

Atopy phenotypes in the Childhood Asthma Prevention Study (CAPS) cohort and the relationship with allergic disease

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Clinical & Experimental Allergy

Summary

Background Atopy in early life is heterogeneous in timing of onset, remission and persistence and in the nature of specific sensitization to allergens. However, this heterogeneity is not well characterized.

Objective Our aim was to define longitudinal phenotypes of atopy between ages 1.5 and 8 years, and to assess the relationship of the atopy phenotypes to the risk of asthma, eczema and rhinitis at 8 years of age.

Methods We used latent class analysis (LCA) to define atopy phenotypes using data from skin prick tests that were performed at 1.5, 3, 5 and 8 years in participants in the Childhood Asthma Prevention Study (CAPS).

Results Four phenotypes were defined: late mixed inhalant sensitization; mixed food and inhalant sensitization; house dust mite (HDM) monosensitized; and no atopy. All three atopic phenotypes were associated with asthma, eczema and rhinitis, but the strongest association, particularly for asthma, was with the mixed food and inhalant sensitization phenotype.

Conclusion & clinical relevance We have used a LCA model to define atopy phenotypes empirically. The finding of a strong association between the mixed food and inhalant sensitization class and the presence of asthma and poor asthma control at age 8 years implies that food sensitization in early life may be of greater significance for subsequent risk of asthma than previously thought.

Keywords asthma, atopy, children, latent class analysis, sensitization, skin prick test

Submitted 29 August 2012; revised 14 January 2013; accepted 16 January 2013

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Cite this as: F. L. Garden, J. M.
Simpson, G. B. Marks and for the
CAPS Investigators, *Clinical &*

Experimental Allergy, 2013 (43) 633–
641.

Introduction

There is a known relation of atopy to asthma, eczema and rhinitis. [1–5] Atopy in early life is heterogeneous in the timing of onset, remission and persistence and in the nature of specific sensitization to allergens. It has been shown that sensitization to food allergens occurs early in life and often remits and that sensitization to inhalant allergens tends to occur later in childhood and increases with age. [1, 6, 7]

There is some evidence that the heterogeneity in the manifestations of atopy in early life relates to the risk of associated asthma, eczema and rhinitis. For example, food allergy is associated with eczema [1, 4]; sensitization to outdoor allergens is associated with rhinitis [1]; and house dust mite (HDM) sensitization is associated

with wheeze and asthma [1, 8]. It has also been shown that persistent atopy is associated with an increased risk of asthma. [2, 4, 5]

However, the heterogeneity of atopy is not well characterized. It is not known whether there are patterns or classes of atopy or, alternatively, the various components of atopy, including timing and the nature of the specific sensitization, vary independently. Historically, types of atopy have been defined, using *ad hoc* descriptions, based on the presence or absence of sensitization to specific allergens or groups of allergens (inhalant, ingested, animal) and on the time course of sensitization to allergens during childhood. [2–5, 7] Recently, more sophisticated data-based approaches have been used to define atopy phenotypes. [9, 10] This approach defined atopy groups in an unsupervised manner that

avoided making *a priori* decisions. One of these studies found that children could be grouped into five atopy phenotypes, which differed in age of onset and the nature of specific sensitization. [9] While the five phenotype groups were described in terms of the number of sensitizations a child had to each allergen, and separately the number of sensitizations a child had at each time-point, the study did not show the relationships among specific sensitizations and how these relations changed over time.

Latent class analysis (LCA) is a powerful statistical tool that can be used to examine the heterogeneity of atopy sensitization and to define atopy phenotypes. [11] The LCA model has been used in the field of asthma to define phenotypes of wheeze and cough during childhood. [12–14] The value of this approach is its ability to group individuals within a sample together based on their pattern of responses over several variables. The model is a stochastic model, which does not need any *a priori* decisions as to how individuals are grouped. In other words, classifications are derived entirely from the observed data. The model also yields descriptive information for each phenotype, based on the response patterns of subjects classified as being within that phenotype.

The aim of this study was to empirically identify and define longitudinal phenotypes of atopy between ages 18 months and 8 years and to assess whether the risk of asthma, eczema and rhinitis at 8 years of age differs according to atopy phenotype defined in this way.

Methods

Design

This study used data collected during the first 8 years of life in the Childhood Asthma Prevention Study (CAPS) cohort. CAPS began as a randomized controlled trial (Australian New Zealand Clinical Trial Registry ACTRN12605000042640) investigating the effectiveness of a HDM avoidance and omega-3 fatty acid supplementation intervention from birth to 5 years for the primary prevention of asthma. [15] Subjects who participated in CAPS were also followed up at 8 years of age. Details of the study design, intervention, population, results from the RCT and first 8 years have been described previously. [15–17] The study was approved by the Human Research Ethics Committees of the University of Sydney (Ref: 3294), Western Sydney Area Health Service (HREC96/7/4.17 (154)) and Sydney South West Area Health Services (Ref: 96/80). All participating patients provided informed written consent prior to participation.

Subject selection

Pregnant women whose unborn children were at increased risk of developing asthma, because one or more parents or siblings had current asthma or wheezing, were recruited from antenatal clinics in western Sydney, Australia, from 1997 to 2000. Exclusion criteria included babies from multiple births, those with a pet cat at home and strict vegetarians, who were excluded because the trial intervention included fish-oil supplementation. A total of 616 children were randomized at birth into active intervention or control groups. Six babies were withdrawn from the trial after birth (and randomization) for medical reasons, including birth before 36-weeks gestation, birthweight less than 2.5 kg or other serious neonatal problems. This was because of potential hazard posed by the trial procedures in the context of these conditions. [17] The study population for this analysis included subjects within the CAPS cohort who completed at least the first two skin prick tests (SPT) at 18 months and 3 years.

Skin prick tests

Sensitization to allergens was measured at 18 months, 3, 5 and 8 years of age by using SPT to the ingested allergens peanuts, egg white, egg yolk and cow's milk and the inhalant allergens *Dermatophagoides pteronyssinus* (HDM), cockroach, cat, *Alternaria alternata*, rye grass and a grass mix (Hollister-Stier, Spokane, Wash), as previously described. [18] Glycerol and histamine phosphate, 10 mg/mL, were used as negative and positive controls respectively. Weal sizes were measured after 10 min, and those that were greater than 3 mm and also greater than those elicited by the negative control were classified as positive. We further defined a subject as positive to egg if their results were positive for egg white or egg yolk; and positive to grass if their results were positive to either rye grass or grass mix.

Other measures

We measured spirometric lung function with the child standing, wearing a nose clip and in accordance with American Thoracic Society recommendations [19] as previously described [16]. The outcome variables current asthma, current eczema, current rhinitis, airway hyperresponsiveness, airflow obstruction and poor asthma control at 8 years are as follows. *Current asthma* was defined as wheeze in the last 12 months plus either ever-diagnosed asthma (reported at ages 18 months and 3, 5 or 8 years) or airway hyperresponsiveness (at 8 years). *Current eczema* was defined as flexural rash at the time of physical assessment or a

history of recurrent itchy rash and use of topical treatments in the last 12 months. *Current rhinitis* was defined as a positive response if the child had a problem with sneezing, or a runny, or blocked nose when he/she did not have a cold or the flu in the past 12 months. *Airway hyperresponsiveness* was defined as a PD₂₀FEV₁ (methacholine) value of less than 6.1 µmol or greater than 12% bronchodilator response in children who did not have a methacholine challenge. *Airflow obstruction* was defined as pre-bronchodilator FEV₁/FVC ratio < lower limit of normal. [20] *Poor asthma control* was defined as one or more of the following in the preceding 12 months: more than 12 attacks of wheezing, disturbed sleep due to wheezing at least once per week, wheezing limiting the child's speech to only one or two words at a time between breaths, use of a bronchodilator for night waking or early-morning chest tightness more than three times per week or hospital emergency department visit or admission for asthma.

Statistical analysis

Atopy phenotypes were defined using LCA in Mplus version 6.1. [11, 21] LCA is a statistical model that defines subgroups (classes) within a sample by grouping together subjects with a similar response pattern to a number of binary indicator variables used as inputs into the model. The LCA model outputs the probability each subject is assigned to a class, where the number of classes within a model is chosen by the user. We assigned each subject to the class for which they had the highest probability and then described each class in terms of responses to the binary indicator variables among subjects assigned to that class.

The binary latent class indicator variables used in this LCA were sensitization (yes/no) to each allergen tested by SPT at each assessment period (i.e. 18 months, 3, 5 and 8 years of age). To determine the optimal number of classes required to describe the heterogeneity of atopy, we ran LCA models with 1–6 classes. All models were estimated using a full-information maximum-likelihood estimator with robust standard errors to handle missing data within the sample. We used the following criteria to determine the optimal number of classes (from 1 to 6): convergence, fit indices (bootstrap likelihood ratio test (BLRT); Bayesian Information Criteria (BIC); adjusted BIC; Lo, Mendell and Rubin likelihood ratio test (LMR-LRT); Akaike's Information Criteria (AIC); corrected AIC, entropy, class size and interpretability. [22]

We tested the relationship between the atopy phenotypes and selected attributes using multinomial logistic regression where subjects were assigned to the phenotype with the highest probability of membership. We tested the relationship between the phenotypes derived

from the LCA and asthma outcomes at 8 years using logistic regression within the LCA framework to allow for individuals to be fractional members of all classes, based on the estimated probability of membership of each class. We also tested the relationship between atopy phenotypes and asthma outcomes at 8 years, adjusting for the covariates sex and the study intervention groups, where subjects were assigned to the phenotype with the highest probability of membership. We present the results as odd ratios (OR) with 95% confidence intervals. The OR is the ratio of the odds of having the asthma/allergic outcome in each atopy phenotype compared with the no-atopy phenotype. SAS (version 9.2; SAS Institute Inc., Cary, NC, USA) was used to conduct all statistical analyses, which did not involve using LCA to define the phenotypes or test the associations within a LCA framework. A *P*-value of <0.05 was considered statistically significant.

Results

Of the 616 subjects in the original CAPS study, 509 (83%) subjects were included in the LCA as they had completed at least the first two SPT at 18 months and 3 years. Of these 509 subjects, 476 (94%) completed a SPT at 5 years, 395 (78%) completed a SPT at 8 years and 382 (62%) had completed all four SPTs at 18 months, 3, 5 and 8 years. The percentage of children with at least one positive SPT reaction to an allergen increased with age from 14% to 24%, 38%, and 45% at 18 months, 3 years, 5 years and 8 years respectively. The most common allergen that children were sensitized to at each assessment was HDM (Table 1).

The characteristics of the study population and a comparison between the study sample and the original CAPS cohort are shown in Supplementary Table 1. Compared with the excluded subjects, those included in this study

Table 1. The proportion of children who had a positive skin prick test reaction to each allergen tested at each assessment

Allergen	Assessment			
	1.5 y	3 y	5 y	8 y
	<i>n</i> = 509 %	<i>n</i> = 509 %	<i>n</i> = 476 %	<i>n</i> = 395 %
House dust mite	6.9	18.9	30.0	35.7
Cockroach	0.0	1.8	4.2	5.1
Cat	0.2	1.0	4.4	6.3
Grass	0.2	4.9	11.3	17.7
Alternaria	0.0	0.8	6.5	11.2
Peanuts	4.1	3.7	3.8	4.4
Eggs	6.1	2.4	2.3	1.8
Cow's milk	0.6	0.2	0.4	—

A positive skin prick test was defined as a mean skin weal diameter of greater than 3 mm and also greater than that elicited by the negative control. Cow's milk was not tested at the 8-year assessment.

had older and more highly educated mothers and fathers, fathers who were more likely to be in full-time employment and a smaller proportion of mothers who were born in Australia, but did not differ in other respects.

Table 2 shows the model fit statistics for the LCA model with 1–6 classes. Standard errors could not be estimated validly for the 6-class model, so this was excluded from further consideration. The fit statistics for 2–5 classes did not suggest one clearly best model. Both the AIC and the adjusted BIC were lowest for the 5-class model, the corrected AIC, entropy and LMR were most favourable for the 4-class model and the BIC was lowest for the 3-class model. Given this ambiguity in the analysis of fit statistics, we examined the class structure, the number of subjects assigned to each class and interpretability of the 3-, 4- and 5-class models. The 5-class model resulted in three classes each with less than 10% of the sample, two of which were essentially the same, so we excluded it from further consideration. The 4-class model produced a more interpretable class structure than the 3-class model, so we selected the 4-class model.

The following four classes were identified by examination of the class structure.

Late mixed inhalant sensitization

There were 54 (11%) subjects assigned to this class. Within this class, the proportion of children with a positive SPT to inhalant allergens increased over time. The allergens with the highest prevalence of positive SPT responses at age 8 years were grass, HDM and Alternaria (Figure 1).

Mixed food and inhalant sensitization

There were 22 (4%) subjects assigned to this class, which is characterized by early and persistently high prevalence of positive SPT to peanut; early, but decreasing,

prevalence of positive SPT to egg; and an increasing prevalence of positive SPT to HDM, Alternaria and grass.

HDM monosensitized

There were 87 (17%) subjects assigned to this class, which is characterized by high and increasing prevalence of positive SPT to HDM, but a low prevalence of sensitization to all other allergens.

No atopy

There were 346 (68%) of subjects assigned to this class, which is characterized by low prevalence of positive SPTs to all allergens.

We conducted a sensitivity analysis in which the LCA was performed on those 382 subjects in whom SPT data were available for all four ages (18 months, 3, 5 and 8 years). The results of this analysis were similar to those of the main analysis (which included some subjects with missing data at 5 and 8 years). Specifically, all but five subjects remained in the same class. Three subjects changed to the late mixed inhalant class one from each of the mixed food and inhalant class, HDM monosensitized class and no-atopy class; and two subjects changed to the mixed food and inhalant class from the no-atopy class.

Table 3 shows the relationship between the atopy phenotypes and various attributes. We found no relationship between atopy phenotypes and sex, study interventions groups, breastfeeding ≥ 6 months, presence of older siblings, parents' asthma status or parents' education level ($P > 0.05$). Children whose mothers smoked during pregnancy were less likely to manifest late mixed inhalant sensitization and mixed food and inhalant sensitization than children whose mothers did not smoke (Table 3).

The relationship between the atopy phenotypes and the asthma and allergic disease outcomes at 8 years is

Table 2. Latent class analysis (LCA) model fit statistics for 1–6 classes

Number of classes	Number of Parameters	Log likelihood	AIC	Corrected AIC	BIC	Adjusted BIC	Entropy	BLRT P-value	LMR P-value
1	29	−2694.67	5447.34	5450.97	5570.08	5478.03	—	—	—
2	59	−2186.30	4490.59	4506.36	4740.30	4553.03	0.89	<0.001	<0.001
3	89	−2080.20	4338.40	4376.63	4715.08	4432.59	0.91	<0.001	0.309
4	119	−1993.41	4224.82	4298.24	4728.48	4350.76	0.94	<0.001	0.016
5	149	−1945.55	4189.10	4313.61	4819.73	4346.79	0.93	<0.001	0.017
6	179	—	—	—	—	—	—	—	—

BIC: Bayesian Information Criteria, AIC: Akaike's Information Criteria, BLRT: bootstrap likelihood ratio test; LMR: Lo, Mendell and Rubin likelihood ratio test. The bold figures indicate the optimal value for each fit statistic, that is, within each column. For AIC, corrected AIC, BIC, adjusted BIC and LMR *P*-value, the optimal number of classes is chosen by the minimum value of the fit statistic; for entropy, the optimal number of classes chosen is based on the highest value of entropy. No fit statistics were available for the 6-class model because the model did not converge.

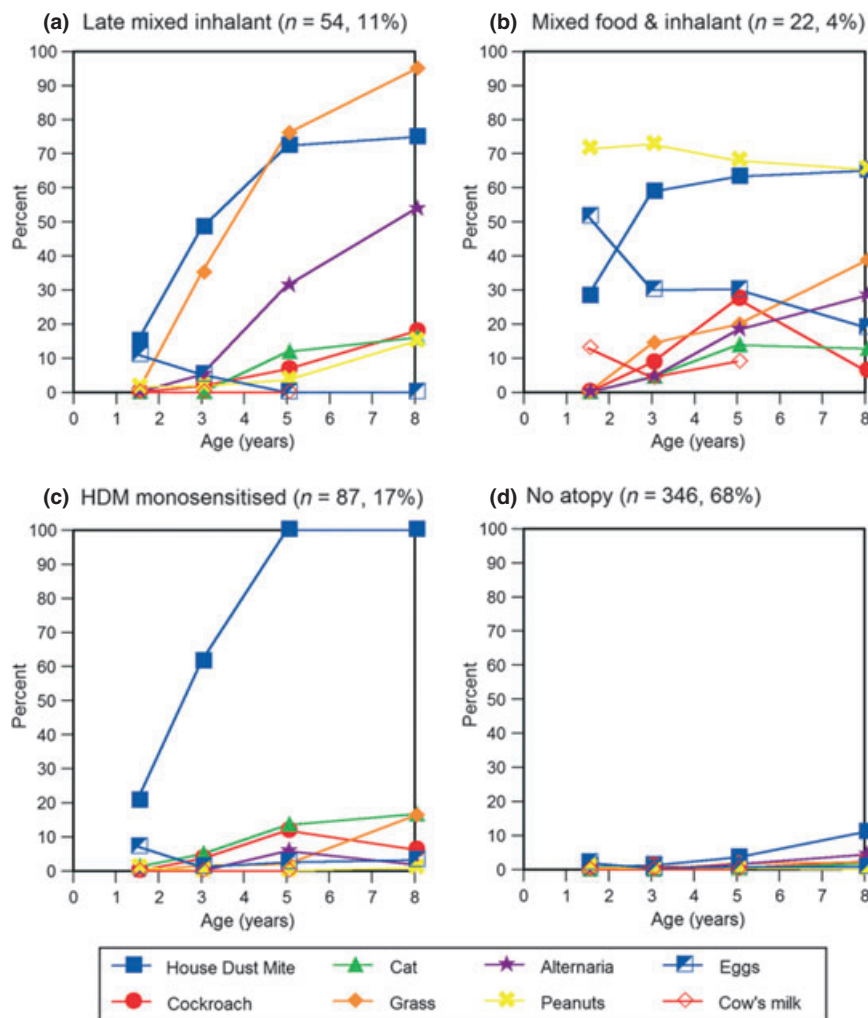


Fig. 1. Percentage of subjects within each of the four atopy phenotypes who had a positive SPT result to each allergen from 1.5 to 8 years. This figure shows the percentage of subjects who were positively sensitized to an allergen at each time point within each latent class. The four classes are shown: (a) Late mixed inhalant, (b) Mixed food and inhalant, (c) HDM monosensitized and (d) No atopy. The number of subjects (n) and the % of subjects of the total sample ($n = 509$) within each class are shown.

shown in Table 4. All atopy phenotypes were significantly associated with asthma, eczema, rhinitis, poor asthma control and airway hyperresponsiveness. However, the strongest association (highest OR) of each asthma outcome was with the mixed food and inhalant sensitization phenotype. The association was stronger for the asthma-related outcomes than for eczema and rhinitis. Although airflow obstruction was uncommon in this cohort, its presence was strongly and significantly associated with both the mixed food and inhalant phenotype and the HDM monosensitized phenotype. All significant associations remained significant after adjusting for sex and the diet and HDM intervention groups.

Discussion

We have defined four phenotypes of atopy characterized by their pattern of sensitization and change over

time: late mixed inhalant; mixed food and inhalant; HDM monosensitized; and no atopy. We have shown that all atopy phenotypes are associated with asthma, eczema, rhinitis and airflow obstruction, but that the strongest association, particularly for asthma, is with a relatively uncommon phenotype, which expressed a mixed food and inhalant pattern of sensitization and which includes a high proportion of subjects with evidence of sensitization at age 18 months.

This analysis has enabled us to resolve a total of 40 allergen-sensitization results, that is, 10 allergen tests on four occasions between 18 months and 8 years, into four stochastically derived classes or phenotypes that meaningfully convey the relevant information. By using a LCA model to define the phenotypes, we were able to base our choice of phenotypes on a data-driven statistical approach and not on an arbitrary decision. The results from the LCA are easy to interpret by examining

Table 3. Relationship between attributes and atopy phenotypes

Attribute		Atopy phenotypes			P-value
		Late mixed inhalant OR* (95% CI)	Mixed food and inhalant OR* (95% CI)	HDM monosensitized OR* (95% CI)	
Sex	Male	1	1	1	0.29
	Female	0.84 (0.47–1.49)	1.08 (0.46–2.57)	0.64 (0.40–1.02)	
HDM intervention group	Control	1	1	1	0.54
	Active	0.71 (0.40–1.26)	1.38 (0.58–3.31)	0.89 (0.56–1.43)	
Diet intervention group	Control	1	1	1	0.41
	Active	0.85 (0.48–1.51)	0.68 (0.29–1.64)	1.34 (0.83–2.15)	
Breastfeeding ≥ 6 m	No	1	1	1	0.08
	Yes	1.33 (0.75–2.37)	2.4 (0.99–5.77)	1.55 (0.97–2.49)	
Mother smoked during pregnancy	No	1	1	1	0.02
	Yes	0.34 (0.14–0.83)	0.13 (0.02–0.99)	0.77 (0.44–1.35)	
Older siblings	No	1	1	1	0.18
	Yes	0.77 (0.42–1.41)	0.73 (0.30–1.80)	0.59 (0.36–0.96)	
Maternal asthma	No	1	1	1	0.64
	Yes	0.91 (0.51–1.61)	1.47 (0.60–3.60)	1.25 (0.77–2.01)	
Paternal asthma	No	1	1	1	0.24
	Yes	1.29 (0.73–2.30)	2.33 (0.97–5.60)	0.99 (0.61–1.60)	
Mother tertiary educated	No	1	1	1	0.51
	Yes	1.37 (0.77–2.43)	1.70 (0.71–4.08)	1.10 (0.69–1.76)	
Father tertiary educated	No	1	1	1	0.69
	Yes	0.88 (0.50–1.58)	1.43 (0.60–3.39)	0.84 (0.52–1.35)	

*The reference group for the OR is the no-atopy phenotype.

the pattern of sensitization to specific allergens and the pattern of change with age. The LCA model can accommodate missing data so we were able to use information from 83% of the original CAPS birth cohort. To our knowledge, this is the first study to use a data-driven approach to define atopy phenotypes in a high-risk asthma cohort.

Our results are similar to those of a recent study from the Manchester Asthma and Allergy Study [9], which used a machine-learning approach to define atopic phenotypes, and also from a recent study of the Isle of Wight cohort, which used cluster analysis. [10] Simpson *et al.* found that a 5-class model was optimal (multiple early, multiple late, dust mite, non-dust mite and no latent vulnerability). [9] The phenotypes in our study are similar to those of Simpson *et al.* for all classes except the non-dust mite sensitized group. In our study, all atopy phenotypes had a high prevalence of HDM sensitization. This may reflect differences in the local environment in which the studies were conducted. Roberts *et al.* found a 4-group cluster analysis optimal at 4 years (HDM; grass, pollen, alternaria and cat; mixed allergens; and no atopy) and similar groupings were found at 10 and 18 years [10]. The clusters in the Isle of Wight study are very similar to those observed in this study. The extent to which findings of these analyses are generalizable beyond the context in which the data were collected remains to be estab-

lished. The findings from these three studies suggest that the findings are broadly, but not completely, generalizable.

One of the key findings of this analysis was the strong association between mixed food and inhalant allergen and asthma at age 8. The association between food sensitization and asthma has been observed in other studies [2, 4, 23–26] and similarly another study has shown that sensitization to food allergy increased the risk of asthma in subjects cosensitized to pollen allergens. [23] Although this was a small subgroup (4%), the majority of these subjects were peanut allergic and this peanut sensitization persisted to age 8, whereas sensitization to other foods (egg and cow's milk) had largely disappeared from the cohort at this age. Peanut sensitization has been found to be a risk factor for asthma in other studies. [26] The results from this study may provide evidence to support the conclusions made by others that food sensitization could be an under-recognized risk factor for asthma. [25]

One of the surprising findings of this analysis is the inverse association between maternal smoking during pregnancy and the membership of the 'late mixed inhalant' and the 'mixed food and inhalant' classes. This inverse association has been observed in previous cohort studies. [27, 28] It is possible that this inverse association is attributable to unmeasured confounding. If it is a true, biological effect, the mechanism is unclear.

Table 4. Relationship between the atopy phenotypes and asthma outcomes at 8 years expressed as odds ratios

	Current asthma				Current eczema				Current rhinitis			
	n/total [†]	row (%)	OR (95%CI)	AOR [‡] (95%CI)	n/total [†]	row (%)	OR (95%CI)	AOR [‡] (95%CI)	n/total [†]	row (%)	OR (95%CI)	AOR [‡] (95%CI)
Overall	96/440	22			63/421	15			120/439	27		
Phenotype												
Late mixed	18/50	36	3.7 (1.8–7.9)	3.8 (1.9–7.6)	9/49	18	2.1 (0.9–5.2)	2.0 (0.9–4.6)	23/50	46	3.8 (1.9–7.6)	3.7(2.0–6.8)
inhalant												
Mixed food	11/16	69	16.4 (5.2–51.7)	14.3 (4.7–44.1)	6/16	38	5.6 (1.8–17.2)	5.6 (1.9–16.8)	8/16	50	5.1 (1.8–14.8)	4.2(1.5–11.7)
and inhalant												
HDM	28/74	38	4.3 (2.2–8.6)	3.8 (2.1–6.8)	19/73	26	3.5 (1.6–7.4)	3.3 (1.7–6.5)	31/73	43	3.6 (1.9–6.9)	3.2(1.8–5.5)
monosensitized												
No atopy	39/300	13	1*	1*	29/283	10	1*	1*	58/300	19	1*	1*
Airway hyperresponsiveness												
Overall	64/351	18			39/440	9			16/405	4		
Phenotype												
Late mixed	8/39	21	1.9 (0.8–4.9)	1.9 (0.8–4.5)	7/50	14	3.3 (1.2–9.4)	3.4 (1.3–9.1)	2/44	5	2.6 (0.5–13.6)	2.6(0.5–13.7)
inhalant												
Mixed food	5/10	50	8.9 (2.3–33.3)	7.4 (2.0–27.6)	5/16	31	9.8 (2.9–33.3)	10.2 (3.1–34.5)	2/16	13	7.7 (1.4–43.2)	7.7(1.4–43.5)
and inhalant												
HDM	21/53	40	4.9 (2.4–9.8)	5.1 (2.6–10.1)	14/74	19	5.6 (2.4–13.2)	4.9 (2.2–11.0)	7/71	10	5.9 (1.8–19.1)	5.5(1.7–18.2)
monosensitized												
No atopy	30/249	12	1*	1*	13/300	4	1*	1*	5/274	2	1*	1*
Airflow obstruction [¶]												
Overall	64/351	18			39/440	9			16/405	4		
Phenotype												
Late mixed	8/39	21	1.9 (0.8–4.9)	1.9 (0.8–4.5)	7/50	14	3.3 (1.2–9.4)	3.4 (1.3–9.1)	2/44	5	2.6 (0.5–13.6)	2.6(0.5–13.7)
inhalant												
Mixed food	5/10	50	8.9 (2.3–33.3)	7.4 (2.0–27.6)	5/16	31	9.8 (2.9–33.3)	10.2 (3.1–34.5)	2/16	13	7.7 (1.4–43.2)	7.7(1.4–43.5)
and inhalant												
HDM	21/53	40	4.9 (2.4–9.8)	5.1 (2.6–10.1)	14/74	19	5.6 (2.4–13.2)	4.9 (2.2–11.0)	7/71	10	5.9 (1.8–19.1)	5.5(1.7–18.2)
monosensitized												
No atopy	30/249	12	1*	1*	13/300	4	1*	1*	5/274	2	1*	1*

*No atopy is the reference category for each OR.

[†]The total number of subjects within each asthma outcome varied because there were some subjects who did not perform clinical tests at the 8-year assessment.[‡]Adjusted OR is adjusted for sex and the study intervention groups.[¶]FEV₁/FVC ratio below Lower Limit Normal.

One potential limitation to the generalizability of the findings of this analysis is that the CAPS cohort is a high-risk cohort for asthma by virtue of the family history of asthma. However, it should be noted that nearly one-third of those screened met the eligibility criteria [29] and at age 8 years, the prevalence of asthma in this cohort is only slightly higher than the general population prevalence of asthma at this age [16].

A further possible limitation of this study is the absence of measures of sensitization status before age 18 months. Several previous investigators have shown that the presence of sensitization to foods and/or inhalant allergens in the first year of life does predict the subsequent onset of asthma. [3, 4, 8] Others have shown that sensitization to foods, particularly cow's milk and egg, in the first year of life is often transient. [6, 30] This study may have missed some cases of transient food sensitization that was no longer evident at age 18 months. However, the very strong association between membership of the mixed food and inhalant class, which was characterized by a high prevalence of sensitization at 18 months, and subsequent asthma indicates that the presence of sensitization at age 18 months does identify a high-risk group.

Cluster analysis has also been used for defining phenotypes. [10] However, LCA has many advantages over cluster analysis. [14, 31] In the LCA approach, subjects are assigned to each class with an estimated probability. In contrast, cluster analysis makes the simplifying assumption that subjects have 100% probability of being in one class and zero probability of being in the other classes. The fit statistics available from the LCA model output can be used to assist in determining the optimal number of classes, whereas in cluster analysis

this requires subjective assessment of alternative cluster structures. Finally, LCA is a stochastic probability model, which can handle categorical variables and appropriately accommodate missing values. One of the limitations of cluster analysis is that the results of the analysis may vary according to which specific distance-minimizing tool is applied. LCA does not require the use of a distance-minimizing tool, so this limitation does not apply to LCA.

In conclusion, we have used a LCA model to empirically define atopy phenotypes. The finding of a strong association between the mixed food and inhalant sensitization class and the presence of asthma, airflow obstruction and poor asthma control at age 8 years implies that food sensitization in early life may be of greater significance for subsequent risk of asthma than previously thought. We suggest that the approach to phenotypic analysis we have presented here will be more informative than previous approaches to defining phenotypes for genome-wide association studies. However, the clinical relevance of the transitions we have identified remains unknown. Further work will be required to understand both the factors that influence these observed transitions and their consequence for prognosis

Acknowledgements

This study was supported by funding from the National Health and Medical Research Council of Australia.

Conflict of interests

The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Characteristics of the subjects included in the Latent Class Analysis sample and those excluded from the analysis out of the original study sample