the principal component threshold set to 0.8. Plate effect was estimated for each peptide by fitting linear models with "plate" as a covariate; then, the coefficients for each plate were subtracted from the original nMFI values.[45]

To assess the reliability of the technical duplicates, a two-way intraclass correlation coefficient (ICC) for agreement was used, with the reliability thresholds defined as excellent (0.75–1.00), good (0.60–0.74), fair (0.40–0.59), or poor (<0.40).[49] Unsupervised hierarchical clustering of the duplicates was performed using Pearson's r correlation as a distance metric and an average-linkage algorithm. The coefficient of variation (CV) was calculated for each pair of replicates as (standard deviation [MFI]/average [MFI])*100. After the reliability assessment, the duplicates were averaged for downstream analyses.

An *es*Ig network was constructed from the Pearson's r using all epitope pairs with the WGCNA algorithm[50]: The epitope-epitope signed correlation matrix was converted to an adjacency matrix using a power function that optimizes a scale-free topology (power = 22). A normalized number of neighbors shared by the nodes on either side of each edge were calculated by transforming the adjacency into a topological overlap matrix (TOM). Then, *es*Igs were joined using the hierarchical clustering with an average linkage of the TOM and split into modules using a hybrid dynamic tree-cut algorithm (height = 0.99). A network layout was generated using the *ForceAtlas2* method in *Gephi*[51] and visualized using the *Cytoscape* software.[52]

The pairwise associations among epitopes of each antibody isotype were measured using Spearman's ρ correlation and cosine similarity. Antibody-level summary statistics for the pairwise correlation were obtained after using the Fisher Z-transformation of the correlation coefficients and reverse-transformed those summary statistics to Spearman's ρ.[53] The association of *es*IgE and $\log_{10}$-normalized egg sIgE was calculated using Spearman's ρ and clustered with *mcquitty* agglomeration algorithm.

Analysis of the egg-allergic (EA) vs atopic children (AC): Comparisons for each *es*Ig were carried out using linear modeling in the *limma* framework, where an empirical Bayes approach allows estimation of the variance parameters acquiring information across all *es*Igs.[54,55] Then, the levels of the significant *es*Igs ($P < .05$) were averaged for the downstream Spearman's ρ estimations; since none of the *es*IgG_4s were significant, the analyses were performed with the average of all 58 *es*IgG_4s. Using either the averages of significant epitope-binding antibodies or the mean of all 58, logistic models were fitted to estimate the area under the curve (AUC) for each immunoglobulin. Similarly, analysis of anaphylaxis and the atopic comorbidities, that is, asthma, eczema, and rhinitis, for only EA children was done using *limma* methodology. Results are summarized as in a Manhattan plot using the $-\log_{10}$ (P-value) for each epitope but including the direction of the association (<0 if negative and >0 if positive). As the resultant plot resembles the skyline of a city by a lake, we have termed "Chicago" plot. Then, for each disease, logistic models were built using a stepwise selection of predictors that included age, atopic comorbidities, and top 5–10 *es*Igs

(based on the smallest *P*-value). Due to a small sample size, the *P*-values were not adjusted for multiple comparisons. Statistical analyses were performed in R version 3.2.2.

3 | RESULTS

3.1 | Characteristics of the study cohort

Thirty-eight egg-allergic (EA) children, with an average egg-white sIgE of 54 kU_A/L, and 6 atopic controls (AC) were included in the study (Table 1). EA children were on average 6.6 (standard deviation (SD) = 3.6) years old, 22 (57%) were male, and 27 (80%) were White/Caucasian. Twenty-four (63%) children in the EA group had a history of egg-related anaphylaxis, 29 (76%) had eczema, 28 (74%) had asthma, and 22 (58%) had rhinitis. The AC group was slightly older, 10.6 (SD = 3.8) years of age, but comparable in other demographics and medical history.

3.2 | BBEA to measure antibodies specific to ovomucoid epitopes

The bead-based epitope assay (BBEA) was developed to measure levels of OVM's *es*Igs. We previously established in the peanut and milk BBEAs that plate effect (that is, technical experimental artifact common to high-throughput assays) can be detected.[45] The IgG_4 experiment was performed on multiple plates that included repeats of the same positive and negative pooled samples. The plate effect accounted for 57.2% of the overall variability and samples cluster by experimental date on a PCA plot (Figure S1A). This effect was estimated and eliminated using linear models, and in the adjusted data, the plate effect was contributing to 1.2% of total variability (Figure S1B).

Experimental reliability of the BBEA was assessed using multiple metrics. First, technical duplicates showed excellent agreement based on the intraclass correlation coefficient (ICC) with the average (±SD) of 0.96 ± 0.07 for *es*IgE, 0.96 ± 0.01 for *es*IgG_4, 0.99 ± 0.003 for *es*IgG_1, 0.77 ± 0.05 for *es*IgA, and 0.97 ± 0.02 for *es*IgD (Figure 1A), which was consistent across individual peptides (Figure S1C). The average coefficient of variation (CV) was below 20% for all antibodies (Figure 1B, Figure S1D). Additionally, using unsupervised hierarchical clustering, duplicates of the same sample clustered together (Figure 1C).

3.3 | IgE shows most epitope specificity and is "connected" to *es*IgG4 and *es*IgD

Epitope-specific antibody levels, presented as a heatmap (Figure 2A), show heterogeneity among patients. *es*IgE had greater intra-patient variability with the lowest average epitope-epitope pairwise correlation (ρ = .68) and cosine similarity (0.74, Figure 2B), preferentially recognizing only a few peptides, that is, IgE epitopes. All 58 IgE antibodies were positively associated with the egg white sIgE, with correlations ranging from 0.20 to 0.61 (Figure 2C). Other immunoglobulin isotypes were present at either low or high levels but with similar specificity to all 58 OVM peptides, as demonstrated by an average pairwise correlation of 0.95 for *es*IgD, 0.87 for *es*IgG_4, 0.84 for *es*IgA, and 0.83 for *es*IgG_1 (Figure 2B).

The relationship across *es*Igs was further investigated through the network analysis, which separates distinct modules based on the overall connectivity. Consistent with the previous observations, *es*IgE antibodies showed the most variability and were split into four modules

(green, black, pink, and red), while other *es*Igs belonged to self-containing groups: brown—*es*IgA, blue—*es*IgG_4, turquoise—*es*IgD, and yellow—*es*IgG_1 (Figure 2D). There was one exception: 015.*es*IgG_1 was assigned to the turquoise (IgD) module, while the rest of the 57 *es*IgG_1 antibodies belonged to the yellow cluster. Interestingly, this *es*IgG_1 antibody had the most connections to both *es*IgD (turquoise) and *es*IgG_1 (yellow) modules, serving as an intermediate link between the two isotypes. The interconnectivity was mostly observed between *es*IgE nodes and *es*IgG_4 or *es*IgD, with no edges connecting *es*IgA to any other *es*Ig.

3.4 | Higher *es*IgE and *es*IgD and lower *es*IgA and *es*IgG1 are associated with egg allergy

To determine which *es*Igs are associated with egg allergy, we compared repertoires of EA and AC groups. Ten *es*IgE and 12 *es*IgD were higher in the EA group ($P < .05$), while 2 *es*IgG_1 and 3 *es*IgA were higher in the AC patients; none of the *es*IgG_4 reached significance (Figure 3A). The averages of these *es*Igs were also significantly different among groups (Figure 3B) and provided better group distinction than averaging all 58 epitopes (Figure S2), with AUCs of 0.97 vs 0.92 for *es*IgE, 0.81 vs 0.69 for *es*IgD, 0.82 vs 0.62 for *es*IgA, and 0.80 vs 0.65 for *es*IgG_1 (Figure 3C).

The associations among *es*Igs were also different in the EA and AC (Figure 3D, Figure S3A). In the EA children, *es*IgD was negatively correlated with *es*IgA ($\rho = -0.32$, $P = .05$), while in the AC group, a strong positive trend was observed between *es*IgE and *es*IgD ($\rho = 0.83$, $P = .06$). Of note, while most patients had some IgD and IgA directed at OVM epitopes, their relative quantities were different: higher levels of one isotype generally meant lower level of the other one, as shown by patients clustering into the opposite groups in Figure S3B.

3.5 | Lower *es*IgD and *es*IgG1 are associated with increased odds of anaphylaxis in egg-allergic patients

Univariate models of *es*Igs and anaphylaxis, as well as asthma, rhinitis, and eczema, showed different profiles of immunoglobulin isotypes. Eight *es*IgD and 14 *es*IgG_1 were lower in patients with a history of anaphylaxis (Figure 4A); and the 017.*es*IgD antibody was significantly associated with reduced odds of anaphylactic reactions (OR = 0.48, $P = .038$) in a multivariable model adjusted for asthma, age, and *es*IgG_1 (AUC = 0.88, Figure 4B). Additionally, nonanaphylactic patients had a positive correlation of *es*IgD with *es*IgE ($\rho = 0.48$, $P = .037$), which was similar to the AC group, while *es*IgD and *es*IgA ($\rho = -0.76$, $P < .001$) and *es*IgE and *es*IgG_1 ($\rho = -0.45$, $P = .057$) were negatively correlated (Figure S4). These associations were in the same direction but with a much lower magnitude in the anaphylactic group.

Of the atopic comorbidities, EA children with rhinitis had 44 *es*IgG_4 antibodies that demonstrated significantly greater levels compared to patients without rhinitis; 5 *es*IgE were higher in children with eczema; and no differences in *es*Igs were observed in patients with or without asthma (Figure 4A,B). In a multivariable model, only a positive association of rhinitis and *es*IgG_4 to epitope #054 remained significant, even when adjusted for age and asthma.

4 | DISCUSSION

Understanding the molecular mechanism of allergic sensitization may help improve diagnostic, prognostic, and treatment approaches in food allergy. Previously, we demonstrated that milk-BBEA and peanut-BBEA have excellent reliability and sensitivity in detecting allergenic epitopes,[45] providing further insights into the role of IgE and IgG4 repertoires in disease phenotypes[44] and prognosis of milk oral immunotherapy outcome.[43] We have now developed an assay, termed egg-BBEA to measure the levels of five antibody isotypes, that is, IgE, IgG_4, IgG_1, IgA, and IgD to peptides covering the entire sequence of a major hen's egg white allergen—ovomucoid. We showed that egg-BBEA, similarly to other high-throughput technologies, has plate effects that can be detected and eliminated; and is a reliable assay with high agreement and low variation among technical replicates (Figure 1, Figure S1).

We have identified IgE directed at sequential peptides associated with egg allergy (Figure 3A), constituting immunogenic IgE epitopes previously identified by other groups[15,23–57] and demonstrating sensitivity of egg-BBEA for epitope detection. We also found that EA children had variable but on average higher *es*IgD, while AC showed greater levels of *es*IgA and *es*IgG_1 antibodies (Figure 3A,B, Figure S3). Furthermore, we found that *es*IgE was highly correlated with *es*IgD in AC but not EA patients (Figure 3D), which may indicate that while atopic patients have low levels of IgE, it is produced by plasma cells coming from a direct isotype switch from IgD^+ B cells, resulting in lower-affinity IgE antibodies.[58] Different antibody isotypes may inhibit IgE-mediated allergic reactions, given they have similar specificity to an antigen,[31] and in this cohort, EA patients had high egg sensitization with median egg sIgE of 49.9 kU_A/L, which might have rendered antibody repertoire profiles different from patients with lower sIgE levels. However, these results provide basis for further studies that should include broader allergic population and a larger number of control participants.

To identify association patterns across repertoires of antibody isotypes, we applied a network analysis and showed that *es*IgE had large variability with four distinct modules and low correlations, suggesting higher epitope specificity of this antibody (Figure 2B,D). On the other hand, the rest of the isotypes were assigned into self-containing modules. Interestingly, across antibodies connectivity was observed between *es*IgE and *es*IgG_4 or *es*IgD, while *es*IgA was disconnected from other modules, and *es*IgG_1 was only connected to *es*IgD via one epitope. This may indicate that IgE-secreting plasma cells come from either sequential isotype switch from the intermediate upstream isotypes (ie, IgG_4^+ B cells) or directly from the IgD^+ B cells. This concept was shown by several groups that sequenced the heavy-chain loci of the human immunoglobulin gene (IgH) and compared the mutation rates of the variable segment, concluding that high-affinity IgE is a product of sequential isotype switch form "antigen-experienced" B cells of the IgG subclasses; while lower-affinity IgE derived from a direct switch from the IgM/IgD B cells.[58–64]

Early food allergy and eczema, especially an extrinsic subtype defined by high IgE levels, are often followed by progression to asthma and allergic rhinitis, commonly referred to as the "atopic march".[65,66] The development of atopic manifestations progresses with age and

having one of the comorbidities is considered a risk factor for the others. Additionally, food-related anaphylaxis was shown to be associated with age as well as the presence of atopic dermatitis.[67,68] We further investigated whether the repertoire of immunoglobulins was associated with atopic diseases and anaphylaxis. While the EA children had higher *es*IgD than AC (Figure 3B), within the EA group patients with lower *es*IgD and *es*IgG_1 had greater odds of anaphylaxis (Figure 4). This finding is consistent with a recent study by Shan et al[36] showing that antigen-bound IgD can attenuate IgE-mediated basophils degranulation, potentially increasing immunity against soluble antigens. High levels of *es*IgG_1 could result in more antigen molecules being cleared through phagocytosis[69,70] rendering them unavailable to IgE. Additionally, while no correlation was observed between *es*IgE and *es*IgD in the EA group (Figure 3D, Figure S3A), after stratifying by history of anaphylaxis, this correlation was positive (Figure S4) among children with no history of anaphylaxis to egg, similarly to that of the atopic controls. Zhang et al[71] observed that antigen-specific IgE was correlated with antigen-specific IgD for several aero-allergens, for example, birch pollen, timothy pollen, and cat dander, with higher IgD levels to these antigens in atopic compared to nonatopic patients, which they hypothesized was indicative of similar regulation of the two antibodies.

Of the atopic comorbidities, high levels of almost all *es*IgG_4 antibodies were associated with rhinitis, and one IgG_4 epitope had significant association in a model adjusted for asthma and age (Figure 4). This association of IgG_4 is not commonly known, but IgG_4 specific to house-dust mite was shown to be on average higher in the rhinitis group than atopic or healthy controls.[72,73] Several *es*IgE antibodies were associated with eczema; and none of the *es*Igs reached significance for asthma.

Despite a small sample size and limited clinical information for these biorepository samples, we showed that examining the relationships across several antibody isotypes, including less studied IgG_1, IgA, and IgD antibodies, could distinguish additional endotypes among egg-allergic children at a molecular level. Further investigation of egg epitope-specific humoral immune profiles in patients with egg allergy confirmed by supervised oral food challenges and a control population with serological sensitization to egg in the absence of clinical allergy is likely to provide insight into the potential severity, natural history, and response to immunotherapy in egg-allergic individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDG MENTS

The authors would like to thank Dr Andrea Cerutti, MD/PhD, for the valuable discussions and insights; and the Food Allergy Research Initiative (FARI) biorepository at the Icahn School of Medicine at Mount Sinai for patients' plasma samples. The FARI repository was supported in part by a grant from Food Allergy Research and Education, a nonprofit organization supporting food allergy research. The study (HS and M.SF.) was funded in part by a grant from National Institute of Allergy and Infectious Diseases (NIAID, AI-66738), the David H. and Julia Koch Research Program in Food Allergy Therapeutics, and AllerGenis LLC. MS was funded by the Integrated Pharmacological Sciences Training Program grant from the National Institute of General Medical Sciences (NIGMS, T32GM062754). Other funding agencies had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Funding information

National Institute of General Medical Sciences, Grant/Award Number: T32GM062754; National Institute of Allergy and Infectious Diseases, Grant/Award Number: AI-66738; David H. and Julia Koch Research Program in Food Allergy Therapeutics; AllerGenis LLC

REFERENCES

1. Eggesbo M, Botten G, Halvorsen R, Magnus P. The prevalence of allergy to egg: a population-based study in young children. Allergy. 2001;56(5):403–411. [PubMed: 11350303]
2. Rona RJ, Keil T, Summers C, et al. The prevalence of food allergy: a meta-analysis. J Allergy Clin Immunol. 2007;120(3):638–646. [PubMed: 17628647]
3. Janzi M, Kull I, Sjoberg R, et al. Selective IgA deficiency in early life: association to infections and allergic diseases during childhood. Clin Immunol. 2009;133(1):78–85. [PubMed: 19541543]
4. Gupta RS, Springston EE, Warrier MR, et al. The prevalence, severity, and distribution of childhood food allergy in the United States. Pediatrics. 2011;128(1):e9–e17. [PubMed: 21690110]
5. Sicherer SH. Epidemiology of food allergy. J Allergy Clin Immunol. 2011;127(3):594–602. [PubMed: 21236480]
6. Osborne NJ, Koplin JJ, Martin PE, et al. Prevalence of challenge-proven IgE-mediated food allergy using population-based sampling and predetermined challenge criteria in infants. J Allergy Clin Immunol. 2011;127(3):668–676.e2. [PubMed: 21377036]
7. Peters RL, Koplin JJ, Gurrin LC, et al. The prevalence of food allergy and other allergic diseases in early childhood in a population-based study: healthNuts age 4-year follow-up. J Allergy Clin Immunol. 2017;140(1):145–153.e8. [PubMed: 28514997]
8. Gupta RS, Warren CM, Smith BM, et al. Prevalence and severity of food allergies among US adults. JAMA Netw Open. 2019;2(1):e185630.
9. Osterlund J, Winberg A, West CE. A 10-year review found increasing incidence trends of emergency egg allergy reactions and food-induced anaphylaxis in children. Acta Paediatr. 2019;108(2):314–320. [PubMed: 29920760]
10. Tordesillas L, Berin MC, Sampson HA. Immunology of food allergy. Immunity. 2017;47(1):32–50. [PubMed: 28723552]
11. Sampson HA, O'Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol. 2018;141(1):11–19. [PubMed: 29307410]
12. Matsuda T, Watanabe K, Nakamura R. Immunochemical and physical properties of peptic-digested ovomucoid. J Agric Food Chem. 1983;31(5):942–946. [PubMed: 6415139]
13. Honma K, Aoyagi M, Saito K, et al. Antigenic determinants on ovalbumin and ovomucoid: comparison of the specificity of IgG and IgE antibodies. Arerugi. 1991;40(9):1167–1175. [PubMed: 1720303]
14. Bernhisel-Broadbent J, Dintzis HM, Dintzis RZ, Sampson HA. Allergenicity and antigenicity of chicken egg ovomucoid (Gal d III) compared with ovalbumin (Gal d I) in children with egg allergy and in mice. J Allergy Clin Immunol. 1994;93(6):1047–1059. [PubMed: 8006309]
15. Cooke SK, Sampson HA. Allergenic properties of ovomucoid in man. J Immunol. 1997;159(4):2026–2032. [PubMed: 9257870]
16. Walsh BJ, Hill DJ, Macoun P, Cairns D, Howden ME. Detection of four distinct groups of hen egg allergens binding IgE in the sera of children with egg allergy. Allergol Immunopathol (Madr). 2005;33(4):183–191. [PubMed: 16045855]
17. Urisu A, Yamada K, Tokuda R, et al. Clinical significance of IgE-binding activity to enzymatic digests of ovomucoid in the diagnosis and the prediction of the outgrowing of egg white hypersensitivity. Int Arch Allergy Immunol. 1999;120(3):192–198. [PubMed: 10592464]
18. Ando H, Moverare R, Kondo Y, et al. Utility of ovomucoid-specific IgE concentrations in predicting symptomatic egg allergy. J Allergy Clin Immunol. 2008;122(3):583–588. [PubMed: 18692888]

19. Andorf S, Borres MP, Block W, et al. Association of clinical reactivity with sensitization to allergen components in multifood-allergic children. J Allergy Clin Immunol Pract. 2017;5(5): 1325–1334.e4. [PubMed: 28351786]
20. Takahashi K, Yanagida N, Sato S, Ebisawa M. Predictive power of ovomucoid and egg white specific IgE in heated egg oral food challenges. J Allergy Clin Immunol Pract. 2018;6(6):2115–2117.e6. [PubMed: 30170161]
21. Berin MC, Grishin A, Masilamani M, et al. Egg-specific IgE and basophil activation but not egg-specific T cells correlate with phenotypes of clinical egg allergy. J Allergy Clin Immunol. 2018;142(1):149–158. e8. [PubMed: 29518422]
22. Shreffler WG, Lencer DA, Bardina L, Sampson HA. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. J Allergy Clin Immunol. 2005;116(4):893–899. [PubMed: 16210066]
23. Jarvinen KM, Beyer K, Vila L, Bardina L, Mishoe M, Sampson HA. Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy. Allergy. 2007;62(7):758–765. [PubMed: 17573723]
24. Flinterman AE, Knol EF, Lencer DA, et al. Peanut epitopes for IgE and IgG4 in peanut-sensitized children in relation to severity of peanut allergy. J Allergy Clin Immunol. 2008;121(3):737–743.e10. [PubMed: 18234310]
25. Urisu A, Ando H, Morita Y, et al. Allergenic activity of heated and ovomucoid-depleted egg white. J Allergy Clin Immunol. 1997;100(2):171–176. [PubMed: 9275136]
26. Strait RT, Morris SC, Finkelman FD. IgG-blocking antibodies inhibit IgE-mediated anaphylaxis in vivo through both antigen interception and Fc gamma RIIb cross-linking. J Clin Invest. 2006;116(3):833–841. [PubMed: 16498503]
27. Lemon-Mule H, Sampson HA, Sicherer SH, Shreffler WG, Noone S, Nowak-Wegrzyn A. Immunologic changes in children with egg allergy ingesting extensively heated egg. J Allergy Clin Immunol. 2008;122(5):977–983 e971. [PubMed: 18851876]
28. Martos G, Lopez-Exposito I, Bencharitiwong R, Berin MC, Nowak-Wegrzyn A. Mechanisms underlying differential food allergy response to heated egg. J Allergy Clin Immunol. 2011;127(4):990–997. e2. [PubMed: 21377717]
29. Leonard SA, Sampson HA, Sicherer SH, et al. Dietary baked egg accelerates resolution of egg allergy in children. J Allergy Clin Immunol. 2012;130(2):473–480.e1. [PubMed: 22846751]
30. Konstantinou GN, Nowak-Wegrzyn A, Bencharitiwong R, Bardina L, Sicherer SH, Sampson HA. Egg-white-specific IgA and IgA2 antibodies in egg-allergic children: is there a role in tolerance induction? Pediatr Allergy Immunol. 2014;25(1):64–70. [PubMed: 24118158]
31. Dodev TS, Bowen H, Shamji MH, et al. Inhibition of allergen-dependent IgE activity by antibodies of the same specificity but different class. Allergy. 2015;70(6):720–724. [PubMed: 25758595]
32. Sugimoto M, Kamemura N, Nagao M, et al. Differential response in allergen-specific IgE, IgGs, and IgA levels for predicting outcome of oral immunotherapy. Pediatr Allergy Immunol. 2016;27(3):276–282. [PubMed: 26764899]
33. Wright BL, Kulis M, Orgel KA, et al. Component-resolved analysis of IgA, IgE, and IgG4 during egg OIT identifies markers associated with sustained unresponsiveness. Allergy. 2016;71(11):1552–1560. [PubMed: 27015954]
34. Natsume O, Kabashima S, Nakazato J, et al. Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial. Lancet. 2017;389(10066):276–286. [PubMed: 27939035]
35. Maeta A, Katahira R, Matsushima M, Onishi H, Nakamura Y, Takahashi K. Stepwise oral immunotherapy for 10 days in an egg-white allergy mouse model did not ameliorate the severity of allergy but induced the production of allergen-specific IgA. Biosci Biotechnol Biochem. 2018;82(12):2176–2179. [PubMed: 30227775]
36. Shan M, Carrillo J, Yeste A, et al. Secreted IgD amplifies humoral T helper 2 cell responses by binding basophils via galectin-9 and CD44. Immunity. 2018;49(4):709–724.e8. [PubMed: 30291028]

37. Lupinek C, Hochwallner H, Johansson C, et al. Maternal allergen-specific IgG may protect the child against allergic sensitization. J Allergy Clin Immunol 2019;144(2):536–554. [PubMed: 30685457]
38. Jimenez-Saiz R, Ellenbogen Y, Koenig JFE, et al. IgG1(+) B-cell immunity predates IgE responses in epicutaneous sensitization to foods. Allergy. 2019;74(1):165–175. [PubMed: 29790165]
39. Leung DYM, Calatroni A, Zaramela LS, et al. The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype. Sci Transl Med. 2019;11(480).eaav2685. 10.1126/scitranslmed.aav2685
40. Gutzeit C, Chen K, Cerutti A. The enigmatic function of IgD: some answers at last. Eur J Immunol. 2018;48(7):1101–1113. [PubMed: 29733429]
41. Klockenbring T, Boese A, Bauer R, Goerlich R. Comparative investigations of wheat and spelt cultivars: IgA, IgE, IgG1 and IgG4 binding characteristics. Food Agric Immunol. 2001;13(3):171–181.
42. Seppo AE, Savilahti EM, Berin MC, Sampson HA, Jarvinen KM. Breast milk IgA to foods has different epitope specificity than serum IgA-Evidence for entero-mammary link for food-specific IgA? Clin Exp Allergy. 2017;47(10):1275–1284. [PubMed: 28449395]
43. Suarez-Farinas M, Suprun M, Chang HL, et al. Predicting development of sustained unresponsiveness to milk oral immunotherapy using epitope-specific antibody binding profiles. J Allergy Clin Immunol. 2018;143(3):1038–1046. [PubMed: 30528770]
44. Sackesen C, Suarez-Farinas M, Sillva R, et al. A new Luminex-based peptide assay to identify reactivity to baked, fermented, and whole milk. Allergy. 2018;74(2):327–336. [PubMed: 30058196]
45. Suprun M, Getts R, Raghunathan R, et al. Novel bead-based epitope assay is a sensitive and reliable tool for profiling epitope-specific antibody repertoire in food allergy. Sci Rep. 2019;9(1):18425. [PubMed: 31804555]
46. Suprun M, Suarez-Farinas M. PlateDesigner: a web-based application for the design of microplate experiments. Bioinformatics. 2018;35(9):1605–1607.
47. Breen EJ, Tan W, Khan A. The statistical value of raw fluorescence signal in luminex xMAP based multiplex immunoassays. Sci Rep. 2016;6:26996. [PubMed: 27243383]
48. Li J, Bushel PR, Chu TM, Wolfinger RD. Principal variance components analysis: estimating batch effects in microarray gene expression data. In: Scherer A, eds. Batch effects and noise in microarray experiments: sources and solutions. UK: John Wiley & Sons, Ltd; 2009:141–154.
49. Cicchetti DV. Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. Psychol Assess. 1994;6(4):284.
50. Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Stat Appl Genet Mol Biol. 2005;4(1):
51. Bastian M, Heymann S, Jacomy M. Gephi: an open source software for exploring and manipulating networks. International AAAI Conference on Weblogs and Social Media. 2009. http://www.aaai.org/ocs/index.php/ICWSM/09/paper/view/154
52. Smoot ME, Ono K, Ruscheinski J, Wang PL, Ideker T. Cytoscape 2.8: new features for data integration and network visualization. Bioinformatics. 2011;27(3):431–432. [PubMed: 21149340]
53. Zar J. Spearman rank correlation: overview. In: Balakrishnan N, Colton T, Everitt B, Piegorsch W, Ruggeri F, Teugels JL, eds: Wiley StatsRef: Statistics Reference Online. Hoboken, NJ: Wiley; 2014. 10.1002/9781118445112.stat05964
54. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data using empirical Bayes methods. Biostatistics. 2007;8(1):118–127. [PubMed: 16632515]
55. Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43(7):e47. [PubMed: 25605792]
56. Kovacs-Nolan J, Zhang JW, Hayakawa S, Mine Y. Immunochemical and structural analysis of pepsin-digested egg white ovomucoid. J Agric Food Chem. 2000;48(12):6261–6266. [PubMed: 11141283]
57. Holen E, Bolann B, Elsayed S. Novel B and T cell epitopes of chicken ovomucoid (Gal d 1) induce T cell secretion of IL-6, IL-13, and IFN-gamma. Clin Exp Allergy. 2001;31(6):952–964. [PubMed: 11422162]

58. Mine Y, Wei ZJ. Identification and fine mapping of IgG and IgE epitopes in ovomucoid. Biochem Biophys Res Commun. 2002;292(4):1070–1074. [PubMed: 11944924]
59. Takagi K, Teshima R, Okunuki H, et al. Kinetic analysis of pepsin digestion of chicken egg white ovomucoid and allergenic potential of pepsin fragments. Int Arch Allergy Immunol. 2005;136(1):23–32. [PubMed: 15591810]
60. Martinez-Botas J, Cerecedo I, Zamora J, et al. Mapping of the IgE and IgG4 sequential epitopes of ovomucoid with a peptide microarray immunoassay. Int Arch Allergy Immunol. 2013;161(1):11–20. [PubMed: 23257567]
61. Looney TJ, Lee JY, Roskin KM, et al. B-cell isotype switching origins of IgE. J Allergy Clin Immunol. 2016;137(2):579–586.e7. [PubMed: 26309181]
62. Xiong H, Dolpady J, Wabl M, Curotto de Lafaille MA, Lafaille JJ. Sequential class switching is required for the generation of high affinity IgE antibodies. J Exp Med. 2012;209(2):353–364. [PubMed: 22249450]
63. Horns F, Vollmers C, Croote D, et al. Lineage tracing of human B cells reveals the in vivo landscape of human antibody class switching. Elife. 2016;5. e16578. 10.7554/eLife.16578
64. Spergel JM, Paller AS. Atopic dermatitis and the atopic march. J Allergy Clin Immunol. 2003;112(6 Suppl):S118–S127. [PubMed: 14657842]
65. Czarnowicki T, Krueger JG, Guttman-Yassky E. Novel concepts of prevention and treatment of atopic dermatitis through barrier and immune manipulations with implications for the atopic march. J Allergy Clin Immunol. 2017;139(6):1723–1734. [PubMed: 28583445]
66. Worm M, Edenharter G, Rueff F, et al. Symptom profile and risk factors of anaphylaxis in Central Europe. Allergy. 2012;67(5):691–698. [PubMed: 22335765]
67. Worm M, Babina M, Hompes S. Causes and risk factors for anaphylaxis. J Dtsch Dermatol Ges. 2013;11(1):44–50. [PubMed: 23181736]
68. Schroeder HW Jr, Cavacini L. Structure and function of immunoglobulins. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S41–S52. [PubMed: 20176268]
69. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol. 2018;18(1):46–61. [PubMed: 29063907]
70. Zhang M, Niehus J, Brunnee T, Kleine-Tebbe J, O'Connor A, Kunkel G. Measurement of allergen-specific IgD and correlation with allergen-specific IgE. Scand J Immunol. 1994;40(5):502–508. [PubMed: 7973457]
71. Aydogan M, Mete N, Yazi D, et al. Comparison of Der p1-specific antibody levels in children with allergic airway disease and healthy controls. Pediatr Allergy Immunol. 2007;18(4):320–325. [PubMed: 17584311]
72. Miranda DO, Silva DA, Fernandes JF, et al. Serum and salivary IgE, IgA, and IgG4 antibodies to Dermatophagoides pteronyssinus and its major allergens, Der p1 and Der p2, in allergic and nonallergic children. Clin Dev Immunol. 2011;2011:302739.

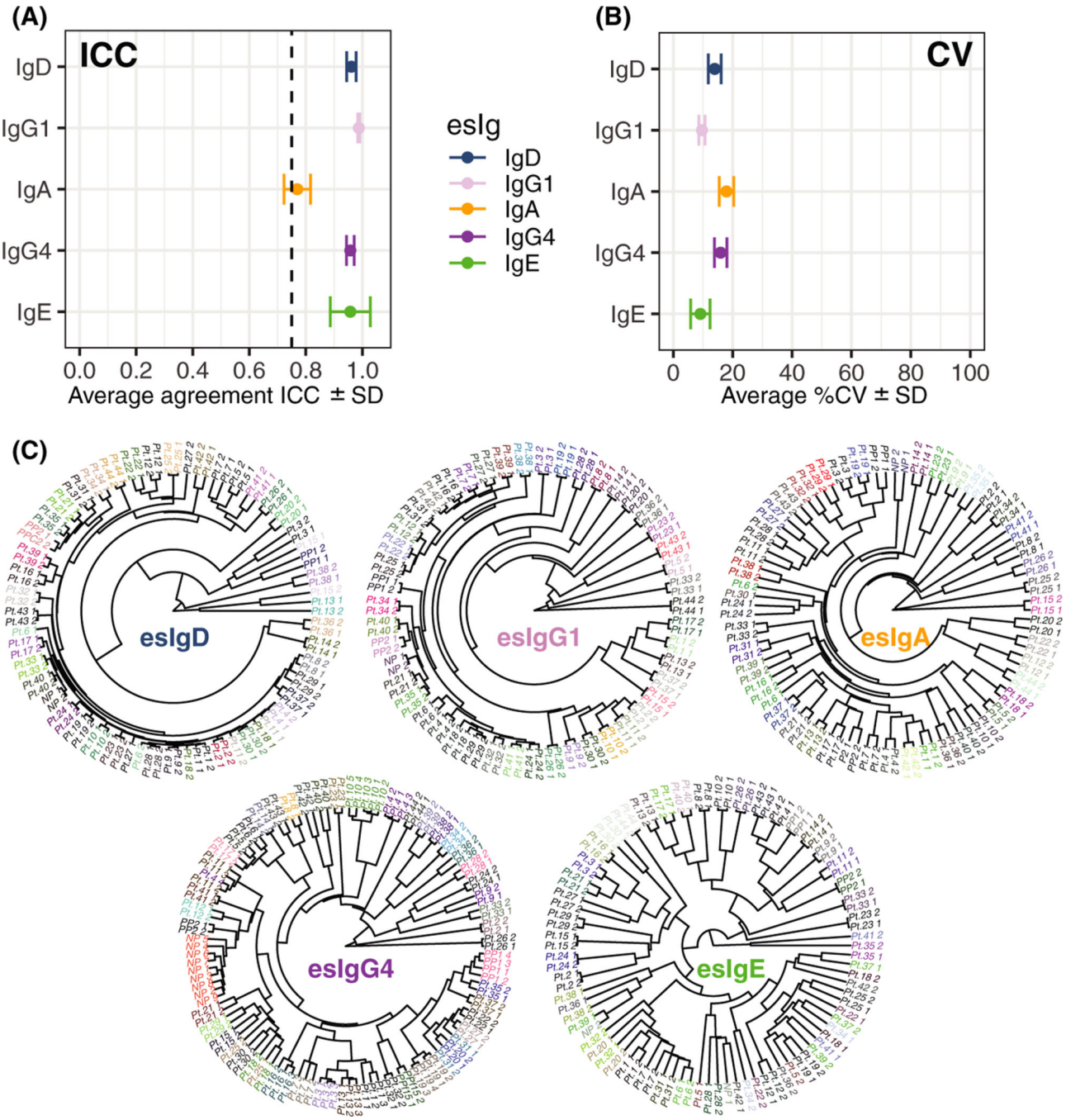


FIGURE 1.

Characterization of egg-BBEA. A, ICC measured among duplicates and averaged across 58 peptides shows high agreement (ICC >0.75) for all antibody isotypes, presented as mean and SD. B, CV averaged across 58 peptides shows low variability among technical replicates, presented as mean and SD C, Unsupervised clustering presented as a phylogenetic tree of the technical duplicates constructed with the Pearson correlation as distance metric and "average" agglomeration algorithm shows that samples from the same patients cluster together

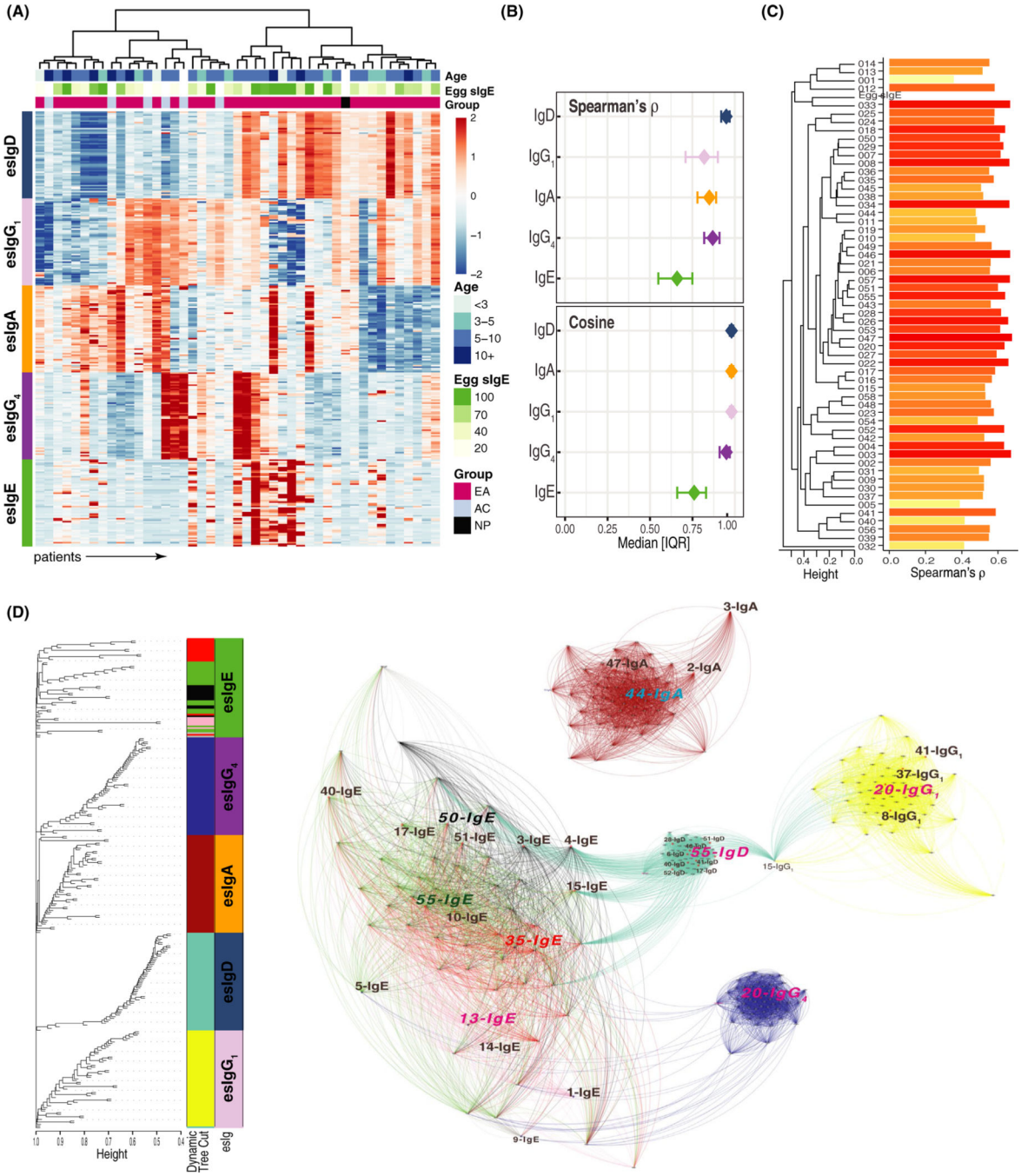


FIGURE 2.

Epitope-specific immunoglobulin repertoire. A, Heatmap representing the (standardized) levels of five antibody isotypes to 58 peptides (rows, ordered by sequential peptide number) for all 44 patients (columns). B, A median and interquartile range of epitope-epitope pairwise Spearman correlations or cosine similarity calculated separately for each antibody isotype. *es*IgEs have lowest correlations, potentially indicating epitope specificity C, Barplot showing that all *es*IgEs have positive Spearman correlation with egg-white sIgE (all *P*-values < 0.05), ordered by unsupervised hierarchical clustering. D, Left, a dendrogram of the

hierarchical clustering of the TOM matrix (colored tips represent module assignment and corresponding classes of the *es*Igs) shows that aside from *es*IgE, other antibody isotypes were assigned to the self-containing modules. Right, a multiscale network of *es*Igs constructed with ForceAtlas2 algorithm and colored by module assignment; larger letters in color (module color for *es*IgE, blue for *es*IgA, and pink of the rest) are most connected nodes, that is, module hubs. *es*Igs in black larger letters highlight epitopes associated with allergy or history of anaphylactic reactions identified in subsequent analysis

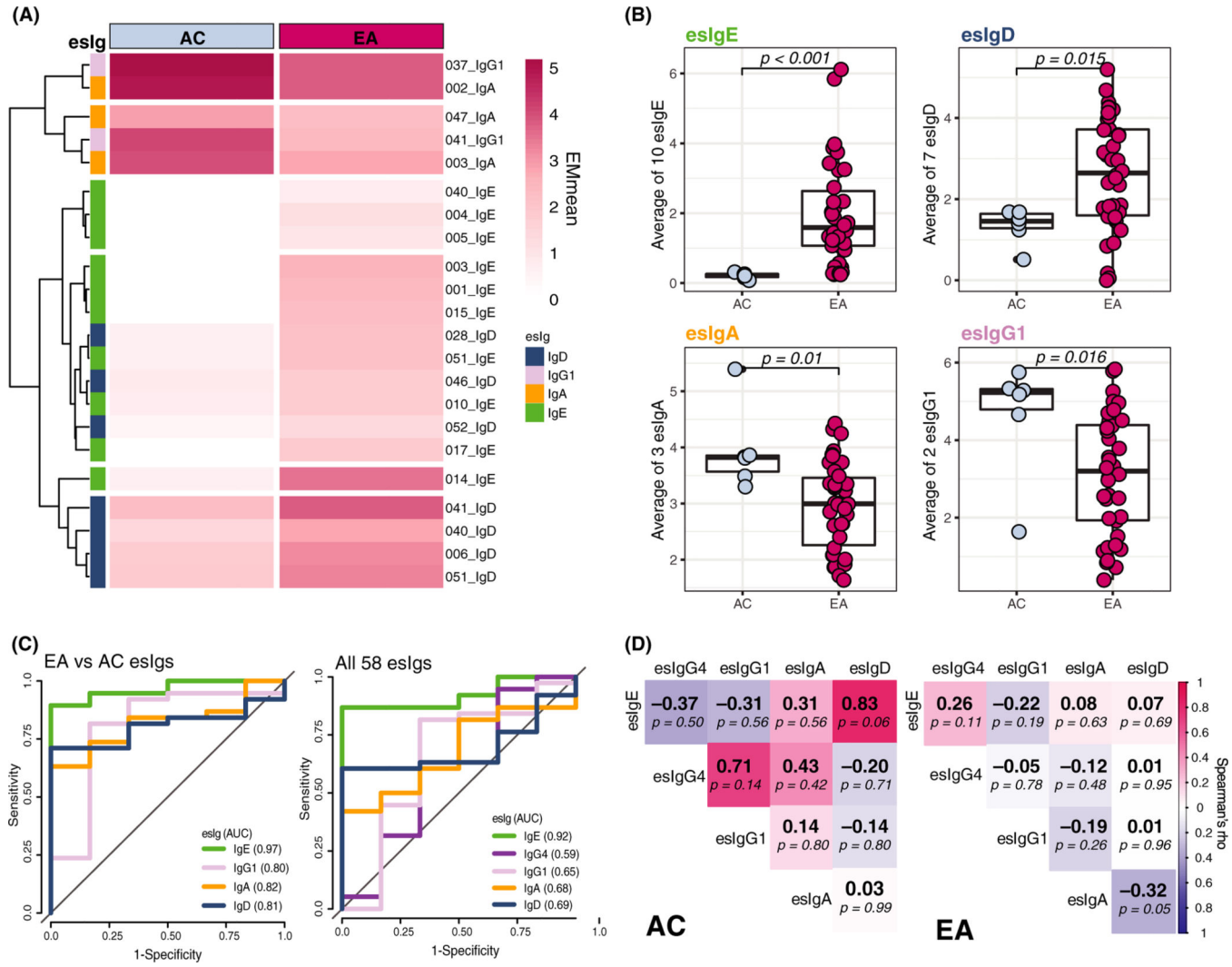


FIGURE 3.

*es*Ig repertoire in EA vs AC groups. A, Heatmap representing the mean levels of *es*Igs that were significantly different among EA and AC groups ($P < .05$): 10 *es*IgE, 7 *es*IgD, 3 *es*IgA, 2 *es*IgG$_1$, and none of the *es*IgG$_4$. B, Boxplots of the average of the significant *es*Igs compared using Wilcoxon-Mann-Whitney *U* test (each point is an individual patient). C, The ROC curves of the logistic models for either the average of only significant *es*Igs (left) or all *es*Igs (right) and their respective AUCs in the legend. D, Spearman correlations of the averages of the significant *es*Igs (and all 58 *es*IgG$_4$s) by group; color represents direction and strength of association, and correlation coefficient is a number in bold, followed by a p-value

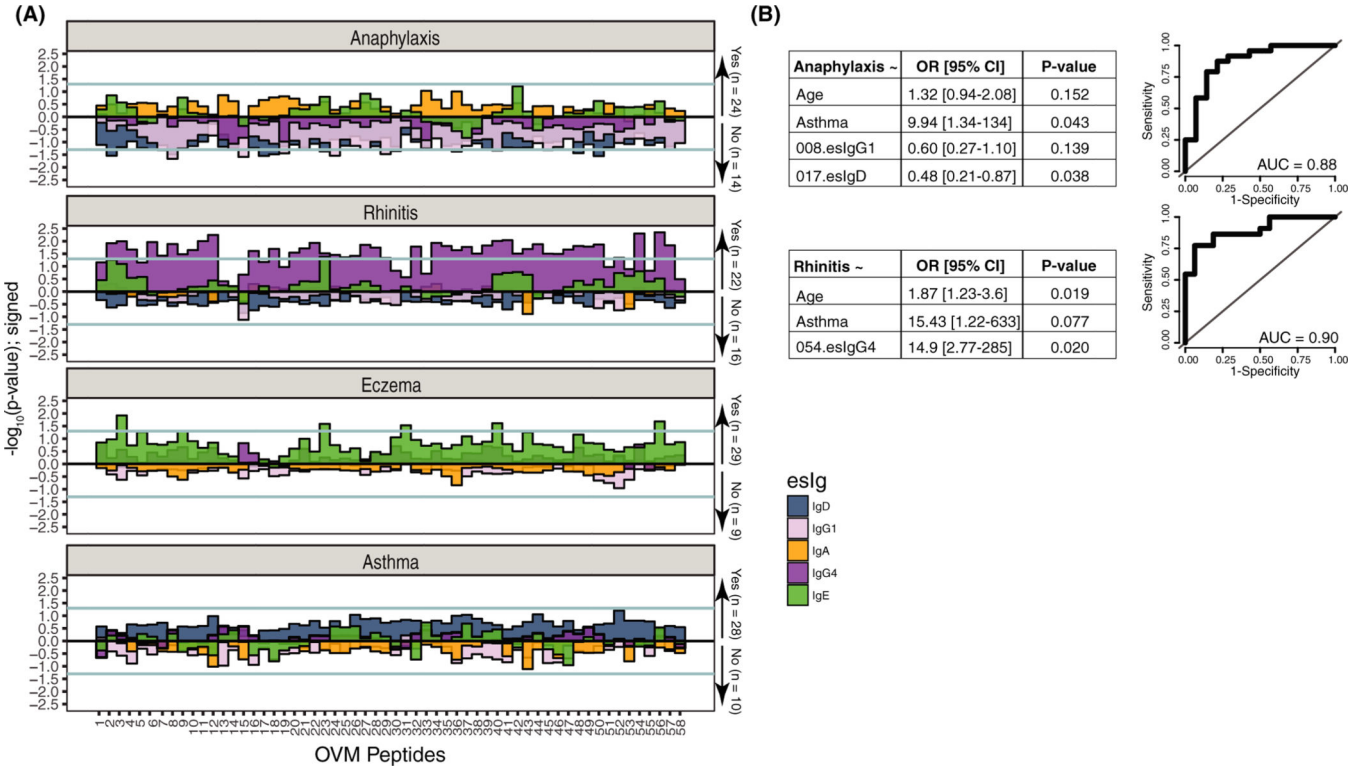


FIGURE 4.

Associations of atopic comorbidities and anaphylaxis with *es*Igs in EA patients. A, "Chicago" plot representing $-\log_{10}(P\text{-value})$ for univariate associations; values above 0 represent positive association and negative association if below 0. B, Multivariable logistic models selected by a stepwise procedure for anaphylaxis (top) and rhinitis (bottom) and their respective ROCs

TABLE 1

Patients' characteristics

	AC (n = 6[a])	EA (n = 38)	*P*-value[b]
Age	10.6 (3.8)	6.6 (3.6)	.016
Male (%)	3 (50.0)	22 (57.9)	.999
Race (%)			
Asian	0 (0.0)	5 (14.7)	.166
Back/African American	1 (20.0)	0 (0.0)	
Other	0 (0.0)	2 (5.9)	
White/Caucasian	4 (80.0)	27 (79.4)	
Not Hispanic or Latino (%)	5 (100.0)	30 (93.8)	.999
Asthma (%)	2 (40.0)	28 (73.7)	.153
Rhinitis (%)	2 (40.0)	22 (57.9)	.64
Eczema (%)	3 (60.0)	29 (76.3)	.589
Anaphylaxis (%)	.	24 (63.2)	
Egg sIgE (kU_A/L)	0.1 [0.1–0.3]	49.9 [22.8–90.5]	<.001

[a] AC group had demographic and medical history data available for five out of six patients.

[b] Continuous variables are presented as mean (SD) or median [1st and 3rd quartile]; categorical variables are reported as frequencies and percentages. Groups were compared using Wilcoxon-Mann-Whitney and Fisher's exact test. AC, atopic controls; and EA, egg allergic.