

Connectivity patterns between multiple allergen specific IgE antibodies and their association with severe asthma

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Background: Allergic sensitization is associated with severe asthma, but assessment of sensitization is not recommended by most guidelines.

Objective: We hypothesized that patterns of IgE responses to multiple allergenic proteins differ between sensitized participants with mild/moderate and severe asthma.

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Methods: IgE to 112 allergenic molecules (components, c-sIgE) was measured using multiplex array among 509 adults and 140 school-age and 131 preschool children with asthma/wheeze from the Unbiased BIOmarkers for the PREdiction of respiratory diseases outcomes cohort, of whom 595 had severe disease. We applied clustering methods to identify co-occurrence patterns of components (component clusters) and patterns of sensitization among participants (sensitization clusters). Network analysis techniques explored the connectivity structure of c-sIgE, and differential network analysis looked for differences in c-sIgE interactions between severe and mild/moderate asthma.

Results: Four sensitization clusters were identified, but with no difference between disease severity groups. Similarly, component clusters were not associated with asthma severity. None of the c-sIgE were identified as associates of severe asthma. The key difference between school children and adults with mild/moderate compared with those with severe asthma was in the network of connections between c-sIgE. Participants with severe asthma had higher connectivity among components, but these connections were weaker. The mild/moderate network had fewer connections, but the connections were stronger. Connectivity between components with no structural homology tended to co-occur among participants with severe asthma. Results were independent from the different sample sizes of mild/moderate and severe groups.

Conclusions: The patterns of interactions between IgE to multiple allergenic proteins are predictors of asthma severity among school children and adults with allergic asthma. (J Allergy Clin Immunol 2020;■■■:■■■-■■■.)

Key words: Asthma, allergic sensitization, cluster, network analysis

Severe asthma affects around 1 in 20 children, adolescents, and adults with asthma, and in these patients the health care resources utilization and mortality are disproportionately higher.¹ Both the diagnosis and treatment of severe asthma are time-consuming and require specialist expertise.² Despite modern pharmacotherapy, including biological agents, there is a significant unmet need in this group of patients.^{3,4}

Many patients with severe asthma have coexisting allergic conditions such as eczema, allergic rhinitis, and food allergy.⁵ Severe asthma is associated with allergic sensitization,⁵⁻⁸ and the US National Institutes of Health expert group recommends a multi-allergen screening as the core biomarker for asthma.⁹ However, the

Abbreviations used

CRD:	Component-resolved diagnostics
c-sIgE:	Component-specific IgE
HDM:	House dust mite
ISU:	ImmunoCAP Immuno-Solid phase Allergy Chip Standardized Units
JDINAC:	Joint density-based nonparametric differential interaction network analysis and classification
SPT:	Skin prick test
U-BIOPRED:	Unbiased BIOMarkers for the PREdiction of respiratory diseases outcomes

assessment of sensitization is not recommended by most clinical guidelines, and its use varies considerably between different countries, specialties, and departments. This is in part because confirmation of sensitization using skin prick tests (SPTs) and specific serum IgE to whole allergen extracts does not prove that patients' symptoms are caused by allergy¹⁰ and in some patients may be a chance finding.¹¹ Furthermore, allergic sensitization may be an agglomerate of several subtypes,^{12,13} some clinically relevant and others not associated with symptomatic disease,¹⁴ which differ in their association with asthma presence and severity.^{15,16} However, identifying clinically relevant sensitization in asthma is challenging,¹⁴ and this limits the value of currently available tests.¹⁷

Some limitations of the tests for evaluating sensitization could potentially be overcome by component-resolved diagnostics (CRD),¹⁸ a method that detects IgE specific to individual allergenic molecules (components; c-sIgE), rather than whole extracts.¹⁹ CRD arrays for multiple components generate complex and rich data sets, which create conditions to application of novel techniques such as machine learning to interrogate the data.²⁰⁻²⁴ These methodologies have identified clusters of c-sIgEs to multiple allergenic proteins associated with high risk of asthma in cross-sectional^{20,21} and longitudinal studies.^{22,23} A recent study investigating the connectivity structure among c-sIgE responses measured by a CRD multiplex array has demonstrated that the key associate of childhood asthma is not the IgE response to any individual molecule but, rather, complex interaction patterns between c-sIgE.²⁴ Of note, the predictive value for asthma of a differential network of pairwise interactions between 18 c-sIgE was high, with the area under the curve of 0.94.²⁴

In this study, we postulate that disambiguation of sensitization using CRD may predict whether sensitized participants with asthma have mild or severe disease, and that this may help the assessment of both childhood and adult severe asthma. Specifically, we hypothesized that connections between IgE responses to multiple allergenic proteins differ among sensitized participants with mild/moderate when compared with those with severe asthma. We also aimed to determine whether these associations differ between children and adults. To address our hypotheses, we measured c-sIgE to 112 allergenic molecules using a multiplex CRD array in serum samples from participants in the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project, which aims to improve our understanding of severe asthma.^{25,26} We used machine learning techniques to explore how different c-sIgEs interact between each other, and to identify sensitization profiles among children and adults with

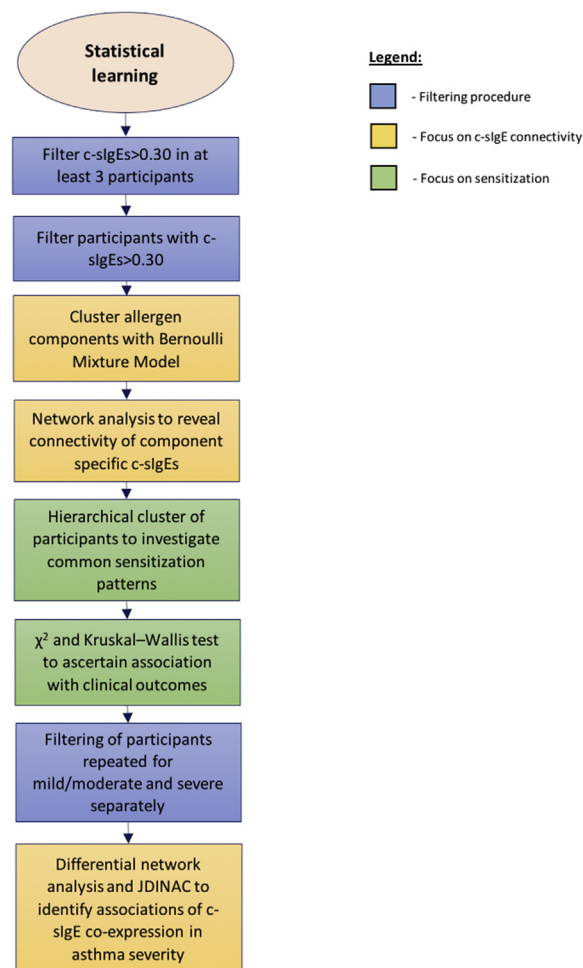


FIG 1. Approach to statistical analysis. The data set was initially restricted to allergen c-sIgE where at least 3 participants showed evidence of sensitization (≥ 0.30) and then to participants with sensitization to at least 1 c-sIgE. Parallel workflows then focused on c-sIgE connectivity and allergen component sensitization patterns.

asthma. Using network analyses, we explored the connectivity structure of c-sIgE in mild/moderate and severe asthma.

METHODS

Study design, setting, and participants

U-BIOPRED is a European multicenter, observational study. The adult cohort was recruited from 16 centers in 11 countries²⁵ and the pediatric cohort from 7 centers in 5 countries.²⁶ The study was approved by the ethics committee for each participating institution and is registered on [ClinicalTrials.gov](https://www.clinicaltrials.gov) (NCT01976767, NCT01982162). All participants and parents/caregivers gave written informed consent.

Three adult patient groups were recruited: (1) severe nonsmoking asthmatic patients, (2) smokers and ex-smokers with severe asthma, and (3) mild/moderate nonsmoking asthmatic patients.²⁵ The 4 pediatric groups were (1) severe school-age asthmatic patients, (2) mild/moderate school-age asthmatic patients, (3) severe preschool wheezers, and (4) mild/moderate preschool wheezers.²⁶ The groups were defined by strict clinical criteria, and both the adult and pediatric participants with severe asthma had poorly controlled disease despite high-dose inhaled corticosteroids and other controller medication, with a proportion requiring maintenance oral corticosteroids.^{25,26}

Data sources/measurements

Full cohort descriptions, inclusion and exclusion criteria, and details of clinical assessment are presented in [Tables E1](#) and [E2](#) in this article's Online Repository at www.jacionline.org. Initial characterization of allergic sensitization was by SPT and/or measurement of serum IgE specific to common allergens, with sensitization defined as a mean wheal diameter greater than or equal to 3 mm or IgE greater than or equal to 0.35 kUA/L, respectively.

Component-resolved diagnostics

We measured c-sIgE to 112 allergenic molecules (components) using ImmunoCAP ISAC (ThermoFisher/Phadia AB, Uppsala, Sweden) where sufficient serum sample was available. The level of c-sIgEs was reported in ImmunoCAP Immuno-Solid phase Allergy Chip Standardized Units (ISU). Depending on the nature of the analysis, we dichotomized c-sIgE using a binary threshold (positive ≥ 0.30 ISU) or used the continuous values.

Statistical learning

The overall approach to analysis is described in [Fig 1](#) (for glossary, see this article's [Online Repository](#) at www.jacionline.org). To account for zero-inflated variables, we analyzed data from participants with at least 1 c-sIgE more than 0.3 ISU and for components with a positive response by at least 3 subjects (described before as active components^{23,24}). Clustering methods (see below) were applied to explore how different c-sIgEs interact (*component clusters* derived by clustering c-sIgEs) and to identify patterns of c-sIgE sensitization among participants (*sensitization clusters* derived by clustering participants). We used chi-square test to evaluate the associations between the identified sensitization and component clusters and asthma severity; all *P* values were adjusted for false-discovery rate. Network analysis techniques were then applied to explore the connectivity structure of c-sIgE, and differential network analysis to ascertain any differences in c-sIgE interactions between severe and mild/moderate asthma. Finally, classification models for asthma severity were investigated.

Statistical grouping of c-sIgEs (component clusters).

Co-occurrence patterns of c-sIgEs were analyzed by grouping the components into homogeneous clusters using Bayesian estimation of a mixture of Bernoulli distributions (Bernoulli Mixture Model)²⁷ (see this article's [Methods](#) section in the Online Repository at www.jacionline.org).

Patterns of sensitization among participants with asthma (sensitization clusters). Participants with similar c-sIgE sensitization patterns were grouped by hierarchical clustering, using the Ward's linkage algorithm on the Jaccard distance matrix computed between binary responses to c-sIgE profiles (see this article's [Methods](#) section in the Online Repository). To determine the number of clusters, dendrogram plots were evaluated and suggested solutions assessed using a bootstrap approach.²⁸

c-sIgE connectivity patterns. To investigate the connectivity structure of c-sIgEs, we used a weighted co-occurrence network analysis (see this article's [Methods](#) section in the Online Repository). Nodes in the network represent c-sIgEs, and pairs of c-sIgEs simultaneously present in at least 1 participant are connected by an edge. Edges are weighted by the co-occurrence frequency of the component pairs. To compare connectivity structures between adults and children, we considered network size and density. To assess the importance and position of each c-sIgE (ie, node) in the network, we used 3 centrality indices, namely, strength, closeness, and betweenness.

Difference in c-sIgE interactions between severe and mild/moderate asthma. We compared network structure between nonsmoking adults with severe and mild/moderate asthma; in children, we focused on school-age asthma. To reduce the noise due to c-sIgEs with no positive response, c-sIgEs with fewer than 3 positive responses were filtered out in the mild/moderate and severe asthma groups separately,²³ and we used those c-sIgEs present in both groups.

The networks were built by connecting c-sIgEs whose pairwise expression similarity, based on Spearman correlation coefficient, was statistically significant ($P < .05$). Centrality measures were used to compare the networks and the role of specific components.²¹ To investigate whether there are differences in

c-sIgE connections between severe and mild/moderate asthma, we applied differential correlation analysis (see this article's [Methods](#) section in the Online Repository), which computes the correlations in each severity group and uses the difference in z-transformed correlation coefficients to assess statistical significance.²⁹ We assessed the stability of the inferred network using a bootstrap approach. We performed sensitivity analyses to evaluate comparability of the mild/moderate and severe networks and assess the impact of the different sample sizes (see this article's [Methods](#) section in the Online Repository).

Classification model for severe asthma

We evaluated the ability of c-sIgE pairwise interactions to classify asthma severity by applying the joint density-based nonparametric differential interaction network analysis and classification (JDINAC; see this article's [Methods](#) section in the Online Repository).³⁰ This model identifies differential interaction patterns of network activation of c-sIgEs that are most closely related to asthma severity, and builds a classification model using the network biomarkers. To ascertain whether c-sIgE pairwise interactions are more informative than individual components to classify asthma severity, we compared the performances of JDINAC with penalized logistic regression. To ensure robustness of the results, we ran both models with 10-fold cross-validation in 20 independent repetitions. In this analysis, sIgEs values were log-transformed ($\log(x + 1)$). To reduce the effect of imbalanced data, we included class weights in both models.

All statistical analyses were run in the programming language R. Allergen components clusters were estimated using packages *BayesBinMix*²⁷ and *label.switching*.³¹ We used *fpc*³² for computing the number of clusters through bootstrap procedure, and *igraph*³³ for network visualizations. Differential correlation analysis was carried out through package *DGCA*.²⁹ JDINAC scripts were made available by the authors at <https://github.com/jijiadong/JDINAC>.

RESULTS

Participants

The study population consisted of 780 participants (509 adults, 140 school-age and 131 preschool children). Of these, 595 had severe asthma/whoeze, with the rest having mild/moderate disease. Characteristics of the study population are presented in [Table I](#).

Allergic sensitization (both SPT and IgE) was highly prevalent across the 4 cohorts. The proportion of sensitized participants was similar in the severe and mild/moderate groups ([Table I](#)), with the exception of the adult smoking and ex-smoking severe asthma groups in which fewer participants were sensitized.

Allergic sensitization to allergen components

CRD data were available for 491 of 509 (96.5%) adults, and for 238 of 271 (87.8%) children ([Fig 2](#)). The percentage of participants with at least 1 positive c-sIgE in the adult, school-age, and preschool cohorts was 50.7% (249 of 491), 82.7% (105 of 127), and 27.1% (30 of 111), respectively ([Table E1](#)). The number of active components was similar among adults and school-age children ($n = 58$ and $n = 64$, respectively), and was significantly lower in the preschool group ($n = 13$; see [Table E3](#) in this article's Online Repository at www.jacionline.org). The proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU), or high (>15 ISU) sensitization for each component is shown in [Fig E1](#) in this article's Online Repository at www.jacionline.org.

There were no obvious differences in c-sIgE to individual components across different asthma severity groups in adults and school-age children, with a high prevalence of positive responses to house dust mite (HDM) and grass components ([Fig 3](#)). In

TABLE I. Characteristics of participants

Characteristic	Adult asthma cohorts			School-age asthma cohorts		Preschool wheeze cohorts	
	Mild/moderate	Severe nonsmoker	Severe smokers	Mild/moderate	Severe	Mild/moderate	Severe
Participants number	88	311	110	43	97	54	77
Sex: female	44 (50.0)	205 (65.9)	56 (50.9)	16 (37.2)	46 (47.4)	20 (37.0)	27 (35.1)
Age (y)	41.66 ± 1.65	51.01 ± 0.8	54.51 ± 1.08	11.26 ± 0.48	12.21 ± 0.31	3.46 ± 0.16	3.56 ± 0.14
White ethnicity	82 (93.2)	277 (89.1)	105 (95.5)	32 (74.4)	74 (76.3)	49 (89.9)	62 (80.5)
Smoking history (pack-years)	4 (1, 4) (n = 13)	2 (1, 4) (n = 47)	17.38 (10, 26) (n = 110)	0 (0, 0) (n = 0)	0 (0, 0) (n = 0)	0 (0, 0) (n = 0)	0 (0, 0) (n = 0)
BMI (kg/m ²)	25.73 ± 0.47	29.11 ± 0.36	29.59 ± 0.6	19.21 ± 0.5	21.52 ± 0.5	15.99 ± 0.15	16.56 ± 0.25
Age at diagnosis (y)	14 (6, 32)	20 (7, 38)	38 (20, 48)	2 (2, 5)	2 (1, 4)	1 (1, 2)	2 (1, 2)
Asthma control	1.01 ± 0.07	2.67 ± 0.08	2.62 ± 0.12	20.25 ± 0.81	15.49 ± 0.63	23 ± 0.67	15.2 ± 0.79
Asthma quality of life	5.84 ± 0.1	4.48 ± 0.07	4.44 ± 0.13	5.8 ± 0.19	4.77 ± 0.15	6.04 ± 0.18	4.27 ± 0.18
Exacerbations in previous year	2 (1, 3)	2 (1, 4)	0 (0, 1)	1 (0, 2)	3 (2, 5)	2 (0, 3)	4 (1, 6)
Inhaled corticosteroids	87 of 88 (98.9%)	310 of 311 (99.7%)	110 of 110 (100%)	43 of 43 (100.0%)	97 of 97 (100.0%)	24 of 54 (44.4%)	75 of 77 (97.4%)
Oral corticosteroids	0 of 88 (0%)	135 of 295 (45.8%)	46 of 103 (44.7%)	0 of 43 (0.0%)	23 of 97 (23.7%)	0 of 54 (0.0%)	4 of 77 (5.2%)
Any LABA	2 of 88 (2.3%)	305 of 309 (98.7%)	109 of 110 (99.1%)	25 of 43 (58.1%)	94 of 97 (96.9%)	0 of 54 (0.0%)	34 of 77 (44.2%)
FEV ₁ % predicted	89.5 ± 1.86 (n = 87)	67.5 ± 1.26 (n = 308)	67.2 ± 1.84 (n = 110)	93.51 ± 2.47 (n = 42)	88.68 ± 2.15 (n = 96)	99.23 ± 5.29 (n = 10)	104.34 ± 3.21 (n = 19)
Fraction of exhaled nitric oxide (ppb)	25 (18-54) (n = 87)	26.5 (16-47) (n = 290)	23.5 (12-42) (n = 104)	35.84 (14-41) (n = 38)	33.5 (15.4-62.2) (n = 92)	—	—
Coexisting disease							
Diagnosed eczema	25 of 87 (28.7)	107 of 308 (34.7)	31 of 108 (28.7)	28 of 43 (65.1)	77 of 97 (79.4)	32 of 54 (59.3)	42 of 74 (56.8)
Diagnosed allergic rhinitis	42 of 82 (51.2)	164 of 291 (56.4)	44 of 108 (40.7)	29 of 41 (70.7)	61 of 95 (64.2)	11 of 51 (21.6)	22 of 69 (31.9)
Diagnosed hay fever	46 of 85 (54.1)	135 of 298 (45.3)	51 of 107 (47.7)	33 of 43 (76.7)	75 of 93 (80.7)	13 of 50 (26.0)	30 of 68 (44.1)
Possible food allergy	5 of 86 (5.8)	25 of 286 (8.7)	3 of 105 (2.9)	12 of 41 (29.3)	28 of 92 (30.4)	8 of 48 (16.7)	7 of 74 (9.5)
Highly likely food allergy	1 of 86 (1.2)	8 of 286 (2.8)	2 of 105 (1.9)	8 of 41 (19.5)	15 of 92 (16.3)	2 of 48 (4.2)	2 of 74 (2.7)
Total IgE (kU IgE/L)	23 (9.66)	120 (45.348)	126 (57.350)	615 (240.985)	560 (120.1606)	74 (16.195)	71 (30.209)
Allergic sensitization	78 of 87 (89.7)	231 of 287 (80.5)	66 of 99 (66.6)	35 of 39 (89.7)	79 of 89 (88.8)	20 of 48 (41.7)	27 of 63 (42.9)
Median number of sensitizations (range)	1 (0-6)	3 (0-6)	1 (0-6)	4 (0-6)	4 (0-6)	0 (0-6)	0 (0-5)
Specific allergic sensitization:							
Tree pollen mixture	33 of 67 (49.6)	90 of 208 (43.3)	28 of 82 (34.1)	22 of 38 (57.9)	46 of 85 (54.1)	7 of 50 (14.0)	9 of 65 (13.9)
Grass pollen mixture	56 of 81 (69.1)	119 of 260 (45.8)	32 of 92 (34.8)	30 of 40 (75.0)	57 of 90 (63.3)	10 of 50 (20.0)	9 of 69 (13.0)
Domestic dog	47 of 76 (61.8)	98 of 251 (39.0)	27 of 89 (30.3)	23 of 39 (59.0)	55 of 88 (62.5)	15 of 52 (28.9)	8 of 67 (11.9)
Domestic cat	54 of 81 (66.7)	115 of 260 (44.2)	25 of 86 (29.1)	24 of 39 (61.5)	58 of 87 (66.7)	15 of 52 (28.9)	11 of 69 (15.9)
<i>Dermatophagoides pteronyssinus</i> or <i>D farinae</i>	53 of 79 (67.1)	143 of 274 (52.2)	41 of 96 (42.7)	28 of 40 (70.0)	59 of 90 (65.6)	8 of 52 (15.4)	21 of 67 (31.3)
<i>Aspergillus fumigatus</i>	24 of 73 (32.9)	68 of 242 (28.1)	29 of 88 (33.0)	16 of 38 (42.1)	37 of 87 (42.5)	4 of 48 (8.3)	5 of 63 (7.9)

Values represent n/N (%), mean (±SE) or median (25th, 75th centiles) for each cohort. Where relevant, number (n) of observations presented. Severe nonsmoking participants were nonsmokers for at least the past 12 months and have a pack history of ≤5 pack-years (pack-years = number cigarettes smoked/day × number of years smoked/20). Asthma control assessed using ACQ7 (≥18 y), ACT (>12 y), or CACT (≤12 y). Asthma-related quality of life assessed using the asthma quality of life questionnaire (AQLQ), the paediatric asthma quality of life questionnaire (PAQLQ), or the paediatric asthma caregiver quality of life questionnaire (PACQLQ) according to age. Eczema, allergic rhinitis, and hay fever diagnosed by a doctor reported by participants or parents (updated since publication of baseline articles^{18,19}). Possible food allergy defined as symptoms of food allergy plus evidence of sensitization. Symptoms of food allergy refer to symptoms of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness, or wheeze within 2 hours of contact with food. Highly likely food allergy defined as symptoms of food allergy plus a ≥5-mm SPT wheal or a specific IgE level ≥10.0 kU/L. Allergic sensitization data updated since publication of baseline articles^{4,5} with serum specific IgE assays run on frozen samples where data were previously missing. Sensitization defined as positive specific IgE (≥0.35 kU/L) or positive SPT result (≥3 mm). Atopy defined as any positive specific IgE (≥0.35 kU/L) or SPT result (≥3 mm).

preschool children, positive c-sIgEs were concentrated around the HDM components. The number of positive c-sIgEs did not vary with asthma severity in either adults or children (Table E3).

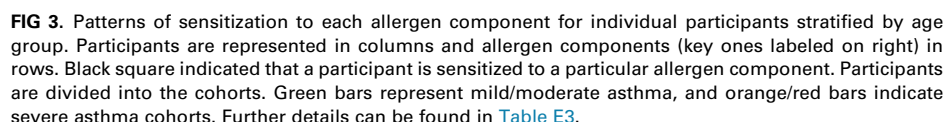
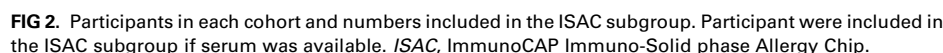
Statistical grouping of allergen components: Component clusters

The Bernoulli Mixture Model inferred 3 component clusters in adults, 5 in school-age, and 2 in preschool children (Fig 4). Cluster membership mapped mostly to the structural homology of proteins and/or their biological sources. For each age group, the algorithm revealed a “Broad” cluster, with sensitization to components from multiple sources, and “HDM” cluster, consisting of 4 HDM components (Der p 1 and Der p 2, Der f1, and Der f 2). In addition, a “Grass” cluster emerged in adults, consisting of 2 components (Phl p 1 and Phl p 5). In the school-age children, 3 additional clusters were found: “Grass/cat,” “Animal/pollen,” and “Class-10 Pathogenesis-related proteins (PR-10)” cluster. There was no significant association between cluster membership, or sensitization to any individual component, and asthma severity (see Table E4 in this article’s Online Repository at www.jacionline.org).

Patterns of sensitization among participants with asthma: Sensitization clusters

The optimal solution for sensitization profiles among study participants identified 4 clusters (see Fig E2 in this article’s Online Repository at www.jacionline.org) in adults and school-age children and 2 in preschool age (Fig 5). On the basis of the profile of components to which sensitization occurred, we qualitatively labeled clusters in adults as multiple (c-sIgE to multiple components across all component clusters), predominantly HDM (c-sIgE mainly to HDM components), predominantly grass pollen (c-sIgE to multiple grass components), and lower-grade sensitization (sensitization to a few of a broad range of components). The same clusters were found in school-age children. In preschool children, we observed only multiple and HDM clusters.

No significant association was found between cluster membership and asthma severity, or allergic comorbidities (see Table E5 in this article’s Online Repository at www.jacionline.org). Among adults, participants in multiple and predominantly HDM clusters had significantly earlier age of onset of symptoms and diagnosis, and those in the predominantly grass cluster had better lung function (significantly higher FEV₁ and FEV₁/forced vital capacity); there was no significant association between



Repository at www.jacionline.org). Comparison of centrality measures revealed similar connectivity and role of components in the adult and school-age networks (see Fig E3 in this article's Online Repository at www.jacionline.org). IgE to HDM components, Phl p 1, and Fel d 1 showed similar behavior, and were among the 10 most important components in the network (see Table E8 in this article's Online Repository at www.jacionline.org). Network centrality measures showed that peanut-related components were more important and connected with a broader range of components in school-age children, indicating that a larger number of participants in this cohort were cosensitized to

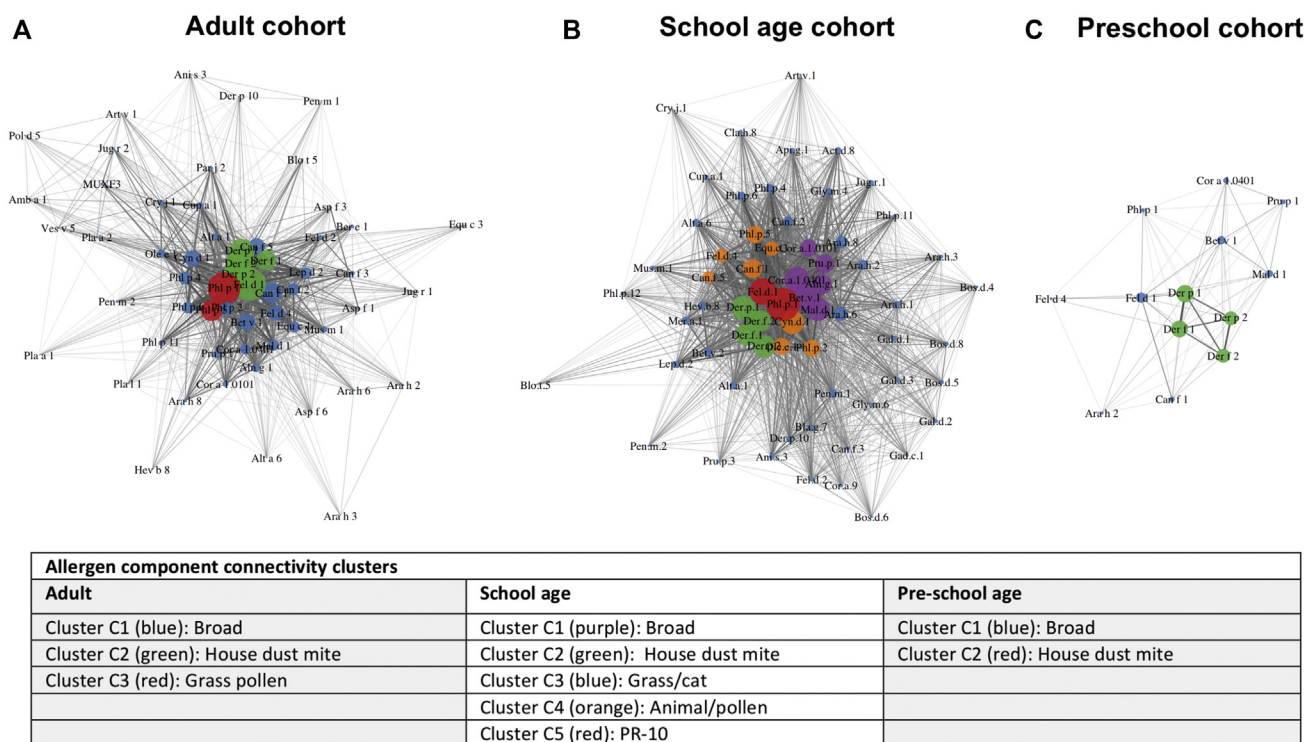


FIG 4. Bernoulli Mixture Model and Co-occurrence network analysis reveal connectivity structure between allergen components. Colors (see table) represent allergen component cluster memberships, node diameter is proportional to the node relative strength, while edge (line) width is proportional to the number of participants sensitized to both components. Participants with no sensitization to allergen components were not included in this step.

peanut-related components and other c-sIgEs. Grass pollen components were more important in the adult network (Fig E3), whereas HDM components and Fel d 1 shared many connections also in the preschool age group (see Fig E4 in this article's Online Repository at www.jacionline.org).

Differential sIgE connectivity structure in mild and severe asthma

After filtering, 32 active c-sIgE components were used in the analysis among nonsmoking adults with severe and mild/moderate asthma, and 41 in school-age children. Table E9 in this article's Online Repository at www.jacionline.org lists components that were removed. Fig 6 shows the connectivity structure stratified by asthma severity in adults and children. There was a markedly different connectivity structure between the severe and mild/moderate asthma networks, with similar patterns of associations in adults and children. Severe asthma networks were characterized by a high number of connections among c-sIgEs, many of which, however, were weak (low values for the Spearman correlation coefficients). In contrast, mild/moderate networks had fewer but stronger connections (Fig 6; see Fig E5 and Table E10 in this article's Online Repository at www.jacionline.org). In mild/moderate networks, we observed the tendency of c-sIgEs to components with structural homology to connect, whereas in severe networks, c-sIgEs interacted with a broader range of components.

The stability analysis (see Fig E6 in this article's Online Repository at www.jacionline.org) showed that connections were very

stable for high-edge weights. Slightly wider error bars were registered for moderate weights, whereas high variability around the bootstrap estimates was observed for low weights, making these connections unstable and difficult to interpret. In the inferred network, these weak connections were filtered out by the thresholding procedure on the basis of significance of the correlation coefficients. The sensitivity analysis excluded the effect of the different sample sizes on the findings (see Fig E7 in this article's Online Repository at www.jacionline.org). Both network structure and density measures suggested that more connections among c-sIgEs were established within the severe group, even when considering samples of reduced size (Figs E6 and E7, C and D).

We proceeded to investigate differences in the connectivity structure between mild/moderate and severe asthma using differential correlation analysis between all pairs of c-sIgEs. There was a significant difference in the connectivity structure between the severe and mild/moderate networks. In adults, 13 c-sIgEs showed significant differential correlations; in school-age children, significant differences were found among 12 c-sIgEs (Fig 7). Connections among animal components showed higher correlations in severe asthma in both adults and children, and connections between peanut allergens characterized mild/moderate asthma. Connections among remaining components also differed, with results suggesting that connections between pairs of components with no structural homology tend to occur in the severe group. The sensitivity analysis showed that results were independent from the different sample sizes of the mild/moderate and severe groups. The pairwise differential c-sIgEs connections were

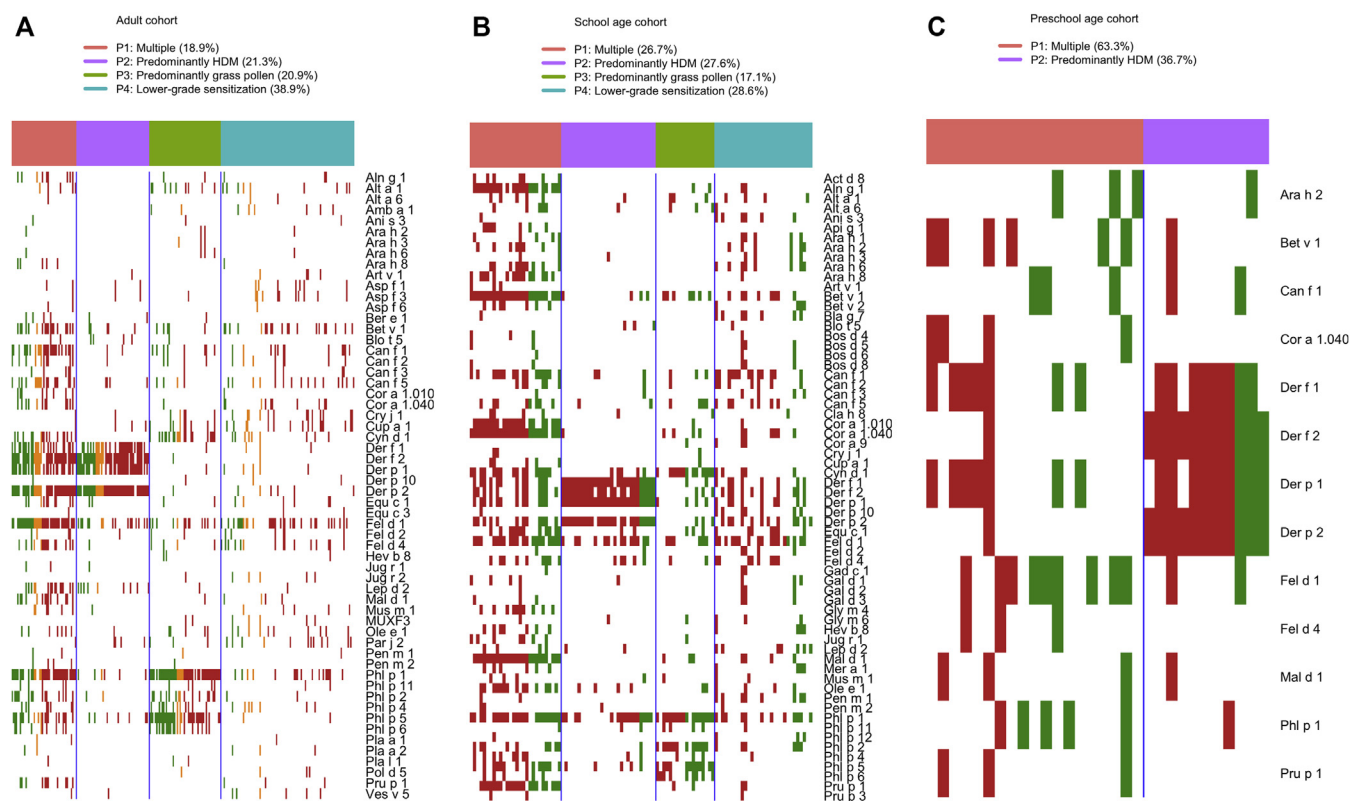


FIG 5. Patterns of IgE responses to allergen components for individual participants within clusters. Rows represent sIgEs, while columns indicate participants. Column colors represent sensitization clusters' membership (participants with no sensitization to allergen components not shown in this and subsequent figures). A colored square represents a c-sIgE greater than 0.3 and colors represent asthma severity.

confirmed by the bootstrap analysis (see Table E11 in this article's Online Repository at www.jacionline.org).

To investigate whether individual c-sIgEs or their pairwise interactions were better discriminators between mild/moderate and severe asthma, we compared the performances of penalized logistic regression and JDINAC. JDINAC was superior in classifying asthma severity in both adults and children (area under the curve 0.95 vs 0.54; and 0.93 vs 0.53, JDINAC and penalized logistic regression, respectively). Both models had high sensitivity and were able to correctly classify the severe group, but had limits in classifying the mild/moderate group (see Table E12 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Our results suggest that among adults and school-age children with allergic asthma, key differences in sensitization between participants with mild/moderate and those with severe disease is different connectivity and interaction between allergen component-specific IgEs. Severe asthma is characterized by higher connectivity among components (ie, higher number of co-occurrences of sensitization between any 2 components) but these connections were weaker than those in mild/moderate asthma. In contrast, in mild/moderate asthma, there are fewer connections between components, but the individual connections are stronger. Connectivity between components with no structural homology tends to co-occur among participants with severe asthma, whereas in those with mild/moderate asthma there is a

tendency of IgE to structurally homologous components to connect. The classification results suggest that global connectivity is much more important than individual component-specific IgE in predicting asthma severity.

Our study provided an opportunity to compare sensitization among patients with asthma across the life-course. Although the prevalence of CRD sensitization was lower in adults with asthma when compared with that among school-age children, among sensitized participants there were striking similarities in the structure of data between adults and school children, with a similar number of active components (58 and 64, respectively), and 4 identical sensitization clusters. The number of active components in preschool children was lower (only 13), with considerably fewer participants being sensitized. Severe wheeze in the preschool cohort was associated with the HDM cluster, but given the small number of preschool children who were sensitized, it is difficult to make definitive conclusions about this age group. This and other studies suggest that specific sensitization patterns in preschool children may be associated with severe wheezing and lung function decline,^{34,35} but whether this can be used as a marker of future risk can be answered only through a long-term follow-up of larger cohorts. Our results suggest that among sensitized individuals, there are considerable similarities between adult and school-age severe asthma and that severe asthma in school-age children is more similar to severe asthma in adults than to troublesome preschool wheezing.

In this study, in both adults and school-age children, there was no relationship between asthma severity and IgE responses to

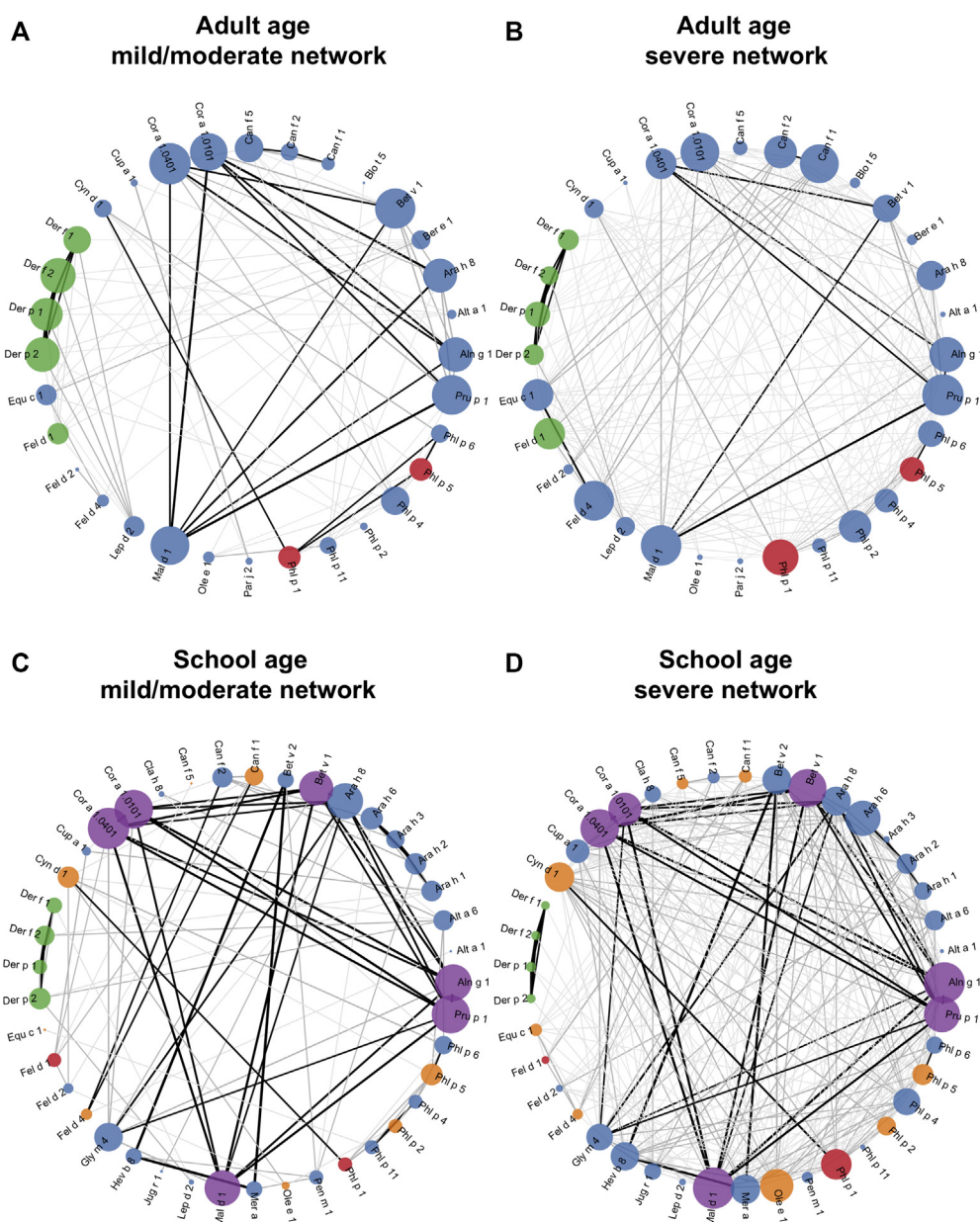


FIG 6. Correlation network for allergen component sensitization in mild/moderate vs severe asthma in the (A and B) adult and (C and D) school-age cohorts. Connections are based on Spearman rank-correlation coefficient (r). Pairs of c-sIgE components are connected if their correlation is significant ($P < .05$). Node colors represent allergen component cluster memberships (as per Fig 3), node diameter is proportional to the scaled connectivity of the specific component, while edge width represents the strength of correlation between pairs of c-sIgE components, while colors capture the differential connectivity. Different shades of gray indicate strength of associations: black edges (line) indicate $r > 0.8$, dark gray $0.6 < r \leq 0.8$, gray $0.4 < r \leq 0.6$, and light gray $r \leq 0.4$.

whole allergen extracts in standard allergy tests (SPT or serum IgE), or any single allergenic molecule tested using CRD. Previous studies have reported that severe asthma was associated with multisensitization to animal component,^{36,37} and that children with asthma respond to more components than do those who do not have asthma.²⁴ By carrying out a thorough assessment of component-specific sensitization using multiplex array in a large number of participants, we have shown no major difference in the number of positive c-sIgEs between mild/moderate and severe asthma, but an increase in the strength of the connections

(measured by the Spearman correlation coefficient) between proteins from furry animals in severe asthma.

We described 4 sensitization clusters (multiple, predominantly HDM, predominantly grasses, and lower-grade sensitization) among participants with asthma. We have previously reported almost identical clusters in a population-based birth cohort (ie, in general population) and have shown that the multiple sensitization cluster was strongly associated with asthma diagnosis.²⁴ In the current study, these sensitization clusters were seen in both severe and mild/moderate asthma, with no difference between the

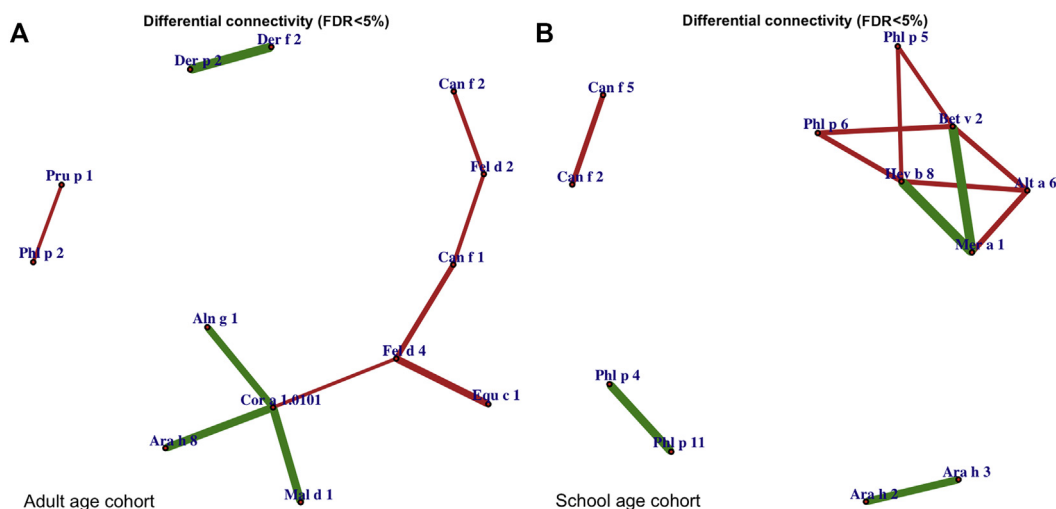


FIG 7. Differential correlation network analysis for allergen component sensitization between severe and mild/moderate asthma in (A) adult and (B) school-age cohorts. Only significant changes in correlation between the mild/moderate and severe asthma are shown. Significance is assessed by controlling the false-discovery rate (FDR) at 5%. Edge (line) colors indicate correlation gain (difference in correlation) in the conditions: green indicates higher absolute correlation value in mild/moderate asthma, while red indicates higher absolute correlation in severe asthma.

severity groups. Similarly, allergen component clusters that we describe here (broad, HDM, grass/cat, PR-10 etc) are comparable to those uncovered in the previous population-level analysis.²³ These component clusters are biologically plausible and likely reflect the sources and structural homogeneity of components within protein families.²¹ However, in contrast to our previous population-based studies in which sensitization to broad component clusters was associated with asthma diagnosis^{21,24} and sensitization to grass/cat cluster predicted asthma development,²³ in the current study of participants with asthma, component clusters were not associated with disease severity. It is reassuring that similar sensitization clusters and allergen component clusters were discovered using data-driven techniques in 2 different types of studies with different biases (population-based and study of participants with asthma), thereby allowing triangulation of findings.^{38,39} Taken together, the results suggest that mechanisms responsible for asthma development differ from those that impact upon disease severity,¹¹ and that specific sensitization patterns that are associated with asthma diagnosis²² and persistence²¹ are not informative about severity.

Severe asthma was characterized by higher connectivity among c-sIgEs, but the connections were weaker; participants with mild/moderate asthma had fewer connections, but connections were stronger. Furthermore, connections between pairs of components with no structural homology tend to occur in severe asthma, whereas in mild/moderate asthma the connectivity was mainly between structurally homologous components. It is tempting to speculate on potential mechanisms that may underpin these observations. Recent data-driven analysis of multiple IFN-related, proinflammatory, and regulatory cytokines induced by rhinovirus described 6 clusters of antiviral responses, 1 of which (characterized by a weak IFN and strong proinflammatory response) was linked with early-onset severe asthma with multiple early allergic sensitization.⁴⁰ IgE in these participants may be produced locally in the airways without cognate T-cell help, to a wide range of structurally unrelated proteins,⁴⁰ resulting in a sensitization pattern characterized by a high number of

connections that are however weak. In participants with mild/moderate asthma, the main pathway to sensitization is via the adaptive T_H2 immunity and allergen exposure, and sensitization is characterized by strong connectivity to structurally homologous proteins from similar sources. This links features of poor respiratory outcomes (impaired antiviral immunity,⁴⁰ severe exacerbations,³⁵ low lung function trajectory,³⁴ and multiple early sensitization^{14,15,19,32,37}) with the severe asthma network of IgE connectivity described here.

One limitation of our study is that some potentially important allergens were not included on the chip that we used, and it is possible that our analyses would provide different solutions if additional components were available. For example, there are relatively few fungal components, which may be important in severe asthma.^{6,7} Our filtering process may have excluded some informative allergens but because zero-inflated variables can reduce the accuracy of cluster analyses and reliability of prediction models, this was a necessary step to account for the effect of measurement errors and noise.

Another limitation is the sparseness of data, and relatively small sample size in preschool children. It is therefore difficult to infer results to the general context and our findings require external validation. However, the number of participants with severe asthma is small and U-BIOPRED is one of largest studies with a focus on severe disease. To our knowledge, there are no other studies with meticulously defined mild/moderate and severe asthma in which CRD data are available. Of note, we demonstrated limited (internal) replication between the school-age and adult cohorts recruited from several European countries and assessed using identical protocols, strengthening our conclusions. It would be helpful to also demonstrate external replication.

We identified only pairwise interactions between component-specific IgEs, and the relation between asthma severity and IgE connectivity structure may be more complex. We suggest that higher-order interactions should be investigated in the future, but such analyses are currently too computationally demanding.

In conclusion, the pattern of connectivity and interactions between IgE to multiple allergenic proteins is a potentially important biomarker of asthma severity among school-age and adult patients with allergic asthma. This study provides new directions for the assessment and interpretation of sensitization in asthma and may help design more reliable diagnostic tools to differentiate clinically important sensitization patterns associated with asthma severity.

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Key messages

- Severe asthma is characterized by higher connectivity among allergen components but these connections are weak, whereas mild/moderate asthma is characterized by fewer but stronger connections.
- It may be possible to develop algorithms to apply to allergen component sensitization data to identify clinically important allergic sensitization patterns that predict asthma severity.

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Connectivity patterns between multiple allergen specific IgE antibodies and their association with severe asthma

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Supplementary materials

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METHODS

Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project is a European Union consortium of academic institutions, pharmaceutical companies and patient organizations.

Clinical assessment

Baseline data included demographics plus detailed asthma and atopic disease histories. Asthma control was assessed using the Asthma Control Test (ACT) (for adults, children ≥ 12 years) [23] or Childhood Asthma Control Test (cACT) (children < 12 years) [24]. Non-scheduled healthcare utilisation was assessed using exacerbations. QoL was assessed using the Asthma Quality of life Questionnaire (adult)[25], Paediatric Asthma Quality of Life Questionnaire (PAQLQ) (school-aged children)[26] and the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ)[27]. Spirometry [28,29] and fraction of exhaled nitric oxide level (FeNO)[30] were performed.

Mild/moderate school-aged asthma cohorts had controlled, or partly-controlled asthma treated with low-dose inhaled corticosteroids (ICS).

Assessment of allergic sensitisation

Skin prick testing

Skin prick testing was performed to common allergens using single headed lancet and positive (histamine 10mg/ml) and negative (saline) controls (ALK-Abello, Horshølm, Denmark). All sites tested *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, domestic cat, domestic dog, grass pollen mixture, tree pollen mixture and *Aspergillus fumigatus*. Where relevant to a specific centre, up to three other allergens, such as German cockroach, *Olea europea* and Parietaria, were additionally included. A positive skin prick test was defined as a wheal ≥ 3 mm with appropriate control results.

Serum specific IgE assessment

Total IgE and specific IgE tests to the six common aeroallergens (*Dermatophagoides pteronyssinus*, domestic cat, domestic dog, grass pollen mixture, tree pollen mixture and *Aspergillus fumigatus*) were measured (Thermo Fisher, Uppsala, Sweden). Allergic sensitisation was defined as ≥ 0.35 kUA/l.

Bernoulli Mixture Model-BMM

We inferred the model parameters, cluster membership and number of clusters using an allocation sampler with an unknown number of mixture components (representing clusters in our terminology). This sampler was embedded in a Metropolis-coupled Markov chain Monte Carlo (MCMC). Identifiability issues due to the label-switching problem were addressed by post-processing the MCMC draws.

The BayesBinMix¹ R package offers a Bayesian framework for clustering binary data by fitting a Bernoulli mixture model (BMM) with an unknown number of clusters. The package allows the joint estimation of the number of clusters and model parameters using Markov chain Monte Carlo sampling, run with parallel, heated chains to accelerate convergence. BayesBinMix also addresses identifiability issues by implementing label switching algorithms.

The observed likelihood for a binary data matrix $\mathbf{x}$ under the Bernoulli mixture model is given by:

$$L_K(\mathbf{p}, \boldsymbol{\theta}; \mathbf{x}) = \prod_{i=1}^n \sum_{k=1}^K p_k \prod_{j=1}^d \theta_{kj}^{x_{ij}} (1 - \theta_{kj})^{1-x_{ij}}$$

where n is the sample size, d is the number of components and K is the number of clusters. θ_{jk} ranges between 0 and 1 and represents the frequency of sensitisation to component j , $j=1,\dots,d$, for subjects in cluster k , with $k=1,\dots,K$, and p_k represents the weight of cluster k which is the prior probability that a subject belongs to that cluster.

The package authors' recommendations for parameters and implementation were followed. This included a Poisson prior distribution for the number of clusters; a uniform distribution for the Bernoulli parameters; and running the sampler with eight heated chains for 2500 MCMC cycles. Default values of 1 for each cluster were used for the Dirichlet prior of the mixture weights, as well as 0.2 for the probability of ejecting empty clusters.

Hierarchical clustering

We investigated common patterns of sensitization using hierarchical clustering (HC), which transforms a distance matrix into a nested series of partitions that can be represented through a treelike graph (dendrogram). By exploring this graph, one can obtain useful information on the hierarchy of the clusters and their similarities. At the lowest level of the hierarchy, each cluster contains a single observation. At the highest level, there is only one cluster containing all of the data. HC algorithms can follow an agglomerative or a divisive approach. Agglomerative strategies start at the bottom and at each level recursively merge a selected pair of clusters into a single cluster. This produces a grouping at the next higher level with one fewer cluster. The pair chosen for merging consist of the two groups with the smallest intergroup dissimilarity. Divisive methods start at the top and at each level recursively split one of the existing clusters at that level into two new clusters. The split is chosen to produce two new groups with the largest between-group dissimilarity. With both paradigms there are $N-1$ levels in the hierarchy. In our analysis, we used the agglomerative procedure combined with the Ward's linkage method, which joins the clusters so that the total within-cluster variance is minimized.

Compared with partitional clustering, HC techniques do not require one to fix the number of clusters a priori, can find different levels of similarity between the sIgE components within the hierarchy of clusters, and, hence, can highlight different patterns of connectivity and biological properties.

Statistical/machine learning and Network Analysis notions and definitions

	Term	Definition
STATISTICAL LEARNING	<i>Feature/predictors</i>	The measurements which represent the data. A feature can be numerical or categorical and it used as input to statistical models.
	<i>Outcome/response</i>	A numerical or categorical variable to predict from features
	<i>Model</i>	a formal representation for a class of processes that allows a means of analysing and summarizing a set of data, for description or prediction\classification.
	<i>Supervised learning</i>	Statistical and Machine Learning models applied to map input to a designated dependent attribute. It is called “supervised” because of the presence of the outcome variable to guide the learning process.
	<i>Unsupervised learning</i>	Statistical and Machine Learning techniques used to explore and infer the inherent structure of our data without using designated outcomes. Only the independent features are observed.
	<i>Algorithm</i>	A strict and logical set of instructions to solve a class of problems.
	<i>Clustering</i>	An unsupervised learning method used to discover the inherent groupings in the data.
	<i>Hierarchical clustering</i>	It is a clustering method that produce hierarchical representations in which the clusters at each level of the hierarchy are created by merging clusters at the next lower level.
	<i>Supervised Classification</i>	It concerns the problem of predict a qualitative outcome starting from a set of features. The model is learnt on the basis of training set of data and it is then validated on a test set.
	<i>Training set</i>	A collection of data containing observations whose category membership is known and used to train a learning algorithm
	<i>K-fold cross-validation</i>	It is a statistical model validation technique that assesses the accuracy of the learning model and how its results will generalize to an independent data set. K-fold cross-validation uses part of the available data to fit the model, and a different part to test it. It does so by partitioning the dataset into complementary subsets, called the folds and by iteratively training the model on one of the subsets (acting as training set) and validating it on the other.
	<i>Accuracy</i>	The proportion of correctly classified observations by the statistical model.
	<i>Sensitivity</i>	The proportion of true positives that are correctly identified by the classification model.
	<i>Specificity</i>	The proportion of true negatives that are correctly identified by the classification model.
	<i>Area under the ROC curve (AUC)</i>	It provides an aggregate measure of performance across all possible classification thresholds.
	<i>Kernel Density Estimation</i>	A nonparametric density estimation technique. The probability density function is obtained by centering a kernel function on each data point and then adding the functions together. Critical is the choice of the kernel function.

NETWORK ANALYSIS	<i>Graph</i>	It provides a visual representation of a network. It consists of: • a set, V, of vertices (nodes) • a collection, E, of pairs of vertices from V called edges/connections. An edge is represented by a line connecting the two vertices. Two vertices are said to be <i>adjacent</i> or <i>neighbours</i> if they are joined by an edge, and an edge is said to be <i>incident</i> to the nodes it joins.
	<i>Undirected network</i>	A network in which a neighbouring relation is valid for both directions and nodes are treated interchangeably
	<i>Directed network</i>	A network in which the orientation is important: the pairs of nodes are ordered. Directed edges can be represented graphically as an arrow drawn between the nodes.
	<i>Weighted network</i>	A network in which each edge has a numerical weight attached to it, indicating the strength of the connection.
	<i>Size</i>	It is given by the total number of connections in the network (number of edges)
	<i>Density</i>	It is the ratio of the number of edges to the number of possible edges in a network.
	<i>Centrality measures</i>	They allow to identify the most prominent nodes within the network.
	<i>Degree of a node</i>	The number of edges connected to the node.
	<i>Strength of a node</i>	The sum of the absolute value of the node's connections with other nodes in the network
	<i>Closeness of a node</i>	It measures the mean distance from a vertex to other vertices. The more central a node is, the closer it is to all other nodes.
	<i>Betweenness of a node</i>	It quantifies the number of times a node acts as a bridge along the shortest path between two other nodes.
	<i>Differential Network Analysis</i>	It compares individual networks inferred from different populations, or groups, to identify group-specific connections/interactions. Differences in the structure of the two networks may denote differences in the underlying biological mechanisms.
TERMS in c-sIgE NETWORK	<i>Connectivity structure</i>	It describes the network configuration. Connectedness in a network describes the way in which network entities/nodes are linked to each other and how they might effect each other.
	<i>c-sIgE interactions/ connections/ associations</i>	In this analysis, we adopt a systematic approach and we borrow terms from gene co-expression networks, where the complex system is often represented as a network which are complex sets of binary interactions (connections) between genes. Here, we indicate edges as interactions, since the levels of one c-sIgE might have an effect on the activity of other c-sIgEs and their co-expression (the simultaneous expression of two or more c-sIgEs) can alter or affect the risk of developing asthma.

Differential correlation analysis

Differential Gene Correlation Analysis (DGCA)² has recently been proposed to perform differential correlation analysis to investigate the difference in gene-gene interactions between various conditions of interest. DGCA computes the correlations in each condition and uses the difference in z-transformed correlation coefficients to assess statistical significance. Correlations can be computed using Pearson product-moment correlation or the Spearman's rank correlation.² In this section, we describe the model for the Spearman coefficient adopted in the study.

Let $D = \{(y_l, \mathbf{x}_l)\}_{l=1, \dots, n}$ be a dataset of n observations and p c-variables. In this study, $\mathbf{x}_l$ represents the level of the component specific IgEs for the l -th child and y_l denote the binary variable defined as:

$$y_l = \begin{cases} 0, & l \text{ is mild/moderate asthmatic} \\ 1, & l \text{ is severe asthmatic} \end{cases}$$

DGCA computes the correlations between pairs of c-sIgEs under the two conditions separately:

$$r_{ij}^k = \text{cor}(\mathbf{x}_i, \mathbf{x}_j)$$

for $i, j = 1, \dots, p, j \neq i$ and $k = \{0, 1\}$ distinguishing between asthma severity groups.

Then, to stabilize the variance of sample correlation coefficients in each condition, the Fisher z-transformation is applied²:

$$z_{ij}^k = \text{arctanh}(r_{ij}^k) = \frac{1}{2} \ln \left(\frac{1+r_{ij}^k}{1-r_{ij}^k} \right)$$

with $k = \{0, 1\}$ and arctanh being the arc-tangent hyperbolic function.

For the Spearman correlation coefficient, assuming that the underlying distribution is a bivariate normally distributed, then the variance can be calculated as^{2,3}:

$$s_{z_{ij}^k} = \text{var}(r_{ij}^k) = \frac{1.06}{n_k - 3}$$

with $k = \{0, 1\}$, n_k being the sample size of the group under the k -th condition.

Finally, the difference in z-scores between the two conditions can then be calculated as

$$d_z = \frac{z_{ij}^0 - z_{ij}^1}{\sqrt{|s_{z_{ij}^0}^2 - s_{z_{ij}^1}^2|}}$$

To test whether the difference in correlation between the two conditions is statistically different from 0, we can compute a two-sided p-value using the computed difference in z-scores d_z and the standard normal distribution.

Stability and sensitivity analysis

We assessed the accuracy of the network parameters using a bootstrap approach. The analyses were based on the following steps:

1. Observations in the data were resampled with replacement to create a new dataset.
2. The network structure was inferred on the basis of the Spearman correlation coefficients: pairs of components, whose correlation coefficient was significant, were connected by an edge. Edges were weighted by the absolute value of the correlation coefficient.

3. Density was computed for the estimated network.

The procedure was repeated 100 times, then mean and standard deviation of the bootstrap estimates were computed to assess stability.

Furthermore, to evaluate comparability of the mild/moderate and severe networks and assess the impact of the different sample sizes, we performed a sensitivity analysis.

We used downsampling to create new severe datasets with sample size equal to the mild/moderate group. We then performed the bootstrap analysis on the downsampled datasets and evaluated edge-weight accuracy and bootstrapped density.

A sensitivity analysis was performed also for the differential correlation analysis. We adopted the following steps:

1. Observations in the data were downsampled from the severe cohorts to create a new dataset.
2. The differential correlation analysis was run comparing mild/moderate and downsampled severe datasets.

The procedure was repeated 100 times. Stability was assessed on the basis of the number of times the pairs of c-sIgEs showed significant differential correlations.

Joint Density-based Nonparametric Differential Interaction Network analysis and Classification

The joint density-based nonparametric differential interaction network analysis and classification (JDINAC)⁴ identifies pairwise differential interactions among predictors that are most closely related to the outcome of interest and, ultimately, builds a classification model. JDINAC has the advantage of capturing nonlinear relations between component-specific IgEs without the need for parametric assumption on their probability distribution. In our study, we are interested in the association between the pairwise interactions of component-specific IgEs with asthma severity. JDINAC has the main advantages of capturing non-linear relations between components without the need of parametric assumption on the probability distribution of the predictors. Specifically, the main assumption of this model is that network-level difference between two conditions comes from the collective effect of differential pairwise components interactions. Here, the interactions are characterized by the conditional joint density of pairs the predictors⁴, estimated through a non-parametric kernel method.

Let P denote the probability of being in class 1, $P = \Pr(y_i = 1)$ and $\mathbf{v}_i$ denote the i -th c-sIgE. In our study we used JDINAC logistic regression-based approach to test the model:

$$\text{logit}(P) = \alpha_0 + \sum_{i=1}^p \sum_{j>i} \beta_{ij} \ln \frac{f_{ij}^1(\mathbf{v}_i, \mathbf{v}_j)}{f_{ij}^0(\mathbf{v}_i, \mathbf{v}_j)}, \quad s. t. \sum_{i=1}^p \sum_{j>i} |\beta_{ij}| \leq c, c > 0$$

where $f_{ij}^1(\mathbf{v}_i, \mathbf{v}_j)$ and $f_{ij}^0(\mathbf{v}_i, \mathbf{v}_j)$ denote the class conditional joint density of $\mathbf{v}_i$ and $\mathbf{v}_j$ for severe asthma and mild/moderate asthma, respectively. The conditional joint densities $f_{ij}^1(\mathbf{v}_i, \mathbf{v}_j)$ indicate the strength of association between of $\mathbf{v}_i$ and $\mathbf{v}_j$ in severe asthma and parameters $\beta_{ij} \neq 0$ indicate differential dependency patterns between condition-specific groups⁴. The estimation procedure of JDINAC is based on a multiple splitting and prediction averaging procedure, which guarantees robust and accurate results.

JDINAC algorithm:⁴

1. Given the data of n observations $D = \{(y_l, \mathbf{x}_l)\}_{l=1, \dots, n}$. Randomly split the data into two parts: $D = (D1, D2)$.
2. Estimate the joint kernel density functions $\hat{f}_{ij}^1$ and $\hat{f}_{ij}^0$ with $i, j = 1, \dots, p, j > i$ on the first partition $D1$.
3. On $D2$, fit the $L1$ penalized logistic regression using cross validation to get the best penalty parameter.
4. Repeat steps 1-3 T times, on t -th repetition obtain predicted probability $\hat{P}_t$ and coefficient $\hat{\beta}_{ij,t}$, $t = 1, 2, \dots, T$.
5. Apply averaging procedure to compute the final classification $\hat{P} = T^{-1} \sum_{t=1}^T \hat{P}_t$. The differential dependency weight of each pair $(\mathbf{v}_i, \mathbf{v}_j)$ between two groups, are calculated as $w_{ij} = I(\hat{\beta}_{ij,t} \neq 0)$ with $i, j = 1, \dots, p, j > i$. $I(\cdot)$ indicates the indicator function.

Table E1. Inclusion and exclusion criteria for adult cohorts

A Severe asthma (non-smokers)(SAn)	B Severe asthma (current smokers or ex-smokers)(SAs/ex)	C Mild to moderate asthma (MMA)	D Healthy volunteers (HV)
Inclusion criteria			
<i>General:</i>			
1. Able to give written informed consent prior to participation in the study, which includes ability to comply with the requirements and restrictions listed in the consent form. Informed consent must be obtained prior to undertaking any study procedures.			
2. Male or female subject aged 18 years or older at screening.			
3. Able to complete the study and all measurements.			
4. Able to read, comprehend, and write at a sufficient level to complete study related materials.			
5. Subjects will be allowed to enrol in other studies while taking part on this study. However, Permission from the Scientific Board must be obtained to enrol or allow the continued participation of a subject enrolled in another study.			
<i>Cohort specific:</i>			
- Subjects must have been under the follow up of a respiratory specialist for at least 6 months prior to enrolment in the study. During this time appropriate investigations should have been performed to exclude other diagnoses and steps taken to optimise asthma control including treatment of co-morbidities, assessment of adherence, and reduction in allergen exposure in sensitised subjects. - Subjects must be classified as non-smokers with a pack history of ≤ 5 years. Definition of non-smokers: A non-smoker for at least the past 12 months with a pack history ≤ 5 pack years. Pack years = No cigarettes smoked/day x No of years smoked / 20	- Subjects must have been under the follow up of a respiratory specialist for at least 6 months prior to enrolment in the study. During this time appropriate investigations should have been performed to exclude other diagnoses and steps taken to optimise asthma control including treatment of co-morbidities, assessment of adherence, and reduction in allergen exposure in sensitised subjects. - Current or ex-smoker: Definition of a smoker: current smoker in the past 12 months irrespective of pack years Definition of an ex-smoker: a non-smoker for at least the past 12 months with a pack history of > 5 years. Pack years = No cigarettes smoked/day x number of years smoked / 20	A non-smoker for at least the past 12 months with a pack history < 5 pack years. Definition of non-smokers: A non-smoker for at least the past 12 months with a pack history ≤ 5 pack years Pack years = No cigarettes smoked/day x number of years smoked / 20	- Healthy; defined as individuals who are free of significant cardiovascular, pulmonary, gastrointestinal, hepatic, endocrine, renal, haematological, neurological and psychiatric disease as determined by medical history, physical examination and clinical chemistry/haematology/urinalysis investigation. - Pre bronchodilator FEV1 $\geq 80\%$ predicted - A non-smoker for at least the past 12 months with a pack history < 5 pack years. Pack years = No cigarettes smoked/day x No of years smoked / 20

A Severe asthma (non-smokers)(SAn)	B Severe asthma (current smokers or ex-smokers)(SAs/ex)	C Mild to moderate asthma (MMA)	D Healthy volunteers (HV)
Exclusion criteria			
<i>General:</i>			
1. As a result of medical interview, physical examination or screening investigation the physician responsible considers the subject unfit for the study either because of the risk to the subject due to the study or the influence this may have on the study results.			
2. The subject has a history of recreational drug use or other allergy, which, in the opinion of the responsible physician, contra-indicates their participation.			
3. Subject is female who is pregnant or lactating or up to 6 weeks post partum or 6 weeks cessation of breast feeding. If a woman is subsequently found to have been pregnant at the time of an assessment data from that assessment will not be included in the analyses.			
4. The subject has participated within 3 months of the first dose in a study using a new molecular entity, or the first dose in any other study investigating drugs or having participated within three months in a study with invasive procedures. Any U-BIOPRED assessments should be deferred until 3 months after the first dose or invasive procedure. Permission from the Scientific Board must be obtained to enroll or allow the continued participation of a subject enrolled in another study.			
5. Those who, in the opinion of the investigator, have a risk of non-compliance with study procedures.			
6. The subject has a recent history of incapacitating psychiatric			
<i>Cohort specific:</i>			
• Subject has changed asthma medication within the 4 weeks prior to screening (except those using the Symbicort maintenance and reliever therapy (SMART) regime). • Subject had a severe asthma exacerbation (requiring high dose OCS or a doubling of maintenance OCS for ≥ 3 days) in the previous month. • Significant alternative diagnoses that may mimic or complicate asthma, in particular dysfunctional breathing, panic attacks, and overt psychosocial problems (if these are thought to be the major problem rather than in addition to severe asthma) • Significant other primary pulmonary disorders in particular pulmonary embolism, pulmonary hypertension, interstitial lung disease, lung cancer • Subjects with emphysema and bronchiectasis should only be excluded if thought to be the major pulmonary disorder rather than severe asthma • Diagnosis or current investigation of occupational asthma • Diagnosis of chronic inflammatory diseases other than asthma • (inflammatory bowel disease, rheumatoid arthritis) • Any subjects currently participating, or having participated within 3 months of the first dose in a study using a new molecular entity, or the first dose in any other study investigating drugs or having participated within three months in a study with invasive procedures will not be eligible for the telemonitoring study or to attend the exacerbation visits.	• Subject has changed asthma medication within the 4 weeks prior to screening. • Subject had a severe asthma exacerbation (requiring high dose OCS or a doubling of maintenance OCS for at least 3 days) in the previous month.	None	

A Severe asthma (non-smokers)(SAn)	B Severe asthma (current smokers or ex-smokers)(SAs/ex)	C Mild to moderate asthma (MMA)	D Healthy volunteers (HV)
Asthma diagnosis - Improvement in FEV1 $\geq$ 12% or 200ml predicted after inhalation of 400 mcg salbutamol OR - Airway hyper-responsiveness (PC20 <8mg/ml) OR - Diurnal variation in PEF: amplitude % mean of twice daily PEF > 8% OR - Decrease in FEV1 >12% and >200mls within 4 weeks after tapering treatment with one or more of the following drugs: ICS, OCS, LABA, SABA - PLUS a history of wheeze occurring spontaneously or on exertion			No history of asthma
Asthma treatment - High dose ICS $\pm$ /or OCS ($\geq$1000mcg FP daily or equivalent) - PLUS one other controller medication			- Low to moderate dose ICS ($\leq$500mcg FP daily or equivalent) - None
Asthma control Uncontrolled (GINA guidelines)[85], three or more of the following present in any week in the preceding 4 weeks: - Daytime symptoms more than twice per week - Any limitation of activities - Nocturnal symptoms once or more per week - Need for reliever treatment more than twice per week - Pre bronchodilator FEV1 <80% predicted or personal best AND / OR - Frequent severe exacerbations ($\geq$2 per year requiring high dose OCS or doubling of maintenance dose for at least three days or requiring hospitalisation)			- No asthma - Pre bronchodilator FEV1 $\geq$80% predicted
			Controlled (GINA guidelines)[85], all of the following in preceding 4 weeks: - Daytime symptoms $\leq$2/ week - No limitation to activities - No nocturnal symptoms - Need for reliever treatment $\leq$2/ week - Pre bronchodilator FEV1 > 80% predicted OR Partly controlled (GINA guidelines)[85], one or two present in any week in the preceding 4 weeks: - Daytime symptoms >2/week - Any limitations to activities - Any nocturnal symptoms - Need for reliever treatment $\geq$2/week - Pre bronchodilator FEV1 < 80% predicted
Healthy volunteers did not form part of this analysis.			

Table E2. Inclusion and exclusion criteria for paediatric cohorts

Severe school-aged asthma (Cohort SA)	Mild - moderate school-aged asthma (Cohort MMA)	Severe pre-school wheeze (Cohort SW)	Mild - moderate pre-school wheeze (Cohort MMW)
Inclusion criteria			
<i>General:</i>			
1. Parent / legal guardian able to give written informed consent			
2. Assent obtained from all children where appropriate			
3. Parent / legal guardian, or where appropriate the child, able to complete the study and all measurements			
4. Parent / legal guardian, or where appropriate the child, able to read, comprehend, and write at a sufficient level to complete study related materials			
<i>Cohort specific:</i>			
1. Aged between 6 – 17 years at screening	1. Aged between 6 – 17 years at screening	1. Aged between 1 – 5 years at the time of screening	1. Aged between 1 – 5 years at the time of screening
2. Under follow up with a respiratory specialist for at least 6 months prior to enrolling in the study		2. Under follow up with a respiratory specialist for at least 6 months prior to enrolling in the study	
3. Previous assessment to exclude other diagnoses and optimise asthma control including treatment of co-morbidities, assessment of adherence and reduction in allergen exposure in sensitised individuals		3. Previous assessment to exclude other diagnoses and optimise asthma control including treatment of co-morbidities, assessment of adherence and reduction in allergen exposure in sensitised individuals	
Exclusion criteria			
<i>General:</i>			
1. As a result of medical interview, physical examination or screening investigation the physician responsible considers the child unfit for the study			
2. History of allergy, which, in the opinion of the responsible physician, contra-indicates participation			
3. Participant is female who is pregnant or lactating or up to 6 weeks post partum or within 6 weeks cessation of breast feeding.			
4. Participation within 3 months of the first dose in a study using a new molecular entity, or the first dose in any other study investigating drugs or having participated within three months in a study with invasive procedures (permission to enroll required from Scientific Board)			
5. Risk of non-compliance with study procedures			
6. Prematurity ≤35 weeks gestation			
7. Change in asthma medication within 4 weeks of the screening assessment (except those using the Symbicort maintenance and reliever therapy			
8. History or current evidence of an upper or lower respiratory infection or symptoms (including common cold) within 2 weeks of baseline assessment (assessment deferred)			
9. Severe exacerbation within 4 weeks of the baseline assessment (assessment deferred)			

Severe school-aged asthma (Cohort SA)	Mild - moderate school-aged asthma (Cohort MMA)	Severe pre-school wheeze (Cohort SW)	Mild - moderate pre-school wheeze (Cohort MMW)
Exclusion criteria			
<i>Cohort specific:</i> 1. Significant alternative diagnoses that may mimic or complicate asthma, in particular dysfunctional breathing, panic attacks, and overt psychosocial problems (if these are thought to be the <i>major</i> problem rather than <i>in addition</i> to severe asthma) 2. Significant other primary pulmonary disorders in particular cystic fibrosis, interstitial lung disease 3. Patients with bronchiectasis only excluded if this is thought to be the major pulmonary disorder rather than <i>in addition</i> to severe asthma 4. Diagnosis of chronic inflammatory diseases other than asthma (inflammatory bowel disease, rheumatoid arthritis)		1. Significant other primary pulmonary disorders in particular cystic fibrosis, interstitial lung disease 2. Patients with bronchiectasis should only be excluded if this is thought to be the major pulmonary disorder rather than <i>in addition</i> to severe asthma 3. Diagnosis of chronic inflammatory diseases other than asthma (inflammatory bowel disease, rheumatoid arthritis)	
Asthma diagnosis Any one or more of the following: - Improvement in FEV1 ≥ 12% or 200ml predicted after 400 mcg salbutamol - Airway hyper-responsiveness (PC20 <8mg/ml) - Spontaneous variability in FEV1 of > 12% or 200 ml over the past year - Diurnal variability in peak flow in a properly conducted trial of > 15%, with a compatible clinical picture - Persistent airflow limitation: post steroid trial (either 2 weeks of high dose OCS or intramuscular triamcinolone) post bronchodilator z score <-1.96 (cohort a only)		History of breathlessness and wheeze	
Asthma treatment			
High dose ICS (≥500mcg FP or ≥800mcg BUD daily or equivalent) ± OCS PLUS a trial of at least two other controller medications (long acting beta agonists, leukotriene receptor antagonists or theophylline).	Low to moderate dose ICS (≤250mcg FP daily or ≤400mcg BUD or equivalent) PLUS none or one other controller medication (long acting beta agonists, leukotriene receptor antagonists or theophylline)	High dose ICS (≥200mcg FP or ≥400mcg BUD daily or equivalent) and a leukotriene receptor antagonist, or a failed trial of these	No treatment or low dose ICS (≤100mcg FP or ≤200mcg BUD daily or equivalent) and/or a leukotriene receptor antagonist

Severe school-aged asthma (Cohort SA)	Mild - moderate school-aged asthma (Cohort MMA)	Severe pre-school wheeze (Cohort SW)	Mild - moderate pre-school wheeze (Cohort MMW)
Asthma control Any one or more of the following: 1. Persistent symptoms , both of the following for 3 of the past 6 months: - Persistent symptoms (at least 50% of days) - Need for reliever treatment ≥ 3 times per week because of asthma symptoms 2. Frequent severe exacerbations ≥ 2 in the past year or ≥ 3 in the past 2 years requiring hospital attendance or high dose OCS - or ≥ 1 in the past year requiring PICU admission) 3. z score FEV1 <-1.96 - post bronchodilator post steroid trial 4. Prescribed daily or alternate day oral steroids and/or omalizumab - irrespective of level of symptom control	Controlled asthma. All of the following in the last 4 weeks: - Daytime symptoms twice per week or less - No limitation to activities - No nocturnal symptoms - Need for reliever treatment twice per week or less - FEV1 > 80% predicted OR Partially controlled asthma. One or two of the following in the previous 4 weeks: - Daytime symptoms more than twice per week - Any limitation of activities - Any nocturnal symptoms - Need for reliever treatment twice per week or more - Pre bronchodilator FEV1 < 80% predicted	Any one or more of the following: 1. Persistent symptoms , both of the following for 3 of the past 6 months: - Persistent symptoms of wheeze, cough or breathlessness (at least 50% of days) - Need for reliever treatment ≥ 3 times per week because of asthma symptoms 2. Frequent severe exacerbations (≥ 2 in the past year or ≥ 3 in the past 2 years requiring hospital attendance or course of high dose ICS or oral prednisolone - or ≥ 1 in the past year requiring PICU admission; 3. Prescription of daily or alternate day OCS - irrespective of level of symptom control	Controlled symptoms (wheeze, cough or breathlessness). All of the following in the last 4 weeks: - Daytime symptoms twice per week or less - No limitations to activities - No nocturnal symptoms - Need for reliever treatment twice per week or less OR Partially controlled symptoms. One or two of the following in the previous 4 weeks: - Daytime symptoms more than twice per week - Any limitation of activities - Any nocturnal symptoms - Need for reliever treatment twice per week or more

Table E3. Number of participants sensitized to allergen components and number of active allergen components in each group

Age groups	Number of active components ¹	Number of sensitized participants ²		Level of sensitisation ³		Number of active components in the severity groups	
		Mild/moderate	Severe	Mild/moderate	Severe	Mild/moderate	Severe
Adults	58	66/86 (76.7%)	Smokers and ex-smokers: 33/108 (30.6%)	5.00 (3.00, 8.00)	Smokers and ex-smokers: 4.00 (2.00, 8.00)	49	Smokers and ex-smokers: 43
			Non-smokers: 150/297 (50.5%)		Non-smokers: 4.00 (2.00, 7.00)		Non-smokers: 58
School age	64	32/38 (84.2%)	73/89 (82.0%)	10.50 (4.75, 15.00)	8.00 (4.00, 12.00)	61	64
Pre-school age	13	14/44 (31.8%)	16/67 (23.9%)	3.00 (1.00, 5.75)	4.00 (3.00, 4.00)	13	12

¹Active allergen components defined as a positive response by at least three subjects in the whole age group.

²Allergic sensitisation defined as at least one allergen component sIgE>0.3 ISU.

³Level of sensitisation described as median of the number of total components >0.3 ISU (25th, 75th centile).

⁴Number of active components per severity group defined as a positive response by at least three subjects stratified by age group and severity.

Table E4. Allergen component cluster memberships in the adult, school and preschool age groups and proportions of sensitized participants stratified by severity.

Allergens components	Cluster	Number of sensitized participants				p-value	FDR
							adjusted p- value
		Mild/moderate asthma		Severe asthma			
		N	%	N	%		
ADULT COHORT							
Phl p 1	Grass pollen	29	43.9%	67	44.7%	1.000	1.000
Phl p 5	Grass pollen	26	39.4%	32	21.3%	0.010	0.552
Aln g 1	Broad	5	7.6%	6	4.0%	0.444	1.000
Alt a 1	Broad	5	7.6%	14	9.3%	0.873	1.000
Alt a 6	Broad	2	3.0%	1	0.7%	0.462	1.000
Amb a 1	Broad	3	4.5%	4	2.7%	0.763	1.000
Ani s 3	Broad	0	0.0%	1	0.7%	1.000	1.000
Ara h 2	Broad	1	1.5%	3	2.0%	1.000	1.000
Ara h 3	Broad	0	0.0%	3	2.0%	0.599	1.000
Ara h 6	Broad	1	1.5%	4	2.7%	0.978	1.000
Ara h 8	Broad	1	1.5%	3	2.0%	1.000	1.000
Art v 1	Broad	2	3.0%	2	1.3%	0.761	1.000
Asp f 1	Broad	3	4.5%	5	3.3%	0.965	1.000
Asp f 3	Broad	3	4.5%	8	5.3%	1.000	1.000
Asp f 6	Broad	2	3.0%	0	0.0%	0.170	1.000

<i>Ber e 1</i>	Broad	1	1.5%	3	2.0%	1.000	1.000
<i>Bet v 1</i>	Broad	12	18.2%	28	18.7%	1.000	1.000
<i>Blo t 5</i>	Broad	1	1.5%	6	4.0%	0.594	1.000
<i>Can f 1</i>	Broad	16	24.2%	27	18.0%	0.382	1.000
<i>Can f 2</i>	Broad	9	13.6%	12	8.0%	0.299	1.000
<i>Can f 3</i>	Broad	4	6.1%	5	3.3%	0.579	1.000
<i>Can f 5</i>	Broad	17	25.8%	21	14.0%	0.058	0.839
<i>Cor a 1.0101</i>	Broad	2	3.0%	3	2.0%	1.000	1.000
<i>Cor a 1.0401</i>	Broad	7	10.6%	11	7.3%	0.593	1.000
<i>Cry j 1</i>	Broad	5	7.6%	12	8.0%	1.000	1.000
<i>Cup a 1</i>	Broad	6	9.1%	20	13.3%	0.512	1.000
<i>Cyn d 1</i>	Broad	11	16.7%	30	20.0%	0.699	1.000
<i>Der p 10</i>	Broad	1	1.5%	1	0.7%	1.000	1.000
<i>Equ c 1</i>	Broad	8	12.1%	9	6.0%	0.206	1.000
<i>Equ c 3</i>	Broad	1	1.5%	2	1.3%	1.000	1.000
<i>Fel d 2</i>	Broad	5	7.6%	7	4.7%	0.591	1.000
<i>Fel d 4</i>	Broad	11	16.7%	17	11.3%	0.393	1.000
<i>Hev b 8</i>	Broad	2	3.0%	1	0.7%	0.462	1.000
<i>Jug r 1</i>	Broad	0	0.0%	2	1.3%	0.864	1.000
<i>Jug r 2</i>	Broad	3	4.5%	3	2.0%	0.549	1.000
<i>Lep d 2</i>	Broad	5	7.6%	16	10.7%	0.648	1.000
<i>Mal d 1</i>	Broad	4	6.1%	11	7.3%	0.961	1.000
<i>Mus m 1</i>	Broad	5	7.6%	5	3.3%	0.310	1.000
<i>MUXF3</i>	Broad	3	4.5%	4	2.7%	0.763	1.000

<i>Ole e 1</i>	Broad	5	7.6%	15	10.0%	0.755	1.000
<i>Par j 2</i>	Broad	2	3.0%	13	8.7%	0.226	1.000
<i>Pen m 1</i>	Broad	0	0.0%	1	0.7%	1.000	1.000
<i>Pen m 2</i>	Broad	1	1.5%	2	1.3%	1.000	1.000
<i>Phl p 11</i>	Broad	3	4.5%	8	5.3%	1.000	1.000
<i>Phl p 2</i>	Broad	9	13.6%	20	13.3%	1.000	1.000
<i>Phl p 4</i>	Broad	10	15.2%	15	10.0%	0.390	1.000
<i>Phl p 6</i>	Broad	14	21.2%	13	8.7%	0.019	0.552
<i>Pla a 1</i>	Broad	1	1.5%	2	1.3%	1.000	1.000
<i>Pla a 2</i>	Broad	4	6.1%	1	0.7%	0.053	0.839
<i>Pla l 1</i>	Broad	1	1.5%	2	1.3%	1.000	1.000
<i>Pol d 5</i>	Broad	2	3.0%	1	0.7%	0.462	1.000
<i>Pru p 1</i>	Broad	3	4.5%	7	4.7%	1.000	1.000
<i>Ves v 5</i>	Broad	3	4.5%	5	3.3%	0.965	1.000
<i>Der f 1</i>	HDM	15	22.7%	36	24.0%	0.977	1.000
<i>Der f 2</i>	HDM	25	37.9%	54	36.0%	0.912	1.000
<i>Der p 1</i>	HDM	22	33.3%	41	27.3%	0.465	1.000
<i>Der p 2</i>	HDM	31	47.0%	58	38.7%	0.321	1.000
<i>Fel d 1</i>	HDM	28	42.4%	59	39.3%	0.782	1.000
<i>SCHOOL COHORT</i>							
<i>Aln g 1</i>	PR-10	11	33.3%	16	21.9%	0.313	1.000
<i>Bet v 1</i>	PR-10	18	54.5%	28	38.4%	0.178	1.000
<i>Cor a 1.0101</i>	PR-10	8	24.2%	16	21.9%	0.989	1.000
<i>Cor a 1.0401</i>	PR-10	13	39.4%	25	34.2%	0.770	1.000

<i>Mal d 1</i>	PR-10	13	39.4%	20	27.4%	0.313	1.000
<i>Pru p 1</i>	PR-10	9	27.3%	16	21.9%	0.723	1.000
<i>Fel d 1</i>	Grass/cat	18	54.5%	39	53.4%	1.000	1.000
<i>Phl p 1</i>	Grass/cat	25	75.8%	41	56.2%	0.087	1.000
<i>Der f 1</i>	HDM	16	48.5%	34	46.6%	1.000	1.000
<i>Der f 2</i>	HDM	16	48.5%	34	46.6%	1.000	1.000
<i>Der p 1</i>	HDM	17	51.5%	37	50.7%	1.000	1.000
<i>Der p 2</i>	HDM	16	48.5%	37	50.7%	1.000	1.000
<i>Act d 8</i>	Broad	2	6.1%	5	6.8%	1.000	1.000
<i>Alt a 1</i>	Broad	4	12.1%	9	12.3%	1.000	1.000
<i>Alt a 6</i>	Broad	5	15.2%	5	6.8%	0.320	1.000
<i>Ani s 3</i>	Broad	1	3.0%	6	8.2%	0.566	1.000
<i>Api g 1</i>	Broad	3	9.1%	4	5.5%	0.786	1.000
<i>Ara h 1</i>	Broad	5	15.2%	5	6.8%	0.320	1.000
<i>Ara h 2</i>	Broad	6	18.2%	8	11.0%	0.479	1.000
<i>Ara h 3</i>	Broad	3	9.1%	4	5.5%	0.786	1.000
<i>Ara h 6</i>	Broad	8	24.2%	10	13.7%	0.289	1.000
<i>Ara h 8</i>	Broad	7	21.2%	9	12.3%	0.373	1.000
<i>Art v 1</i>	Broad	0	0.0%	3	4.1%	0.583	1.000
<i>Bet v 2</i>	Broad	5	15.2%	6	8.2%	0.459	1.000
<i>Bla g 7</i>	Broad	2	6.1%	6	8.2%	1.000	1.000
<i>Blo t 5</i>	Broad	0	0.0%	4	5.5%	0.412	1.000
<i>Bos d 4</i>	Broad	1	3.0%	3	4.1%	1.000	1.000
<i>Bos d 5</i>	Broad	2	6.1%	3	4.1%	1.000	1.000

<i>Bos d 6</i>	Broad	2	6.1%	1	1.4%	0.474	1.000
<i>Bos d 8</i>	Broad	2	6.1%	3	4.1%	1.000	1.000
<i>Can f 2</i>	Broad	6	18.2%	9	12.3%	0.617	1.000
<i>Can f 3</i>	Broad	4	12.1%	2	2.7%	0.138	1.000
<i>Cla h 8</i>	Broad	2	6.1%	5	6.8%	1.000	1.000
<i>Cor a 9</i>	Broad	1	3.0%	3	4.1%	1.000	1.000
<i>Cry j 1</i>	Broad	1	3.0%	2	2.7%	1.000	1.000
<i>Cup a 1</i>	Broad	3	9.1%	3	4.1%	0.566	1.000
<i>Der p 10</i>	Broad	2	6.1%	6	8.2%	1.000	1.000
<i>Fel d 2</i>	Broad	3	9.1%	3	4.1%	0.566	1.000
<i>Gad c 1</i>	Broad	1	3.0%	2	2.7%	1.000	1.000
<i>Gal d 1</i>	Broad	3	9.1%	5	6.8%	0.994	1.000
<i>Gal d 2</i>	Broad	2	6.1%	2	2.7%	0.779	1.000
<i>Gal d 3</i>	Broad	3	9.1%	3	4.1%	0.566	1.000
<i>Gly m 4</i>	Broad	5	15.2%	5	6.8%	0.320	1.000
<i>Gly m 6</i>	Broad	3	9.1%	3	4.1%	0.566	1.000
<i>Hev b 8</i>	Broad	5	15.2%	6	8.2%	0.459	1.000
<i>Jug r 1</i>	Broad	4	12.1%	4	5.5%	0.423	1.000
<i>Lep d 2</i>	Broad	5	15.2%	8	11.0%	0.772	1.000
<i>Mer a 1</i>	Broad	5	15.2%	5	6.8%	0.320	1.000
<i>Mus m 1</i>	Broad	2	6.1%	5	6.8%	1.000	1.000
<i>Pen m 1</i>	Broad	4	12.1%	6	8.2%	0.781	1.000
<i>Pen m 2</i>	Broad	0	0.0%	4	5.5%	0.412	1.000
<i>Phl p 11</i>	Broad	3	9.1%	5	6.8%	0.994	1.000

<i>Phl p 12</i>	Broad	2	6.1%	2	2.7%	0.779	1.000
<i>Phl p 4</i>	Broad	6	18.2%	8	11.0%	0.479	1.000
<i>Phl p 6</i>	Broad	7	21.2%	8	11.0%	0.271	1.000
<i>Pru p 3</i>	Broad	1	3.0%	2	2.7%	1.000	1.000
<i>Can f 1</i>	Animal/pollen	10	30.3%	25	34.2%	0.860	1.000
<i>Can f 5</i>	Animal/pollen	9	27.3%	12	16.4%	0.302	1.000
<i>Cyn d 1</i>	Animal/pollen	15	45.5%	24	32.9%	0.305	1.000
<i>Equ c 1</i>	Animal/pollen	8	24.2%	16	21.9%	0.989	1.000
<i>Fel d 4</i>	Animal/pollen	6	18.2%	18	24.7%	0.626	1.000
<i>Ole e 1</i>	Animal/pollen	8	24.2%	16	21.9%	0.989	1.000
<i>Phl p 2</i>	Animal/pollen	12	36.4%	16	21.9%	0.185	1.000
<i>Phl p 5</i>	Animal/pollen	15	45.5%	16	21.9%	0.025	1.000
<i>PRE-SCHOOL COHORT</i>							
<i>Der f 1</i>	HDM	5	35.7%	10	58.8%	0.357	0.775
<i>Der f 2</i>	HDM	4	28.6%	8	47.1%	0.496	0.806
<i>Der p 1</i>	HDM	5	35.7%	11	64.7%	0.213	0.691
<i>Der p 2</i>	HDM	4	28.6%	8	47.1%	0.496	0.806
<i>Ara h 2</i>	Broad	1	7.1%	3	17.6%	0.741	0.876
<i>Bet v 1</i>	Broad	2	14.3%	5	29.4%	0.568	0.821
<i>Can f 1</i>	Broad	2	14.3%	3	17.6%	1.000	1.000
<i>Cor a 1.0401</i>	Broad	0	0.0%	4	23.5%	0.160	0.691
<i>Fel d 1</i>	Broad	6	42.9%	5	29.4%	0.688	0.876
<i>Fel d 4</i>	Broad	2	14.3%	1	5.9%	0.859	0.931
<i>Mal d 1</i>	Broad	0	0.0%	4	23.5%	0.160	0.691

<i>Phl p 1</i>	Broad	5	35.7%	1	5.9%	0.102	0.691
<i>Pru p 1</i>	Broad	0	0.0%	3	17.6%	0.297	0.771

P-values are based on the χ^2 test.

Table E5. Proportion of each subgroup that belongs to each allergic sensitisation cluster

		Allergic sensitisation clusters					
		Multiple	Predominantly house dust mite	Predominantly grass pollen	Lower-grade sensitization	p-value	FDR adjusted p-value
Adult participants							
Asthma	Severe non-smokers	16.7%	22.0%	18.0%	43.3%	0.101	0.303
	Severe smokers	18.2%	18.2%	15.2%	48.5%		
	Mild/moderate	24.2%	21.2%	30.3%	24.2%		
Eczema	Yes	23.6%	24.7%	16.9%	34.8%	0.224	0.311
	No	16.4%	18.5%	24.0%	41.1%		
Allergic rhinitis	Yes	21.0%	17.9%	21.0%	40.1%	0.311	0.311
	No	15.1%	27.4%	21.9%	35.6%		
School age participants							
Asthma	Severe	24.7%	32.9%	12.3%	30.1%	0.103	0.309
	Mild/moderate	31.2%	15.6%	28.1%	25.0%		
Eczema	Yes	30.9%	25.9%	16.0%	27.2%	0.308	0.462
	No	10.0%	35.0%	25.0%	30.0%		
Allergic rhinitis	Yes	28.9%	25.0%	18.4%	27.6%	0.725	0.725
	No	20.0%	36.0%	16.0%	28.0%		

Allergic sensitisation defined as at least one allergen component sIgE>0.3 ISU. P-values represent a Chi square test.

Table E6. Baseline clinical measures stratified by allergic sensitisation clusters

Allergic sensitisation clusters						
	Multiple	Predominantly house dust mite	Predominantly grass pollen	Lower-grade sensitization	p- value	FDR adjusted p- value
Adult participants						
Age of onset breathing problems, years	6.00 [2.00;14.00]	6.00 [3.00;12.00]	13.00 [6.50;28.50]	19.00 [4.00;34.00]	<0.001	0.003
Age of asthma diagnosis, years	6.00 [3.00;15.00]	8.00 [4.00;17.75]	18.00 [10.00;30.00]	22.00 [6.00;40.00]	<0.001	<0.001
Number of exacerbations in last year	1.00 [0.00;2.00]	1.00 [0.00;3.00]	1.00 [0.00;2.00]	1.00 [0.00;3.00]	0.314	0.366
FEV ₁ % predicted (L)	71.29 [60.86;90.88]	70.66 [50.58;89.04]	80.58 [62.80;94.47]	67.42 [51.23;88.07]	0.023	0.044
FEV ₁ /FVC ratio	64.30 [57.4;75.36]	64.38 [53.01;76.57]	71.62 [62.14;79.92]	63.26 [53.79;71.12]	0.025	0.044
FEV ₁ absolute change, %	9.46 [4.94;14.45]	8.68 [5.57;13.04]	8.67 [5.96;14.09]	7.61 [3.14;12.46]	0.569	0.569
FeNO, ppb	30.00 [20.50;70.00]	24.25 [14.00;37.00]	21.00 [14.00;42.5.]	25.00 [15.00;43.00]	0.087	0.122
School age participants						
Age of onset breathing problems, years	1.00 [1.00;2.25]	1.00 [1.00;3.00]	1.00 [1.00;2.00]	1.00 [1.00;2.00]	0.947	0.947
Age of asthma diagnosis, years	2.00 [1.00;3.00]	3.00 [2.00;4.25]	3.00 [2.00;7.00]	2.00 [1.00;4.00]	0.102	0.432
Number of exacerbations in last year	3.00 [2.00;5.75]	2.50 [2.00;4.00]	2.00 [2.00;3.00]	2.00 [2.00;4.00]	0.485	0.595

FEV₁ Pct (L)	85.51 [74.32;98.22]	94.39 [80.95;104.91]	88.96 [74.80;94.51]	85.78 [78.10;92.88]	0.407	0.595
FEV₁/FVC ratio	78.92 [70.49;84.64]	78.86 [71.52;85.42]	76.47 [62.88;80.93]	77.94 [72.09;82.31]	0.510	0.595
FEV₁ absolute change, %	7.76 [5.75;14.45]	8.36 [4.35;14.81]	9.20 [2.14;15.92]	14.30 [7.67;22.43]	0.158	0.432
FeNO, ppb	47.00 [29.00;65.00]	32.50 [15.37;56.62]	38.00 [17.00;54.00]	21.00 [12.25;90.00]	0.185	0.432

Median and interquartile range (in brackets) are reported. P-values are based on the Kruskal–Wallis test. FDR: false detection rate.

Table E7. Comparison of adult and school age networks through network measure

	Number of nodes	Number of edges	Density
<i>Adult cohort</i>	58	1001	0.61
<i>School age cohort</i>	64	1665	0.83
<i>Preschool age cohort</i>	13	64	0.82

Network density is defined as the ratio of the number of edges to the number of possible edges in a network.

Network measures in adult and paediatric cohorts show that the network in the latter is bigger but tighter. More connections appear in the school age group.

Table E8. Top 10 c-sIgE components ranked by their strength in adult and school age cohorts

Adult cohort		School age cohort	
<i>c-sIgE</i>	<i>strength</i>	<i>c-sIgE</i>	<i>strength</i>
<i>Fel d 1</i>	1.000	<i>Phl p 1</i>	1.000
<i>Phl p 1</i>	0.980	<i>Bet v 1</i>	0.832
<i>Der p 2</i>	0.916	<i>Fel d 1</i>	0.806
<i>Der f 2</i>	0.869	<i>Cor a 1.0401</i>	0.771
<i>Der p 1</i>	0.771	<i>Der p 1</i>	0.722
<i>Phl p 5</i>	0.639	<i>Cyn d 1</i>	0.712
<i>Der f 1</i>	0.615	<i>Mal d 1</i>	0.711
<i>Can f 1</i>	0.610	<i>Der f 1</i>	0.690
<i>Bet v 1</i>	0.514	<i>Der p 2</i>	0.682
<i>Can f 5</i>	0.494	<i>Der f 2</i>	0.673

Table E9. c-sIgE components excluded from the differential network analysis.
c-sIgE with less than 3 positive responses in the mild/moderate and severe group.

	Alt a 6	Amb a 1	Ani s 3	Ara h 2	Ara h 3	Ara h 6	Art v 1
Adult cohort	Asp f 1	Asp f 3	Asp f 6	Can f 3	Cry j 1	Der p 10	Equ c 3
	Hev b 8	Jug r 1	Jug r 2	Mus m 1	MUXF3	Pen m 1	Pen m 2
	Pla a 1	Pla a 2	Pla l 1	Pol d 5	Ves v 5		
School age cohort	Act d 8	Ani s 3	Api g 1	Art v 1	Bla g 7	Blo t 5	Bos d 4
	Bos d 5	Bos d 6	Bos d 8	Can f 3	Cor a 9	Cry j 1	Der p 10
	Gad c 1	Gal d 1	Gal d 2	Gal d 3	Gly m 6	Mus m 1	Pen m 2
	Phl p 12	Pru p 3					

Table E10. Comparison of mild/moderate (MMA) versus severe asthma (SA) in adult and school age cohorts through network measure (for explanation see page 7.)

		Number of nodes	Number of edges	Density
Adult cohort	<i>MMA</i>	32	92	0.18
	<i>SA</i>	32	197	0.40
School age cohort	<i>MMA</i>	41	98	0.12
	<i>SA</i>	41	290	0.36

When comparing cohort stratified by asthma severity, we observe that networks in the severe groups are smaller and tighter with higher connectivity among the c-sIgEs. There were insufficient data to replicate the analysis in the pre-school cohorts.

Table E11. Stability of differential correlation through bootstrap approach.

Pairwise c-slgEs connection which were found to have significant differential correlation in at least 30% of the bootstrap runs are presented in the table

Adult cohort	Schoo-age cohort
<i>Ara h 8—Cor a 1.0101</i>	<i>Alt a 6—Bet v 2</i>
<i>Can f 1—Fel d 2</i>	<i>Alt a 6—Hev b 8</i>
<i>Can f 1—Fel d 4</i>	<i>Alt a 6—Mer a 1</i>
<i>Can f 2—Fel d 2</i>	<i>Bet v 2—Mer a 1</i>
<i>Cor a 1.0101—Fel d 4</i>	<i>Bet v 2—Phl p 6</i>
<i>Cor a 1.0101—Mal d 1</i>	<i>Can f 2—Can f 5</i>
<i>Cor a 1.0101—Phl p 2</i>	<i>Cla h 8—Cup a 1</i>
<i>Der f 2—Der p 2</i>	<i>Hev b 8—Mer a 1</i>
<i>Equ c 1—Fel d 4</i>	<i>Hev b 8—Phl p 6</i>
	<i>Phl p 11—Phl p 4</i>

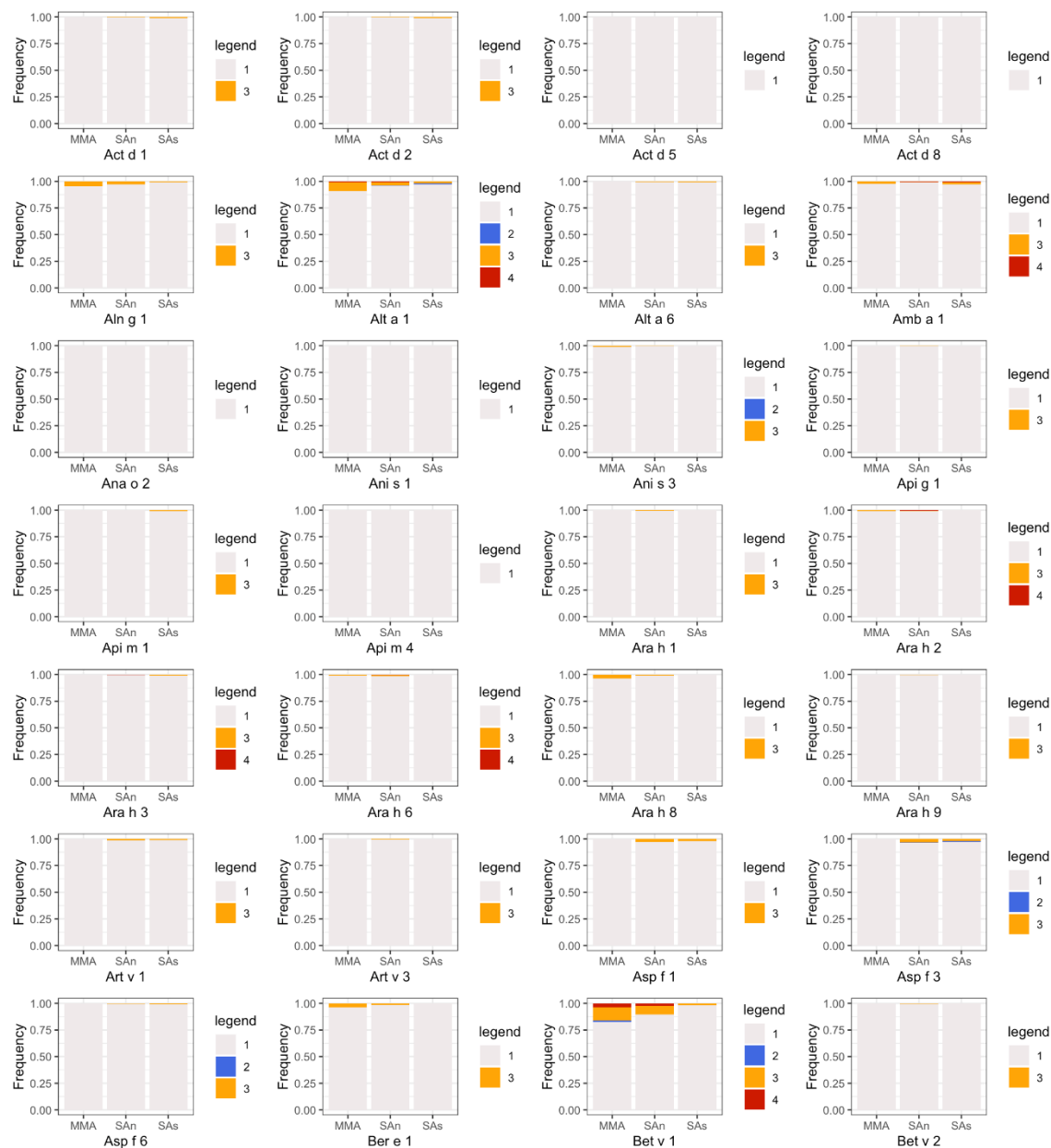
Table E12. The ability of penalised logistic regression with individual components and JDINAC with pairwise interactions of c-slgE allergens to predict asthma severity

		AUC	Accuracy	Sensitivity	Specificity	Kappa
Adult cohort	<i>JDINAC</i>	0.945	0.856	1.000	0.530	0.611
	<i>pLR</i>	0.541	0.653	0.800	0.318	0.126
School age cohort	<i>JDINAC</i>	0.928	0.867	1.000	0.562	0.641
	<i>pLR</i>	0.516	0.648	0.795	0.312	0.114

Table presents a comparison of performance in predicting severe versus mild/moderate asthma in the adult and school age cohorts. The performance of penalised logistic regression with individual components and JDINAC with pairwise interactions of c-slgE allergens are presented separately.

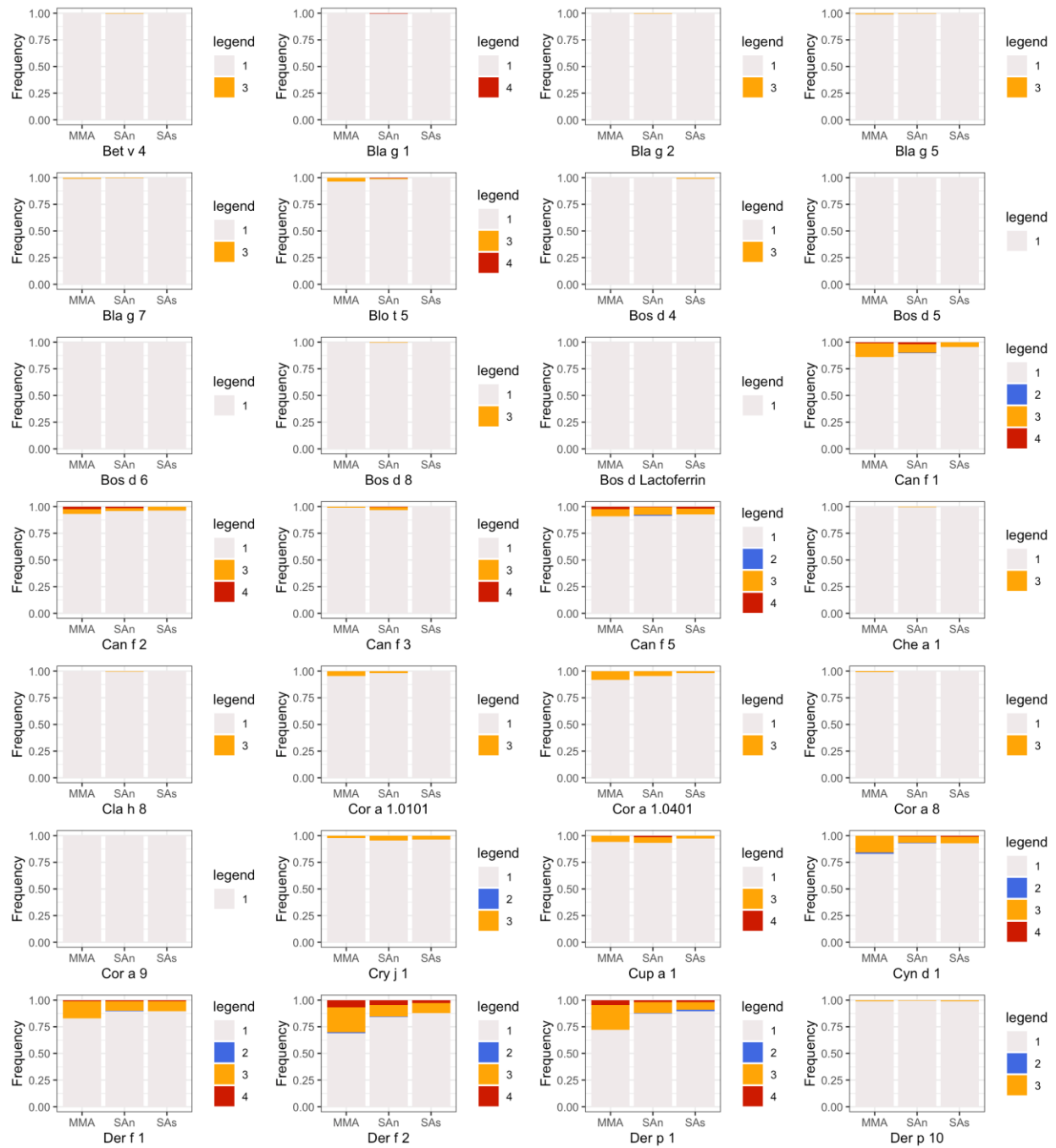
Figure E1. Sensitization to individual allergen components in (a) adult and (b) paediatric cohorts

(a) Adult cohorts



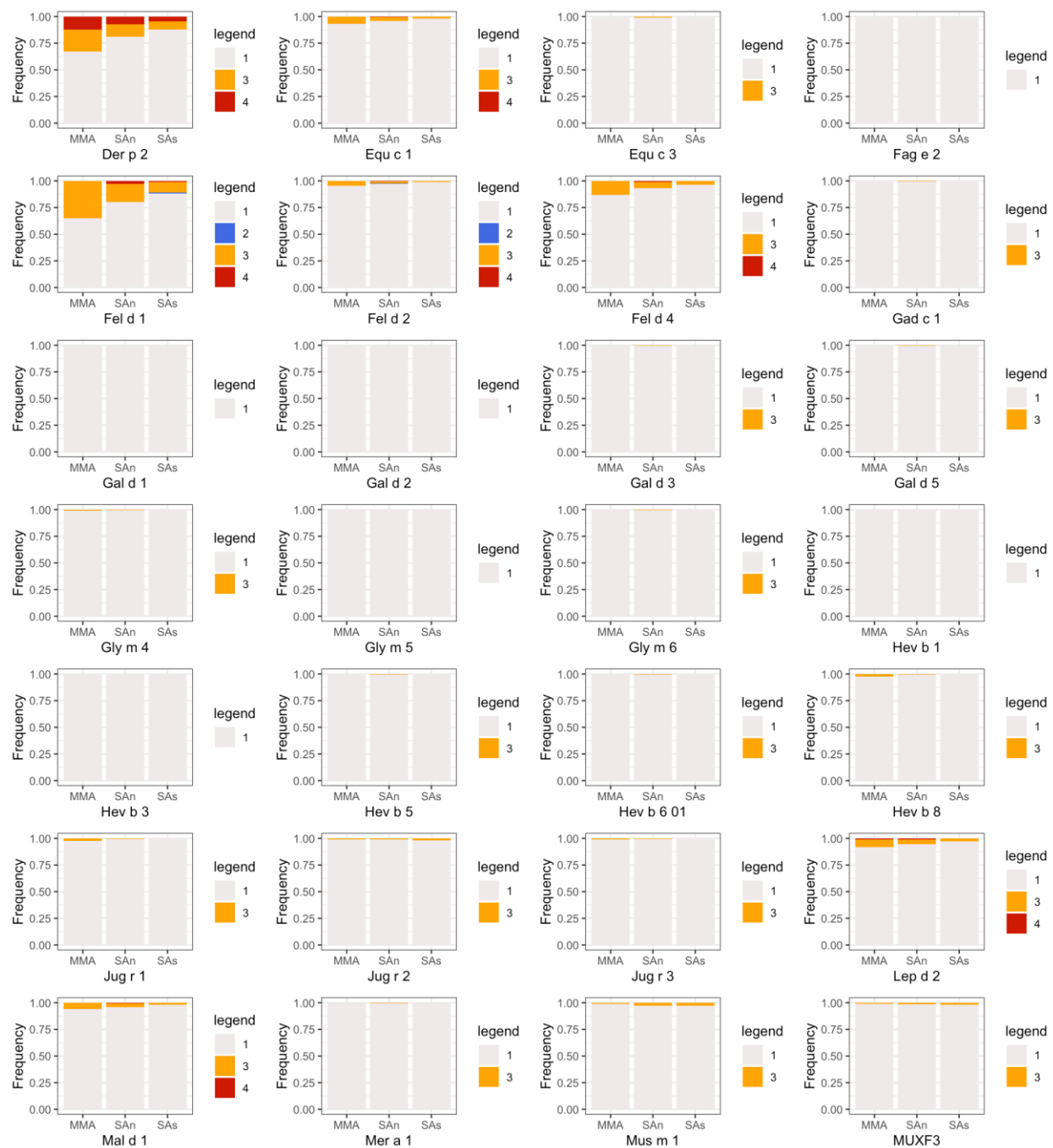
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(a) Adult cohorts continued



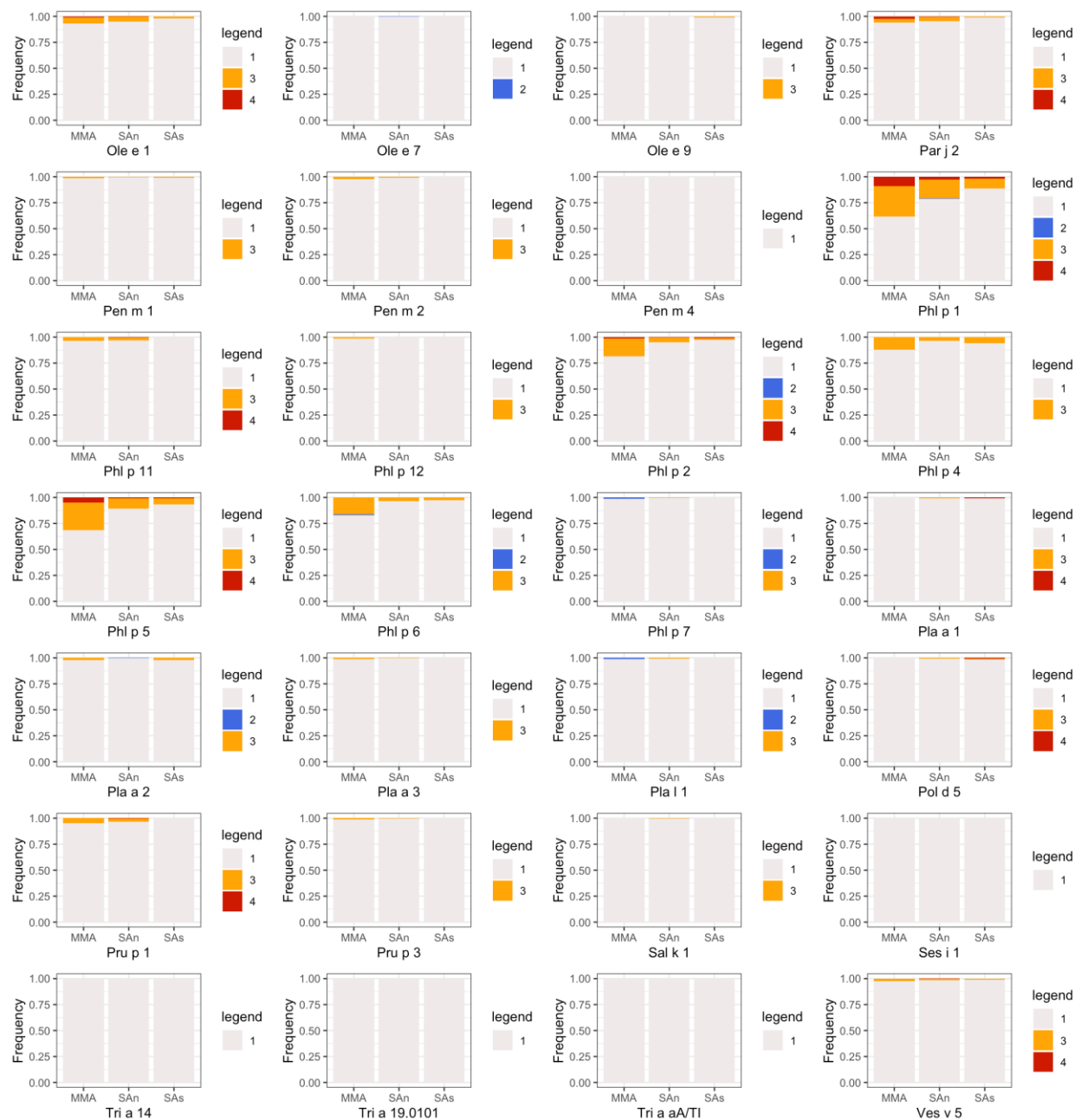
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(a) Adult cohorts continued



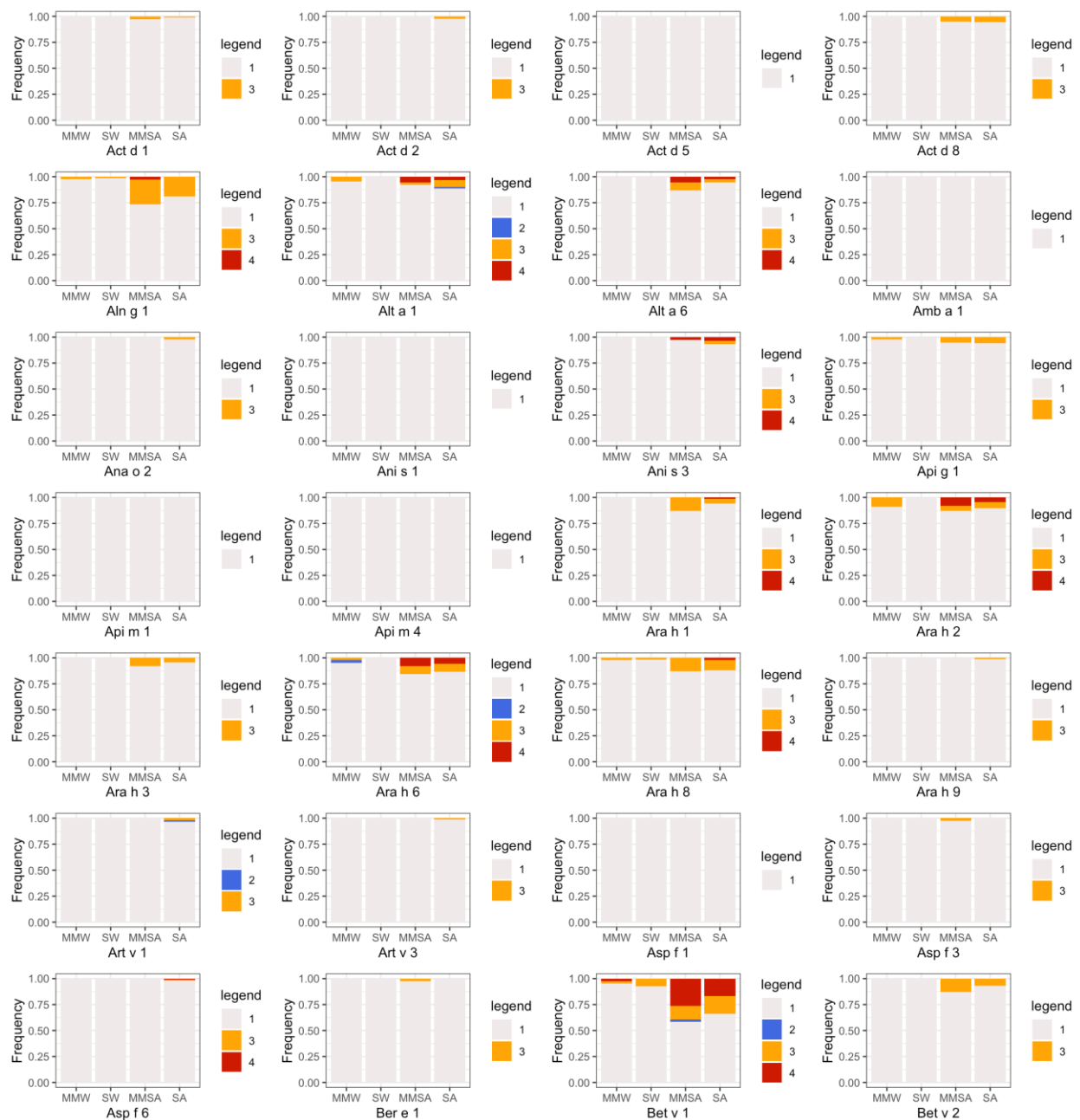
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(a) Adult cohorts continued



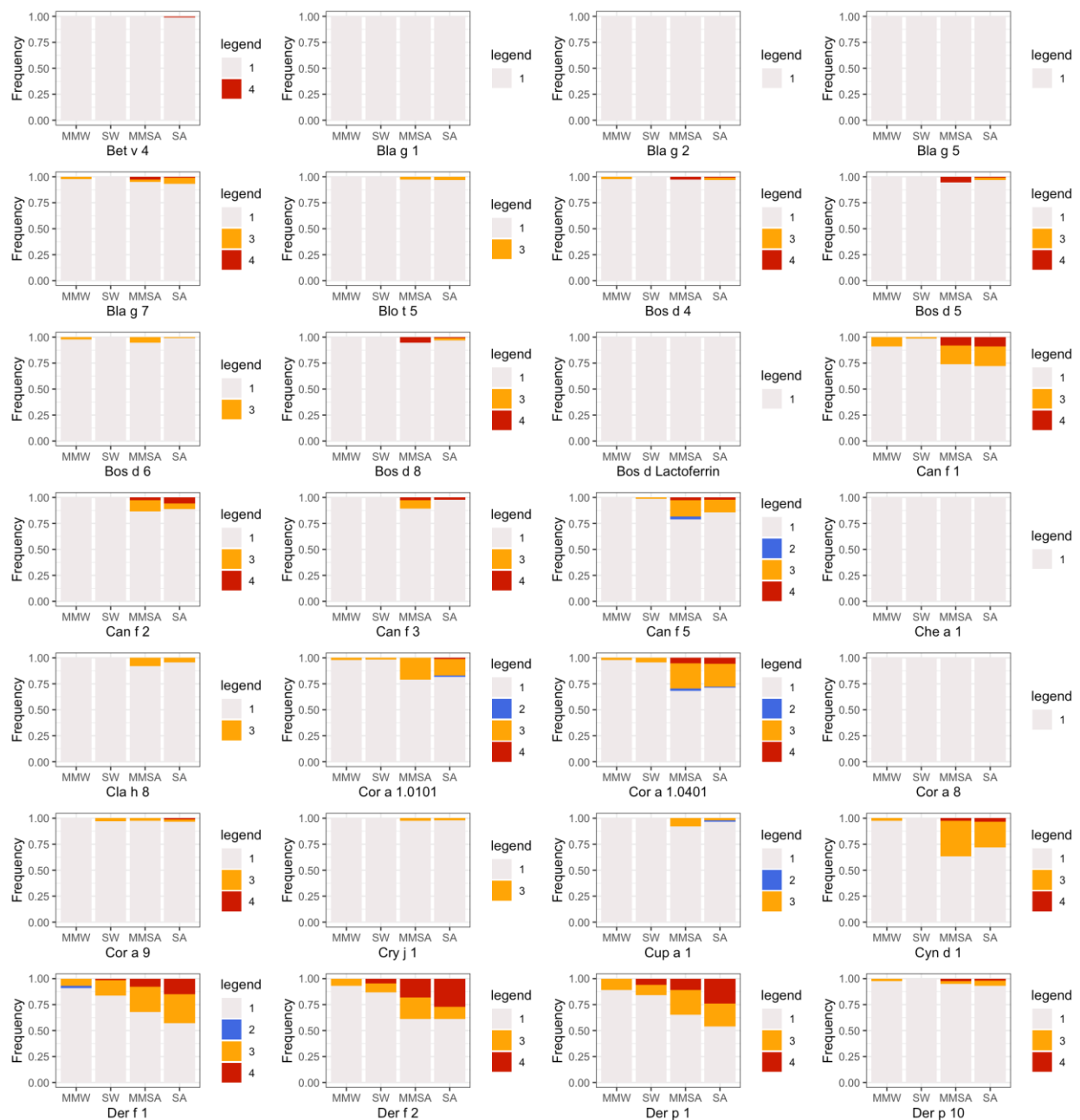
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(b) Paediatric cohorts



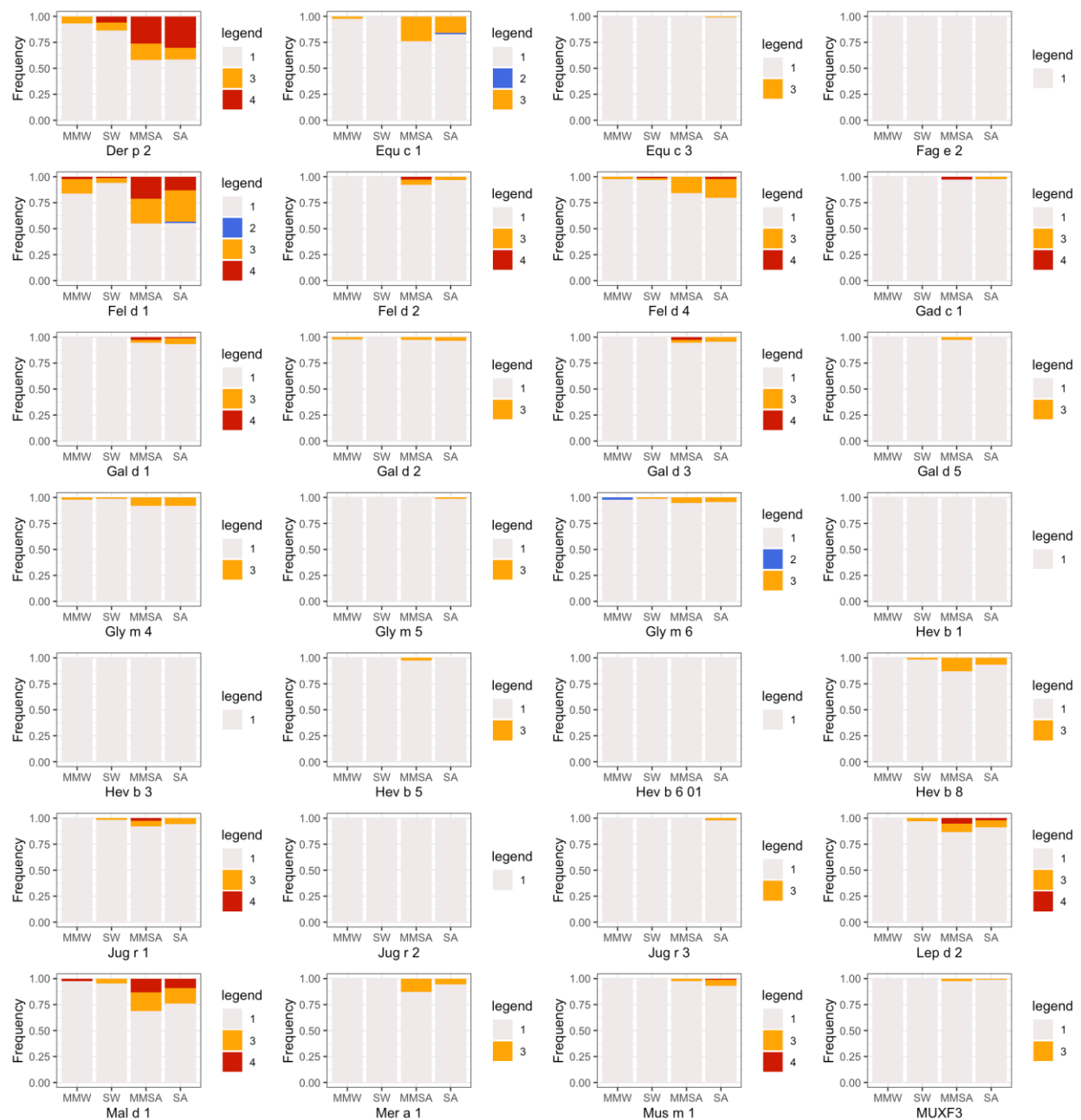
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(b) Paediatric cohorts continued



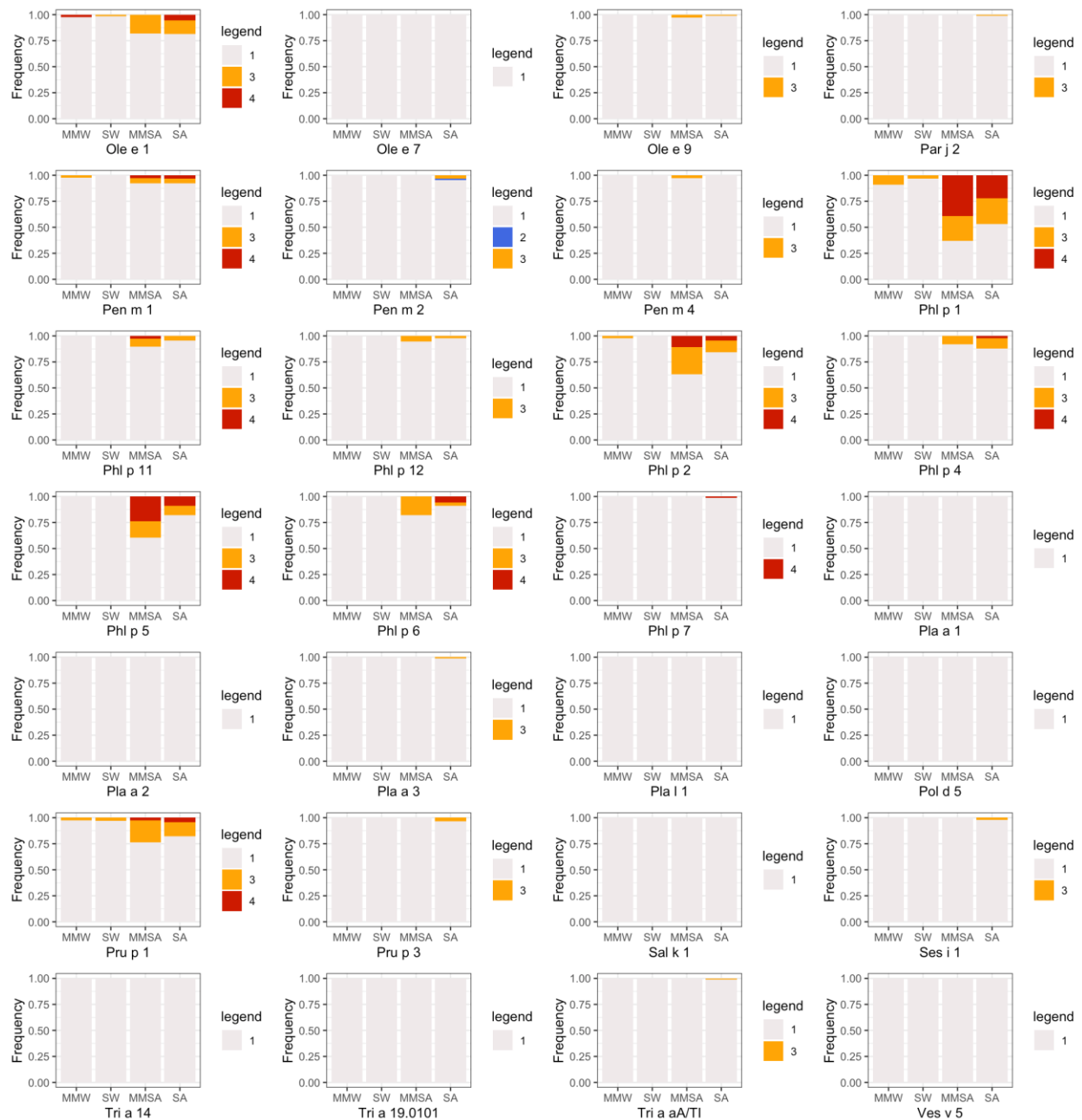
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(b) Paediatric cohorts continued



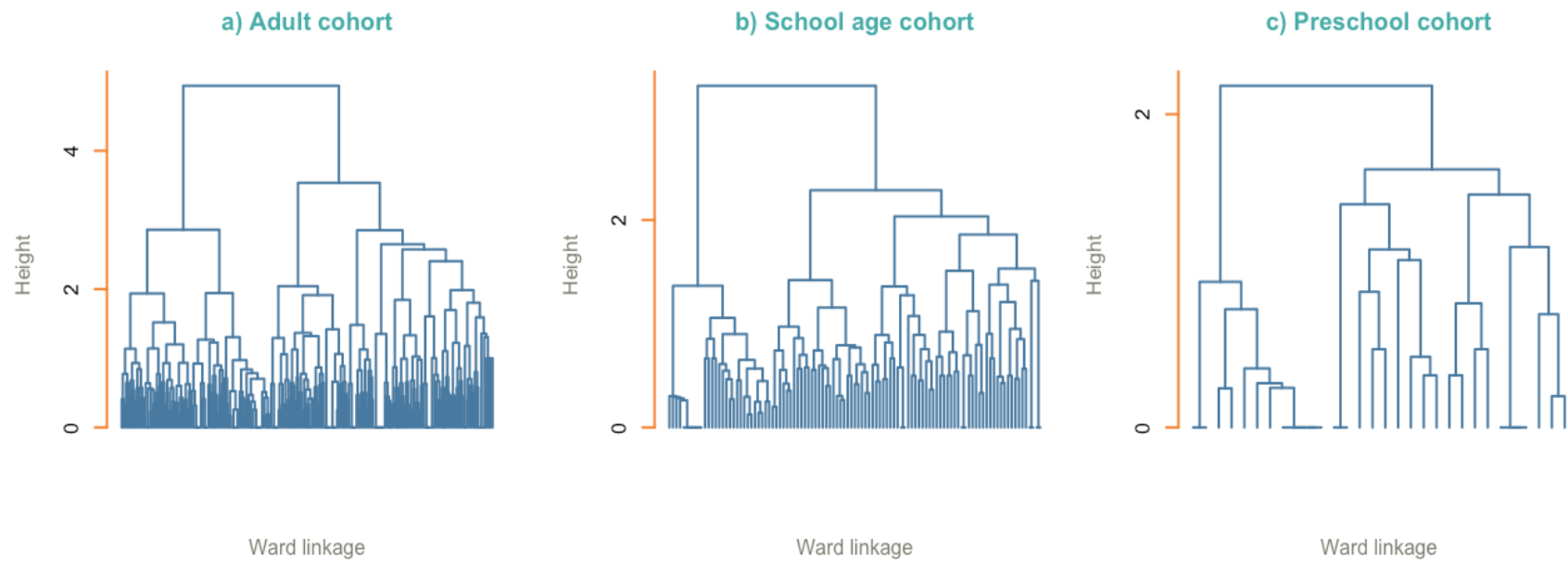
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

(b) Paediatric cohorts continued



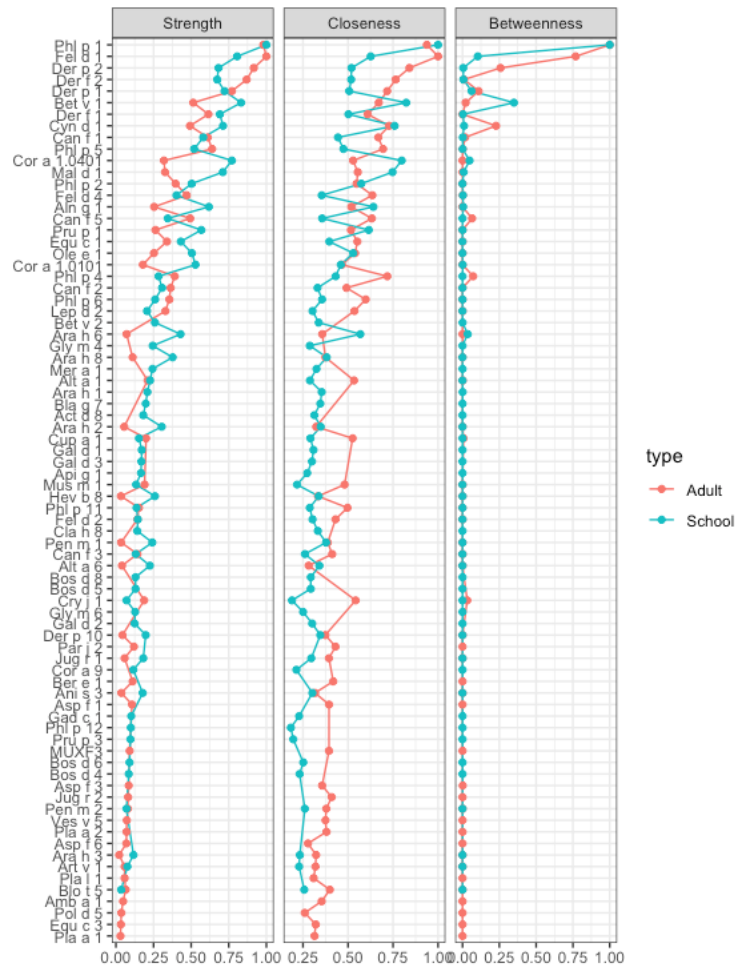
Stacked bar charts representing the proportion of participants in each cohort with no (<0.3 ISU), low (0.3-1 ISU), medium (1-15 ISU) or high (>15 ISU) sensitization for each ISAC allergen component. All participants included.

Figure E2. Dendrograms and bootstrap estimate of the clustering instability for the selection of number of clusters



COHORT	Number of clusters		
	2	3	4
Adult	0.131	0.149	0.122
School age	0.120	0.123	0.099
Preschool age	0.087	0.105	0.095

Figure E3. Comparisons of component-specific IgEs centrality measures in adult and school age networks



See page 7 for explanation.

Figure E4. Component-specific IgEs centrality measures in preschool network

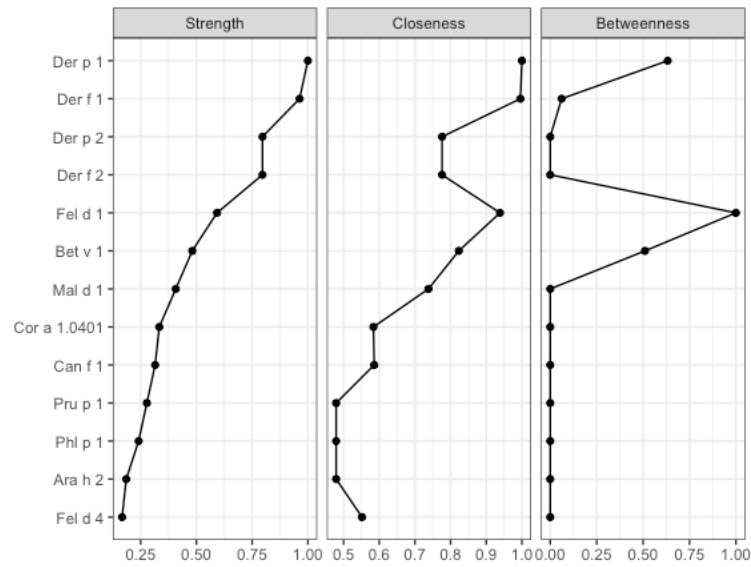


Figure E5. Comparisons of component-specific IgEs in mild/moderate versus severe networks in (a) adult and (b) school age cohorts

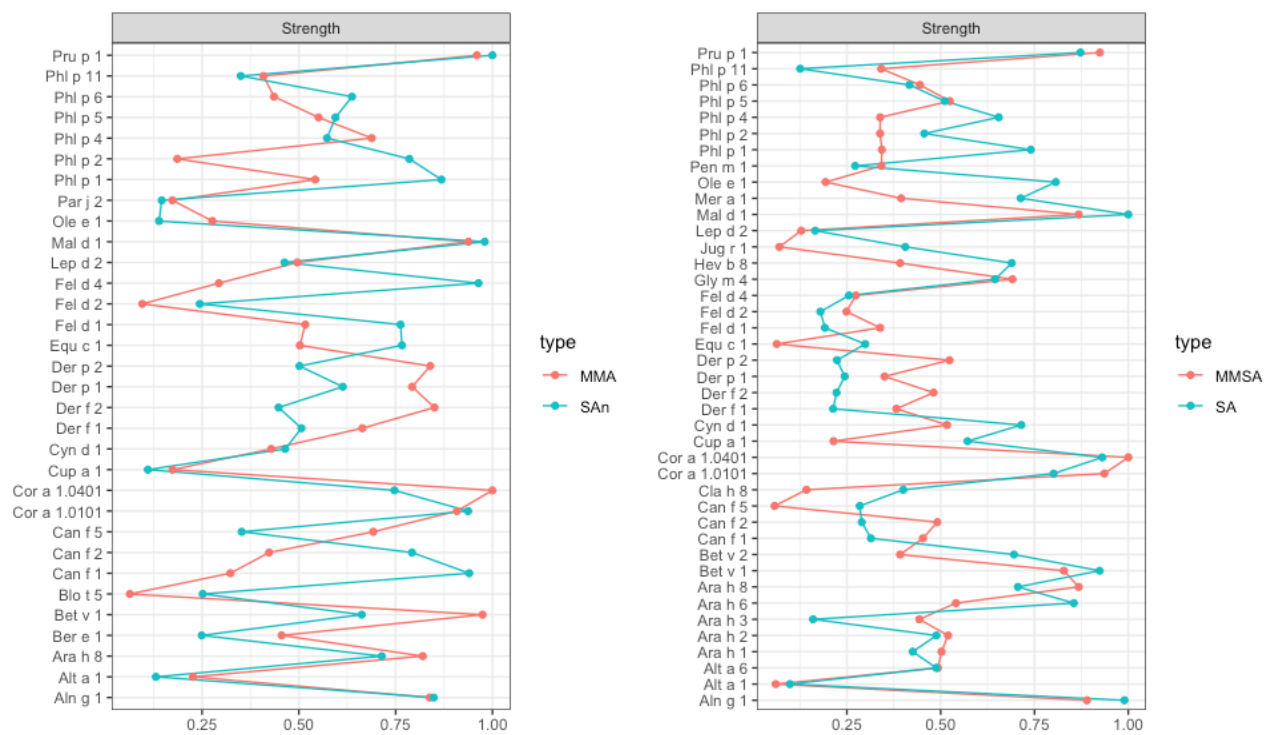
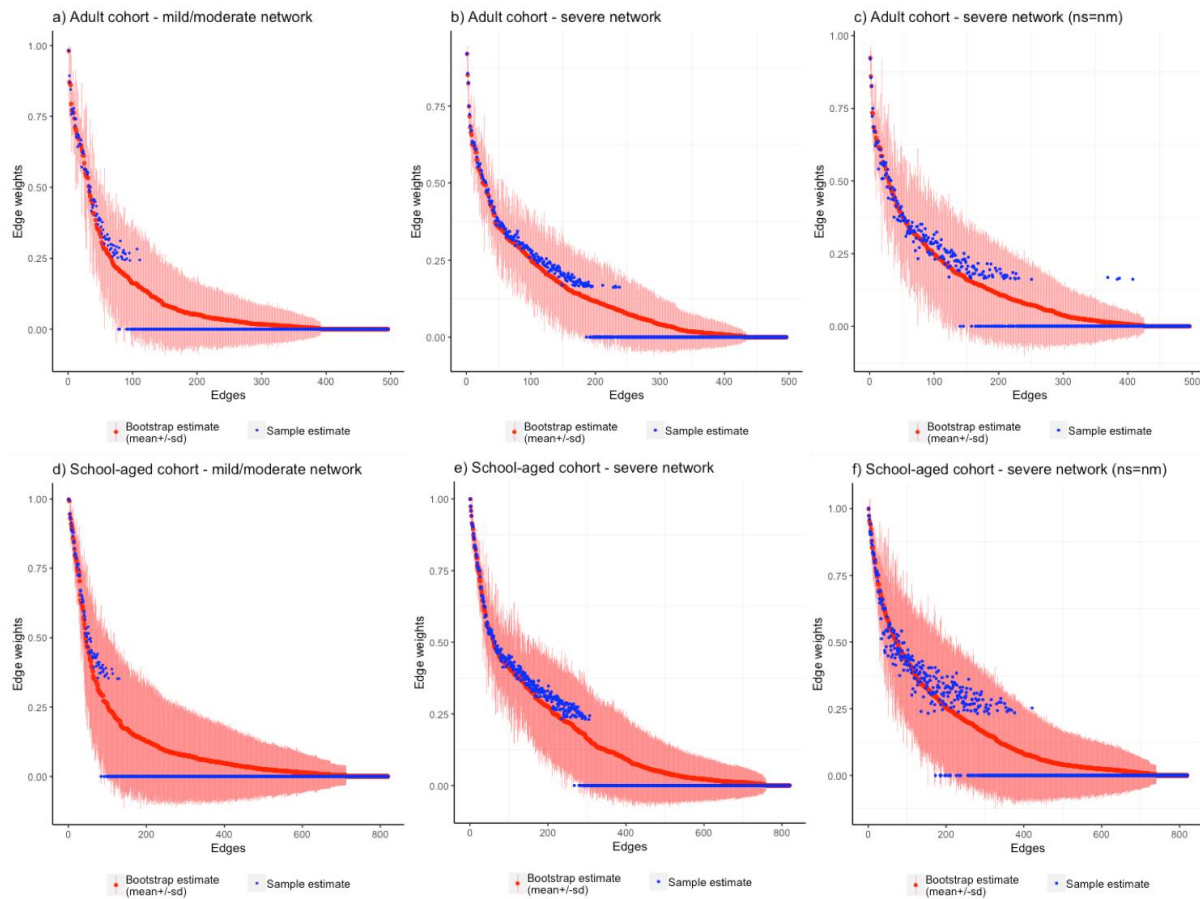


Figure E6. Network stability: edge-weight accuracy computed through bootstrap approach.

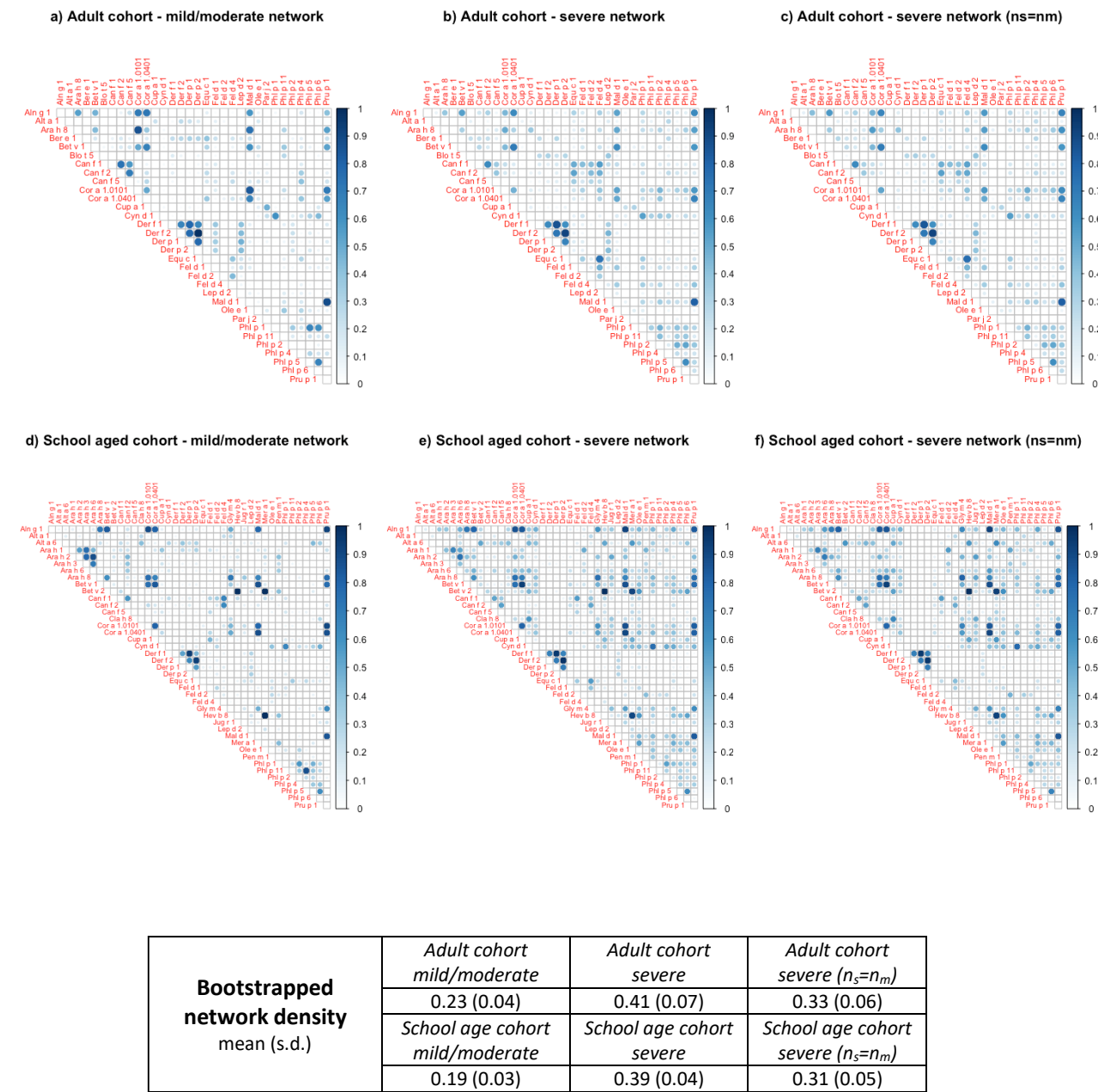


Blue dots represent the pairwise correlation coefficients estimated using the original samples. Red dots indicate the bootstrap mean estimate for the pairwise correlation coefficients, while red lines represent error bars (mean $\pm$ standard deviation).

Panels a and d are referred to the mild/moderate asthma in the adult ($n_m=66$) and school-aged ($n_m=32$) participants, panels b and e indicated the severe asthma in the adult ($n_s=150$) and school-aged ($n_s=73$) participants while c and d panels are estimated using the downsampling procedure on the severe cohorts. In c, bootstrap estimates were obtained from 100 datasets created by sampling 66 out of the 150 severe adult participants. In d, 32 participants were drawn from the 73 severe school-aged participants.

See page 8 for explanation.

Figure E7. Correlation plot representing the network structure and density measure computed through bootstrap approach.



Blue circles represent the absolute value of the Spearman correlation coefficient between pairs of c-slgEs. Estimates of the correlation coefficients and network density were obtained from the 100 datasets defined in the bootstrap procedure.

See page 8 for explanation.

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