BEYOND ATOPY: MULTIPLE PATTERNS OF SENSITIZATION IN RELATION TO ASTHMA IN A BIRTH COHORT STUDY

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In epidemiological studies and clinical practice, children are classified as atopic if they have a positive IgE or skin prick test. By adopting a machine learning approach, we have identified that IgE antibody responses do not reflect a single phenotype of atopy, but multiple different atopic vulnerabilities. We have demonstrated that only one of these atopic classes (Multiple Early Atopic Vulnerability) predicts asthma.

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ABSTRACT

Background: The pattern of IgE response (over time or to specific allergens) may reflect different atopic vulnerabilities which are related to the presence of asthma in a fundamentally different way from current definition of atopy.

Methods: In a population-based birth cohort in which multiple skin and IgE tests have been taken throughout childhood, we used a machine learning approach to cluster children into multiple atopic classes in an unsupervised way. We then investigated the relation between these classes and asthma (symptoms, hospitalizations, lung function and airway reactivity).

Results: A five-class model indicated a complex latent structure, in which children with atopic vulnerability were clustered into four distinct classes (*Multiple Early* [112/1053, 10.6%]; *Multiple Late* [171/1053, 16.2%]; *Dust Mite* [47/1053, 4.5%]; and *Non-dust Mite* [100/1053, 9.5%]), with a fifth class describing children with *No Latent Vulnerability* [623/1053, 59.2%]. The association with asthma was considerably stronger for Multiple Early compared to other classes and conventionally defined atopy (odds ratio [95% CI]: 29.3 [11.1-77.2] vs. 12.4 [4.8-32.2] vs. 11.6 [4.8-27.9] for Multiple Early class vs. Ever Atopic vs. Atopic age 8). Lung function and airway reactivity were significantly poorer amongst children in Multiple Early class. Cox regression demonstrated a highly significant increase in risk of hospital admissions for wheeze/asthma after age 3 years only amongst children in the Multiple Early class (HR 9.2 [3.5-24.0], p<0.001).

Conclusions: IgE antibody responses do not reflect a single phenotype of atopy, but several different atopic vulnerabilities which differ in their relation with asthma presence and severity.

Key words: asthma, atopy, unsupervised clustering, Bayesian inference, machine learning in epidemiology

INTRODUCTION

Atopy is a term describing the tendency to become IgE-sensitized to common allergens to which most people are exposed but don't have a prolonged IgE antibody response(1, 2). In most literature, atopic sensitization is defined as a positive allergen-specific serum IgE (sIgE) test or skin prick test (SPT) to any common food or inhalant allergen(s), and atopic sensitization thus defined remains the single strongest risk-factor for asthma in the western world(3-5). Although evidence from twin and family studies suggests a strong genetic component of atopy(6), more than a decade of intensive work has failed to identify causal associations with genetic variants that are consistently replicated(7). Similarly, the increase in prevalence of atopy since the 1960s suggests an important environmental component, but no environmental exposure has consistently been associated with the development of the atopy(8). We propose that one reason for this is phenotypic heterogeneity, as the diagnostic label of "atopy" may encompass many different phenotypes with different aetiologies, not all of which are associated with symptomatic disease. The conventional epidemiological approach does not reflect the complexities of disease; consequently, reproducible genetic and environmental studies remain elusive.

We speculate that the presence of positive 'allergy test' (either sIgE or SPT) does not equate to the atopic phenotype associated with symptomatic allergic disease. We hypothesize that more useful information may be obtained by identifying common underlying statistical clusters that are characterized by IgE responses. Several recent publications have demonstrated the utility of using a clustering approach in multidimensional data to identify different asthma phenotypes(9-12). Results of latent class analysis on a large dataset collected annually over a 7 year period identified six childhood wheezing phenotypes, two of which had not been described previously(10). Unsupervised hierarchical cluster analysis identified five distinct clinical phenotypes of

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adult asthma, emphasising the need for new approaches for classification of disease

phenotypes(11). We conducted a Principle Component Analysis using answers to

multiple questions relating to wheeze to identify five syndromes of coexisting symptoms

which are likely to reflect different underlying pathophysiologic processes(12).

Ideally, one should aim to model all available data (i.e. multiple measurements at

multiple time points) to identify latent variables which best describe the structure of the

data. Such models would need to be tailored to individual datasets, to precisely encode

prior knowledge and to scale up to large volumes of data. A machine learning approach

using Bayesian inference for unsupervised learning of latent variables to identify

structure within the data is used commonly by computer scientists for problems in many

other fields, and is ideally suited to this task. We applied this approach to a large

complex data-set from a population-based birth cohort in which measures of allergic

sensitization (both sIgE and SPT) to multiple inhalant and food allergens have been

taken throughout childhood, to assign children to atopic latent classes in an

unsupervised way, thus avoiding constraints placed by pre-specified ideas of the nature

and number of such classes. We sought to investigate whether these different latent

atopic classes were related to the presence or absence of asthma, in ways that are

fundamentally different from current diagnostic categories.

METHODS

Study design, setting and participants

Manchester Asthma and Allergy Study is a population-based birth cohort(13-16) (detailed description in Online supplement). Participants were recruited prenatally and followed prospectively, attending review clinics at ages 1, 3, 5 and 8 years. The study is registered as ISRCTN72673620 and approved by the Local Research Ethics Committee (04/Q1403/45). Written informed consent was obtained from all parents, and children gave their assent.

Definition of variables

Atopic sensitization: We ascertained atopic sensitization by skin-prick tests (Hollister-Stier, VA, USA) and measurement of sIgE (ImmunoCAP, Phadia, Uppsala, Sweden) at each time point to a panel of inhalant and food allergens (summarized in Table E2). We defined allergen-specific sensitization as mean wheal diameter at least 3mm greater than the negative control and/or specific IgE≥0.35 kU/l. The conventional definition considered a child to be atopic if (s)he had allergen-specific sensitization to at least one allergen. Children with any positive test (SPT or sIgE) at any time point were considered to be "Atopic ever".

Wheeze: A validated questionnaire was interviewer-administered to collect information on parentally-reported symptoms, physician-diagnosed illnesses and treatments received. Current wheeze was defined as wheeze in the past 12 months. Based on prospectively collected data, children were assigned to the following wheeze phenotypes: *No wheezing*–no wheezing ever at any follow-up by age 8 years; *Transient early wheezing*–wheezing only during the first 3 years; *Late-onset wheezing*–wheezing started after age 3 years; *Persistent wheezing*–wheezing during the first 3 years, wheezing in the previous 12 months at ages 5 and 8 years. *Intermittent wheezing*–wheezing at one time point during the first 5 years, wheezing at age 8 years.

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Lung function: We measured specific airway conductance (sG_{aw}) using whole-body

plethysmography at age 3 and 5 years(15, 17) and FEV_1 using spirometry at age 8 years

(online supplement).

Airway hyper-reactivity (AHR-methacholine challenge): Assessed at age 8 years in a 5-

step protocol using quadrupling doses of methacholine (Table E1) according to ATS

guidelines(18). A dose-response ratio was calculated and transformed as previously

described(19).

Asthma: We used a stringent epidemiological definition of asthma at age 8 years as

symptomatic airway hyper-reactivity (i.e. presence of current wheeze and positive

methacholine challenge)(20).

Hospital admission for asthma/wheeze: A trained physician reviewed the written and

computerized primary care medical records and extracted the data on hospitalizations for

wheeze or asthma(21).

Data analysis

We took a machine learning approach to the data analysis. Using a Hidden Markov

Model (HMM)(22), all available SPTs and sIgEs (collected at review clinics at ages 1, 3,

5 and 8 years) were used to infer one multinomial latent variable per child so as to

cluster the children in an unsupervised manner into different sensitization classes (Figure

1). At the core of the model are the 4 dichotomous latent *Acquired Sensitization*

variables for each allergen which are linked together in a Markov chain across the 4 time

points. We inferred time-dependent transition probabilities (i.e. the probabilities of

gaining and losing sensitization at each age) which were assumed to be shared by all

children in each sensitization class, but were allowed to differ between classes.

Inference: Inference was performed using Infer.NET

(http://research.microsoft.com/infernet), a Microsoft-owned library for large-scale

Bayesian inference, which is now freely available for research purposes. We used

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Infer.NET to infer the false and true positive rates of the SPTs and sIgEs, missing values, the class-specific state-transition probabilities, the observation (emission) probabilities, the acquired sensitization variables and also the sensitization class for each child. An approximate Bayesian inference method (Variational Message Passing-VMP)(23) was used to perform the inference in an efficient manner.

Robustness and reproducibility: Robust and reproducible clustering was achieved by training the sensitisation HMM multiple times on different subsets of children and selecting the clustering which both gave good predictions on the remaining children and which was robust to the subset of children selected. Reproducibility was confirmed by computing confusion matrices between different replications of the clustering process (see detailed description and confusion matrices in Online supplement).

Handling the missing data: Variables corresponding to missing data values were included in the model but treated as unobserved. Distributions over these missing data values were computed using VMP based on the available measurements.

Sensitization Class: This is a multinomial variable indicating to which sensitization class each child belongs (out of between 2 and 5 classes). The model assumes that each child belongs to one of these classes. We investigated a two-class and a five-class model (see Online supplement). No assumptions were made about the nature of each class. During inference, a distribution was computed for each child giving the probability of their belonging in each class. For further analysis, we assumed the child belonged to the highest probability class.

We then investigated the association between the classes we had inferred in a completely unsupervised manner and the clinical outcomes using appropriate statistical methods (chi-squared test, logistic regression, Kaplan-Meier univariate estimates and Cox regression multivariate estimates of survival/clinical status). Results are presented as the main effect with 95% confidence intervals (CI).

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RESULTS

Of the 1186 participants with any evaluable data, 133 who were randomized into the primary prevention study(24, 25) were excluded from the analysis of the association between clinical outcomes and inferred sensitization class. All remaining children with available clinical outcomes were included at each time point (Table E2). There was no difference in parental history of allergic disease between children with or without missing data on clinical outcomes (data available on request).

At age 8 years, 18% (163/905) of children had current wheeze; 13.7% (124/905) were persistent wheezers and 8.1% (45/555) had asthma (symptomatic AHR). Data collected from primary care records revealed that 16.7% (136/814) of children had been admitted to hospital with wheezing/asthma on at least one occasion during the first eight years of life. Using conventional definitions, of 827 children who had either SPT or sIgE measured at age 8 years, 322 (38.9%) were considered atopic; 1029 children had at least one assessment of atopic status throughout the duration of the follow-up, of whom 441 (42.9%) were considered to be atopic ever.

Sensitization Class

The structure of the classes was inferred in a completely unsupervised manner using all data (SPT and sIgE) from all four time points with missing data inferred using Variational Message Passing(23) (i.e. we did not assume beforehand how the children will be clustered, the unsupervised learning algorithm automatically discovered the latent structure) under the assumption that data was missing at random.

We present the results with the sensitization state being considered to have two classes (best reflecting a conventional assignment to atopy/no atopy), and five classes (which better captured the underlying structure of the data).

Two-class model: The children were assigned as having either a Latent atopic vulnerability (280/1053, 26.6%) or No latent atopic vulnerability (773/1053, 73.4%)

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(Figure E1); 161 of 440 children (36.6%) who were sensitized on at least one occasion were classified as not vulnerable. Compared with conventional definitions, there was complete agreement in 86.0% (Atopy age 8 years) and 84.0% of cases (Atopy ever).

Five-class model: This model indicated a more complex latent structure incorporating time-varying probabilities of the gain and the loss of sensitization (Figure E2). The children with latent atopic vulnerability were clustered into four distinct sensitization classes, which, based on our interpretation of the characteristics of each class, we assigned as the following:

(1) *Non-dust Mite Atopic Vulnerability* (100/1053, 9.5%)

(2) *Dust Mite Atopic Vulnerability* (47/1053, 4.5%)

(3) *Multiple Late Atopic Vulnerability* (171/1053, 16.2%)

(4) *Multiple Early Atopic Vulnerability* (112/1053, 10.6%)

The final class comprised children with *No Latent Vulnerability* (623/1053, 59.2%). In this model, 61/440 (13.9%) children who were atopic ever were classified as having No Latent Vulnerability; amongst 322 children who were atopic at age 8, 36 (11.2%) were classified as having No Latent Vulnerability. All but one child in the Multiple Early class were atopic at age 8 years using conventional definition, but the Multiple Early class comprised only 28.0% of those atopic at age 8 years (Table E3).

To determine the appropriate number of classes, differing numbers of clusters were tested as to their ability to predict the sensitization state of children where that state was artificially made missing. This imputation process suggests that between 3 and 5 clusters were justified (Figure E3 in the Online supplement) and so a 5-class model was selected since it exposed the most information about the structure of the data set. The choice of 5 classes was also validated when considering the confusion matrices found when the clustering process was replicated (see Tables E4 and E5 in the Online supplement). For the 5-class case, there was very little confusion between different clusterings, indicating

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that the 5-class clustering is robust. For example, for the Multiple-Early class 111 of the

112 children assigned to this class in the reference clustering were repeatedly assigned

to the same class in other 5-class clusterings.

Sensitization class and clinical outcomes

We went on to ascertain relationships between atopy defined conventionally (atopic ever,

atopic at age 8 years), the novel latent classes (two-class and five-class models) and

clinical phenotypes associated with asthma (current wheeze, persistent wheeze,

symptomatic AHR, hospital admission with asthma/wheeze), adjusting for gender. The

results are presented in Figures 2 and E4 and Table E6. The relationships with clinical

outcomes for ever atopic, atopic at age 8 years and the two-class model were not

materially different. However, for the five-class model, it was apparent that there were

marked differences between the four classes of atopic vulnerability, in that the

associations with clinical outcomes were considerably stronger for Multiple Early

compared to other classes, the two-class model and conventionally defined atopy (e.g.

for symptomatic AHR, odds ratio [95% CI]: 29.3 [11.1-77.2] vs. 12.4 [4.8-32.2] vs. 11.6

[4.8-27.9] vs. 9.2 [4.5-18.9] for Multiple Early class vs. Ever Atopic vs. Atopic age 8 vs.

Latent Atopic vulnerability-two-class model; Table E6). There was a very strong

association between Multiple Early class and persistent wheezing (12.9 [6.8-24.4].

These finding indicated that IgE antibody responses do not reflect a single phenotype of

atopy, but several atopic vulnerabilities which differ in their relationship with asthma. To

further test this, we proceeded to investigate the relationship between markers of asthma

severity (objective measures of lung function and airway reactivity, hospital admissions)

within the five-class model.

Lung function, airway reactivity and hospital admissions in the five-class model

In the univariate analysis we found a significant association between sG_{aw} at age 3 and 5

years, FEV_1, FEV_1/FVC ratio and DRR at age 8 years and five-class latent variable

(Table E7). Multiple comparison test (Tukey) revealed that for all measures of lung function and airway reactivity, lung function was significantly poorer amongst children in Multiple Early class compared to those with No Latent Atopic Vulnerability, with little differences between the other three classes and the No Latent Vulnerability class (Table E7, Figures E5-E9).

In the multiple ANOVA models adjusted for gender, maternal smoking and wheezing (sG_{aw}, FEV_1/FVC ratio and DRR) and gender, wheezing, maternal smoking and height (FEV_1), children in the Multiple Early class had significantly poorer lung function compared to those in the No Latent Vulnerability class (sG_{aw} age 3, p=0.02; sG_{aw} age 5, p=0.01; age 8 FEV_1, FEV_1/FVC ratio and DRR: p<0.001; Table 1). There were no significant differences in lung function between the other three classes and the No Latent Vulnerability class, apart from airway reactivity (DRR) being significantly higher in the Multiple Late class (p=0.05, Table 1).

Kaplan-Meier plots demonstrating the age of the first hospital admission with wheeze/asthma in relation to the five-class model are presented in Figure 3A. The results of a Cox regression that included the five classes, gender and maternal smoking indicated a highly significant association between the risk of hospital admission and five-class model (P<0.0001), with a risk of hospital admission increasing amongst children in the Multiple Early class (hazard ratio (HR) [95% CI], 5.1 [2.8-9.3], p<0.001), Dust Mite class (3.4 [1.4-8.2.7], p=0.004) and Non-dust Mite (2.5 [1.2-5.3, p=0.02]), but not those in the Multiple Late class (1.3 [0.6-2.9, p=0.4]). In order to remove the effect of hospital admission for wheeze caused only by early-life virus infections, we have reanalyzed the data on the time to the first hospital admission with wheeze/asthma amongst children who had a hospital admission after age 3 years (Kaplan-Meier plot, Figure 3B). Cox regression demonstrated a highly significant increase in risk only amongst children in the Multiple Early class (HR 9.2 [3.5-24.0], p<0.001).

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Discussion

Principal findings

We have demonstrated that genuinely novel phenotypes of atopy can be revealed by adopting a machine learning approach which takes full advantage of the data-intensive environment provided by a birth cohort study. Machine learning techniques identified latent structures within the data which may accurately reflect "unbiased" phenotypes of atopy and avoid constraints of investigator-imposed classifications. Our results suggest that IgE antibody responses do not reflect a mere presence or absence of atopy, but instead multiple atopic vulnerability classes. The validity of these classes was tested by examining their relations to the presence and severity of asthma and measures of lung function, which demonstrated that different atopic vulnerabilities (i.e. different phenotypes of atopy) differ markedly in their relationship with asthma. It is not the presence or absence of specific IgE antibodies, but the pattern of the response (age at development, type and number of specific allergens involved) that has a fundamental effect on the clinical expression of asthma. It is of note that less than a third of children who would have been considered atopic at age 8 years using conventional diagnostic criteria were in the class most strongly associated with asthma (Multiple Early), whereas there was little appreciable increase in risk of asthma amongst those in the other classes. We propose that positive specific IgE or positive skin prick tests do not equate to atopy, but should be viewed as intermediate phenotypes of a true atopic vulnerability. This may be analogous to asthma, where a collection of intermediate phenotypes can objectively be measured (e.g. peak flow variability, airway hyper-reactivity, or an obstructive spirometric pattern), but individually their presence does not equate to a diagnosis of asthma(26).

Strengths and limitations

We recognize that Bayesian learning applied to a longitudinal dataset is exploratory and hypothesis generating, rather than confirmatory. However, the classes we identified

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seem intuitively correct (i.e. have face validity), and we have demonstrated significant

relationships with asthma, lung function and airway reactivity (i.e. have content validity).

We acknowledge the computational complexity and intensity of this analysis. It is

important to emphasise that this is not a simple "black box" or the "data-mining"

approach; the analysis is informed by and capitalizes on the wealth of knowledge which

already exist on the problem. Once determined, the classes may become clinical

outcome variables in their own right and can be used in further analyses. Such

dimensionality reduction reduces the need for repeated cross-sectional analysis, as often

seen in longitudinal datasets, and reduces the need for multiple testing.

A strength of our model is that is generative, enabling missing measurements to be

handled meaningfully. A further strength of the study is that the prevalence of atopic

sensitization among the parents of the children in our cohort(27, 28) is similar to that of

young adults in the UK(29), suggesting that the cohort is representative of the general

population. However, it would be of great value and importance to explore similar

approaches in the other large birth cohort studies. We recognize that the number of

relevant classes might be different to the five reported here, and further replications

would be desirable.

We acknowledge that our findings do not have an immediate impact on clinical practice.

However, we argue that our approach to data analysis will advance our understanding of

the etiology of asthma.

Interpretation of the study

The study of asthma at the population level to date has been predominantly hypothesis

driven, often focussing on ill-defined, over-simplified phenotypes, using reductionist

approaches to causality. Whilst identifying some major independent determinants of

disease, this approach does not fully reflect the complexity of disease. Furthermore, it

fails to take full advantage of the richness of the available datasets collected in birth

cohort studies. We propose that one of the reasons for contradictory findings reported by

a number of genetic and environmental studies aiming to elucidate the mechanisms of

asthma is phenotypic heterogeneity and poor phenotype definition.

In epidemiological studies of allergic diseases investigators collect large volumes of

information, often at multiple time points. Data on sensitization collected over a time

series may be used to assign a phenotype based on distinctive patterns of results (e.g.

early, late or very late IgE sensitization(30), mono- or poly-sensitization(30), remission or

persistence(30), declining, flat or increasing pattern(31)). These categories are often

imposed by the investigators, and do not necessarily reflect the substructure within the

dataset. Ideally, one should aim to model all the data to identify a single multinomial

latent variable which best describes the structure of the data. By using a machine

learning approach, we have demonstrated that diagnostic label of "atopy" encompasses

several different phenotypes which may have different etiologies.

Since these classes better reflect the presence and severity of disease, we propose that

further efforts be made to develop new diagnostic tests that will allow clinicians to better

differentiate between the true atopic classes than the currently available tests. Current

reagents for skin testing and specific IgE measurement are based on whole extracts

containing multiple proteins, many of which are recognized by IgE antibodies(32) (e.g.,

for dust mite *D pteronyssinus* there are >20 recognized allergens(33)). We speculate

that response to different individual proteins within an allergen may be associated with

different atopic classes (and consequently different clinical phenotypes). Utilization of this

component-based approach may offer novel diagnostic possibilities and improve the

value of allergy diagnosis, allowing practicing physicians more accurate diagnosis based

on a single measurement at the time of presentation.

We have previously extended the observation that sensitization to inhalant allergens is a

risk factor for wheezing by demonstrating that the level of specific IgE antibodies offers

more information than just the presence of IgE(34). The current paper introduces the concept of different atopic vulnerabilities with distinct characteristics in terms of their association with disease. We have demonstrated that only one of the atopic classes (Multiple Early) predicts asthma. This may in part explain the huge variability in the relationship between "atopy" and asthma observed in the different parts of the world (e.g. the fraction of wheeze attributable to sensitization ranges from 0% in Turkey to 94% in China(35)), as the relative contribution of different atopic vulnerabilities to "atopy" may differ in each location consequent to differences in genetic predisposition and environmental exposures.

Conclusions

Viewing atopic sensitization as a dichotomous trait in its relationship to asthma may be an oversimplification. Our data suggest that IgE antibody responses do not reflect a single phenotype of atopy, but several different atopic vulnerabilities which differ in their relationship with asthma. One of these atopic vulnerability classes (Multiple Early, comprising approximately one quarter of children who would be considered atopic using conventional definition) predicts not only the presence, but also persistence and severity of childhood asthma.

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LEGEND FOR FIGURES

Figure 1. Graphical representation of a Hidden Markov Model: all available SPTs and sIgEs were used to infer one multinomial latent variable per child to cluster the children in an unsupervised manner into different sensitization classes

Figure 2. Association between atopy defined conventionally (atopic ever, atopic at age 8 years), the novel latent classes (two-cluster and five-cluster models) and clinical phenotypes associated with asthma ascertained by age 8 years: regression analysis adjusted for gender. Results expressed as adjusted odds ratios and 95% confidence intervals

Figure 3. Kaplan-Meier Estimates of Cumulative Risk of hospital admission with wheeze/asthma during the first 8 years of life stratified on Five-class model

Panel A: Age at first hospital admission for children with hospital admission with wheeze/asthma at any age

Panel B: Age at first hospital admission amongst children who had a hospital admission after age 3 years

Table 1. Lung function (specific airway conductance at ages 3 and 5 years; FEV_1 and FEV_1/FVC ratio at age 8 year) and airway reactivity (dose-response ratio – DRR) in children with different latent atopic vulnerabilities in the five-class model

Estimated marginal means and 95% CIs from multiple ANOVA models adjusted for gender, maternal smoking and wheezing (sG_{aw} and FEV_1/FVC ratio) and gender, wheezing, maternal smoking and height (FEV_1)

	No Latent atopic vulnerability	Non-dust Mite	Dust mite	Multiple late	Multiple early
sGaw age 3, kPa/s	0.90 0.87-0.92	0.90 0.83-0.98	0.97 0.89-1.07	0.88 0.83-0.94	0.83 0.78-0.88
sGaw age 5, kPa/s	0.84 0.82-0.87	0.82 0.79-0.86	0.84 0.79-0.90	0.85 0.82-0.88	0.81 0.78-0.84
FEV_1 age 8, (L/sec)	1.58 1.54-1.62	1.62 1.54-1.70	1.60 1.54-1.66	1.57 1.53-1.61	1.47 1.42-1.52
FEV_1/FVC age 8 (%)	86.01 84.76-87.25	86.86 84.38-89.34	85.97 84.02-87.92	85.32 84.08-86.56	83.82 82.40-85.24
DRR age 8	8.40 6.61-10.49	6.33 3.61-10.42	7.64 5.07-11.28	9.73 7.55-12.52	13.32 9.96-18.03

Figure 1

Figure 2

Figure 3A

(a)

Risk of hospital admission vs. Age of the first hospital admission for wheeze/asthma (days)

Clusters:
- No latent atopic vulnerability
- Atopic vulnerability, not dust mite
- Atopic vulnerability, dust mite
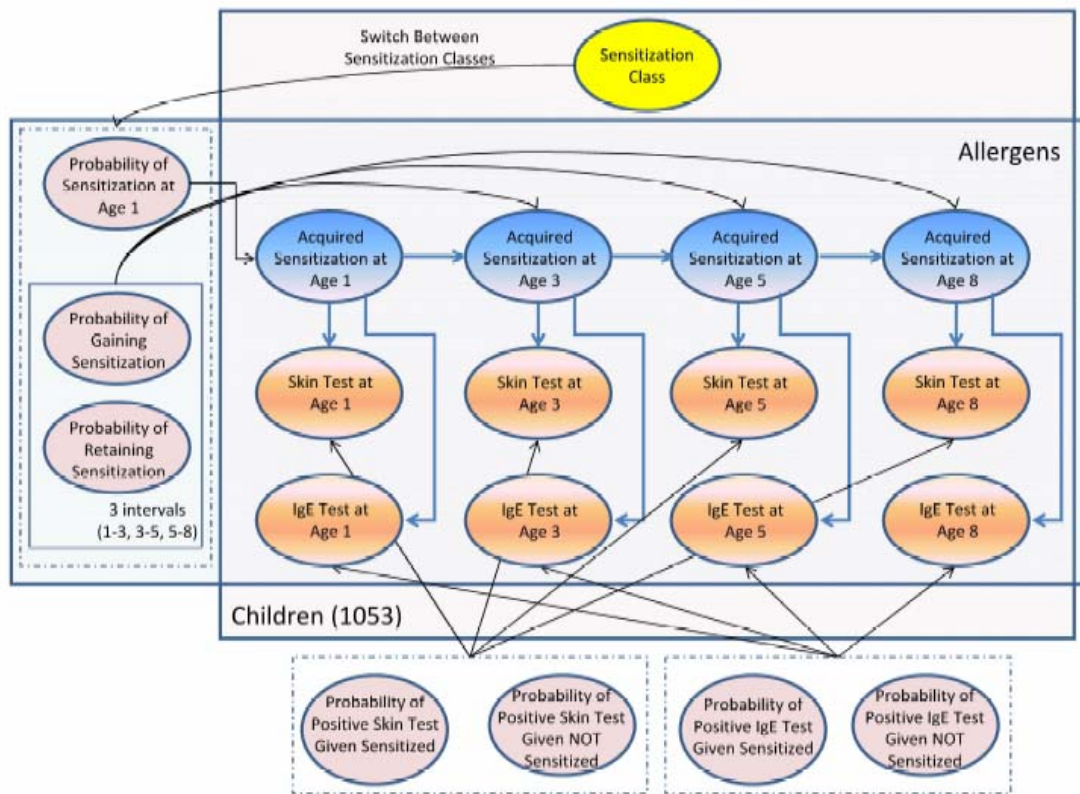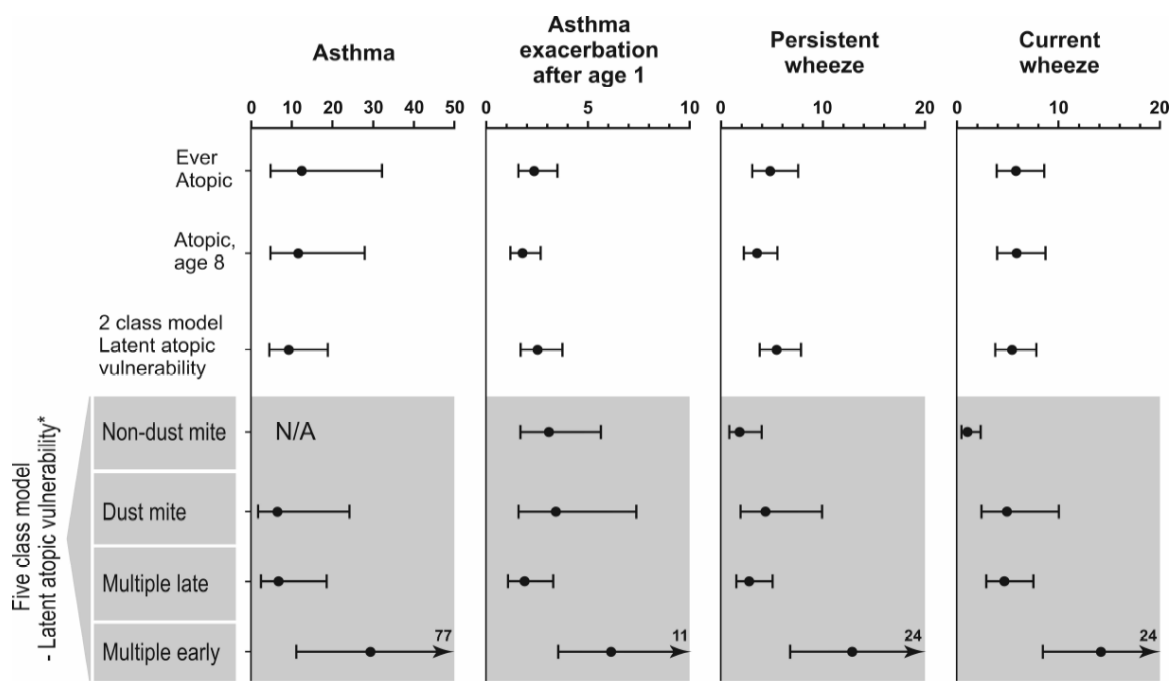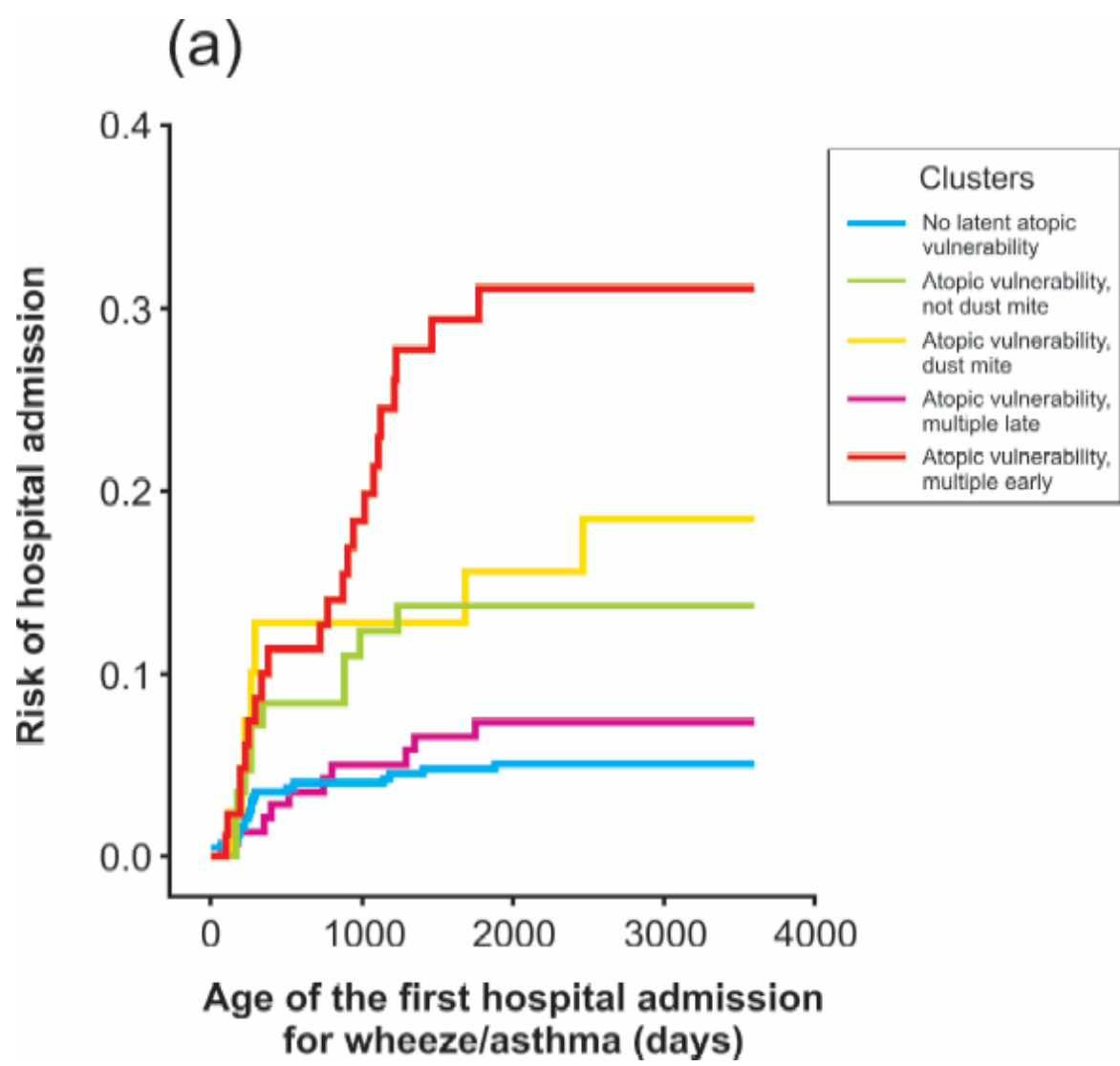- Atopic vulnerability, multiple late
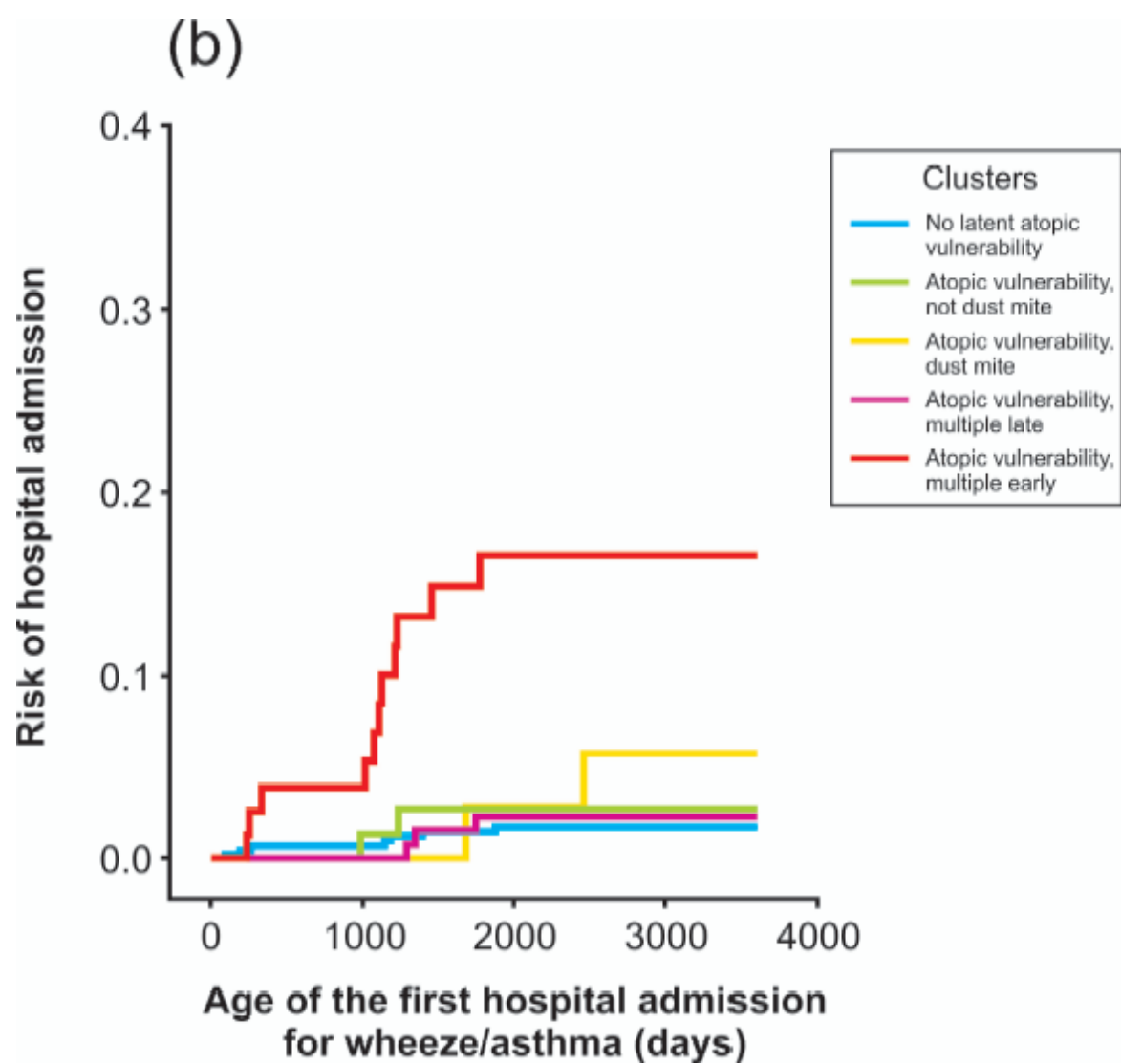- Atopic vulnerability, multiple early

Figure 3B

BEYOND ATOPY: MULTIPLE PATTERNS OF SENSITIZATION IN RELATION

TO ASTHMA IN A BIRTH COHORT STUDY

ONLINE DATA SUPPLEMENT

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METHODS

Study populations

The Manchester Asthma and Allergy Study is an unselected, population-based prospective study which follows the development of asthma and other atopic disorders in a cohort of children. The setting is the maternity catchment area of Wythenshawe and Stepping Hill Hospitals, comprising of 50 square miles of South Manchester and Cheshire, UK, a stable mixed urban-rural population. Study was approved by the Local Research Ethics Committee. Informed consent was obtained from all parents.

Screening & Recruitment

All pregnant women were screened for eligibility at 'Booking' antenatal visits (8th-10th week of pregnancy). The study was explained to the parents, and informed consent for initial questionnaires and skin prick testing was obtained. Both parents completed a questionnaire about their and their partner's history of asthma and allergic diseases and smoking habits.

If the pregnant woman's partner was not present at the antenatal clinic visit, an invitation was sent for him to attend an open-access evening clinic for skin prick testing and questionnaire. Once both parents had completed questionnaires and skin prick testing, a full explanation of the proposed future follow-up for the child was given.

Of the 1499 couples who met the inclusion criteria ($\leq$10 weeks of pregnancy, maternal age $\geq$18 years, questionnaire and skin test data available for both parents), 288 declined to take part in the study.

Of the 1186 participants with any evaluable data, 133 who were randomized into the primary prevention study were excluded from the analysis of the association between clinical outcomes and inferred sensitization class.

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Follow-up

The children have been followed prospectively, and attended review clinics at ages 1, 3, 5 and 8 years (±4 weeks). At age 1 year, only high and low risk children were invited to attend for clinical follow up. At all other time points for all other measures all children were invited to participate.

Definitions of exposures and outcomes

Atopic sensitization

Atopic sensitization was ascertained by skin prick testing at age 1, 3, 5 and 8 years (*D pteronyssinus*, cat, dog, grasses, moulds, milk, egg [Bayer, Elkahrt, IN, USA]). We defined sensitization as a mean weal diameter 3mm greater than negative control to at least one of the allergens tested. We also measured specific serum IgE to mite, cat, dog, grasses, milk, egg and peanut by ImmunoCAP^{TM} (Phadia, Uppsala, Sweden) collected at the four time points. We defined allergen-specific sensitization as mean wheal diameter at least 3mm greater than the negative control and/or specific IgE≥0.35kU/l. Conventional definition considered a child to be atopic if he/she had allergen-specific sensitization to at least one allergen. Children with any positive test (skin test or IgE) at any time point were considered to be atopic ever, and those with no evidence of sensitisation as never atopic.

Wheeze

A validated ISAAC questionnaire was administered by a trained interviewer to collect information on parentally reported symptoms, physician-diagnosed illnesses and treatments received.

Lung function

At age 3 and 5 years we carried out measurements of specific airway conductance (sG$_{aw}$) to assess airway function in all children who were willing to cooperate. Measurements were made using a constant volume whole body plethysmograph

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(Masterscreen Body 4.34; Jaeger, Würzburg, Germany). Flow and volume were measured with a heated differential pressure screen-type pneumotachograph with a resistance of 0.036 $kPa^{-1}{\cdot}s$ and a dead space of 160mls. Pressure measurements were made with a pressure transducer (Nr.660.99007; Hube Control AG, Wuerenlos, Switzerland) with an input range of ±100 Pa, a resolution of 0.05 Pa and a linear response up to 10 Hz. The plethysmograph was calibrated daily. Sensors in an ambient unit supplied with the plethysmograph recorded ambient data on temperature, humidity and barometric pressure. The pneumotachograph was volume calibrated according to the American Thoracic Society recommendations using a 2 L syringe at flow rates of 0–1.5, 1.5–5 and >5 l/s. The half value period was calibrated to ensure a specific leakage in the box of 4-7 seconds.

The pressure transducer was calibrated using a 50 mL motor driven piston pump to generate sinusoidal variations of plethysmographic pressure. Electronic body temperature, pressure, and saturation (BTPS) compensation was applied throughout, using a time-shift of 60 ms.

sG_{aw} is measured by a single-step procedure from the simultaneously measured changes of respiratory flow and changes of plethysmographic pressure, omitting the measurement of TGV. Measurements were carried out during tidal breathing using a facemask, which was adapted by fitting a standard paediatric facemask with a non-compressible mouthpiece made from silicone tubing. The end of the tubing was made rigid with an aluminium splint. The purpose of this was to maintain stable airway opening, prevent nose breathing and support the cheeks. The procedure was explained to the accompanying adult and the use of the facemask demonstrated to the child. The children were encouraged to sit in the plethysmograph alone but if they refused, the accompanying adult, usually a parent, accompanied the child in the plethysmograph

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cabinet with the child seated on their knee. The door of the plethysmograph was closed

and the subject asked to breathe through the facemask.

Children were encouraged to breathe at a rate of 30-45 breaths per minute. If a parent

accompanied the child, the adult was asked to inhale and hold their breath for

approximately 20 seconds. sG_{aw} measurements were made once a stable breathing

pattern had been re-established. Once a stable breathing pattern was established, at

least three measurements of sG_{aw} were performed, and each was calculated from the

means of 5 consecutively measured technically acceptable loops (each child performed

at least 15 loops). The median of these 3 measurements of *effective* sG_{aw} was used in

the analysis. The measured values of sG_{aw} were corrected for the influence of the

pneumotachograph screen and for the volume displacement caused by the subject (or

subject + parent).

Children were asymptomatic at the time of assessment of lung function.

Airway reactivity - Methacholine Challenge

Airway reactivity was assessed using a 5 step protocol performed according to ATS

guidelines. The methacholine (acetyl-β-methylcholine chloride) solutions were prepared

with sterile normal saline (Stockport Pharmaceuticals, UK). Quadrupling doses of

methacholine (0.0625 – 16.0 mg/mL) were delivered to subjects via a DeVilbiss 646

nebuliser (Sunrise Medical HHG, Somerset, PA) and a KoKo dosimeter (Pulmonary Data

Services, Doylestown, PA) calibrated to deliver 0.009 mL per 0.6s actuation. The dosing

schedule is described in Table E1. The test was explained to the subject and the best

baseline FEV_1 measurement performed in the wedge bellow spirometer was recorded.

The predicted FEV_1 was calculated and if the measured values was <1.0 l or less than

60% predicted the test was not performed. If the child was unable to produce

reproducible FEV_1 measurements the procedure was abandoned. Assuming the child

met the criteria to continue, the 20% drop from the child's baseline value was calculated

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so that the operator would know when to stop the test. After normal tidal expiration to

FRC (functional residual capacity) the dosimeter was triggered at the onset of

inspiration, and the subject asked to inhale slowly and deeply over 6 s. Subjects were

instructed to hold their breath for 5 s, followed by slow exhalation for 5 s. FEV_1 was

measured 30 and 90 seconds after 5 inhalations of each dose of methacholine. The

challenge was stopped when either a 20% fall in FEV_1 was observed, or the maximum

methacholine concentration had been administered with a fall of less than 20% in FEV_1.

Children were categorized as having a positive or a negative challenge based on

whether or not they reached a 20% fall in FEV_1 by the final dose of the challenge

(16mg/ml).

 Hospital admission for asthma/wheeze:: The UK health care system ensures that a

single medical record is held by the primary care physician which provides a full record

of all encounters with health professionals. GPs are legally required to maintain accurate

records of all medical encounters of their patients, including retention of all records of

hospital encounters. A trained physician reviewed the written and computerized primary

care medical records and extracted the data on hospitalisations for wheeze or asthma.

Data analysis

We took a machine learning approach to the data analysis. Using a Hidden Markov

Model, the available physiological measurements of skin prick tests (SPTs) and serum

specific IgE tests (SITs) to a panel of allergens were used to infer one multinomial latent

variable per child to cluster the children in an un-supervised manner into different

sensitization classes (the model is shown in Figure 1).

At the core of the model are the 4 binary latent variables for each allergen labelled

'Acquired Sensitization' and these are linked together in a Markov chain across the 4

time points. We inferred time-dependent transition probabilities (i.e. the probabilities of

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gaining and losing sensitization at each age) which were assumed to be shared by all

children in each sensitization class, but differing between classes. In our model, for

ease of inference, we placed *conjugate* priors on all the variables that were to be inferred

- using beta priors as the variables of interest were binary and beta is conjugate to the

binomial distribution [E1]. We also observed that our results were insensitive to the choice

of hyperparameters (the parameters that define the prior distributions).

Inference

Inference was performed using Infer.NET (http://research.microsoft.com/en-

us/um/cambridge/projects/infernet/), a Microsoft-owned library of statistical algorithms for

large-scale Bayesian inference. We inferred the false and true positive rates of the SPT

and IgE tests, missing SPT and IgE values, the state-transition and observation

(emission) probabilities, the acquired sensitization variable and finally, the sensitization

class. An approximate Bayesian inference method (Variational Message Passing-

VMP)[18] was used to perform the inference in an efficient manner.

Handling the missing data

Variables corresponding to missing data values were included in the model but treated

as unobserved. Distributions over these missing data values were also computed using

VMP based on the available measurements. This approach assumes that the missing

values are missing completely at random (MCAR).

Training and validation data sets with multiple imputations and assessment of the
robustness of the clustering

To determine the appropriate number of classes, differing numbers of clusters were

tested as to their ability to predict the sensitization state of children where that state was

artificially made missing. The process starts by randomly dividing the data so that 80%

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formed a training set and the remaining 20% formed a validation set. Using the training

set, a clustering is learned by computing posterior distributions over the parameters of

the sensitisation HMM using the variational message passing (VMP) inference algorithm.

Hence, for each cluster, distributions were learned over the probability of initial

sensitisation and the probabilities of gaining and retaining sensitisation for each cluster.

In addition, common distributions were learned over probabilities of positive tests given

sensitisation or lack of sensitisation.

This learned clustering was validated using an imputation experiment, where removed

data values were predicted under the learned clustering. In each run of the experiment,

the posterior distributions learned in the initial clustering (using the training data) were

used as corresponding prior distributions in a new clustering model, used to cluster the

validation data. 20% of the values in the validation data were removed at random and

then predicted using the posterior distributions of the new clustering model.

 Because VMP can be sensitive to its random initialisation, the training process was

repeated for 10 different such initialisations and the clustering with the best score

selected. To avoid bias due to the training/validation splits itself, the entire process was

repeated for 10 different random training/validation data splits. The imputation score was

computed as the sum of the log probability of the removed values under their inferred

posterior distributions, averaged across the 10 runs. Results for models with 1–7 clusters

are shown in Figure E3; note that the baseline in the figure has been adjusted so that the

(unique) single cluster model has a score of zero.

The robustness and repeatability of the clustering process to small changes in the data

set was assessed by comparing the clusterings given by the 10 random

training/validation splits. For each clustering with a given number of clusters, a

confusion matrix was computed indicating how frequently children were assigned to the

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same cluster in the other nine clusterings. The clustering with the best confusion matrix (defined as the matrix with the largest sum of diagonal elements) was selected as the reference clustering for the given number of clusters. The confusion matrices for the reference clusterings with 2 and 5 clusters is shown in Tables E4 and E5, demonstrating no confusion between clusters in the 2-class case and very little confusion between the clusters in the 5-class case.

Sensitization Class

This is a multinomial variable indicating to which sensitization class each child belongs (out of between 2 and 5 classes). The model assumes that each child belongs to one of these classes. We investigated a two-class and a five-class model. The number of clusters was chosen as the maximum that contained sufficient observations for a statistically credible inference, and had a structure that was plausible. For example, if the number of clusters was set to 6, the number of children in some clusters will be very small. In some cases, some clusters may even not contain a single individual.No assumptions were made about the nature of each class. We assumed only that children in different classes have different state-transition probabilities, but they have the same observation probabilities across time. During inference, a distribution was computed for each child giving the probability of their belonging in each class. For further analysis, we assumed the child belonged to the highest probability class - a maximum a-posteriori approach.

We then investigated the association between the clinical outcomes and the classes which had been inferred in a completely unsupervised manner. The relation between each class and relevant clinical outcomes were tested in models that adjusted for known confounding factors, effect modifiers and multiple testing.

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TABLES

Table E1. Dosing schedule for methacholine challenge

Step	*Methacholine concentration (mg/ml)*	*Methacholine dose (mg)*	*Cumulative methacholine dose (mg)*
1	0.0625	0.003	0.003
2	0.25	0.011	0.014
3	1.0	0.045	0.059
4	4.0	0.180	0.239
5	16.0	0.720	0.959

Table E2. Number of children with available outcomes at each time point

	1 year	3 years	5 years	8 years
Atopic status				
Skin prick tests (Mite, cat, dog, grass, milk, egg)	377	857	849	817
Skin prick tests (peanut)				815
Specific IgE to mite, cat, dog, milk, egg	186	175	534	511
Specific IgE to grass pollen, peanut			534	511
Clinical outcomes				
Current wheeze (questionnaire)	1020	981	950	905
Wheeze phenotypes			950	905
Asthma exacerbations (primary care records)				814
Lung function and airway hyperreactivity				
sG_{aw} (whole-body plethysmography)		560	829	
FEV_1 (spirometry)				695
Methacholine challenge				555

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Table E3. Relationship between conventional definition of atopic sensitisation at age 8 years and latent atopic vulnerabilities in the five-class model

	Five-class model					Total
	No latent vulnerability	Non-dust mite	Dust Mite	Multiple Late	Multiple Early	
Not atopic	406	79	9	9	1	504
	80.6%	15.7%	1.8%	1.8%	0.2%	100.0%
Atopic	36	17	38	141	90	322
	11.2%	5.3%	11.8%	43.8%	28.0%	100.0%
Total	442	96	47	150	91	826
	53.5%	11.6%	5.7%	18.2%	11.0%	100.0%

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Table E4. Robustness and confidence: The confusion matrix for the reference clusterings for 2 clusters. The rows corresponds to cluster assignments under the reference clustering (numbers in parentheses give the number of children assigned to the cluster) and the columns correspond to average cluster assignments, along with their standard deviations, computed over the remaining training/validation 9 clusterings. The-matrix is diagonal, indicating that all clusterings are in complete agreement.

	No Latent Vulnerability	**Latent Vulnerability**
No Latent Vulnerability (773)	773.0 ± 0.0	0.0 ± 0.0
Latent Vulnerability (280)	0.0 ± 0.0	280.0 ± 0.0

Table E5. Robustness and confidence: The confusion matrix for the reference clusterings for 5 clusters. The rows corresponds to cluster assignments under the reference clustering (numbers in parentheses give the number of children assigned to the cluster) and the columns correspond to average cluster assignments, along with their standard deviations, computed over the remaining training/validation 9 clusterings. This matrix indicates that there is little disagreement between clusters.

	No Vulnerability	**Non-Dust Mite**	**Dust Mite**	**Multiple Late**	**Multiple Early**
No Vulnerability (623)	617.8 ± 8.9	4.7 ± 9.5	0.6 ± 2.3	0.0 ± 0.0	0.0 ± 0.0
Non-Dust Mite (100)	15.8 ± 44.6	83.4 ± 45.6	0.0 ± 0.0	0.8 ± 1.3	0.0 ± 0.0
Dust Mite (47)	0.1 ± 0.7	0.7 ± 2.6	45.2 ± 6.0	1.0 ± 4.2	0.0 ± 0.0
Multiple Late (171)	0.0 ± 0.0	0.2 ± 1.3	2.9 ± 7.2	167.4 ± 6.6	0.4 ± 2.7
Multiple Early (112)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.7	111.1 ± 0.7

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Table E6. Association between atopy defined conventionally (atopic ever, atopic at age 8 years), the novel latent classes (two-cluster and five-cluster models) and clinical phenotypes associated with asthma: regression analysis adjusted for gender

aOR-Adjusted odds ratio, CI-Confidence interval; *index category – No latent atopic vulnerability; **index category – never wheezers

	Ever atopic		Atopic, age 8		2 cluster model Vulnerable		Five cluster model - Latent atopic vulnerability* Non-dust Mite		Dust mite		Multiple Late		Multiple Early	
	aOR 95%CI	P	aOR 95%CI	P	aOR 95%CI	P	aOR 95%CI	P	aOR 95%CI	P	aOR 95%CI	P	aOR 95%CI	P
Asthma	12.43 4.80-32.17	<0.001	11.57 4.79-27.93	<0.001	9.24 4.52-18.91	<0.001	N/A		6.49 1.72-24.20	0.006	6.74 2.44-18.59	<0.001	29.33 11.14-77.24	<0.001
Asthma exacerbation	2.03 1.41-2.92	<0.001	1.74 1.19-2.54	0.004	2.29 1.51-3.20	<0.001	2.75 1.57-4.82	0.001	3.21 1.57-6.58	<0.001	1.71 1.02-2.97	0.04	4.42 2.85-8.14	<0.001
Exacerbation after age 1	2.36 1.59-3.50	0.001	1.78 1.19-2.68	0.006	2.53 1.70-3.75	<0.001	3.09 1.69-5.64	0.001	3.43 1.60-7.38	0.002	1.89 1.08-3.30	0.026	6.14 3.55-10.63	<0.001
Exacerbation after age 3	2.95 1.91-4.79	<0.001	2.31 1.43-3.72	0.001	3.17 2.01-4.99	<0.001	2.77 1.33-5.78	0.007	3.86 1.61-9.24	0.002	2.60 1.37-4.91	0.003	6.65 3.56-12.44	<0.001
Persistent wheeze**	4.84 3-09-7.59	<0.001	3.54 2.27-5.54	<0.001	5.46 3.80-7.85	<0.001	1.82 0.83-4.01	0.14	4.37 1.93-9.91	<0.001	2.77 1.52-5.06	0.001	12.86 6.78-24.41	<0.001
Current wheeze	5.83 3.94-8.63	<0.001	5.90 3.98-8.75	<0.001	5.46 3.80-7.85	<0.001	1.07 0.48-2.36	0.88	4.96 2.45-10.06	<0.001	4.70 2.92-7.56	<0.001	14.20 8.49-23.75	<0.001

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Table E7. Lung function (specific airway conductance at ages 3 and 5 years; FEV_1 and FEV_1/FVC ratio at age 8 year) and airway reactivity in children with different latent atopic vulnerabilities in the five-class model
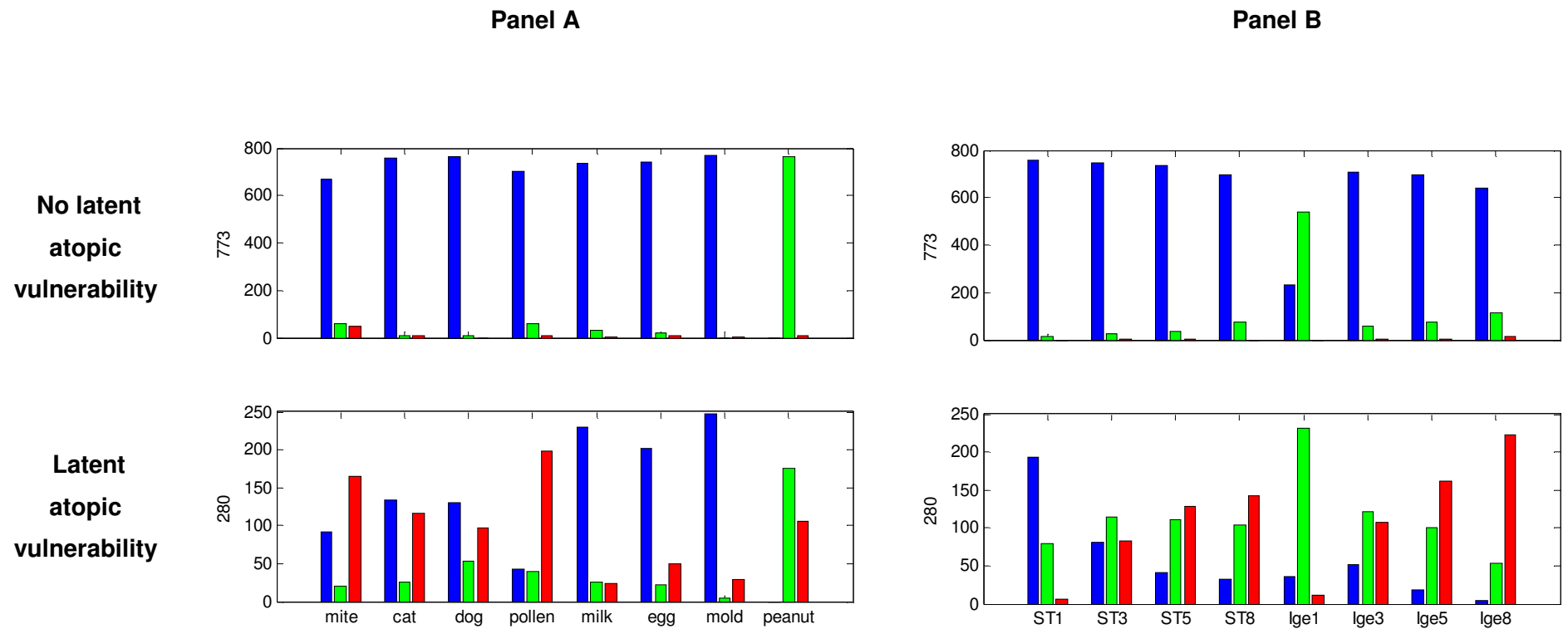
	No Latent atopic vulnerability	Non-dust Mite	Dust mite	Multiple late	Multiple early
sGaw age 3, kPa/s	0.92	0.90	0.94	0.89	0.83
GM, 95% CI	0.90-0.94	0.83-0.97	0.86-1.02	0.85-0.93	0.79-0.88
sGaw age 5, kPa/s	0.87	0.84	0.85	0.86	0.80
GM, 95% CI	0.85-0.88	0.80-0.87	0.80-0.90	0.83-0.88	0.77-0.83
FEV_1% pred, age 8,	102.63	101.64	103.03	101.01	94.12
mean, 95% CI	101.39-103.86	98.90-104.40	99.24-106.82	98.85-103.16	91.40-96.86
FEV_1/FVC, age 8, %	87.11	86.78	86.04	86.03	83.49
mean, 95% CI	86.52-87.71	85.44-88.11	84.20-87.87	84.20-87.07	82.18-84.82
DRR age 8	6.90	6.04	7.67	10.12	15.54
mean, 95% CI	6.09-7.79	4.45-8.02	5.27-10.96	8.20-12.50	11.82-20.82

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Figure E1. The structure of the two clusters (No latent atopic vulnerability and Latent atopic vulnerability)

Panel A: number of sensitization a child has to each specific allergen

Panel B: number of sensitizations at each time point (for skin tests and IgE).

The blue, green and red bars denote the number of children who have fewer than 1, between 1 and 2 and more than 2 sensitizations respectively.
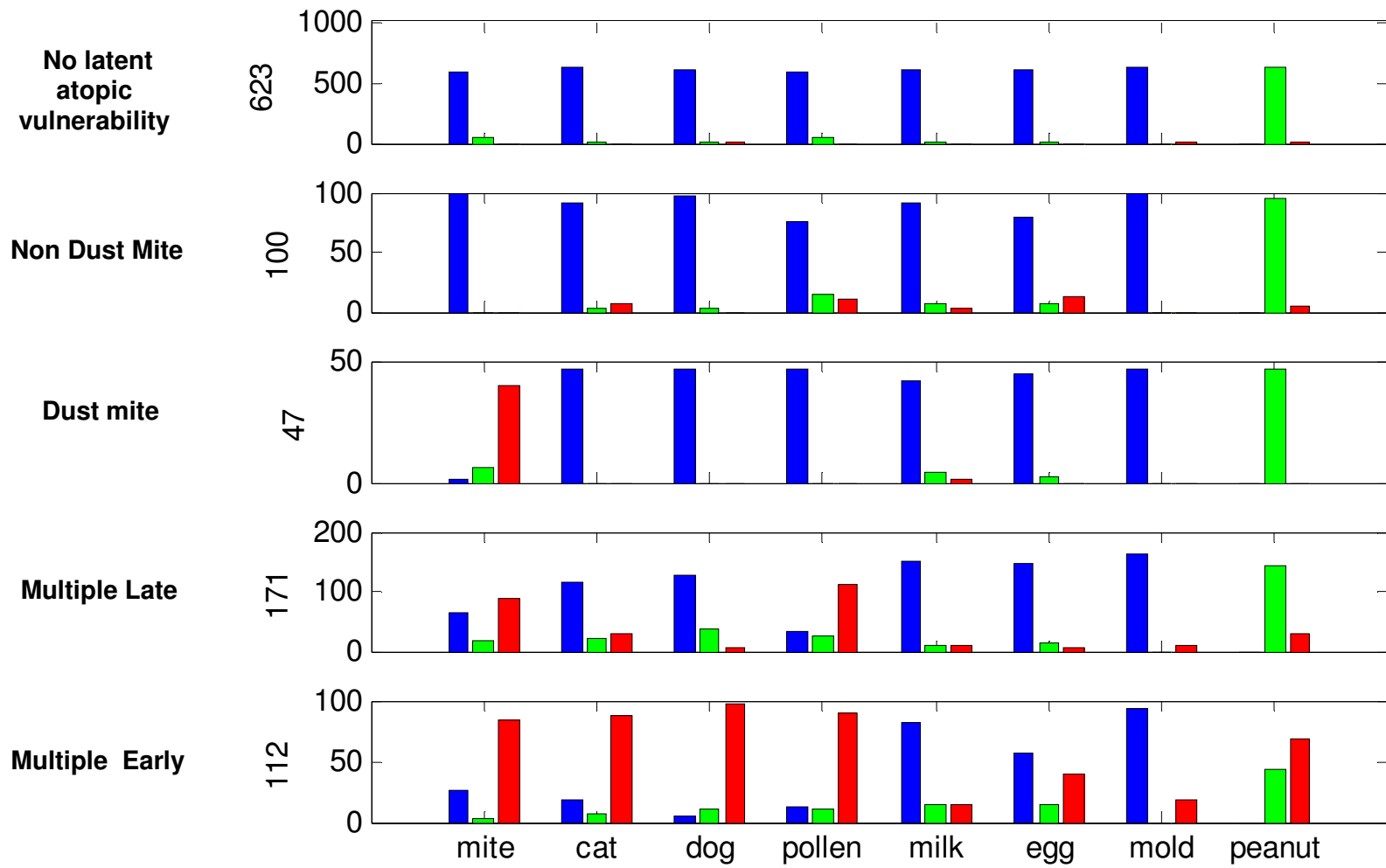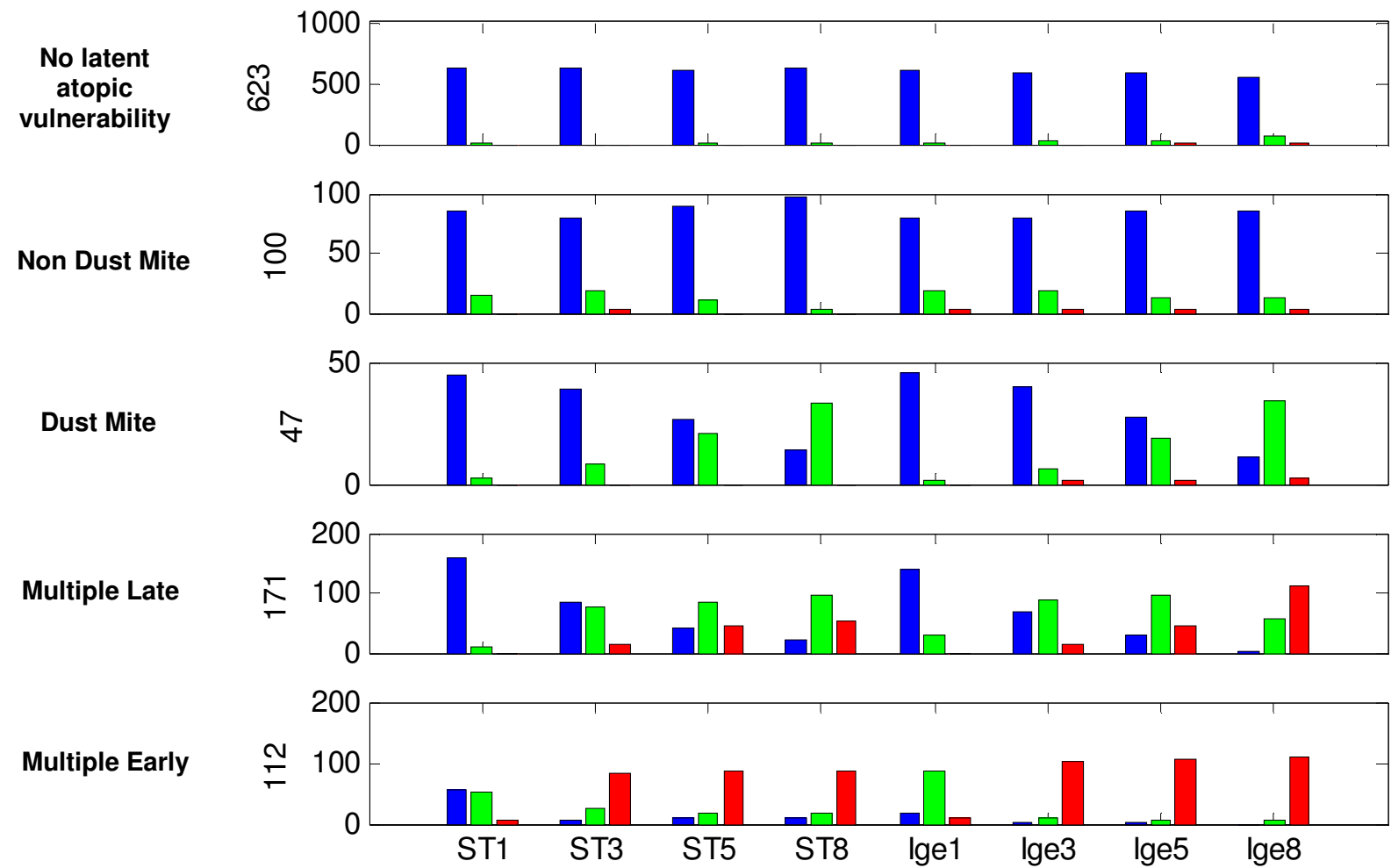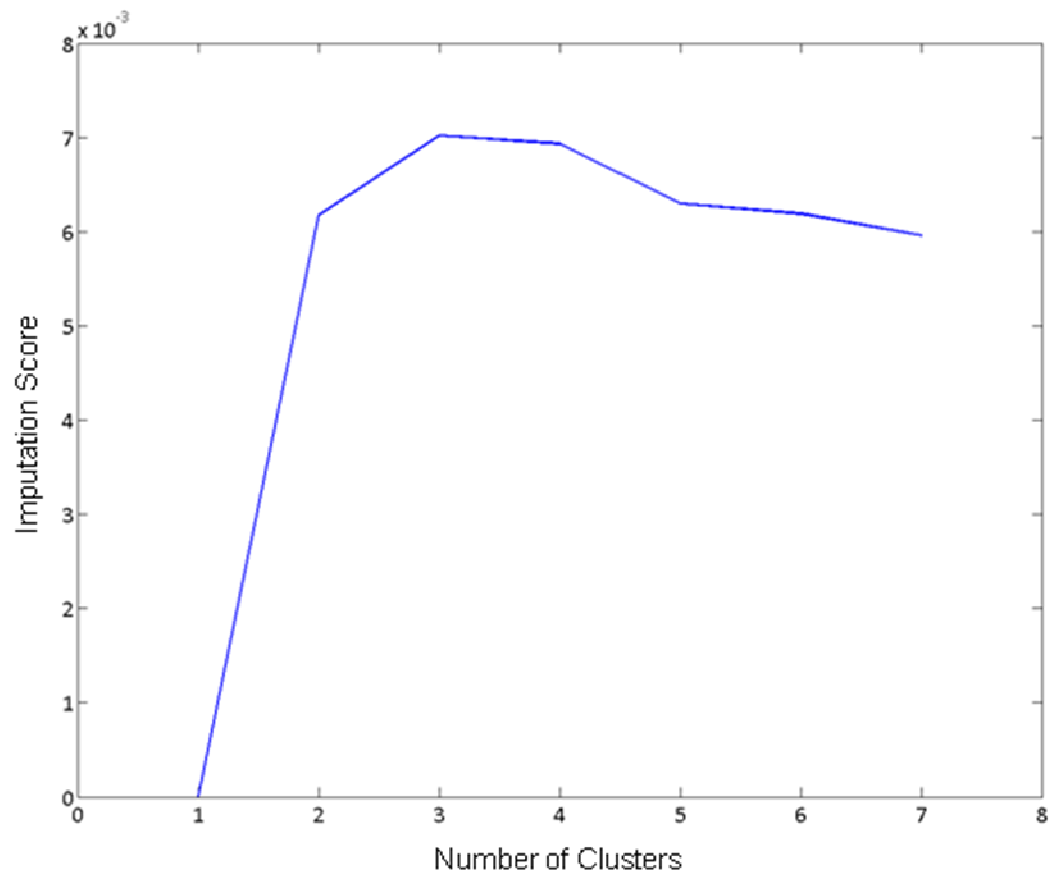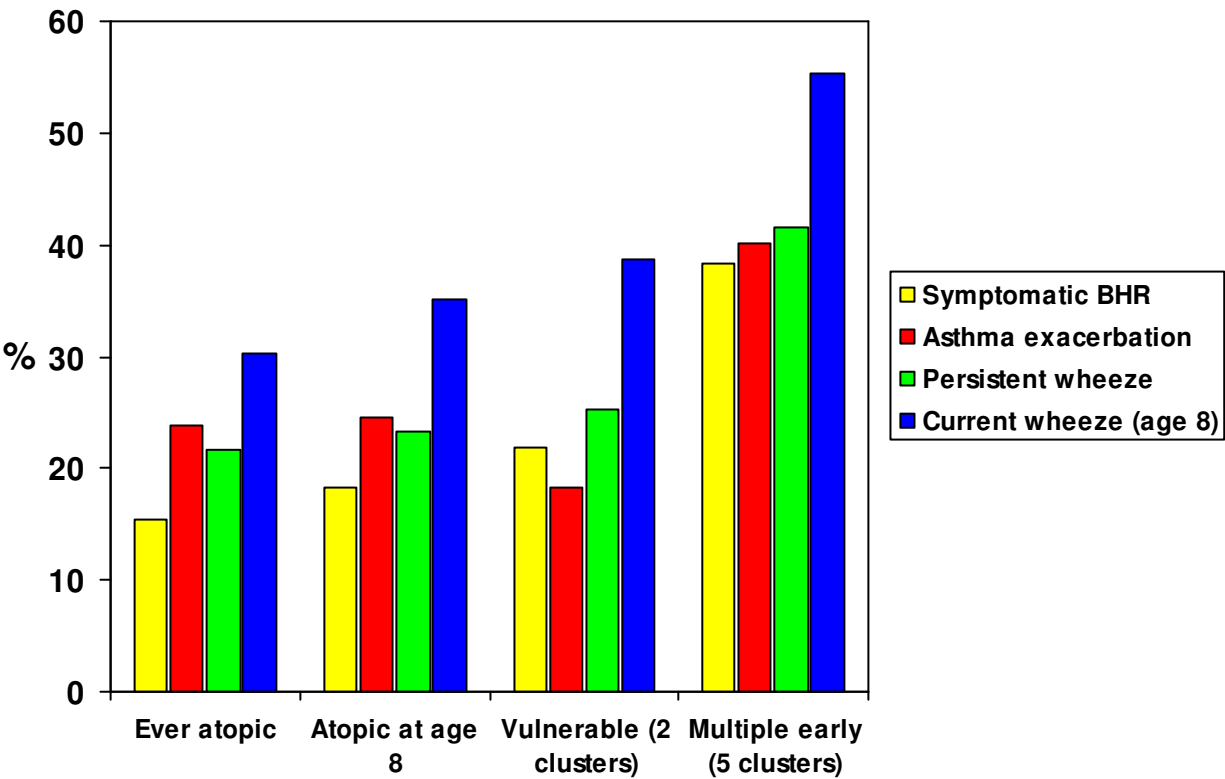
Panel A   **Panel B**

Figure E2. The structure of the five clusters (No latent atopic vulnerability, Non-dust Mite Atopic Vulnerability, Dust Mite Atopic Vulnerability, Multiple Late Atopic Vulnerability, Multiple Early Atopic Vulnerability)

Panel A: number of sensitization a child has to each specific allergen

Panel B: number of sensitizations at each time point (for skin tests and IgE).

The blue, green and red bars denote the number of children who have fewer than 1, between 1 and 2 and more than 2 sensitizations respectively.

Figure E2, PANEL A

Figure E2, PANEL B

Figure E3. Average imputation score for models with 1–7 clusters; note that the score baseline has been adjusted (see text for details).
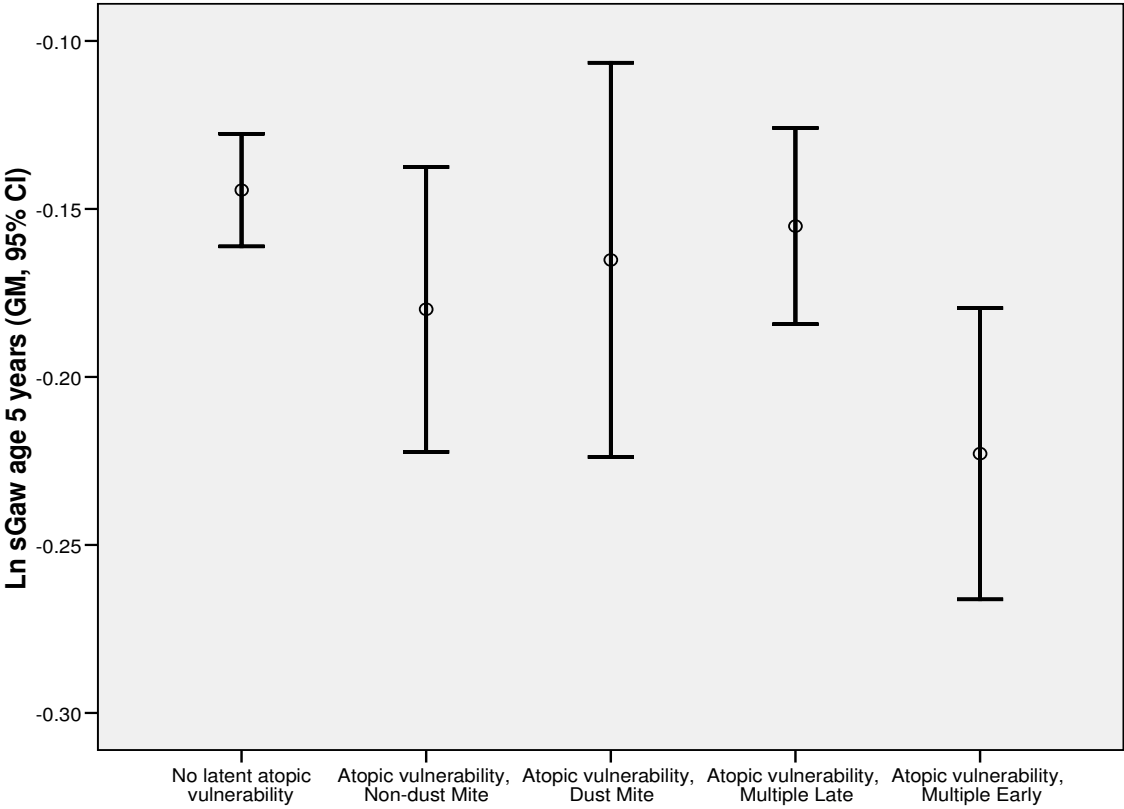
Figure E4. Proportion of children with different clinical outcomes within those considered "atopic" by four different classifications

Figure E5. Specific airway conductance at age 3 in children with different latent atopic

vulnerabilities in the five-class model

Figure E6. Specific airway conductance at age 5 years in children with different latent

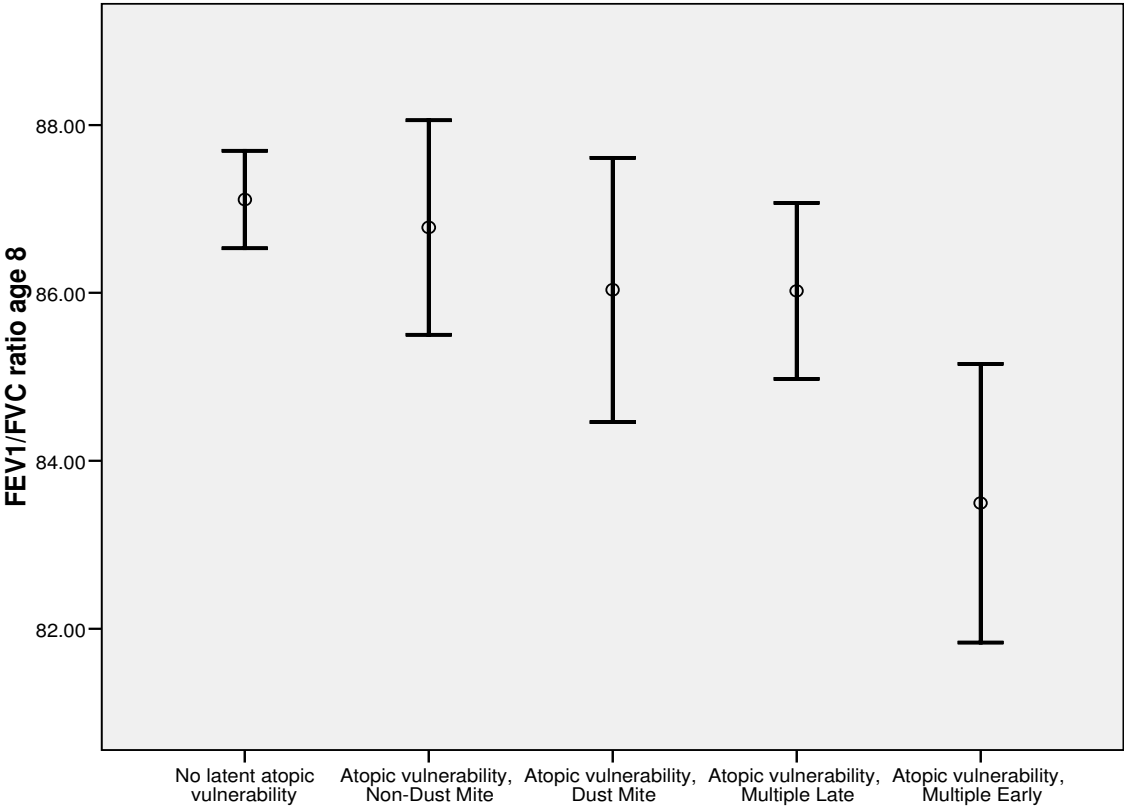atopic vulnerabilities in the five-class model

Figure E7. FEV_1 (% predicted) at age 8 year in children with different latent atopic
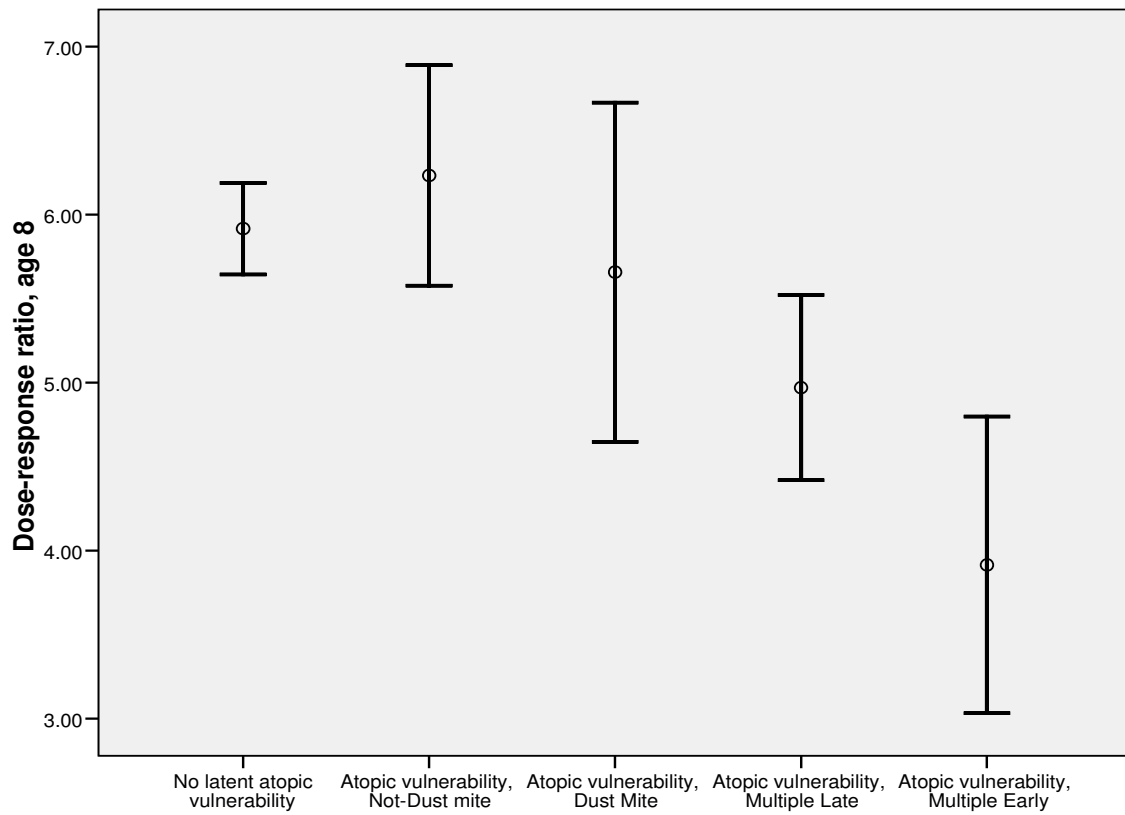
vulnerabilities in the five-class model

Figure E8. FEV$_1$/FVC ratio at age 8 year in children with different latent atopic

vulnerabilities in the five-class model

Figure E9. Airway reactivity (dose-response ratio) at age 8 years in children with

different latent atopic vulnerabilities in the five-class model

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