Classification of childhood asthma phenotypes and long-term clinical responses to inhaled anti-inflammatory medications

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Background: Although recent studies have identified the presence of phenotypic clusters in asthmatic patients, the clinical significance and temporal stability of these clusters have not been explored.

Objective: Our aim was to examine the clinical relevance and temporal stability of phenotypic clusters in children with asthma.

Methods: We applied spectral clustering to clinical data from 1041 children with asthma participating in the Childhood Asthma Management Program. Posttreatment randomization follow-up data collected over 48 months were used to determine the effect of these clusters on pulmonary function and treatment response to inhaled anti-inflammatory medication.

Results: We found 5 reproducible patient clusters that could be differentiated on the basis of 3 groups of features: atopic burden, degree of airway obstruction, and history of exacerbation. Cluster grouping predicted long-term asthma control, as measured by the need for oral prednisone ($P < .0001$) or additional controller medications ($P = .001$), as well as longitudinal differences in pulmonary function ($P < .0001$). We also found that the 2 clusters with the highest rates of exacerbation had different responses to inhaled corticosteroids when compared with the other clusters. One cluster demonstrated a positive response to both budesonide ($P = .02$) and nedocromil ($P = .01$) compared with placebo, whereas the other cluster demonstrated minimal responses to both budesonide ($P = .12$) and nedocromil ($P = .56$) compared with placebo.

Conclusion: Phenotypic clustering can be used to identify longitudinally consistent and clinically relevant patient subgroups, with implications for targeted therapeutic strategies and clinical trials design. (J Allergy Clin Immunol 2014;133:1289-300.)

Key words: *Childhood asthma, asthma phenotypes, inhaled corticosteroids, cluster analysis, asthma classification, longitudinal study*

Asthma is characterized by chronic airway inflammation, airways hyperresponsiveness, and reversible airflow limitation.[1] The Global Initiative for Asthma guidelines[2] and multiple large-scale clinical trials[3-5] have helped to guide current evidence-based treatments. The current stepwise therapeutic approach is designed to maximize the overall level of asthma control and medication adherence while minimizing treatment cost and adverse side effects. However, it has been observed that subsets of asthmatic children not only respond differently to medications[6-8] but also exhibit markedly different disease trajectories, with some children outgrowing their asthma by early adolescence and others having disease progression[9-12] or decreased lung function in adulthood.[13] The lack of distinct histologic features or reliable quantitative biomarkers suggests that asthma might represent a collection of diverse disorders with distinct causes and natural histories. Such heterogeneity poses significant clinical challenges, particularly in regard to long-term prognostication and treatment decision making.

Numerous classification schemes have been proposed to account for this heterogeneity.[8,14-16] Although tailored treatment strategies are suggested for patients with distinct forms of disease, most classification schemes have limited utility in guiding management strategies or reliably predicting long-term morbidity. Recognition of these limitations has motivated the development of multivariate models that consider many patient characteristics simultaneously.[17-19] More recently, newer data generation procedures that leverage sophisticated statistical approaches have been applied to large asthma cohorts, with early success in

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Abbreviations used
AD: Atopic dermatitis
AOE: Atopy-Obstruction-Exacerbation
CAMP: Childhood Asthma Management Program
ED: Emergency department
FVC: Forced vital capacity
SARP: Severe Asthma Research Program

defining previously unrecognized clusters of asthmatic patients.[20-22] However, these studies were limited by their lack of prospective follow-up data, precluding assessment of the utility of these classification schemes in informing treatment decisions or disease prognostication.

It is in this context that we present the results of a phenotype-based cluster analysis of asthmatic children aged 5 to 12 years who participated in the 48-month Childhood Asthma Management Program (CAMP) trial[3,23] and demonstrate that computational approaches can define meaningful clusters with both longitudinal consistency and differing responses to medical therapy.

METHODS

Study population

The CAMP study design and primary outcomes have been described.[3,23] Subjects aged 5 to 12 years were eligible if they (1) had a history of mild-to-moderate persistent asthma defined by the presence of symptoms at least twice weekly, the use of an inhaled bronchodilator at least twice weekly, or the use of daily medication for asthma; (2) had greater than 7 days of symptoms or decreased peak expiratory flow rates during the 28-day run-in period when taking only albuterol as needed; (3) exhibited airway hyperresponsiveness to methacholine; and (4) had no other clinically significant conditions. Participants were randomized to receive 200 μg of budesonide twice daily (n = 311; Pulmicort; AstraZeneca, Westborough, Mass), 8 mg of nedocromil sodium (n = 312; Tilade; Rhone–Poulenc Rorer, Collegeville, Pa), or matching placebo (n = 418). Subjects were evaluated every 4 months for 48 months. Asthma exacerbations were treated with short courses of oral prednisone. The addition of beclomethasone dipropionate (168 μg twice daily; Vanceril, Schering-Plough, Kenilworth, NJ) was allowed if asthma control was inadequate. If control remained unsatisfactory, replacement or additional medications were allowed.

Variable selection

Asthma history interviews were conducted before and spirometry and methacholine challenge testing were performed after a 28-day screening period off all anti-inflammatory asthma medications. From an initial list of 48 clinical variables (see Table E1 in this article's Online Repository at www.jacionline.org), we selected 18 variables as representative of each child's objective risk factors for increased asthma burden as inputs for the clustering algorithm. We excluded several clinical variables that were included in prior cluster analysis studies, including asthma symptoms. These symptom variables were purposely excluded from the model for several reasons, including the following: (1) asthma symptoms were not evaluated as an outcome in the original CAMP study, (2) descriptions of asthma symptoms were obtained from daily symptom diaries maintained by study participants and subject to reporting bias, and (3) there was increased variability in the response rate and number of missing values for the symptoms described in the diaries.

Also, in contrast to prior cluster analyses, potential asthma risk factors, such as sex, ethnicity, and environmental exposure, were purposely excluded from cluster model building. Our rationale for excluding this demographic information was our desire to test the hypothesis that sex and ethnic factors play a role in the pathogenesis of childhood asthma. Cluster analysis provided the opportunity to evaluate the segregation of these variables independent of the model-building process. For this reason, we excluded sex and ethnicity for our model and instead included hypothesis testing of these variables after building the phenotypic clusters.

Cluster analysis

We used spectral clustering to perform cluster analysis.[24] In recognition that clustering is dependent on user-defined inputs, we used an iterative approach over a range of clusters (1 cluster up to a maximum of 8 clusters) to define the optimal number of clusters to form. For each iteration, cluster centers were generated by means of random sampling of the data eigenvector matrix. We used the gap statistic to select the optimal number of clusters. The gap statistic reflects the difference between within-cluster dispersion and that expected under an appropriate null distribution.[25] Further details of the cluster-building methodology are provided in the Methods section in this article's Online Repository at www.jacionline.org.

Statistical analysis of phenotypic clusters

We explored between-cluster differences in baseline clinical, demographic, and environmental covariates that were not included in the original model by using Kruskal-Wallis and χ^2 tests and used Kaplan-Meier estimates of cumulative probability and Cox proportional hazards models to evaluate 2 prospective outcomes, including time to first course of prednisone and time to initiation of alternate antiasthma therapies. We used linear mixed-effects models to examine between-cluster differences in prospective measures of lung function and PC_{20}.

RESULTS

Cluster analysis

The baseline characteristics for all 1041 participants are presented in Table E2 in this article's Online Repository at www.jacionline.org. As previously reported,[23] the demographic composition of the CAMP cohort is consistent with that of patients with childhood asthma in North America, including a higher proportion of boys, early age of onset, and high prevalence of atopic features.

We performed spectral clustering of 18 baseline phenotypic characteristics and optimized our model selection by calculating a gap statistic for different clustering models. Our results demonstrated 5 distinct phenotypic clusters.

All but 5 of the variables included in the final model were distributed differentially across clusters (Table I). Despite their prominence in previously described asthma classification schemes,[20-22] no between-cluster differences were observed in this study for anthropomorphic measures or circulating leukocyte levels (Table I).

Phenotypic characterization of asthma clusters

The essential features of each cluster can be characterized with respect to 3 groups of factors: (1) atopic burden (prevalence of atopic dermatitis (AD), allergic rhinitis or skin reactivity, and total serum IgE and circulating eosinophil levels); (2) lung function and airway lability (prebronchodilator FEV_1, FEV_1/forced vital capacity [FVC] ratio, bronchodilator response, and methacholine airways hyperresponsiveness [PC_{20}]); and (3) baseline exacerbation history (prior hospitalization and emergency department [ED] visits; Box 1). From these groupings, we constructed an Atopy-Obstruction-Exacerbation (AOE) scoring scheme, assigning low, medium, or high scores

TABLE I. Distribution of traits across clusters

	Cluster 1 (n = 300)	Cluster 2 (n = 202)	Cluster 3 (n = 218)	Cluster 4 (n = 225)	Cluster 5 (n = 96)
AOE classification	**LLL**	**HLL**	**HHM**	**MHH**	**HHH**
Asthma history					
Age of asthma onset (y)	3.52 ± 2.63	3.09 ± 2.40	3.66 ± 2.62	2.21 ± 1.89	2.27 ± 1.80
No. of subjects ever hospitalized for asthma (%)	0 (0)	0 (0)	1 (0.46)	225 (100)	94 (97.9)
ED visits for asthma (no./100 person-years)	44.3	47.0	70.2	75.6	101
Atopic features					
History of AD (%)	0 (0)	202 (100)	2 (0.1)	0 (0)	94 (97.9)
History of hay fever (%)	61 (20.3)	132 (65.3)	191 (87.6)	119 (52.9)	54 (56.3)
History of positive skin test responses (%)	230 (76.7)	185 (91.6)	209 (95.9)	198 (88)	92 (95.8)
Total serum IgE levels (log_{10})	2.37 ± 0.70	2.72 ± 0.72	2.79 ± 0.58	2.64 ± 0.61	2.81 ± 0.63
Spirometry					
Prebronchodilator FEV_1 (% predicted)	96.4 ± 12.7	97.7 ± 14.8	89.7 ± 13.9	91.4 ± 13.8	92.0 ± 16.1
Prebronchodilator FEV_1/FVC (% predicted)	81.8 ± 7.68	81.5 ± 7.59	77.6 ± 8.54	77.8 ± 8.24	78.6 ± 9.60
Prebronchodilator peak flow	276.1 ± 67.3	274.3 ± 73.3	276.7 ± 69.1	276.4 ± 70.8	255.6 ± 73.3
Airway responsiveness					
Methacholine PC_{20} (natural log)	0.71 ± 1.03	0.14 ± 1.11	−0.54 ± 1.00	0.038 ± 1.14	−0.23 ± 1.17
FEV_1 bronchodilator response (L)	0.077 ± 0.07	0.097 ± 0.08	0.12 ± 0.11	0.12 ± 0.11	0.16 ± 0.14
Anthropomorphic features					
BMI (kg/m^2)	18.1 ± 3.46	18.6 ± 3.83	18.5 ± 3.66	17.8 ± 3.19	17.6 ± 3.38
Waist/hip ratio	0.882 ± 0.06	0.885 ± 0.07	0.881 ± 0.06	0.874 ± 0.05	0.877 ± 0.07
Peripheral blood counts					
Eosinophils (log_{10})	2.35 ± 0.55	2.54 ± 0.53	2.57 ± 0.52	2.50 ± 0.49	2.71 ± 0.41
Lymphocytes (%)	42.1 ± 11.5	40.9 ± 9.82	41.1 ± 10.9	41.7 ± 10.5	40.8 ± 9.78
Neutrophils (%)	45.5 ± 12.2	44.5 ± 11.0	44.6 ± 11.5	44.7 ± 11.5	43.1 ± 11.0

for each factor group (Table I). For clarity of subsequent discussion, although prospective long-term asthma control was not considered in the clustering procedures (only baseline variables were considered), the cluster groups are also numbered in ascending rank order of poor long-term asthma control (ie, 1 = best control and 5 = worst control, as defined by need for oral steroid therapy during the 48 months of follow-up observation, see below).

The largest group of patients (cluster 1, 28.8% of cohort) represents the mildest cases with the fewest prior exacerbations, the lowest prevalence of atopic features, and preserved lung function (AOE classification LLL). The smallest cluster, cluster 5 (9.3%), consists of the most severe cases with the highest report of prior exacerbation, a very high atopic burden, and reduced lung function (AOE group HHH). The 3 remaining clusters reflect subsets with intermediate levels of severity and more heterogeneous clinical features. Cluster 2 (19.3%) includes those subjects with high atopic burden but preserved lung function (relative to the other groups) and intermediate airways hyperresponsiveness. This group has a low baseline ED visit rate and no reports of prior hospitalization (AOE group HLL). Patients in cluster 3 (20.9%) have high atopy burden, the most compromised lung function, and extreme airways hyperresponsiveness but have intermediate ED visit rates and only 1 report of prior hospitalization (AOE group HHM). In contrast, although patients in cluster 4 (21.6%) are substantially less atopic than those in cluster 2 and have reduced lung function at levels similar to those in cluster 3, they report higher ED visit rates, and nearly all report prior hospitalization for asthma (AOE group MHH). It is clear that no single feature is sufficient to characterize these groups.

When we developed the phenotypic clusters, we did not include multiple demographic, environmental, and symptom variables in the development of our model. However, we explored the presence of between-cluster differences for these variables and found that with the exception of age, for which statistically significant differences were observed across cluster groups, demographic variables, including sex and socioeconomic indicators, did not differ. This finding differed from previously reported cluster analyses, which found between-cluster differences in demographic variables. Details of these findings are reported in the Results section in this article's Online Repository at www.jacionline.org.

Temporal stability of phenotypic clusters

We evaluated the temporal stability of the phenotypic clusters by assessing longitudinal differences in exacerbation rates and multiple measures of pulmonary function between different clusters. We assessed the clinical and prognostic relevance of the derived cluster designations by performing survival analysis of time to asthma exacerbation over 48 months of follow-up. Because the original CAMP study involved the randomization of medical therapies, we were able to make an unbiased assessment of between-cluster differences in response to treatment. Kaplan-Meier analysis confirmed that cluster grouping was predictive of time to the first course of oral prednisone (log-rank $P < .0001$; Fig 1, *A*) and time to initiation of additional asthma controller therapy ($P < .0001$; Fig 1, *B*). Within the first 12 months after randomization, in clusters 4 and 5 a minority of subjects (34% [95% CI, 29% to 42%] and 36% [95% CI, 25% to 44%], respectively) had not required at least 1 course of oral steroids compared with 44% (95% CI, 38% to 51%) and 46% (95% CI, 39% to 52%) of subjects in clusters 3 and 2 and 54% (95% CI, 48% to 60%) of subjects in cluster 1. These established trends persisted for the remainder of the trial, with greater separation of cluster groupings over time. At 4 years, the 2 most extreme

Box 1. Summary of clinical characteristics of asthma clusters (AOE classification)

Cluster 1: Mild asthma with low atopy, obstruction, and exacerbation rate (LLL)

- Largest subgroup of patients (28.8%)
- No history of AD, lowest prevalence of hay fever or skin prick test reactivity, lowest IgE levels
- Preserved lung function (highest FEV_1/FVC ratio)
- Lowest bronchodilator response, intermediate airway hyperresponsiveness
- No prior hospitalization for asthma and lowest reported prevalence of ED visits
- Lowest risk of exacerbation*

Cluster 2: Atopic asthma with low levels of obstruction and medium rates of exacerbation (HLL)

- Universally report AD, high prevalence of allergic rhinitis and skin test reactivity
- Preserved lung function (highest FEV_1)
- Intermediate bronchodilator response and airways hyperresponsiveness
- No prior hospitalization, low rates of prior ED visits
- Low-to-intermediate risk of exacerbations*

Cluster 3: Atopic asthma with high levels of obstruction and medium rates of exacerbation (HHM)

- Rarely report AD (in contrast to HLM cluster) but highest prevalence of allergic rhinitis and skin test reactivity
- Most reduced lung function (lowest FEV_1 and FEV_1/FVC ratio)
- High bronchodilator response and most severe airways hyperresponsiveness
- Few prior hospitalizations but intermediate rates of prior ED visits (similar to HLM cluster)
- Intermediate risk of exacerbations*

Cluster 4: Moderately atopic asthma with high levels of obstruction and high exacerbation rates (MHH)

- No history of AD, intermediate prevalence of hay fever (52.9%), lower IgE levels
- Reduced lung function (low FEV_1/FVC ratio, similar to HHH cluster)
- High bronchodilator response and high airways hyperresponsiveness
- Most reports of prior hospitalization
- Intermediate-to-high risk of exacerbation*

Cluster 5: Highly atopic asthma with high levels of obstruction and high exacerbation rates (HHH)

- Smallest subgroup of patients (9.3%)
- Nearly universal AD, highest prevalence of skin test reactivity, highest IgE levels, highest eosinophilia, intermediate prevalence of allergic rhinitis
- Reduced lung function (low FEV_1/FVC ratio, similar to MHH cluster)
- Highest bronchodilator response and severe airways hyperresponsiveness
- Most reports of prior hospitalization and highest rate of ED visits
- Highest risk of exacerbation*

*Poor long-term asthma exacerbation risk is defined from prospective survival analysis of time to first course of oral prednisone. This variable was derived by using the defined cluster groupings and was therefore not considered in spectral cluster analyses used to define the clusters.

groups exhibited a 3-fold difference in their lack of requirement for oral prednisone (32% [95% CI, 27% to 37%] in cluster 1 vs 11% [95% CI, 5% to 18%] in cluster 5, $P < .0001$). Similar relationships were noted for time to initiation of additional asthma controller therapies (Fig 1, *B*).

With 4 years of prospective follow-up as part of the CAMP clinical trial, we were able to assess the longitudinal consistency in airway hyperresponsiveness and pulmonary function across the 5 identified clusters using linear mixed-effects models. For methacholine PC_{20}, prebronchodilator and postbronchodilator FEV_1, and prebronchodilator and postbronchodilator FEV_1/FVC ratio, we observed statistically significant ($P < .0001$) between-cluster differences across time (Fig 2). Values for methacholine PC_{20}, FEV_1, and FEV_1/FVC ratio demonstrated similar temporal patterns, with cluster 3 (HHM) having relatively lower values than cluster 1 (LLL) across time and minimal overlap in the trajectories of the clusters with the most extreme values. These temporal differences are aligned with baseline assessment of airway obstruction (Table I) and differ from baseline assessments of exacerbation rates, where cluster 5 (HHH) had the highest rate of exacerbations and cluster 1 (LLL) had the lowest rate.

Cluster grouping correlates with prospective long-term asthma control and response to specific inhaled anti-inflammatory controller medications

We next assessed whether treatment response to specific inhaled anti-inflammatory controller medications differed by cluster group. As originally reported in the primary outcomes assessment of the CAMP trial,[3] use of inhaled budesonide significantly reduced the number of asthma exacerbations compared with placebo in all phenotypic clusters. A further finding was that nedocromil did not significantly reduce exacerbation rates or additional controller therapies compared with placebo. However, in a *post hoc* evaluation stratified by cluster grouping, we observed heterogeneity in treatment

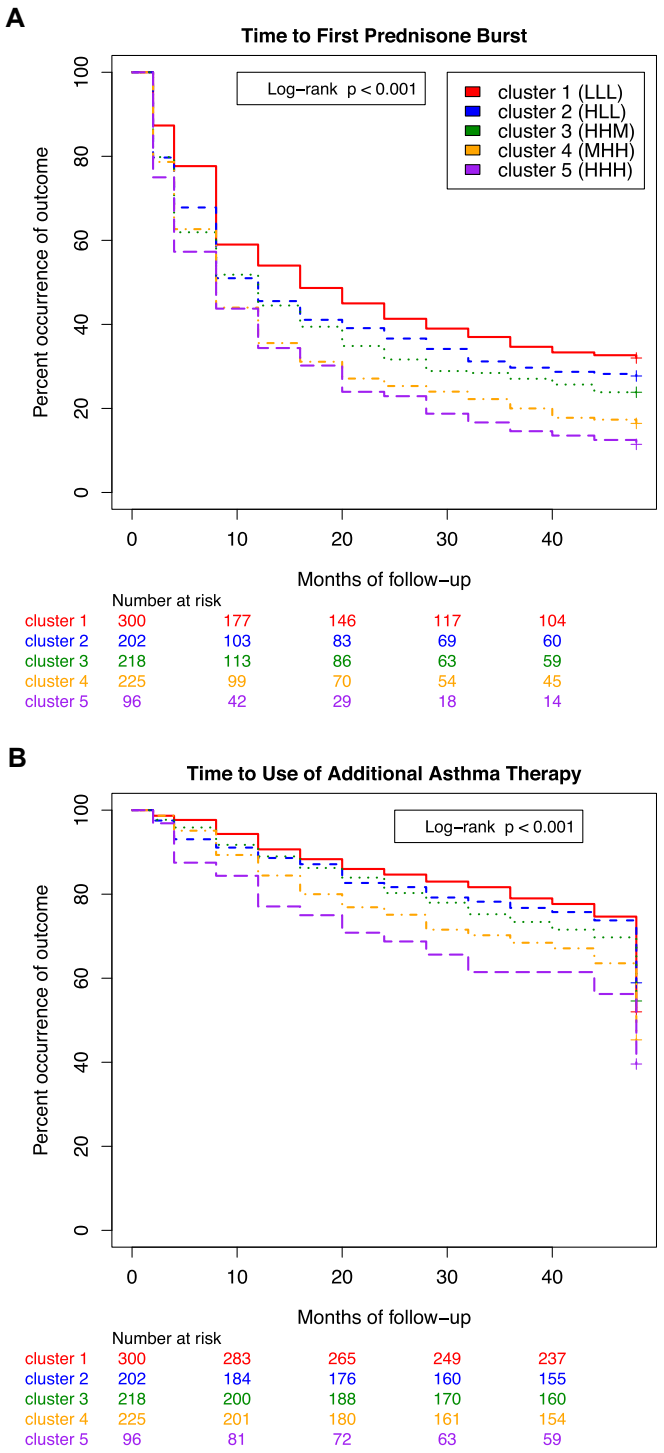

FIG 1. Kaplan-Meier plots by cluster of the cumulative probability of a first course of prednisone **(A)** or initiation of additional asthma controller therapies (beclomethasone or other; **B**) during the 4-year follow-up period of the CAMP trial.

response rates to both medications (Fig 3 and Table II; and see Table E3 in this article's Online Repository at www.jacionline.org). We found that subjects stratified to the 3 more mild clusters (clusters 1, 2, and 3) demonstrated treatment response patterns similar to those reported in the cohort as a whole. However, in the 2 most severe clusters (clusters 4 and 5), we found that the therapeutic efficacy of nedocromil appeared to be more similar to that of budesonide. Subjects in cluster 4, those with the lowest atopic burden, worst lung function, and high baseline exacerbation rates, demonstrated a significant reduction in exacerbation rates when randomized to nedocromil (1.7-fold [95% CI, 1.4-fold to 2.2-fold] reduction compared with placebo at 12 months and 1.6-fold [95% CI, 1.1-fold to 1.6-fold] reduction at 4 years) that was similar (log-rank $P = .96$ for difference) to the reductions observed among those randomized to budesonide (1.6-fold [95% CI, 1.3-fold to 2.0-fold] reduction compared with placebo at 12 months and 1.4-fold [95% CI, 1.0-fold to 1.5-fold] reduction at 4 years). For subjects in cluster 5, those with a high atopic burden, low lung function, and the highest baseline exacerbations, there was no decrease in exacerbation rates for subjects randomized to either nedocromil or budesonide (compared with placebo; log-rank $P = .56$ and .12, respectively).

Comparisons with previously reported cluster groupings of asthma

To assess the generalizability of our cluster analysis, we compared our results with those of 2 prior cluster analyses of subjects from the Severe Asthma Research Program (SARP) cohort, one in a pediatric population[20] and one in a cohort of pediatric and adult subjects.[22] Although both the SARP study and current study used unsupervised multivariate statistical methods for cluster determination, the variables included in the initial clustering model were different. The SARP studies included a mixture of both demographic and disease descriptive variables, whereas the current study included only disease descriptive variables in the initial model. In addition, the CAMP cohort was the only one that controlled for the use of inhaled corticosteroids (Table III), allowing for a formal test of differential response to therapy between clusters.

The asthma subgroups defined by both the SARP childhood study and the current CAMP study were similar with respect to the degree of atopy, airway obstruction, and exacerbation rates present within each of the subgroups (Table III). However, several differences exist between the 2 studies, including the absence in the SARP cohorts of a cluster with a low degree of atopy and a high degree of both airway obstruction and exacerbation rates (cluster 4 in the CAMP study).

Furthermore, differences in the degree of atopy between clusters are more pronounced in the CAMP cohort than the SARP cohorts. For example, for the CAMP clusters, there are multiple clusters in which almost 100% of the subjects have a history of AD (cluster 2, 100%; cluster 5, 97.9%) and multiple clusters in which almost none of the subjects have a history of AD (cluster 1, 0%; cluster 4, 0%; cluster 3, 0.1%). For the SARP clusters, the difference in the history of AD is comparatively less, with within-cluster incidence ranging from 47% to 72%. In addition, the degree of spread for the serum IgE variable is different between the SARP and CAMP clusters, with a greater degree of spread present among the CAMP clusters when compared with the SARP clusters (234-645 vs 105-405 kU/L, respectively).

There are also many similarities between the CAMP clusters and the clusters generated from a cluster analysis of adults from the SARP study. Similar to the CAMP and childhood SARP clusters, the mixed adult and pediatric SARP clusters are comparable with respect to the degree of airway obstruction and exacerbation rates present within the

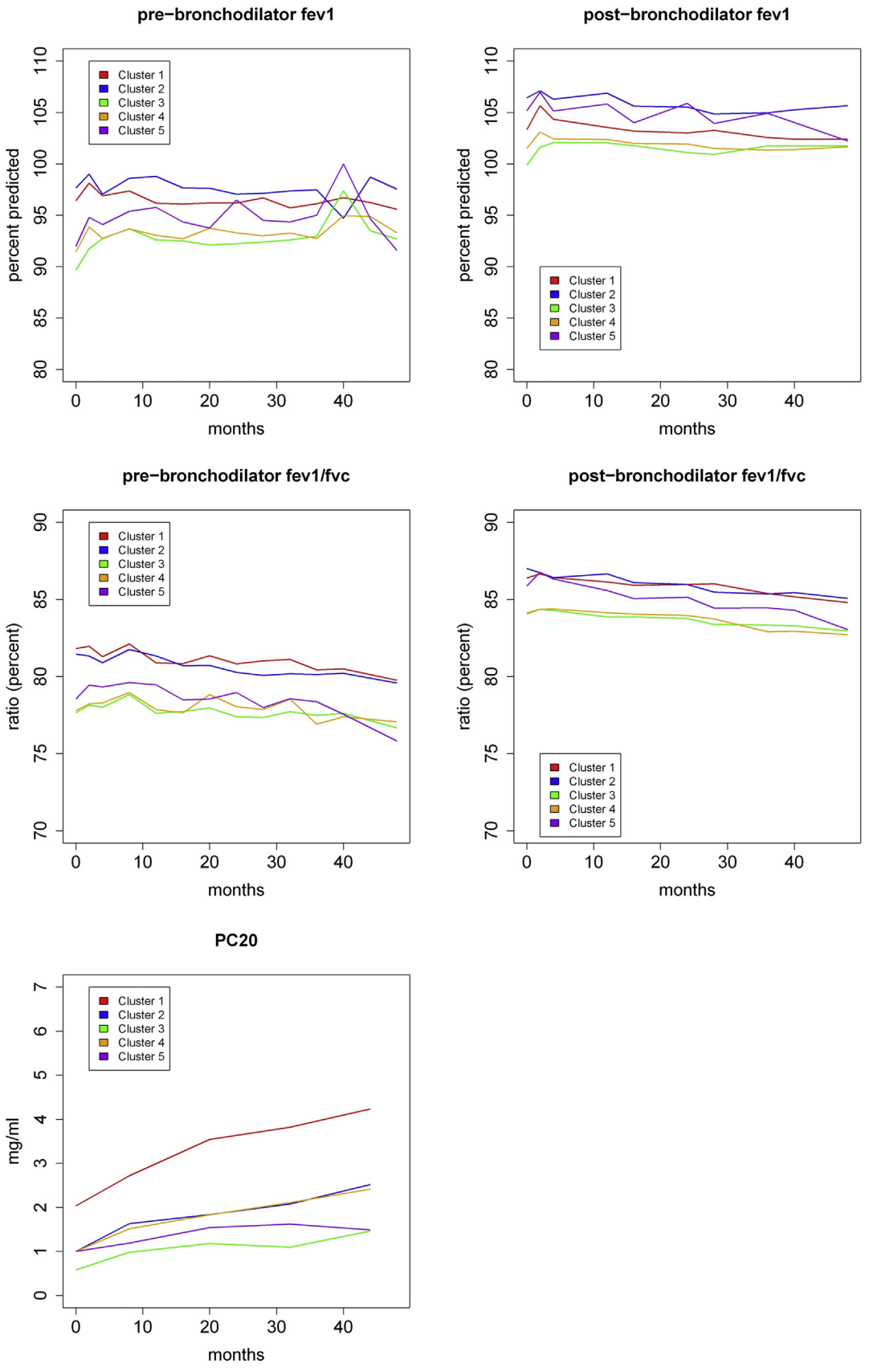

FIG 2. Mean pulmonary function measurements for each phenotypic cluster evaluated over 48 months of follow-up. *P* values for between-cluster differences among all longitudinal measures were less than .0001, as calculated by using linear mixed-effects models.

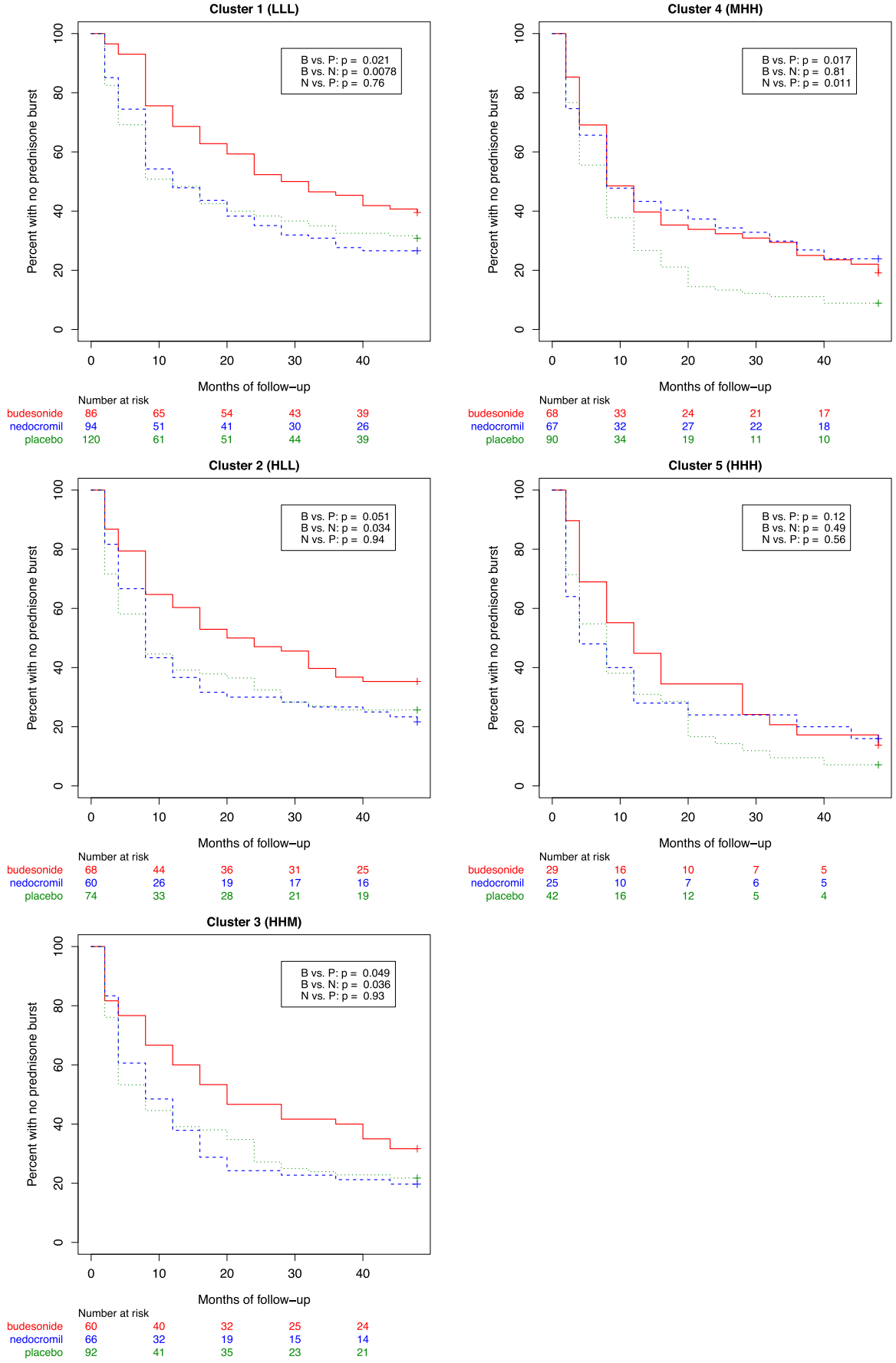

FIG 3. Kaplan-Meier estimate by treatment group of the cumulative probability of prednisone use during 4 years of follow-up stratified by phenotypic cluster. *B*, Budesonide; *N*, Nedocromil.

TABLE II. Number of prednisone bursts over time stratified by cluster and treatment group

AOE classification	Cluster 1 (n = 300) LLL	Cluster 2 (n = 202) HLL	Cluster 3 (n = 218) HHM	Cluster 4 (n = 225) MHH	Cluster 5 (n = 96) HHH	*P* value
Budesonide (n = 311 [29.9%])						
2 mo	0.06 ± 0.34	0.19 ± 0.61	0.18 ± 0.39	0.30 ± 0.90	0.19 ± 0.19	.13
4 mo	0.12 ± 0.22	0.30 ± 0.31	0.34 ± 0.41	0.54 ± 0.46	0.58 ± 0.36	.003
8 mo	0.39 ± 0.63	0.53 ± 0.52	0.71 ± 0.72	1.09 ± 0.82	0.92 ± 0.36	.01
12 mo	0.61 ± 0.67	0.72 ± 0.54	0.91 ± 0.58	1.44 ± 0.64	1.29 ± 0.31	.004
16 mo	0.78 ± 0.44	0.95 ± 0.65	1.21 ± 0.76	1.63 ± 0.42	2.22 ± 0.78	.01
20 mo	0.85 ± 0.38	1.15 ± 0.38	1.46 ± 0.58	1.87 ± 0.69	2.70 ± 0.44	.01
24 mo	1.07 ± 0.89	1.35 ± 0.72	1.57 ± 0.48	2.07 ± 0.44	3.04 ± 0.37	.04
28 mo	1.24 ± 0.46	1.47 ± 0.36	1.76 ± 0.50	2.39 ± 0.93	3.40 ± 0.38	.04
32 mo	1.39 ± 0.45	1.69 ± 0.58	1.96 ± 0.68	2.54 ± 0.47	3.63 ± 0.27	.05
36 mo	1.61 ± 0.62	1.98 ± 0.54	2.19 ± 0.48	2.85 ± 0.73	4.05 ± 0.42	.03
40 mo	1.75 ± 0.46	2.13 ± 0.39	2.33 ± 0.48	3.14 ± 0.74	4.52 ± 0.28	.03
44 mo	1.78 ± 0.31	2.23 ± 0.36	2.56 ± 0.49	3.03 ± 0.59	4.71 ± 0.20	.02
48 mo	1.94 ± 0.46	2.42 ± 0.63	2.86 ± 0.48	3.38 ± 0.52	5.00 ± 0.28	.04
Nedocromil (n = 312 [30.0%])						
2 mo	0.21 ± 0.55	0.29 ± 0.65	0.22 ± 0.60	0.35 ± 0.65	0.54 ± 0.83	.91
4 mo	0.37 ± 0.43	0.55 ± 0.64	0.54 ± 0.56	0.61 ± 0.61	1.13 ± 1.18	.06
8 mo	1.03 ± 1.03	0.94 ± 0.52	1.12 ± 1.14	1.02 ± 0.68	1.59 ± 0.73	.78
12 mo	1.38 ± 0.70	1.26 ± 0.95	1.52 ± 0.78	1.46 ± 0.95	2.14 ± 0.99	.66
16 mo	1.71 ± 0.92	1.76 ± 1.11	1.94 ± 0.70	1.89 ± 0.80	2.78 ± 1.10	.60
20 mo	2.05 ± 0.51	2.10 ± 0.69	2.38 ± 0.79	2.15 ± 0.61	3.00 ± 0.52	.55
24 mo	2.33 ± 0.41	2.42 ± 0.69	2.92 ± 1.93	3.37 ± 0.60	3.50 ± 1.15	.55
28 mo	2.48 ± 0.47	2.74 ± 0.95	3.42 ± 2.07	2.53 ± 0.47	3.91 ± 0.83	.62
32 mo	2.65 ± 0.45	3.20 ± 0.94	3.74 ± 0.68	2.82 ± 0.65	4.29 ± 0.51	.56
36 mo	2.87 ± 0.60	3.27 ± 0.39	4.09 ± 0.65	3.29 ± 1.26	4.85 ± 0.58	.28
40 mo	3.14 ± 0.63	3.51 ± 0.40	4.29 ± 0.60	2.53 ± 0.63	5.15 ± 0.76	.26
44 mo	3.25 ± 0.35	3.78 ± 0.69	4.46 ± 0.67	3.80 ± 0.64	5.40 ± 0.64	.22
48 mo	3.56 ± 0.50	4.17 ± 0.67	4.67 ± 0.36	4.02 ± 0.82	5.42 ± 0.63	.34
Placebo (n = 418 [40.1%])						
2 mo	0.30 ± 0.77	0.45 ± 0.86	0.37 ± 0.72	0.34 ± 0.71	0.41 ± 0.72	.49
4 mo	0.53 ± 0.52	0.84 ± 0.75	0.77 ± 0.65	0.83 ± 0.84	0.76 ± 0.69	.16
8 mo	1.07 ± 0.92	1.32 ± 0.81	1.21 ± 0.73	1.45 ± 0.96	1.61 ± 1.25	.29
12 mo	1.47 ± 0.93	1.80 ± 0.95	1.56 ± 0.66	2.12 ± 0.88	2.29 ± 1.47	.04
16 mo	1.97 ± 0.81	1.92 ± 0.50	1.71 ± 0.43	2.85 ± 0.97	2.77 ± 0.78	.01
20 mo	2.37 ± 1.03	2.30 ± 0.88	1.99 ± 0.58	3.65 ± 1.02	3.80 ± 1.72	<.001
24 mo	2.81 ± 0.95	2.84 ± 0.98	2.54 ± 1.06	4.23 ± 0.93	4.26 ± 0.78	<.001
28 mo	3.38 ± 2.47	3.14 ± 0.55	2.82 ± 0.51	4.75 ± 0.95	4.86 ± 0.92	<.001
32 mo	3.79 ± 0.96	3.42 ± 0.54	3.10 ± 0.70	5.30 ± 0.96	5.46 ± 0.92	<.001
36 mo	3.98 ± 0.51	4.09 ± 2.78	3.30 ± 0.43	6.01 ± 2.98	5.89 ± 0.70	<.001
40 mo	4.18 ± 0.49	4.34 ± 0.69	3.53 ± 0.53	6.32 ± 0.65	6.40 ± 0.71	<.001
44 mo	4.42 ± 0.63	4.64 ± 0.67	3.75 ± 0.55	6.65 ± 0.71	6.82 ± 0.78	<.001
48 mo	4.69 ± 0.52	4.72 ± 0.78	3.91 ± 0.46	6.89 ± 0.53	7.42 ± 0.86	<.001
P values						
Budesonide vs nedocromil	.0006	.008	.054	.96	.22	
Budesonide vs placebo	.0007	.001	.022	.005	.13	
Nedocromil vs placebo	.39	.45	.65	.006	.82	

The cumulative number of subjects experiencing asthma exacerbations, as demonstrated by the need for oral prednisone therapy, at each study time point stratified by treatment group is shown. Shown are the mean ± SD cumulative number of prednisone bursts per person from the onset of the study period and *P* values for between-cluster differences in outcome (far right) and pairwise comparisons of within-cluster differences in outcomes (bottom level).

clusters (Table III). However, the SARP clusters with a higher proportion of adults differ from both the CAMP and childhood SARP clusters in that the overall degree of atopy present, as determined by serum IgE level, is less for the adult than the childhood populations. The lowest IgE level for the childhood clusters (105 kU/L for childhood SARP cluster 1) is almost as high as the highest IgE level for the adult clusters (141 kU/L for adult SARP cluster 1). Additionally, the degree of airway obstruction present in the adult population is much greater in the adult population, with FEV_1/FVC ratios for each cluster ranging from 0.57 to 0.78 in the adult population versus 0.78 to 0.82 in the CAMP and 0.72 to 0.82 in the childhood SARP populations.

DISCUSSION

The clinical heterogeneity of asthma has motivated the use of machine-learning algorithms for the classification of patients using data-driven unbiased criteria. Although earlier work[20-22] established the feasibility of this approach, many important questions remain unaddressed, including issues of reproducibility, generalizability, and clinical relevance. Without longitudinal

TABLE III. Comparison of study design and results from independent asthma phenotype clustering analyses

	CAMP (current study)	SARP childhood study	SARP adult study
Sample size	1041	161	726
Age distribution (y)	5-13	6-17	12-80
Percentage non-Hispanic white	68	39	64
Inclusion criteria	Dx, AHR, BDR No active symptoms	Dx, AHR, BDR No active symptoms	Dx, nonsmokers
ICS at time of evaluation	No	Yes × 6 mo	Yes
Spectrum of severity at enrollment	Mild to moderate	Mild to severe	Mild to severe
Variables included in model	18	12	34
No. of clusters	5	4	5
AOE cluster subtypes			
1. LLL	Cluster 1 (28.8%)	Cluster 1 (29.8%)	Cluster 1 (15.2%)
Descriptor	Mild asthma with a low atopic burden	Late onset, normal lung function	Young female subjects with atopic asthma and normal lung function
Age of asthma onset (y)	3.52	6.08	
Total serum IgE (kU/L)	234	105	141
AD	0%	50%	
Pre-BD FEV_1/FVC ratio	0.82 ± 0.8	0.82 ± 0.11	0.78 ± 0.1
Exacerbation rate (rank)	Lowest	Lowest	Lowest
2. HLL	Cluster 2 (19.4%)	Cluster 2 (32.3%)	Cluster 2 (44.2%)
Descriptor	Atopic with preserved lung function	Early onset, atopic, normal lung function	Older female subjects with atopic asthma and normal lung function
Age of asthma onset (y)	3.09	2.5	
Total serum IgE (kU/L)	524	405	125
AD	100%	56%	
Pre-BD FEV_1/FVC ratio	0.81 ± 0.8	0.79 ± 0.09	0.74 ± 0.1
Exacerbation rate (rank)	Second lowest	Second lowest	Second lowest
3. HHM	Cluster 3 (20.9%)	Cluster 3 (19.9%)	Cluster 4 (16.5%)
Descriptor	High obstruction, severe AHR	Early onset, atopic, mild airflow limitation	Childhood-onset atopic asthma, severe obstruction, high BDR
Age of asthma onset (y)	3.66	1.2	
Total serum IgE (kU/L)	616	216	132
AD	0.1%	47%	
Pre-BD FEV_1/FVC ratio	0.78 ± 0.8	0.72 ± 0.10	0.64 ± 0.1
Exacerbation rate (rank)	Third lowest	Third lowest	Third lowest
4. MHH	Cluster 4 (21.6%)	Not represented	Cluster 5 (16.0%)
Descriptor	Low atopy, high obstruction, high exacerbation		Childhood-onset atopic asthma, severe obstruction, low BDR
Age of asthma onset (y)	2.21		
Total serum IgE (kU/L)	436		98
AD	0%		
Pre-BD FEV_1/FVC ratio	0.78 ± 0.08		0.57 ± 0.1
Exacerbation rate (rank)	Second highest		Second highest
5. HHH	Cluster 5 (9.2%)	Cluster 4 (18.0%)	Cluster 3 (8.13%)
Descriptor	Exacerbation	Early onset, atopic, advanced airflow limitation	Older women, high BMI, low atopy, low BDR
Age of asthma onset (y)	2.68	1.6	
Total serum IgE (kU/L)	645	361	54
AD	97.9%	72%	
Pre-BD FEV_1/FVC ratio	0.79 ± 0.09	0.73 ± 0.10	0.74 ± 0.1
Exacerbation rate (rank)	Highest	Highest	Highest

AHR, Airway hyperresponsiveness; *BD*, bronchodilator; *BDR*, bronchodilator response; *ICS*, inhaled corticosteroid; *Dx*, diagnosis.

follow-up, which was unavailable in prior reports, it is unclear whether the defined clusters have clinical utility. It is in this context that the results of our phenotypic clustering efforts and longitudinal analysis can be summarized.

First, we demonstrate the longitudinal consistency of our phenotypic clusters. When we developed the clusters, we limited ourselves to the clinical data obtained during baseline assessment of CAMP participants. Next, we evaluated for changes in cluster membership over the 48-month study period and found remarkable consistency in phenotypic distributions over time, particularly with regard to airway hyperresponsiveness, obstruction, and exacerbation rates. These findings echo those of a recent longitudinal cluster analysis that found membership in phenotypic clusters to be extremely stable over time.[26] An additional finding of our study was that different inhaled anti-inflammatory medications appeared to have no statistically significant effect on cluster membership over time, suggesting that although these medications might affect day-to-day symptoms,

they have minimal effect on the natural history of childhood asthma.

Second, we demonstrate the therapeutic benefit of our phenotypic clusters. We found important between-cluster differences in response to inhaled asthma therapies, with one cluster (cluster 4) showing decreased rates of exacerbations with both budesonide and nedocromil therapy and another cluster (cluster 5) showing poor response with both budesonide and nedocromil therapy. Our data suggest that although inhaled corticosteroids, such as budesonide, should serve as the primary treatment choice for asthma control in children with mild-to-moderate asthma, there are several subgroups of patients, including those with the poorest level of baseline asthma control, who appear to respond to nedocromil at levels similar to budesonide. Given safety concerns, particularly in children, regarding the long-term exposure to inhaled glucocorticoid therapy, identification of phenotypic clusters that could benefit similarly from nonsteroidal therapies would be of great value. Although the retrospective nature of the current study and the small size of several of the clusters limits our ability to draw firm clinical conclusions about the current results, our findings serve as the foundation for future prospective clinical trials investigating personalized responses to inhaled anti-inflammatory medications.

Finally, despite notable differences in the compositions of the patient populations, the computational algorithms used, and the variables considered in generating the clusters, our results show remarkable consistency with those obtained in the pediatric and adult SARP populations, both with respect to the number of phenotypic clusters identified (5-6 clusters in CAMP and SARP cohorts) and the characteristics of individual clusters.[20-22] The similarity of our phenotypic clusters to those of other cohorts provides further evidence for the potential generalizability of clustering as a method of phenotyping asthmatic patients. Observed differences in the degree of atopy and airway obstruction present in the pediatric compared with adult clusters lend further support to the hypothesis of etiologic differences between childhood and adult asthma.

Our study had several limitations. First, we evaluated only children, and reports have shown that pediatric and adult asthma might represent 2 different disease states with different pathogenic mechanisms and natural histories.[27] For this reason, the clinical implications of this cluster analysis might not be widely applicable to an adult asthmatic population.

Second, our study did not include patients with severe childhood asthma. Because our original population was ascertained for the purposes of a clinical trial, it included children with mild-to-moderate persistent asthma and specifically excluded those with more severe asthma. Thus there is a possibility that there is a severe childhood asthmatic phenotypic that was missed with our analysis, although the strong similarities in observed clusters with the childhood SARP study (which included a broader spectrum of disease severity) provide reassurance that the results of our cluster analysis are more widely applicable.

Third, the conclusions that we can draw from the clinical outcomes of our clusters are limited because of their small sample size and modest differences. For example, the children in cluster 5 had a limited response to inhaled budesonide and nedocromil compared with placebo, suggesting that the children in this cluster might have some resistance to corticosteroid therapy. However, because there were only 96 children in this cluster, we were underpowered to draw more clinically meaningful conclusions from this particular analysis. It will be necessary to validate some of these preliminary findings in future prospective studies to determine whether the children in this cluster are truly steroid resistant.

In conclusion, our results support the use of computationally inferred phenotypic classifications of asthma as having clinical utility. These models define subsets of patients with unique clinical attributes, discrete clinical trajectories, and variable responsiveness to antiasthma controller medications. Recognition of these clusters and their clinical relevance should motivate novel strategies in both the research and clinical settings. More refined phenotypic classification might better inform treatment decisions: as suggested by the results of our treatment responsiveness analysis, cluster assignment identifies 2 subsets of patients who respond similarly to both budesonide and nedocromil, providing clinicians with viable treatment options for patients at risk for corticosteroid-related complications. The observed between-cluster differences in environmental and genetic factors suggest that important etiologic differences underlie the configuration of different asthma subgroups. Future studies that consider more homogenous subsets of patients should improve research precision in characterizing the genetic and environmental causes. Thus in addition to helping inform clinical management, these more refined phenotypic classification schemes should help accelerate research efforts in defining the molecular and environmental underpinnings of this complex airways disease.

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Clinical implications: Childhood asthma is heterogeneous. The current cluster analysis suggests that heterogeneous phenotypic clusters of asthmatic children remain stable over time, even after treatment with different medical therapies.

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METHODS

All statistical analysis was performed with R 2.10.1 software.

Model building

Variable selection. Of 48 variables initially considered, 10 were excluded because of an excess of missing data (>10% of values missing). Because of the inherent strong correlation between prebronchodilator and postbronchodilator spirometric measures, we considered only one of the 2 measurements (either prebronchodilator or postbronchodilator) for FEV_1, FVC, FEV_1/FVC ratio, and peak flow. Because of the subjective nature of many of the symptom-provoking variables, such as "animal dander worsens asthma," these variables were excluded from further analysis. After variable selection, missing values were imputed by using a *k*-nearest neighbor algorithm from the *pamr* package of Bioconductor 2.5.[E1] Given that clustering results might be affected by differences in scale among variables,[E2] vector normalization was performed to scale each variable to a unit vector.

Cluster analysis. To this set of 18 variables, we applied a spectral clustering algorithm, as implemented in the *specc* function of the *kernlab* package.[E3] We specified a range of 1 to 8 clusters for the algorithm to define. The cluster centers were randomly initialized by using the spectral clustering algorithm. We used an iterative approach, adding additional variables one at a time to the model, and used the gap statistic[E4] to calculate the optimal number of variables for partitioning of the data.

Spectral clustering with 18 baseline phenotypic characteristics yielded many models with high gap statistics over the range of cluster numbers considered; however, the maximum statistic (gap = 0.86) was observed for 5 clusters (Fig E1, *A*). By using leave-out-one variable cross-validation, the importance of all 18 variables in the final model was confirmed because exclusion of any single variable resulted in substantial subgroup fragmentation and inferior model performance (data not shown). We also repeated the clustering analysis, restricting ourselves to 711 self-reported white subjects (the largest ethnic subgroup), and found no difference in cluster assignment (data not shown). Hence our final model is optimal with respect to the number of variables considered and clusters defined and does not appear to be confounded by ethnic-specific phenotypic differences.

Fig E1, *B*, presents a heat map of the clinical phenotypes grouped by cluster. Several variables, such as the history of hay fever, AD, prior hospitalizations, and PC_{20}, segregate discretely by cluster, suggesting that these variables were primary drivers of the clustering.

Cluster validation. We assessed the influence of including individual single variables on determining the final cluster assignments. Instead of using a multivariate model with 18 variables for cluster analysis, we used each variable separately to perform the clustering. For the continuous variables, we specified the formation of 5 clusters to allow for comparison to the multivariate model. The categorical variables led to the formation of 2 clusters. To compare the univariate and multivariate approaches to clustering, we evaluated the ability of both approaches to predict future exacerbations (ie, the time to first use of oral prednisone).

To evaluate the reproducibility of our cluster assignments, we repeated the unsupervised analysis in the CAMP cohort using the clustering algorithm used in the classification studies in the SARP adult[E5] and childhood[E6] cohorts, with hierarchic clustering with Ward minimum distance as an agglomeration method. We used the *hclust* function of the *stats* package in R to generate 5 clusters and compared the composition of these new clusters with our original cluster assignments. We also performed an outcomes analysis with these new clusters and compared this with our original outcomes analysis.

RESULTS

Demographic, environmental, and familial determinants of cluster grouping

Descriptions of demographic, environmental, and familial clinical variables across phenotypic clusters are presented in Table E4. Although trends for higher proportions of non-Hispanic white subjects in the mildest group and black subjects in the most severe groups were noted, these differences were not statistically significant. In contrast, enlightening differences across clusters were observed for numerous environmental and familial factors. For example, although environmental tobacco smoke exposure was reported by subjects in all 5 clusters, the prevalence was greatest among subjects in clusters 4 and 5 (ie, those with the highest baseline exacerbation rates). However, among subjects in the less severe clusters, a direct relationship between severity and smoke exposure was not observed: those with the lowest childhood smoke exposure (cluster 2, 30.2%) had higher baseline exacerbation and greater airways hyperresponsiveness than subjects in cluster 1, who had significantly higher childhood smoke exposure (39.7%), and exacerbation rates, lung function, and airways responsiveness were markedly different between clusters 1 and 3, despite very similar childhood smoke exposure rates (39.7% vs 37.6%, respectively). Similarly, although differences in aeroallergen exposure and in familial burden of both asthma and atopy were observed across the 5 phenotypic clusters, obvious linear correlations between risk factor exposure and severity of disease were not observed.

Comparison of univariate versus multivariate cluster analysis

We compared our multivariate cluster analysis using 18 variables with a univariate approach by using each of the 18 variables by itself. To evaluate the difference in the ability of each of these methods to predict future exacerbations, as measured by the time to first use of prednisone, we performed survival analysis for each of the models. We found that the single variable with the best predictive accuracy for future exacerbations was that of history of prior hospitalization for asthma exacerbations (Fig E2). This variable was the only single variable to outperform the multivariate phenotypic clusters in terms of its ability to predict future exacerbations (as measured by r^2). However, as a dichotomous variable, this factor provided only gross partitioning of the 3 lowest-risk and 2 highest-risk groups.

Reproducibility of cluster assignments by using different clustering algorithms

Using hierarchical clustering, we were able to generate clusters quite similar in composition to our original clusters in terms of AOE grouping (Fig E3, *A*). To assess whether the new cluster assignments also demonstrated longitudinal consistency similar to the original clusters, we repeated our survival analysis of time to asthma exacerbation using the 4 years of follow-up data generated as part of the CAMP clinical trial. For the survival analysis, we found that the clusters generated by using hierarchical clustering demonstrated a similar natural history to our original clusters (Fig E3, *B*).

Cluster grouping correlates with prospective long-term asthma control and response to specific inhaled anti-inflammatory controller medications

We performed a Cox regression analysis stratified by treatment group. The primary outcome of interest was exacerbation rate, defined as the initiation of therapy with oral prednisone. We found that for all clusters, with the exception of cluster 2, therapy with budesonide resulted in significantly fewer exacerbations when compared with placebo (Table E5). We also found that for clusters 3, 4, and 5, therapy with nedocromil resulted in fewer

exacerbations when compared with placebo. We also performed a formal test of interaction between cluster and treatment group that demonstrated a nominally significant interaction ($P = .05$) between the phenotypic cluster and the study drug for cluster 4 and both nedocromil (with placebo as reference) and budesonide (with nedocromil as reference), indicating that this cluster showed some modest response to nedocromil that was not detected in the original CAMP study when subjects were not evaluated by cluster (see Table E6).

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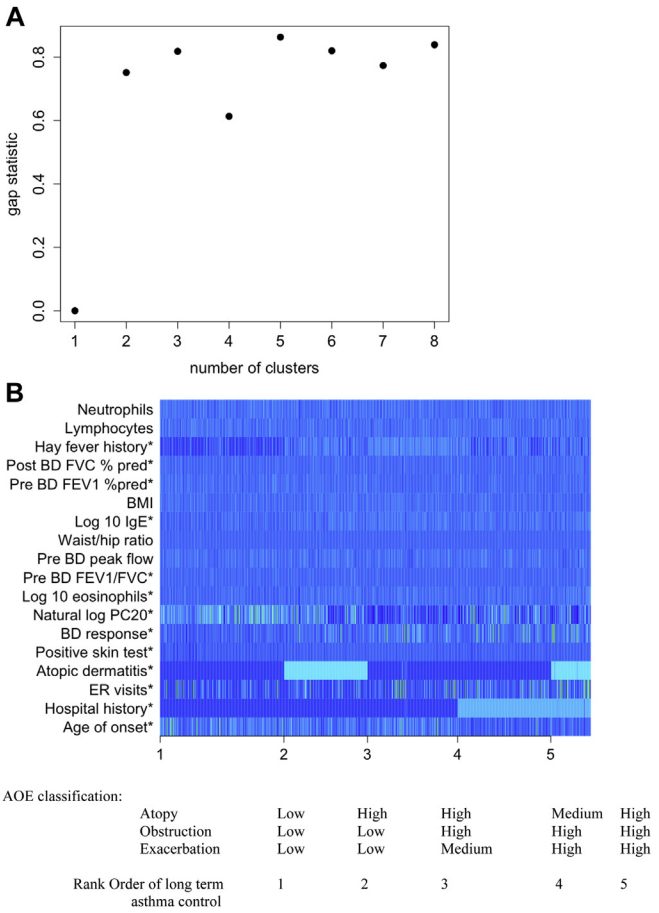

AOE classification:

Atopy	Low	High	High	Medium	High
Obstruction	Low	Low	High	High	High
Exacerbation	Low	Low	Medium	High	High
Rank Order of long term asthma control	1	2	3	4	5

FIG E1. A, The gap statistic as a function of the number of clusters. A higher gap statistic indicates greater between-cluster separation. **B,** Heat map depicting the differences among normalized clinical variables used for clustering and the different phenotypic clusters. The cluster assignments are grouped along the *horizontal axis*, and the variables used to determine the cluster assignments appear along the *vertical axis*. The lighter shades of blue denote relatively higher magnitudes for each variable, and the darker shades denote relatively lower magnitudes. *$P < .0001$ for difference in distribution across clusters. Variables demonstrating more distinct between-cluster differences in magnitude, such as hospital history, AD, PC_{20}, and hay fever history, were the primary drivers of the cluster assignments. *BD*, Bronchodilator.

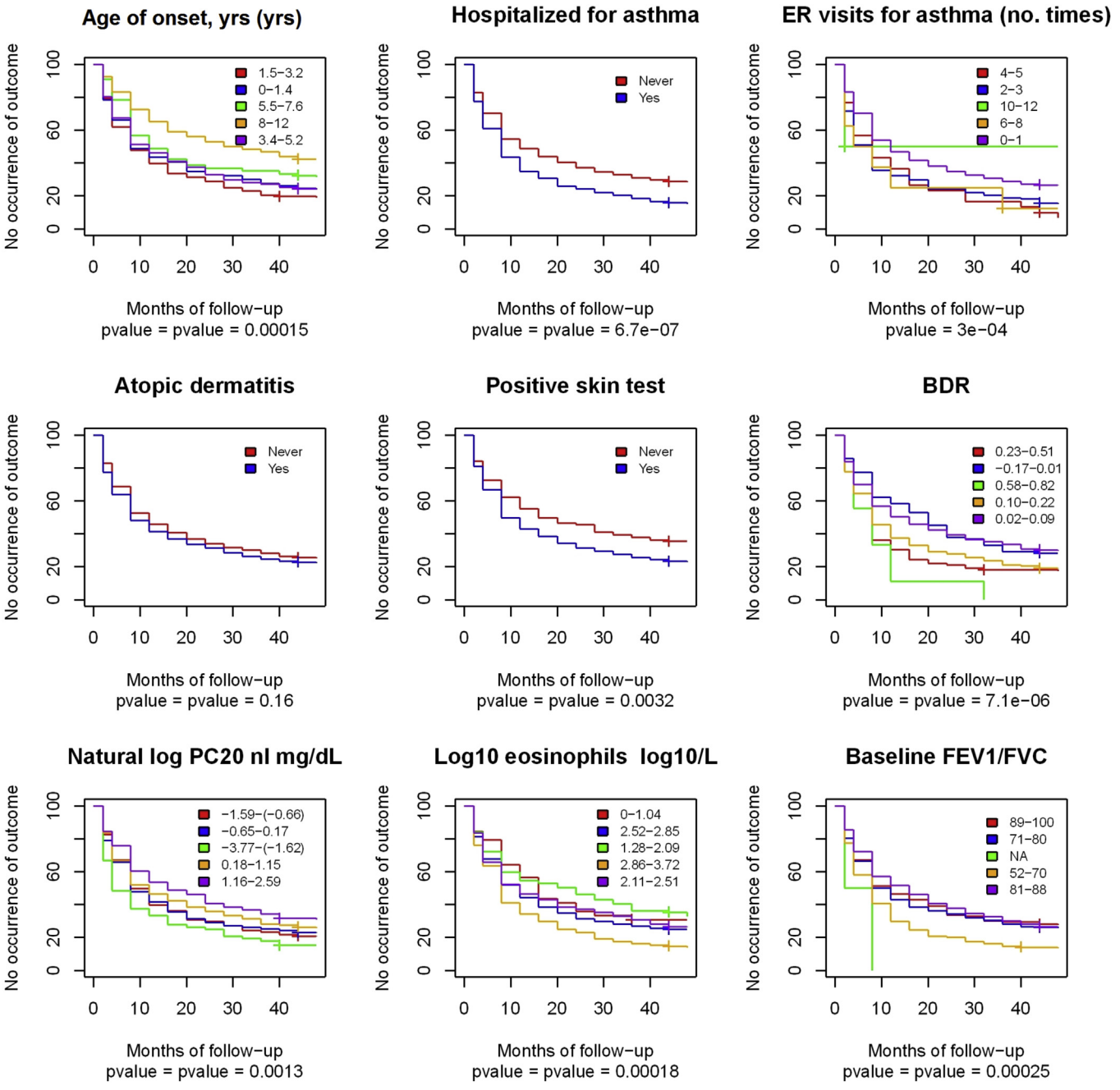

FIG E2. Kaplan-Meier plots by cluster of the cumulative probability of a first course of prednisone during the 4-year follow-up period of the CAMP trial. Clusters were determined based on a single clinical variable indicated at the *top* of each figure.

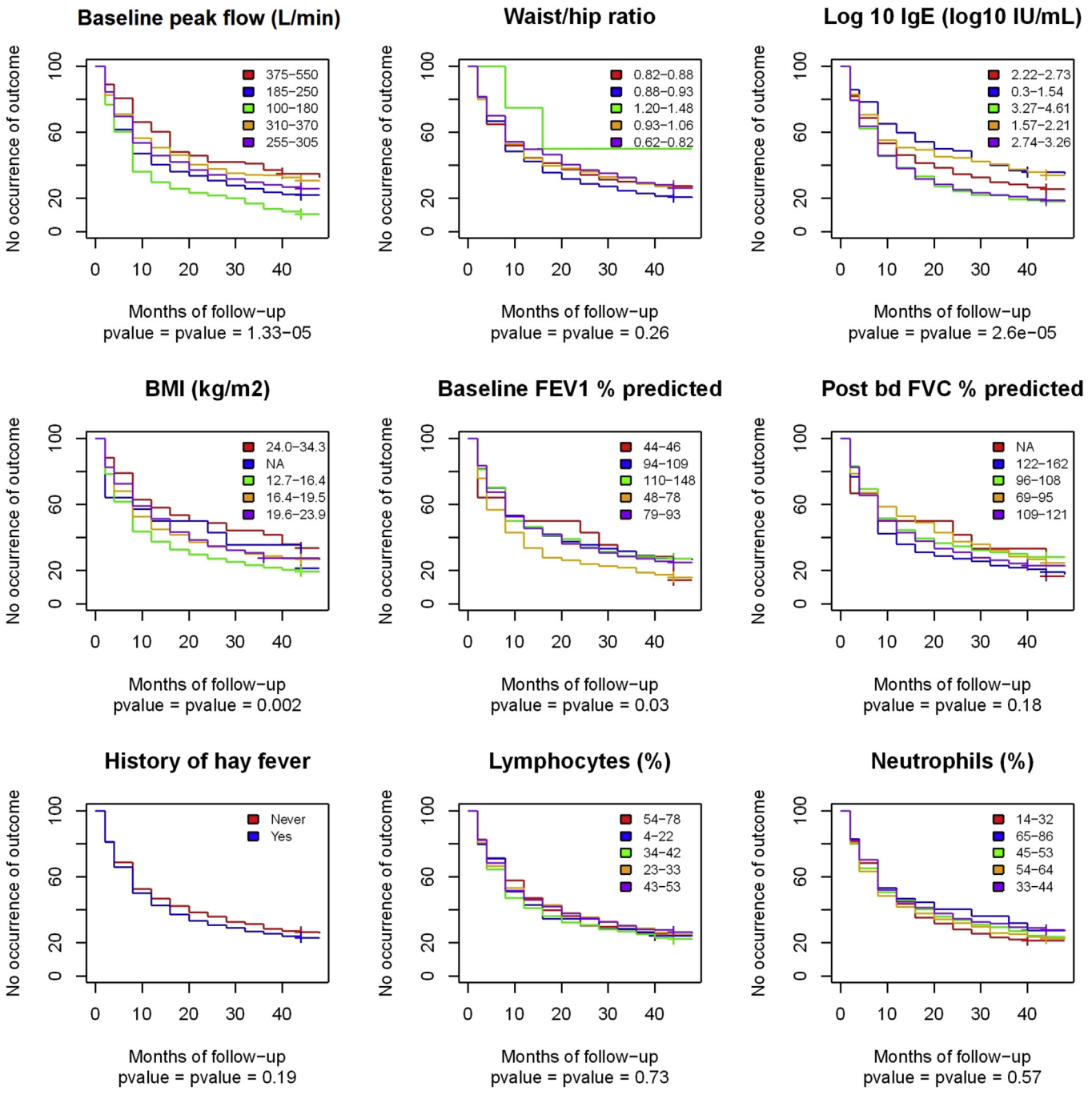

FIG E2. *(Continued)*

A

	New Cluster	1	2	3	4	5
Old Cluster	**1**	188	2	7	29	2
	2	0	164	0	37	1
	3	57	0	130	27	4
	4	3	1	1	3	217
	5	0	0	0	20	76

B

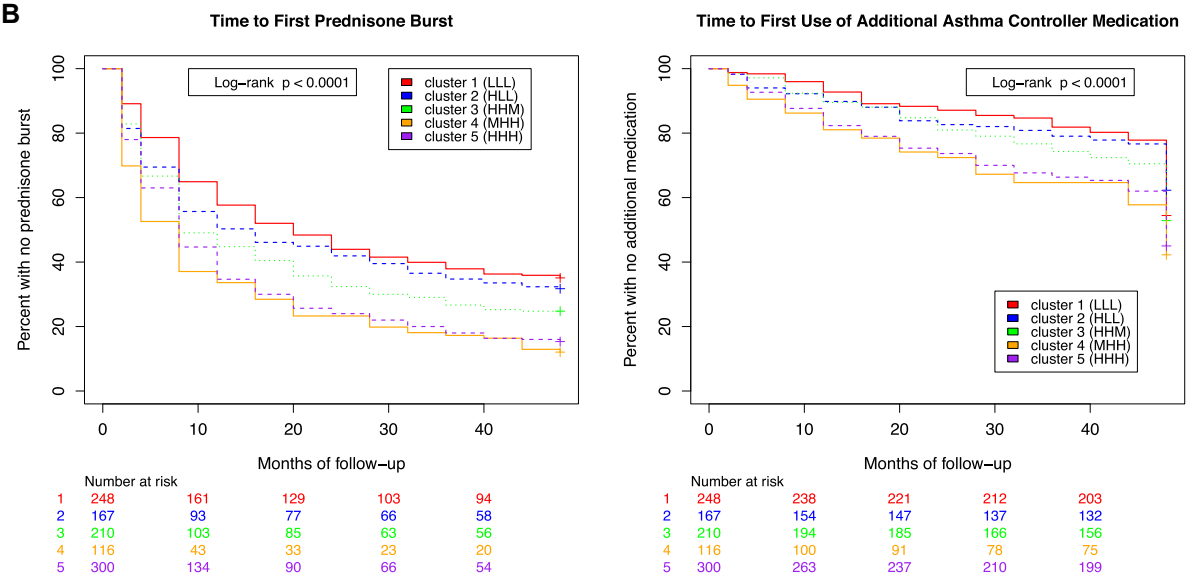

FIG E3. Comparison of phenotypic clusters generated by means of hierarchical clustering (new clusters) versus spectral clustering (old clusters). **A,** Comparison of study subject cluster assignments. **B,** Survival analysis for time to first use of prednisone *(left)* and time to use of additional asthma controller therapy *(right)* for hierarchical clusters.

TABLE E1. Baseline clinical variables considered for cluster analysis

Medical history	Reason for exclusion
• Hay fever • Positive allergy skin test result • AD • ED visits for asthma • Ever hospitalized for asthma • Age of asthma onset CAMP study coordinator's assessment of asthma	All variables were included in the model, except for caregiver's assessment, which was considered too subjective.
Asthma worsened by house dust or animals or tobacco smoke or chemicals or emotional factors or exercise or certain foods or respiratory tract infections or dampness or changes in the weather or cold air or aspirin	>10% missing values (cold air and aspirin). The remainder were considered too subjective.
Clinical presentation Provoked by exercise Provoked by allergy Age at first symptoms	These variables were considered to be too subjective for use in the clustering model.
Symptom burden	
Age when child began wheezing with shortness of breath	>10% missing values
Prior awakening from sleep because of cough or wheeze	>10% missing values
Awakening from sleep in past 6 mo	>10% missing values
Awakening from sleep in past month	>10% missing values
Awakening from sleep in past week	>10% missing values
Cough or wheeze during the day unrelated to exercise	>10% missing values
Cough or wheeze during the day caused by exercise	>10% missing values
Cough or phlegm with or without an upper respiratory tract infection	overly subjective
Wheezing present on most days	overly subjective
Wheezing present with or without an upper respiratory tract infection	overly subjective
Wheezing present with shortness of breath	>10% missing values
Two or more episodes of wheezing with shortness of breath	>10% missing values
Received a prescription medication for wheezing with shortness of breath	>10% missing values
Normal breathing between attacks of wheezing with shortness of breath	
Anthropomorphic measurements • BMI • Waist/hip ratio	All variables were included in the model.
Pulmonary function • Post-BD FVC as a percentage of the predicted value (post-BD FVC percent predicted) • Pre-PD FEV_1 as a percentage of predicted value (pre-BD FEV_1 percent predicted) • Pre-BD peak expiratory flow rate • Pre-BD FEV_1/FVC ratio • Methacholine PC_{20} (natural log) • Post-BD FEV_1 − pre-BD FEV_1/pre-BD FEV_1 (BDR)	All variables were included in the model.
Peripheral blood measures • Absolute serum neutrophil count • Absolute serum lymphocyte count • Total serum IgE level (log_{10}) • Absolute serum eosinophils (log_{10})	All variables were included in the model.

Baseline clinical variables were considered for cluster analysis. From an initial list of 48 variables shown in the table, we selected 18 clinical variables (denoted by *asterisks*) as inputs to the spectral clustering algorithm.
BDR, Bronchodilator response.

TABLE E2. Baseline features of 1041 CAMP asthmatic subjects

Variable	Count or mean
Sex	
Male (%)	621 (59.7)
Female (%)	420 (40.3)
Age (y)	8.94 ± 2.12
Self-reported race	
White (%)	711 (68.3)
Black (%)	138 (13.3)
Hispanic (%)	98 (9.41)
Other (%)	94 (9.03)
Family history of asthma (%)	
Yes	574 (55.1)
No	444 (42.7)
Missing	23 (2.21)
Family history of atopy (%)	724 (69.5)
History of tobacco smoke exposure (%)	439 (42.2)
Household income	
<$30,000	242 (23.2)
≥$30,000	758 (72.8)
Missing	41 (3.94)
Age of asthma onset (y)	3.07 ± 2.44
Hospitalized for asthma (%)	320 (30.7)
ED visits for asthma, no./100 person-years	648 ± 62.2
History of AD (%)	298 (28.6)
History of hay fever (%)	557 (53.5)
History of positive skin test result (%)	914 (87.8)
Prebronchodilator FEV_1 (L [range])	1.65 (0.42-3.31)
Prebronchodilator FEV_1/FVC ratio (range)	80 (52-100)
FEV_1 bronchodilator response (L [range])	0.11 (−0.17 to 0.82)
Methacholine PC_{20} (natural log mg/dL [range])	0.10 (−3.77 to 2.59)
Total serum IgE level (IU/L [range])	484 (0-5304)
Peripheral blood eosinophil count (log_{10}/L [range])	2.50 (0-3.72)
Waist/hip ratio	0.88 ± 0.061
BMI (kg/m^2)	18.2 ± 3.52

TABLE E3. Number of patients needing additional or replacement asthma controller medications

	Cluster 1 (n = 300)	Cluster 2 (n = 202)	Cluster 3 (n = 218)	Cluster 4 (n = 225)	Cluster 5 (n = 96)	
AOE classification	**LLL**	**HLL**	**HHM**	**MHH**	**HHH**	***P* value**
Budesonide (n = 311 [29.9%])						
2 mo	0.01 ± 0.11	0.04 ± 0.21	0.03 ± 0.18	0.00 ± 0.71	0.00 ± 0.72	.32
4 mo	0.01 ± 0.11	0.05 ± 0.21	0.03 ± 0.18	0.00 ± 0.84	0.04 ± 0.69	.43
8 mo	0.01 ± 0.12	0.06 ± 0.30	0.03 ± 0.18	0.06 ± 0.96	0.04 ± 1.25	.66
12 mo	0.03 ± 0.16	0.06 ± 0.39	0.07 ± 0.26	0.11 ± 0.88	0.13 ± 1.47	.24
16 mo	0.04 ± 0.20	0.06 ± 0.40	0.11 ± 0.31	0.13 ± 0.97	0.17 ± 0.78	.21
20 mo	0.05 ± 0.28	0.10 ± 0.43	0.13 ± 0.33	0.16 ± 1.02	0.17 ± 1.72	.43
24 mo	0.06 ± 0.29	0.10 ± 0.43	0.13 ± 0.33	0.18 ± 0.93	0.17 ± 0.78	.38
28 mo	0.10 ± 0.34	0.10 ± 0.43	0.15 ± 0.36	0.25 ± 0.95	0.26 ± 0.92	.46
32 mo	0.11 ± 0.36	0.10 ± 0.43	0.19 ± 0.44	0.26 ± 0.96	0.26 ± 0.92	.43
36 mo	0.18 ± 0.52	0.11 ± 0.45	0.21 ± 0.50	0.35 ± 2.98	0.30 ± 0.70	.52
40 mo	0.24 ± 0.64	0.11 ± 0.45	0.27 ± 0.63	0.51 ± 0.65	0.36 ± 0.71	.27
44 mo	0.25 ± 0.65	0.13 ± 0.47	0.31 ± 0.67	0.54 ± 0.71	0.38 ± 0.78	.18
48 mo	0.66 ± 0.92	0.37 ± 0.64	0.50 ± 0.71	0.82 ± 0.53	0.70 ± 0.86	.27
Nedocromil (n = 312 [30.0%])						
2 mo	0.00 ± 0.0	0.02 ± 0.13	0.00 ± 0.0	0.02 ± 0.0	0.04 ± 0.0	.33
4 mo	0.00 ± 0.0	0.09 ± 0.35	0.02 ± 0.12	0.05 ± 0.0	0.17 ± 0.20	.002
8 mo	0.06 ± 0.23	0.19 ± 0.62	0.12 ± 0.38	0.11 ± 0.24	0.23 ± 0.20	.20
12 mo	0.13 ± 0.43	0.28 ± 0.94	0.14 ± 0.39	0.17 ± 0.36	0.36 ± 0.34	.17
16 mo	0.22 ± 0.60	0.41 ± 1.42	0.18 ± 0.46	0.26 ± 0.38	0.59 ± 0.48	.15
20 mo	0.30 ± 0.78	0.48 ± 1.58	0.23 ± 0.56	0.30 ± 0.55	0.68 ± 0.48	.24
24 mo	0.35 ± 0.93	0.52 ± 1.61	0.45 ± 0.94	0.40 ± 0.56	0.82 ± 0.48	.27
28 mo	0.38 ± 0.99	0.64 ± 1.80	0.62 ± 1.12	0.37 ± 0.67	0.86 ± 0.75	.29
32 mo	0.42 ± 1.11	0.69 ± 1.85	0.79 ± 1.36	0.49 ± 0.75	1.00 ± 0.75	.19
36 mo	0.48 ± 1.72	0.76 ± 1.95	0.86 ± 1.46	0.60 ± 0.88	1.15 ± 0.82	.23
40 mo	0.59 ± 1.44	0.84 ± 2.10	0.95 ± 1.59	0.69 ± 1.15	1.15 ± 1.00	.29
44 mo	0.65 ± 1.50	1.00 ± 2.45	1.05 ± 1.87	0.75 ± 1.13	1.15 ± 1.02	.48
48 mo	0.94 ± 1.66	1.27 ± 2.59	1.20 ± 1.79	1.00 ± 1.20	1.26 ± 1.22	.92
Placebo (n = 418 [40.1%])						
2 mo	0.03 ± 0.16	0.01 ± 0.12	0.05 ± 0.26	0.03 ± 0.15	0.08 ± 0.35	.82
4 mo	0.05 ± 0.27	0.10 ± 0.34	0.09 ± 0.39	0.10 ± 0.34	0.18 ± 0.46	.26
8 mo	0.10 ± 0.36	0.20 ± 0.65	0.13 ± 0.43	0.18 ± 0.44	0.25 ± 0.50	.21
12 mo	0.16 ± 0.47	0.28 ± 0.82	0.17 ± 0.49	0.26 ± 0.58	0.43 ± 0.85	.12
16 mo	0.18 ± 0.50	0.31 ± 0.85	0.20 ± 0.55	0.41 ± 0.79	0.54 ± 0.98	.03
20 mo	0.25 ± 0.67	0.39 ± 0.95	0.24 ± 0.75	0.53 ± 0.87	0.83 ± 1.27	.001
24 mo	0.38 ± 0.97	0.45 ± 0.98	0.39 ± 0.96	0.58 ± 0.97	0.91 ± 1.34	.01
28 mo	0.45 ± 1.06	0.55 ± 1.09	0.43 ± 1.11	0.65 ± 1.02	1.06 ± 1.39	.004
32 mo	0.49 ± 1.09	0.62 ± 1.21	0.52 ± 1.33	0.78 ± 1.26	1.26 ± 1.54	.002
36 mo	0.56 ± 1.24	0.75 ± 1.49	0.56 ± 1.34	0.85 ± 1.44	1.37 ± 1.66	.002
40 mo	0.64 ± 1.38	0.82 ± 1.58	0.65 ± 1.56	0.95 ± 1.61	1.43 ± 1.69	.005
44 mo	0.74 ± 1.61	0.95 ± 1.74	0.71 ± 1.79	1.04 ± 1.77	1.74 ± 1.80	.003
48 mo	1.04 ± 1.60	1.02 ± 1.67	1.03 ± 2.04	1.44 ± 1.93	2.06 ± 1.84	<.001
P values						
Budesonide vs nedocromil	.32	.78	.003	.24	.28	
Budesonide vs placebo	.81	.45	.75	.13	.97	
Nedocromil vs placebo	.56	.71	.07	.65	.49	

The cumulative number of asthma exacerbations (ie, symptom worsening requiring additional or replacement asthma controller medications) at each study time point stratified by treatment group is shown. Shown are mean ± SD cumulative number of exacerbations from the onset of the study period and *P* values for between-cluster differences in outcome (far right) and pairwise comparisons of within-cluster differences in outcomes (bottom level).

TABLE E4. Distribution of nonclassifying features across asthma clusters

	Cluster 1 (n = 300)	Cluster 2 (n = 202)	Cluster 3 (n = 218)	Cluster 4 (n = 225)	Cluster 5 (n = 96)	
AOE classification	**LLL**	**HLL**	**HHM**	**MHH**	**HHH**	***P* value**
Sex						.45
Male	173 (57.7%)	115 (56.9%)	130 (59.6%)	146 (64.9%)	57 (59.4%)	
Female	127 (42.3%)	87 (43.1%)	88 (40.4%)	79 (35.1%)	39 (40.6%)	
Age at trial enrollment (y), mean ± SD						.003
	8.79 ± 2.05	8.83 ± 2.12	9.38 ± 2.13	9.03 ± 2.08	8.46 ± 2.25	
Self-reported race						.19
White	217 (72.3%)	137 (67.8%)	140 (64.2%)	150 (66.7%)	67 (69.8%)	
Black	36 (12.0%)	27 (13.4%)	26 (11.9%)	35 (15.6%)	14 (14.6%)	
Hispanic	27 (9.0%)	13 (6.4%)	31 (14.2%)	21 (9.3%)	6 (6.3%)	
Other	20 (6.7%)	25 (12.4%)	21 (9.6%)	19 (8.4%)	9 (9.4%)	
Annual household income						.31
<$30,000	70 (23.3%)	40 (19.8%)	45 (20.6%)	66 (29.3%)	21 (21.9%)	
Highest household education						.13
Less than high school	1 (0.33%)	1 (0.50%)	2 (0.92%)	1 (0.44%)	0 (0.00%)	
High school	5 (1.7%)	5 (2.5%)	4 (1.8%)	5 (2.2%)	4 (4.2%)	
Higher education	125 (41.7%)	78 (38.6%)	87 (39.9%)	101 (44.9%)	48 (50.0%)	
Family history						
Asthma (any)	154 (51.3%)	114 (56.4%)	137 (62.8%)	110 (48.9%)	59 (61.5%)	.07
Asthma (maternal)	64 (21.3%)	46 (22.8%)	70 (32.1%)	50 (22.2%)	32 (33.3%)	.02
Asthma (paternal)	40 (13.3%)	49 (24.3%)	58 (26.6%)	46 (20.4%)	15 (15.6%)	.0009
Atopy (any)	189 (63.0%)	158 (78.2%)	162 (74.3%)	139 (61.8%)	76 (79.2%)	.0004
Atopy (maternal)	114 (38.0%)	110 (54.5%)	112 (51.4%)	98 (43.6%)	53 (55.2%)	.0009
Atopy (paternal)	87 (29.0%)	90 (44.6%)	84 (38.5%)	73 (32.4%)	37 (38.5%)	.05
Environmental exposures						
Tobacco smoke	119 (39.7%)	61 (30.2%)	82 (37.6%)	105 (46.7%)	46 (47.9%)	.01
Dust mite	60 (20.0%)	36 (17.8%)	61 (28.0%)	44 (19.6%)	16 (16.7%)	.30
Cockroach	1 (0.33%)	3 (1.49%)	1 (0.46%)	0 (0.00%)	0 (0.00%)	.04
Randomized treatment arm in CAMP clinical trial						.91
Budesonide	86 (28.7%)	68 (33.7%)	60 (27.5%)	68 (30.2%)	29 (30.2%)	
Nedocromil	94 (31.3%)	60 (29.7%)	66 (30.3%)	67 (29.8%)	25 (26.0%)	
Placebo	120 (40.0%)	74 (36.6%)	92 (42.2%)	90 (40.0%)	42 (43.8%)	
Long-term asthma control rank*	1	2	3	4	5	

*Long-term asthma control rank was subsequently determined in prospective survival analysis of time to first course of oral prednisone.

TABLE E5. Summary of *P* values for Cox proportional hazards modeling of the risk of an asthma exacerbation

Initiation of oral prednisone	Treatment with budesonide (placebo as reference)	Treatment with nedocromil (placebo as reference)	Treatment with budesonide (nedocromil as reference)
Cluster 2	.12	.12	.17
Cluster 3	.03	.03	.06
Cluster 4	<.001	<.001	.006
Cluster 5	<.001	<.001	<.001

Cox proportional hazards models for decrease in risk of future asthma exacerbations by using cluster assignment and different treatment group comparisons as predictor variables under an additive model. Cluster 1 was used as the reference group for cluster assignment. Shown are the *P* values for the degree of risk contributed by each variable to the model. For example, for cluster 4, the risk of initiation of oral prednisone is significantly decreased ($P < .001$) in the budesonide group (compared with placebo), significantly decreased ($P < .001$) in the nedocromil group (compared with placebo), and significantly decreased in the budesonide group ($P = .006$) compared with nedocromil.

TABLE E6. Summary of *P* values for Cox proportional hazards modeling of drug-by-cluster interaction

Initiation of oral prednisone	Treatment with budesonide (placebo as reference)	Treatment with nedocromil (placebo as reference)	Treatment with budesonide (nedocromil as reference)
Drug*Cluster 2	.94	.93	.99
Drug*Cluster 3	.93	.87	.97
Drug*Cluster 4	.98	.05	.05
Drug*Cluster 5	.92	.60	.50

Cox proportional hazards models for decrease in risk of future asthma exacerbations using cluster assignment and treatment group as predictor variables under an interaction model are shown. Cluster 1 was used as the reference group for cluster assignment. Shown are *P* values for the interaction terms. For example, for cluster 4, when considering the risk of initiation of oral prednisone, there is a nominally significant ($P = .05$) interaction between cluster membership and the study drug when budesonide is considered with the nedocromil group as a reference and when nedocromil is considered with the placebo group as a reference, suggesting that there is some differential response to medical therapy that exists within this phenotypic cluster compared with the other clusters.