Childhood asthma clusters and response to therapy in clinical trials

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Background: Childhood asthma clusters, or subclasses, have been developed by computational methods without evaluation of clinical utility.
Objective: To replicate and determine whether childhood asthma clusters previously identified computationally in the Severe Asthma Research Program (SARP) are associated with treatment responses in Childhood Asthma Research and Education (CARE) Network clinical trials.
Methods: A cluster assignment model was determined by using SARP participant data. A total of 611 participants 6 to 18 years old from 3 CARE trials were assigned to SARP pediatric clusters. Primary and secondary outcomes were analyzed by cluster in each trial.
Results: CARE participants were assigned to SARP clusters with high accuracy. Baseline characteristics were similar between SARP and CARE children of the same cluster. Treatment response in CARE trials was generally similar across clusters. However, with the caveat of a smaller sample size, children in the early-onset/severe-lung function cluster had best response with fluticasone/salmeterol (64% vs 23% 2.5× fluticasone and 13% fluticasone/montelukast in the Best ADd-on Therapy Giving Effective Responses trial; $P = .011$) and children in the early-onset/comorbidity cluster had the least clinical efficacy to treatments (eg, −0.076% change in FEV_1 in the Characterizing Response to Leukotriene Receptor Antagonist and Inhaled Corticosteroid trial).
Conclusions: In this study, we replicated SARP pediatric asthma clusters by using a separate, large clinical trials network. Early-onset/severe-lung function and early-onset/comorbidity clusters were associated with differential and limited response to therapy, respectively. Further prospective study of therapeutic response by cluster could provide new insights into childhood asthma treatment. (J Allergy Clin Immunol 2014;133:363-9.)

Key words: *Asthma, clustering, clinical trials, replication, pediatric, therapy response*

Asthma is likely not a single disease, but rather a syndrome comprising multiple complex phenotypes.[1] Researchers have recognized this and have attempted to subclassify asthma by using expert opinion or computational techniques such as clustering.

Expert panels have also subclassified asthma. For example, the National Asthma Education and Prevention Program Expert

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Abbreviations used
BADGER: Best ADd-on Therapy Giving Effective Responses
CARE: Childhood Asthma Research and Education
CLIC: Characterizing Response to Leukotriene Receptor Antagonist and Inhaled Corticosteroid
LDA: Linear discriminant analysis
PACT: Pediatric Asthma Controller Trial
QDA: Quadratic discriminant analysis
SARP: Severe Asthma Research Program

Panel Report 3[2] has classified asthma severity as intermittent, mild-persistent, moderate-persistent, and severe-persistent.[2] Once therapy is initiated, asthma control is defined on the basis of symptoms and lung function. A similar classification has been used in the Global Initiative for Asthma.[3] In both the Expert Panel Report 3 and the Global Initiative for Asthma, asthma phenotypes, applicable to large patient groups, are defined on the basis of the amount of therapy necessary to achieve adequate control. However, one phenotype may consist of subphenotypes, each with a different optimal treatment.

While asthma guidelines have led to improvements in asthma care, it has been argued that they do not reflect the heterogeneous nature of the disease. Miller et al[4] identified a lack of classification agreement among guidelines, physician assessment, and health care usage. Wenzel[1] proposed new asthma phenotype definitions based on clinical history, triggers, and inflammatory markers.

There is extensive literature on asthma clustering for phenotype identification using clinical, genetic, and imaging data.[5-13] For example, Moore et al[6] studied adults from the Severe Asthma Research Program (SARP) and identified 5 adult asthma clusters. Few studies have focused on clustering in childhood asthma. Fitzpatrick et al[14] studied 6- to 17-year-old SARP children (N = 161), roughly one-half with severe asthma. The authors described 4 pediatric clusters distinct from the adult clusters: cluster 1 had late-onset (mean age, 73 months) symptomatic asthma with normal lung function (late-onset/normal-lung); cluster 2 had early-onset (mean age, 30 months) atopic asthma with mild airflow limitation (early-onset/normal-lung); cluster 3 had earliest-onset (mean age, 14 months) atopic asthma with mild airflow limitation and greater comorbidity (early-onset/comorbidity); and cluster 4 had early-onset (mean age, 17 months) atopic asthma with advanced airflow limitation and the greatest medication use (early-onset/severe-lung). The SARP analysis[14] was intended to identify pediatric asthma clusters but was unable to evaluate the clinical utility of this differentiation.

Although clustering methodology has provided an additional perspective in asthma phenotypes, computationally derived phenotypes have not been evaluated for applicability to other asthma populations or clinical utility. Therefore, we used a large well-characterized population of children who participated in the Childhood Asthma Research and Education (CARE) Network clinical trials to determine, first, whether SARP pediatric asthma clusters could be replicated in a new population and, second, whether these clusters were associated with response to therapy.

METHODS

The study population consisted of 6- to 18-year-old children with asthma (N = 611) enrolled in 3 CARE Network clinical trials.[15-17] The trials are summarized in Table I. Briefly, the Pediatric Asthma Controller Trial (PACT)[16] was a 3-arm (1× fluticasone, 0.5× fluticasone plus salmeterol, or montelukast) double-blind study of children with mild-moderate asthma and used percentage of asthma control days as the primary outcome. The Characterizing Response to Leukotriene Receptor Antagonist and Inhaled Corticosteroid (CLIC)[15] trial was a crossover study comparing fluticasone 100 μg 1 inhalation twice daily and montelukast in children with mild-moderate asthma and used percent change in FEV_1 as its primary outcome. The Best ADd-on Therapy Giving Effective Responses (BADGER)[17] trial was a triple crossover study evaluating step-up therapy for children with mild-moderate asthma uncontrolled on low doses of inhaled corticosteroids (100 μg of fluticasone twice daily = 1×). Treatments included 2.5× fluticasone, 1× fluticasone plus salmeterol, and 1× fluticasone plus montelukast. The primary outcome was the best treatment based on a composite evaluation considering prednisone usage for exacerbations, asthma control days, and percent change in FEV_1. The present post hoc analysis was submitted to the University of Wisconsin Institutional Review Board and determined exempt from review.

Cluster assignment procedure

Linear discriminant analysis (LDA) was the model used by Fitzpatrick et al[14] to classify participants into SARP clusters with percent-predicted FEV_1, asthma duration, and number of controller medications as variables. The CARE data set, which the model would later be applied to, did not contain the number of controller medications. Therefore, leave-one-out cross-validation[18,19] was used to evaluate LDA and quadratic discriminant analysis (QDA) using SARP data FEV_1 and asthma duration variables. The LDA models with 2 and 3 variables were compared with the Wilks' lambda *F* test. (See Methods, LDA and QDA, in the Online Repository at www.jacionline.org for assumptions and risks of LDA and QDA.)

Compared with LDA, the QDA classification model had better performance and was used to assign CARE participants to SARP pediatric clusters. Missing data were replaced by using multiple imputation.[20] Three participants in the BADGER trial were missing FEV_1 percent-predicted measurements. (See Methods, Multiple imputation).

Demographics and run-in clinical characteristics were summarized with complete nonmissing data by using descriptive statistics and compared across clusters by using ANOVA for continuous measures and Fisher exact test for categorical measures.

Association of clusters with clinical trials outcome

We analyzed the association of clusters and treatment outcomes for the PACT, the CLIC trial, and the BADGER trial. Possible interactions between treatment and cluster were evaluated for the primary outcome and secondary outcomes (percent asthma control days, percent change in FEV_1, and time to first exacerbation) for each trial. Percent asthma control days in the PACT were analyzed by using a quasi-binomial generalized linear model with a logit link; percent asthma control days in the CLIC and BADGER trials were analyzed by using a quasi-binomial generalized estimating equations model with an independent working correlation matrix.[21] Linear regression models were used to analyze percent change in FEV_1 for the PACT; mixed-effect linear models were used to analyze repeated measurements of percent change in FEV_1 for CLIC and BADGER trials. Time to first exacerbation was analyzed by using a Cox proportional hazards model for all 3 studies; frailty models[22] accounted for repeated measurements on the same participant in CLIC and BADGER trials. Differences in best treatment by cluster in the BADGER trial were assessed by using a Monte Carlo test based on Pearson's χ^2 statistic for independence in a 2×2 table.

RESULTS

Classification model and assignment

For early-onset/normal-lung, late-onset/normal-lung, early-onset/comorbidity, and early-onset/severe-lung clusters, cross-validated QDA recall using SARP data with FEV_1 and asthma duration was 96%, 94%, 97%, and 90%, while precision was 96%, 94%, 94%, and 93%, respectively (see Table E1 in this

TABLE I. Summary of 3 CARE Network clinical trials

Trial	Participants	N	Treatment	Primary outcome	Results
PACT	Mild-moderate persistent asthma, 6-14 y	285	1 of 3 double-blind: 1× ICS (fluticasone), 0.5× ICS + LABA (salmeterol), or LTRA (montelukast)	Percent asthma control days	1× fluticasone and 0.5× fluticasone + salmeterol
CLIC	Mild-moderate persistent asthma, 6-17 y	144	Crossover: ICS (fluticasone), LTRA (montelukast)	Percent change FEV_1	Fluticasone
BADGER	Mild-moderate asthma not controlled by low-dose ICS (fluticasone), 6-17 y	182	Triple crossover: 2× ICS (fluticasone), 1× ICS + LABA (salmeterol), 1× ICS + LTRA (montelukast)	Differential period response for prednisone usage, asthma control days, and percent change FEV_1	1× fluticasone + salmeterol

ICS, Inhaled corticosteroid; *LABA*, long acting beta-agonist; *LTRA*, leukotriene receptor antagonist.

article's Online Repository at www.jacionline.org). Using SARP data with the same 2 variables, cross-validation LDA recall was 90%, 96%, 94%, and 90% and precision was 98%, 89%, 94%, and 90%, respectively. These results were similar to the original SARP LDA model using percent-predicted FEV_1, asthma duration, and number of controller medications, which had recall between 86% and 100% and precision between 86% and 96%. It is possible for stepwise LDA to identify 3 instead of 2 significant variables ($P = 4.54 \times 10^{-5}$) while cross-validation shows little difference between LDA with 2 or 3 variables because the former is based on the *F* test and the latter is based on precision and recall cross-validation.

CARE trial participants were assigned to SARP pediatric asthma clusters by using the 2-variable QDA model because precision and recall were slightly improved compared with LDA. The number of PACT, CLIC trial, and BADGER trial participants assigned to SARP pediatric clusters is shown in Table II. Most of the participants were assigned to the early-onset/normal-lung cluster (41%) or late-onset/normal-lung cluster (40%). The early-onset/comorbidity cluster had the fewest participants (7%), and the early-onset/severe-lung cluster had slightly more (12%).

TABLE II. Numbers of participants in each cluster

Trial	Late-onset/ normal-lung (1)	Early-onset/ normal-lung (2)	Early-onset/ comorbidity (3)	Early-onset/ severe-lung (4)
PACT	109	133	9	34
CLIC	61	48	15	20
BADGER	78	62	20	22

CLIC and BADGER trials were double and triple crossover trials, respectively. The effective number of participants given each treatment is roughly double and triple.

Demographics and clinical characteristics of clusters

Table III summarizes baseline demographic and clinical characteristics of all participants. As expected, FEV_1 percent-predicted ($P < .001$) and asthma duration ($P < .001$), the 2 variables used for cluster assignment, were significantly different among clusters. The early-onset/normal-lung cluster had the shortest mean asthma duration and the highest FEV_1 percent-predicted, whereas the early-onset/severe-lung cluster had the longest mean asthma duration and the lowest FEV_1 percent-predicted. Body mass index was highest in the early-onset/comorbidity cluster at 23 kg/m^2. There was no difference in race among clusters. Although FEV_1 percent-predicted in the early-onset/comorbidity cluster was slightly higher than in the late-onset/normal-lung cluster, the FEV_1/forced vital capacity was slightly lower in the early-onset/comorbidity cluster. The early-onset/comorbidity cluster had the highest percentage of positive skin test results, total IgE, and fractional exhaled nitric oxide.

Baseline demographic and clinical characteristics of SARP children in the clusters described by Fitzpatrick et al[14] were similar in CARE children assigned to SARP clusters. Asthma duration and FEV_1 had similar trends across clusters. Late-onset/normal-lung and early-onset/normal-lung clusters were similar in sex and lung function. CARE and SARP participants in the early-onset/comorbidity cluster had the highest body mass index but had contrasting methacholine responsiveness. The early-onset/severe-lung cluster had lower lung function. There were fewer black participants in CARE trials for this cluster.

Clusters and clinical trial outcomes

The association of clusters and treatment response for step 2 therapy was examined in the PACT and the CLIC trial. For the PACT, the cluster and treatment interaction was not significant for the primary outcome, percent asthma control days (Table IV). However, treatment responses were significantly different in the late-onset/normal-lung cluster ($P = .008$), with montelukast being the least beneficial for these children. Across all treatment arms, percent asthma control days was lowest in the early-onset/comorbidity cluster (36%), with all other clusters having 55% or more asthma control days, although this difference was not statistically significant ($P = .10$).

Considering secondary outcomes in the PACT (Table IV), 1× fluticasone was significantly beneficial for percent change in FEV_1 in the late-onset/normal-lung cluster (8.0%, $P = .004$) and the early-onset/normal-lung cluster (6.8%; $P = .005$). The early-onset/comorbidity cluster had the smallest overall percent change in FEV_1 (−0.25%) and the highest rate of exacerbations (54%) (see Table E2 in this article's Online Repository at www.jacionline.org), though not significantly different from other clusters.

In the CLIC trial, while no significant cluster and treatment interaction was found for the primary outcome of percent FEV_1 improvement from baseline, fluticasone was significantly superior to montelukast for the late-onset/normal-lung, early-onset/normal-lung, and early-onset/severe-lung clusters with a 3.6%,

TABLE III. Demographic and run-in clinical characteristics among clusters

Variable	Late-onset/ normal-lung (1)	Early-onset/ normal-lung (2)	Early-onset/ comorbidity (3)	Early-onset/ severe-lung (4)	*P* value
Participants, n	248	243	44	76	<.001
Demographics					
Age at study entry (y)	9.4 ± 2.6	9.9 ± 2.2	15 ± 1.7	13 ± 1.7	<.001
Age asthma onset (y)	5.9 ± 3	2.2 ± 2	1.4 ± 1	1.8 ± 1.6	<.001
Duration (y)	3.5 ± 1.6	7.7 ± 1.2	14 ± 1.4	11 ± 0.83	<.001
Height (cm)	137 ± 15	140 ± 14	165 ± 10	157 ± 13	<.001
Weight (kg)	38 ± 16	40 ± 16	64 ± 16	56 ± 19	<.001
BMI (kg/m^2)	19 ± 4.5	20 ± 4.8	23 ± 5	22 ± 5.2	<.001
BMI >90th percentile, n (%)	76 (31)	84 (35)	13 (30)	29 (38)	.56
Sex: female, n (%)	113 (46)	86 (35)	14 (32)	19 (25)	.004
Ethnicity (non-Hispanic), n (%)	187 (75)	184 (76)	32 (73)	56 (74)	.95
White, n (%)	183 (74)	161 (66)	27 (61)	52 (68)	.18
Black, n (%)	46 (19)	63 (26)	12 (27)	18 (24)	.20
American Indian, n (%)	14 (5.6)	13 (5.3)	4 (9.1)	4 (5.3)	.76
Asian, n (%)	5 (2)	5 (2.1)	0 (0)	1 (1.3)	1.0
Pacific Islander, n (%)	0 (0)	1 (0.41)	1 (2.3)	1 (1.3)	.08
Family history					
Father asthma, n (%)	63 (29)	57 (27)	14 (36)	24 (35)	.47
Father atopic, n (%)	82 (39)	74 (36)	14 (39)	25 (37)	.93
Mother asthma, n (%)	73 (30)	77 (33)	9 (21)	22 (30)	.45
Mother atopic, n (%)	99 (41)	96 (42)	16 (36)	33 (45)	.85
Lung function					
FEV_1 (L)	1.9 ± 0.62	1.9 ± 0.63	3.1 ± 0.61	2.5 ± 0.78	<.001
FEV_1 % predicted	99 ± 13	97 ± 12	98 ± 18	90 ± 11	<.001
FVC (L)	2.4 ± 0.77	2.4 ± 0.79	4 ± 0.79	3.4 ± 1.1	<.001
FVC % predicted	107 ± 12.3	106 ± 12.2	109 ± 13.9	105 ± 11.8	.23
FEV_1/FVC, n (%)	82 (7.4)	81 (7)	78 (7.4)	75 (8.5)	<.001
Max BD % change FEV_1	12 (8.2)	11 (9)	15 (11)	15 (11)	<.001
Average ACD (per week)	2.1 ± 1.7	2.1 ± 1.8	2.3 ± 2	2.1 ± 1.9	.87
# Prednisone (per year)	0.49 (0.92)	0.51 (0.9)	0.59 (0.9)	0.43 (0.77)	.82
PC_{20} (mg/dL), median (range)*	0.97 (0-12)	1.4 (0-37)	1.8 (0-37)	0.44 (0-37)	.36
Allergic biomarkers					
Eczema, n (%)	83 (33)	100 (41)	15 (34)	30 (39)	.33
Positive skin test result, n (%)	190 (77)	187 (77)	37 (84)	65 (88)	.16
IgE (kU/L), median (range)*	170 (1-2713)	190 (1-4929)	310 (5-2871)	250 (1-3026)	.041
ECP (ng/mL), median (range)*	15 (1-136)	15 (1-175)	17 (7-64)	19 (1-316)	.03
Urinary LTE4 (pg/mg), median (range)*	98 (27-525)	110 (25-438)	81 (45-354)	92 (23-363)	.56
F_{ENO} (ppb), median (range)*	15 (2-198)	19 (2-207)	34 (7-216)	29 (4-218)	<.001
Peripheral eosinophils (%), median (range)	4.3 (0-30)	4.8 (0-18)	5 (0-17)	4.7 (1-25)	.50

Data represent mean ± SD or n (%) unless otherwise specified.
ACD, Asthma control days; *BD*, bronchodilator; *BMI*, body mass index; *ECP*, eosinophil cationic protein; *F_{ENO}*, fraction of exhaled nitric oxide; *FVC*, forced vital capacity; *LTE4*, leukotriene E4; *# Prednisone*, number of prednisone courses in the year before enrollment.
*Data were log transformed before analysis.

5.3%, and 8.0% improvement, respectively (Table V). Similar to the primary percent asthma control days outcome in the PACT, CLIC trial children in the early-onset/comorbidity cluster demonstrated no improvement in FEV_1 from baseline, with an average percent FEV_1 change of −0.076% across treatments. Secondary outcomes in the CLIC trial identified consistent significant benefit of fluticasone in the early-onset/normal-lung cluster for percent asthma control days (81%; $P = .003$) (Table V) and time to first exacerbation ($P = .008$) (see Table E2).

Association among clusters and step 3 treatment response was examined in the BADGER trial. In this trial, a significant cluster and best treatment response interaction was not observed ($P = .55$). Table VI shows the percentage of participants within each cluster who had a specific treatment as their best treatment determined by the composite outcome. Fluticasone/salmeterol was most likely to provide the best response (64%) for the early-onset/severe-lung cluster ($P = .01$). Similar to the primary BADGER trial analysis, fluticasone/salmeterol tended to have the greatest chance of best response (~40%) in the other 3 clusters.

Significant treatment benefits were observed in the late-onset/normal-lung and early-onset/severe-lung clusters' secondary outcomes (Table VI). Fluticasone/salmeterol was the best treatment for the late-onset/normal-lung cluster according to percent asthma control days (80%; $P < .001$). The early-onset/severe-lung cluster had the greatest benefit with fluticasone/salmeterol (7.7%) and 2.5× fluticasone (7.6%), with percent change FEV_1 as the outcome ($P = .008$).

DISCUSSION

In this study, we replicated SARP pediatric asthma clusters by demonstrating that CARE Network participants assigned to

TABLE IV. PACT outcomes across treatments and clusters

Treatment	Late-onset/normal-lung 1* (*P* = .008)	Early-onset/normal-lung 2 (*P* = .23)	Early-onset/comorbidity 3 (*P* = .78)	Early-onset/severe-lung 4 (*P* = .44)	All clusters
Percent ACD (*P* = .54)					
0.5× fluticasone + salmeterol	76 (62-86)	52 (40-64)	50 (9.4-91)	66 (45-82)	63 (55-70)
1× fluticasone	69 (56-80)	63 (51-73)	21 (2.7-72)	65 (29-89)	64 (56-72)
Montelukast	*50 (37-62)*	49 (36-61)	40 (11-77)	46 (26-67)	48 (40-57)
All treatments (*P* = .10)	64 (57-71)	55 (48-62)	36 (16-63)	58 (44-71)	
Percent change FEV_1 (*P* = .46)					
Treatment	**1* (*P* = .004)**	**2* (*P* = .005)**	**3 (*P* = .22)**	**4 (*P* = .81)**	**All clusters**
0.5× fluticasone + salmeterol	2.2 (−1.0 to 5.5)	3.5 (−0.030 to 7.0)	4.4 (1.8 to 7.0)	3.4 (−3.3 to 10)	3.1 (0.80 to 5.3)
1× fluticasone	**8.0 (4.6-11)**	**6.8 (4.0-9.5)**	−0.61 (−7.6 to 6.4)	3.4 (−3.3 to 10)	6.8 (4.8-8.9)
Montelukast	−0.026 (−3.5 -to 3.4)	−0.61 (−3.5 to 2.3)	−2.3 (−4.3 to −0.25)	7.3 (−4.9 to 20)	0.66 (−1.9 to 3.2)
All treatments (*P* = .69)	3.4 (1.4-5.5)	3.5 (1.7-5.4)	−0.25 (−3.1 to 2.6)	4.9 (−0.73 to 11)	

Boldface numbers indicate treatments beneficial for asthma. Italicized numbers indicate treatments not beneficial for asthma. 95% CIs are shown in parentheses. *P* values in primary outcome header test cluster and treatment interaction. *P* values in cluster number column headers test treatment effect within that cluster. *P* values in the row header "All treatments" test cluster effect for all participants.

ACD, Asthma control days.

*Clusters in which significant differences (*P* < .05) were identified among treatments.

TABLE V. CLIC trial outcomes across treatments and clusters

Treatment	Late-onset/normal-lung 1* (*P* = .006)	Early-onset/normal-lung 2* (*P* < .001)	Early-onset/comorbidity 3 (*P* = .31)	Early-onset/severe-lung 4* (*P* < .001)	All clusters
Percent change FEV_1 (*P* = .18)					
Fluticasone	**3.6 (0.61-6.6)**	**5.3 (1.7-9.0)**	0.73 (−4.6 to 6.1)	**8.0 (3.5-13)**	4.5 (2.6-6.5)
Montelukast	−0.23 (−3.1 to 2.7)	−0.21 (−2.9 to 2.5)	−0.88 (−5.1 to 3.3)	−0.55 (−3.2 to 2.1)	−0.34 (−2.0 to 1.3)
All treatments (*P* = .58)	1.7 (−0.41 to 3.8)	2.7 (0.35-5.0)	−0.076 (−3.4 to 3.3)	3.7 (0.83-6.6)	
Percent ACD (*P* = .36)					
Treatment	**1 (*P* = .08)**	**2* (*P* = .003)**	**3 (*P* = .21)**	**4 (*P* = .13)**	**All clusters**
Fluticasone	65 (55-74)	**81 (70-88)**	70 (48-85)	62 (45-77)	70 (64-76)
Montelukast	60 (50-69)	66 (55-76)	60 (40-77)	52 (36-69)	61 (54-67)
All treatments (*P* = .25)	63 (56-69)	74 (66-80)	64 (50-77)	57 (45-68)	

Boldface numbers indicate treatments beneficial for asthma. 95% CIs are shown in parentheses. *P* values in primary outcome header test cluster and treatment interaction. *P* values in cluster number column headers test treatment effect within that cluster. *P* values in the row header "All treatments" test cluster effect for all participants.

ACD, Asthma control days.

*Clusters in which significant differences (*P* < .05) were identified among treatments.

SARP clusters had similar characteristics. Furthermore, overall patterns of treatment response in the PACT, the CLIC trial, and the BADGER trial were similar across clusters when compared with responses in entire study populations. Interestingly, however, the early-onset/severe-lung cluster clearly had a best step 3 response with fluticasone/salmeterol in the BADGER trial, while the early-onset/comorbidity cluster had a poor overall step 2 treatment response in the PACT and the CLIC trial.

Using readily available clinical information (asthma duration and FEV_1 percent-predicted), we were able to assign CARE children to the original SARP pediatric clusters with very high recall and precision. We also found that the baseline demographic and clinical characteristics of SARP children in clusters described by Fitzpatrick et al[14] were quite similar to those of CARE children assigned to SARP clusters, with a few exceptions. Asthma duration trended in the same fashion. The late-onset/normal-lung cluster had the highest FEV_1 percent-predicted and the early-onset/severe-lung cluster had the lowest. However, FEV_1 percent-predicted values were globally higher in CARE children as would be expected because many SARP participants had severe asthma and CARE participants included in this study had mild-moderate asthma.

Similar to SARP children, CARE children in the late-onset/normal-lung cluster had the youngest age, greatest percentage of white race, highest percentage of females, lowest total IgE, and lowest FENO. The CARE participant early-onset/normal-lung cluster was similar to the original SARP early-onset/normal-lung cluster, but it had lower total IgE levels. The small number of participants in early-onset/comorbidity and early-onset/severe-lung clusters limited the power to detect differences between clusters, but trends were noted. Similar to the original SARP early-onset/comorbidity cluster, CARE children in this cluster had the highest body mass index. For CARE participants, this cluster had the least methacholine responsiveness, in contrast to SARP cluster participants who had the greatest methacholine responsiveness. This reduced methacholine responsiveness may be related to the higher FEV_1 percent-predicted of 98% in CARE participants versus 90% in SARP participants. The CARE participant early-onset/severe-lung cluster did not contain the highest percentage of black participants as described in the original SARP early-onset/severe-lung cluster, but it did have the worst baseline asthma control as reflected by asthma control days. Male sex, more predominant in this cluster, was associated

TABLE VI. BADGER trial outcomes across treatments and clusters

	Best treatment % (P = .55)				
Treatment	**Late-onset/normal-lung 1 (P = .17)**	**Early-onset/normal-lung 2 (P = .76)**	**Early-onset/comorbidity 3 (P = .78)**	**Early-onset/severe-lung 4* (P = .01)**	**All clusters**
Fluticasone + salmeterol	44 (32-56)	39 (26-51)	41 (21-64)	**64 (41-84)**	44 (37-52)
2.5× fluticasone	26 (16-37)	30 (19-42)	32 (13-54)	23 (7.1-45)	28 (21-35)
Fluticasone + montelukast	30 (20-42)	32 (20-44)	27 (9.9-48)	13 (1.8-312)	28 (21-35)
	Percent change FEV_1 (P = .32)				
Treatment	**1 (P = .77)**	**2 (P = .11)**	**3 (P = .94)**	**4* (P = .008)**	**All clusters**
Fluticasone + salmeterol	1.7 (−0.94 to 4.3)	1.6 (−0.86 to 4.1)	1.5 (−2.2 to 5.2)	7.7 (4.6-11)	2.3 (0.80-3.8)
2.5× fluticasone	1.9 (−1.0 to 4.8)	−0.86 (−3.5 to 1.8)	1.0 (−2.4 to 4.5)	7.6 (1.8-13)	1.6 (−0.18 to 3.3)
Fluticasone + montelukast	0.64 (−1.7 to 3.0)	−0.91 (−4.0 to 2.2)	1.3 (−4.0 to 6.6)	*4.3 (−1.4 to 10)*	1.0 (−1.1 to 2.3)
All treatments* (P = .045)	1.4 (−0.11 to 2.9)	−0.0068 (−1.6 to 1.6)	1.3 (−1.1 to 3.7)	6.6 (3.7-9.5)	
	Percent ACD (P = .62)				
Treatment	**1* (P < .001)**	**2 (P = .35)**	**3 (P = .33)**	**4 (P = .12)**	**All clusters**
Fluticasone + salmeterol	**80 (74-85)**	77 (70-83)	81 (69-89)	81 (69-89)	79 (75-82)
2.5× fluticasone	69 (63-75)	74 (67-80)	78 (66-87)	70 (58-80)	72 (68-76)
Fluticasone + montelukast	74 (68-80)	74 (67-80)	76 (64-85)	70 (58-80)	74 (70-78)
All treatments (P = .82)	74 (71-78)	75 (71-79)	78 (72-84)	74 (67-79)	

Boldface numbers indicate treatments beneficial for asthma. Italicized numbers indicate treatments not beneficial for asthma. 95% CIs are shown in parentheses. P values in primary outcome header test cluster and treatment interaction. P values in cluster number column headers test treatment effect within that cluster. P values in the row header "All treatments" test cluster effect for all participants.

ACD, Asthma control days.

*Clusters in which significant differences (P < .05) were identified among treatments.

with lower lung function as reported in other studies including previous SARP analyses[23] and the Dunedin study.[24]

We next evaluated the ability of pediatric asthma cluster assignment to predict treatment responses. For many outcomes, treatment responses were similar across clusters. However, there were some differences in treatment response by clusters, which are of interest. For example, in the BADGER study investigating step 3 therapy, fluticasone/salmeterol combination therapy provided the greatest likelihood of best response in comparison to 2.5× fluticasone and fluticasone/montelukast for the early-onset/severe-lung cluster.

Children in the early-onset/comorbidity cluster tended to have the least clinical efficacy in the PACT and the CLIC trial investigating step 2 therapy. However, this group was the smallest, decreasing our power to detect differences. Further study of the early-onset/comorbidity cluster is warranted on the basis of these study results and their unique characteristics from the pediatric SARP analysis including high comorbidities (gastroesophageal reflux and chronic sinusitis), high daily oral corticosteroid usage, lower total lung capacity, and increased airway resistance.

Derivation of clusters is dependent on the population, as was evident in different clusters identified from SARP clustering in adults and children. This study attempted to generalize previous childhood clustering results by determining whether the same clusters exist in a new, more prevalent mild to moderate childhood asthma population as a form of validation. The second component of this study, associating cluster assignment with treatment outcome, is hypothesis generating and necessitates validation in future prospective studies in children.

A strength of our analysis is the large and well-characterized patient population that participated in rigorously performed CARE Network clinical trials. Compared with SARP, CARE had adherence monitoring, broad recruitment from both pediatricians and asthma specialists to reduce selection bias, and a racial/ethnic distribution more consistent with US census data allowing better generalization. In addition, rather than creating new clusters, we have taken the "next step" by assessing whether pediatric asthma phenotype clusters could be considered endotypes on the basis of clinical treatment response.[25] The full clinical utility of asthma clusters is yet to be determined.

A limitation of this study is the smaller number of participants assigned to early-onset/comorbidity and early-onset/severe-lung clusters. The larger number of participants assigned to late-onset/normal-lung and early-onset/normal-lung clusters suggest that subclustering these more mild-moderate children may be warranted. In addition, the retrospective nature of the study limited analyzable characteristics.

Overall, we replicated previously developed pediatric asthma clusters in a large, well-defined population. While this study cannot be used to make treatment recommendations, our findings suggest that clusters can provide insight into which patients will have the most beneficial treatment response or potentially no preference, particularly in step 2 or 3 asthma care. Further investigation of these childhood asthma clusters is warranted and could aid clinicians in personalizing treatment regimens.

Clinical implications: Therapeutic responses across pediatric asthma clusters, or subclasses, were similar. One cluster/subclass showed most benefit with fluticasone/salmeterol, and another cluster/subclass showed limited treatment response warranting investigation to facilitate individualizing therapy.

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METHODS

LDA and QDA

Both LDA and QDA are standard classification models that allow assignment of a patient into one of the many (≥2) possible groups. The assumptions are that the distribution of the observations (patients) and the distribution of the variables given a classification are a normal distribution. Because these models have simple assumptions, the risk is that a more complex model may more accurately separate groups of patients. However, training a more complex model may perform less well in a general population.

Multiple imputation

Twenty multiple imputed data sets[E1] for the 3 BADGER trial participants with missing FEV_1 percent-predicted measurements were created. Predictive mean matching[E2] and Bayesian logistic regression[E3] analyses were used for continuous and binary variables, respectively. Other variables used during multiple imputation included family history (eg, parental asthma), demographics (eg, sex, race, and ethnicity), environment (eg, pets), symptomatology (eg, eczema, asthma control days), lung function (eg, forced vital capacity), and inflammatory markers (eg, blood eosinophils). Because each CARE participant was represented as 20 imputed samples, these 20 imputed samples were assigned to each of the 4 SARP clusters by QDA. CARE participants were assigned to the cluster with the highest count.

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TABLE E1. Leave-one-out cross-validation of SARP cluster assignment with QDA

	True cluster				
	1	**2**	**3**	**4**	**Total**
Prediction					
1	46	2	0	0	48
2	2	49	0	1	52
3	0	0	31	2	33
4	0	1	1	26	28
Total	48	52	32	29	

TABLE E2. PACT, the CLIC trial, and the BADGER trial percent exacerbation displayed for time to first exacerbation outcome across treatment and clusters

		PACT ($P = .46$)			
Treatment	**Late-onset/normal-lung 1 ($P = .16$)**	**Early-onset/normal-lung 2 ($P = .15$)**	**Early-onset/comorbidity 3 ($P = .11$)**	**Early-onset/severe-lung 4 ($P = .44$)**	**All clusters**
0.5× fluticasone + salmeterol	57 (40-74)	51 (36-66)	17 (7.2-81)	38 (17-63)	50 (40-60)
1× fluticasone	40 (25-56)	36 (24-50)	38 (0.87-89)	42 (6.8-83)	38 (27-48)
Montelukast	55 (39-70)	52 (37-68)	90 (47-97)	54 (27-79)	56 (46-65)
All treatments ($P = .78$)	50 (41-60)	46 (38-54)	54 (23-84)	45 (29-61)	
		CLIC trial ($P = .11$)			
Treatment	**1 ($P = .17$)**	**2* ($P = .008$)**	**3 ($P = .24$)**	**4 ($P = .25$)**	**All clusters**
Fluticasone	2.6 (0.10-8.3)	**1.1 (0.18-6.3)**	9.4 (0.34-29)	2.5 (0.44-14)	2.9 (0.74-6.4)
Montelukast	7.4 (2.2-15)	12 (4.2-23)	3.1 (0.57-17)	7.1 (0.26-22)	8.4 (4.4-14)
All treatments ($P = .86$)	5.0 (1.8-9.7)	6.6 (2.4-13)	6.3 (0.54-17)	4.9 (0.43-14)	
		BADGER trial ($P = .82$)			
Treatment	**1 ($P = .85$)**	**2 ($P = .84$)**	**3 ($P = .50$)**	**4 ($P = .23$)**	**All clusters**
Fluticasone + salmeterol	17 (9.5-27)	20 (10-31)	12 (1.8-29)	7.1 (0.26-22)	16 (11-22)
2.5× fluticasone	20 (12-30)	22 (12-33)	23 (7.1-43)	26 (9.8-47)	22 (15.8-3)
Fluticasone + montelukast	20 (11-29)	25 (15-36)	26 (9.8-47)	17 (4.0-36)	22 (16-28)
All treatments ($P = .73$)	19 (14-24)	22 (16-28)	20 (11-31)	17 (8.4-27)	

P values refer to time to exacerbation Cox proportional hazard significance testing. Percent exacerbations are displayed for ease of interpretation. 95% CIs are shown in parentheses. Boldface numbers indicate treatments beneficial for asthma. *P* values in primary outcome header test cluster and treatment interaction. *P* values in cluster number column headers test treatment effect within that cluster. *P* values in the row header "All treatments" test cluster effect for all participants.
*Clusters in which significant differences ($P < .05$) were identified among treatments.