

Measuring the corticosteroid responsiveness endophenotype in asthmatic patients

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Background: Inhaled corticosteroids are the most commonly used controller therapies for asthma, producing treatment responses in 6 clinical phenotypes: lung function, bronchodilator response, airway responsiveness, symptoms, need for oral steroids and frequency of emergency department visits and hospitalizations. We hypothesize that treatment response in all of these phenotypes is modulated by a single quantitative corticosteroid responsiveness endophenotype. **Objective:** We sought to develop a composite phenotype that combines multiple clinical phenotypes to measure corticosteroid responsiveness with high accuracy, stability across populations, and robustness to missing data.

Methods: We used principal component analysis to determine a composite corticosteroid responsiveness phenotype that we tested in 4 replication populations. We evaluated the relative accuracy with which the composite and clinical phenotypes measure the endophenotype using treatment effect area under the receiver operating characteristic curve (AUC).

Results: In the study population the composite phenotype measured the endophenotype with an AUC of 0.74, significantly exceeding the AUCs of the 6 individual clinical phenotypes, which ranged from 0.56 ($P < .001$) to 0.67 ($P = .015$). In 4 replication populations with a total of 22 clinical phenotypes available, the composite phenotype AUC ranged from 0.69 to 0.73, significantly exceeded the AUCs of 14 phenotypes, and was not significantly exceeded by any single phenotype.

Conclusion: The composite phenotype measured the endophenotype with higher accuracy, higher stability across populations, and higher robustness to missing data than any clinical phenotype. This should provide the capability to model corticosteroid pharmacologic response and resistance with

increased accuracy and reproducibility. (*J Allergy Clin Immunol* 2015;■■■:■■■-■■■.)

Key words: Asthma, corticosteroids, drug therapy, endophenotype, pharmacogenetics, pharmacologic response

Inhaled corticosteroids (ICSs) are the most commonly used^{1,2} and efficacious controller therapies for asthma. Variation in treatment response to ICSs has been well identified, and within-person variation in ICS treatment response is highly repeatable.³ ICS treatment response has a genetic basis using the change in lung function as the clinical phenotype.^{4,5} Current asthma guidelines characterize treatment response in terms of lung function, symptoms, or exacerbations.² ICSs also produce a significant treatment response in bronchodilator response and airway responsiveness.⁶

Nonresponse to corticosteroids is a common clinical problem, with up to 24% of patients with severe asthma who take oral corticosteroids not responding with a greater than 15% improvement in prebronchodilator FEV₁.⁷ Identifying steroid nonresponse is clinically difficult, rendering it underrecognized, even though steroid nonresponse poses a significant patient risk because patients are more likely to experience adverse outcomes. When patients prescribed ICSs experience adverse outcomes, clinicians often attribute these problems to environmental triggers or medication nonadherence. In reality, these patients might share a group of common phenotypes that suggest steroid nonresponse.

For 15 years, asthma researchers have considered these phenotypes individually; however, this approach presents significant problems. For example, in pharmacogenetic modeling researchers aim to define an interaction between a genomic feature or variation and response to a particular pharmacologic agent. When studying the pharmacogenetics of corticosteroid response in asthmatic patients, there are many potential pharmacologic responsiveness phenotypes from which to choose. Choosing a specific phenotype is typically based on the characteristics of the cohort available. Nevertheless, this choice carries with it many repercussions for the researcher. Different clinical phenotypes will have differing statistical power, and different phenotypes will have different rates of missing data; most importantly, different phenotypes will be assessed in other cohorts with varying frequencies, leading to difficulties in replication of the pharmacogenetic findings. As a result of such factors, the choice of a single clinical phenotype to characterize the ICS treatment response is problematic.

We propose to move away from the focus on single phenotypes to a more holistic approach. We hypothesize that a single quantitative corticosteroid responsiveness endophenotype modulates the asthma disease process (Fig 1). The endophenotype is latent in untreated subjects and active in ICS-treated subjects. Under this hypothesis, clinical treatment response phenotypes are not regulated by separate mechanisms but instead by this

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Abbreviations used

AMSYM:	A clinical phenotype measuring the trend in average morning symptoms as recorded in daily diary card
AUC:	Area under the receiver operating characteristic curve
BDRABPCT:	A clinical phenotype measuring the trend in BDR
BURSTS:	A clinical phenotype measuring the trend in cumulative number of courses of oral corticosteroid treatment
CAMP:	Childhood Asthma Management Program
EDHOS:	A clinical phenotype measuring the trend in cumulative number of emergency department visits and hospitalizations
ICS:	Inhaled corticosteroid
IMPACT:	Improving Asthma Control Trial
LNPC20:	A clinical phenotype measuring the trend in the natural log of PC ₂₀
PACT:	Pediatric Asthma Controller Trial
PC:	Principal component
PCA:	Principal component analysis
PREFEV:	A clinical phenotype measuring the trend in FEV ₁
ROC:	Receiver operating characteristic
SHARP:	Single Nucleotide Polymorphism Health Association-Asthma Resource Project
SOCS:	Salmeterol or Corticosteroids Study

endophenotype that influences the asthma disease process to produce the treatment effect observed in all clinical phenotypes. If this hypothesis is true, the endophenotype should characterize steroid response better than individual phenotypes. For the purpose of pharmacologic modeling, our objective is to measure this endophenotype as accurately as possible in each subject. We interpret the various clinical phenotypes as indirect measurements of the endophenotype, which display varying accuracies between different phenotypes and across populations. We propose a composite corticosteroid responsiveness model that combines clinical phenotypes to produce a composite phenotype that measures the endophenotype with higher accuracy, more stability across populations, and more robustness to missing data than any single clinical phenotype. Our objective in the current work is to develop such a composite corticosteroid responsiveness phenotype for patients with mild-to-moderate asthma.

METHODS

Study design

We designed this study (Fig 2) to have the following steps: (1) select clinical phenotypes known to exhibit significant treatment response; (2) select a well-powered and representative study population and suitable replication populations; (3) use principal component analysis (PCA) to determine the composite corticosteroid responsiveness phenotype in the study population; (4) generalize the study population result to a composite corticosteroid responsiveness phenotype model and test in replication populations; and (5) evaluate and compare the relative endophenotype measurement accuracy of all phenotypes.

Clinical phenotypes

We selected 6 clinical phenotypes that display statistically significant ICS treatment response in patients with mild-to-moderate asthma: symptoms, lung function, airway responsiveness, bronchodilator response, emergency department (ED) visits/hospitalizations, and oral corticosteroid bursts. We defined these phenotypes and determined values for each subject phenotype by

performing simple linear regression of clinical observations, as described in the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Populations

We selected study and replication populations from among the cohorts of the Single-Nucleotide Polymorphism Health Association-Asthma Resource Project (SHARP). SHARP consolidates clinical trial data from 3 National Heart, Lung, and Blood Institute-sponsored asthma clinical research networks: the Childhood Asthma Management Program (CAMP),⁶ the Childhood Asthma Research and Education network,^{8,9} and the Asthma Clinical Research Network.¹⁰⁻¹²

We selected populations from cohorts that contained a treatment group that received ICSs and a treatment group that did not receive ICSs. In each population we created 2 groups: the ICS group contained subjects who received ICSs or a combination therapy including ICSs. The "non-ICS" group contained subjects who received placebo or a non-ICS drug, such as a leukotriene antagonist. Clinical observations were taken from the SHARP phs000166.v2.p1 dbGaP¹³ data set, with the exception of CAMP symptoms, which were taken directly from CAMP trial data sets because the CAMP symptom data were found to be incomplete in the dbGaP data set.

Endophenotype measurement accuracy

We evaluated the relative accuracy with which the composite and clinical phenotypes measure the endophenotype using treatment effect area under the receiver operating characteristic curve (AUC). The rationale for this choice of statistic and the methods used are described in the [Methods](#) section in this article's Online Repository.

Composite corticosteroid responsiveness model

We determined a composite corticosteroid responsiveness phenotype from the pattern of treatment response exhibited by the study population. We selected an unsupervised method, PCA, with the expectation that the endophenotype would primarily modulate the first principal component (PC1) of the treatment response, which would comprise a composite phenotype measuring the endophenotype accurately.

We determined clinical phenotypes as described in the [Methods](#) section in this article's Online Repository. After discarding subjects for whom phenotypes were missing, we performed PCA on the remaining complete sets of 6 phenotypes with scaling to unit variance using the R version 3.1.0 base stats package `prcomp` function. We determined the treatment effect AUC of PCs and clinical phenotypes. We set the comparison direction for clinical phenotypes based on clinical experience. Because the sign of components produced by using PCA is arbitrary, we let the `pROC` package automatically determine the comparison direction for PCs. We analyzed the covariance of PC1 with the covariates typically used in the study of asthma, sex, and age.

We generalized the study population result into a population-independent composite corticosteroid responsiveness model that we tested in each replication population. Clinical phenotypes were determined as described in the [Methods](#) section in this article's Online Repository, and treatment effect AUCs were calculated. Missing phenotypes were imputed by being set equal to the center value of the respective phenotype in the study population. The 6 phenotypes were then centered and scaled by using the study population PCA center and scale coefficients, multiplied by the study population PC1 loading coefficients, and summed to produce the composite phenotype value. Finally, composite phenotype treatment effect AUC was determined.

Sensitivity to missing clinical phenotypes

We investigated the sensitivity of the composite phenotype to missing component phenotypes by forming a pooled replication population comprised of all subjects found in the replication populations that possessed a complete set of clinical phenotypes. We determined the treatment effect AUC of each clinical phenotype for this pooled population. For all possible combinations of

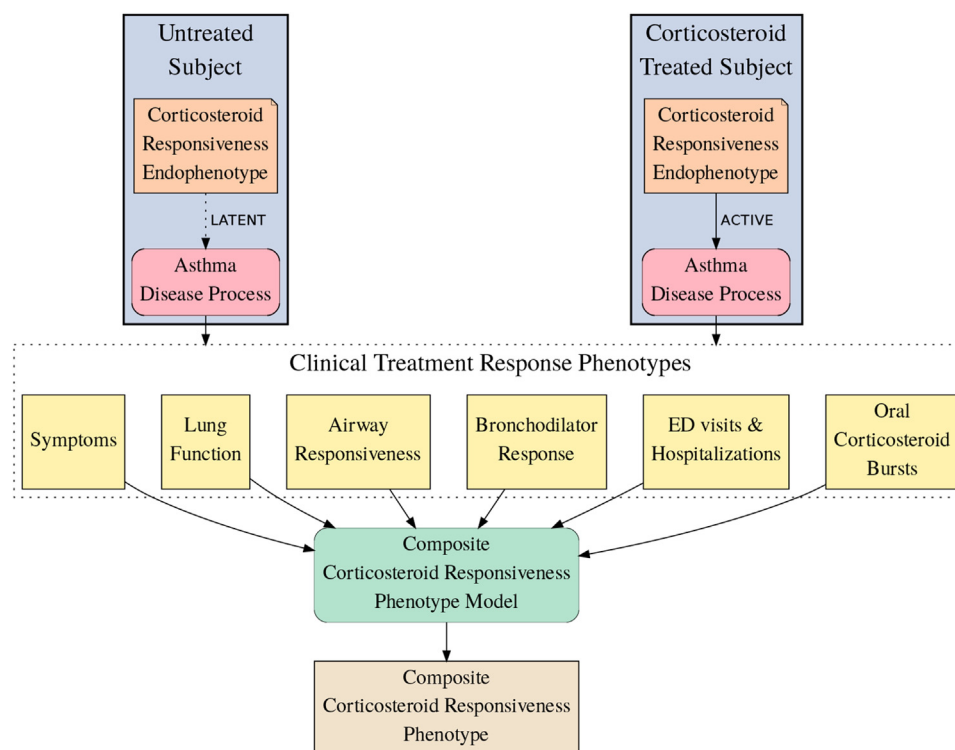


FIG 1. Measuring corticosteroid responsiveness. We hypothesize a corticosteroid responsiveness endophenotype that modulates the asthma disease process, is latent in untreated subjects, and is active in ICS-treated subjects. We propose a composite corticosteroid responsiveness phenotype model that combines 6 clinical phenotypes into a composite phenotype to measure the endophenotype.

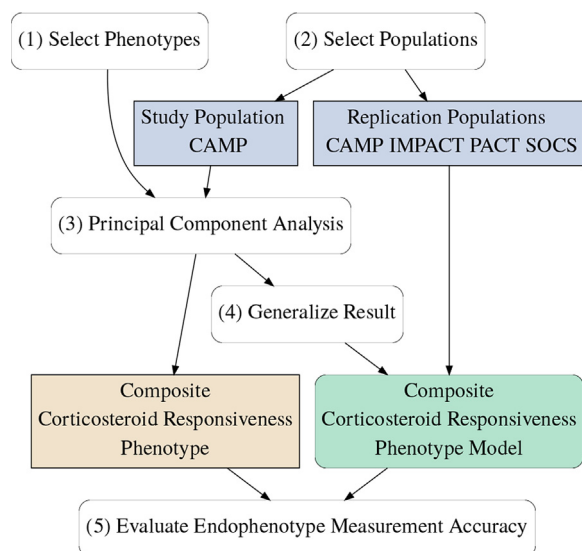


FIG 2. Study design. We selected clinical phenotypes known to exhibit significant treatment response and representative study and replication populations. We used PCA to determine the composite corticosteroid responsiveness phenotype in the study population. We generalized this result to produce the composite phenotype model for testing in replication populations. We evaluated the relative measurement accuracy of all phenotypes.

0, 1, 2, 3, or 4 phenotypes missing from this population, we determined the composite phenotype, its treatment effect AUC, and single-sided *P* values with respect to each clinical phenotype used in its calculation.

RESULTS

Population characteristics

The study population consisted of white children from CAMP. Four replication populations were selected from CAMP, the Childhood Asthma Research and Education network, and the Asthma Clinical Research Network (Table 1). The CAMP replication population consisted of nonwhite children. The replication populations included both children (CAMP and the Pediatric Asthma Controller Trial [PACT]) and adults (the Salmeterol or Corticosteroids Study [SOCS] and Improving Asthma Control Trial [IMPACT]). Subjects in the populations had relatively normal lung function. In each population subjects used a single corticosteroid inhaler drug. Collectively, the populations represent a variety of different corticosteroid inhalers. Bronchodilator response was relatively consistent across the populations, but airway responsiveness and symptoms were more variable. To increase sample size, we included non-ICS drugs in the non-ICS group and ICS combination therapy in the ICS group. In the SOCS population we grouped long-acting β_2 -adrenergic receptor agonist therapy (salmeterol) with placebo. In PACT we grouped salmeterol/ICS combination therapy with ICS-only therapy. In PACT, in which placebo treatment was not available, the non-ICS group consisted of subjects treated with a leukotriene receptor antagonist (montelukast).

Study population

We determined clinical phenotypes for the CAMP study population, as described in the Methods section in this article's Online Repository (Fig 3 shows phenotypes for 1 subject). We

TABLE I. Population characteristics

Population	CAMP study	CAMP replication	PACT replication	SOCS replication	IMPACT replication
Trial	CAMP	CAMP	PACT	SOCS	IMPACT
Network	CAMP	CAMP	CARE	ACRN	ACRN
No.	388	188	142	56	80
Race(s)	White	African American, Hispanic, other	White	White	White
Age (SD)	8.8 (2.1)	9.2 (2.1)	9.8 (2.2)	30 (9.8)	34 (11)
Sex (male)	228 (59%)	112 (60%)	92 (65%)	17 (30%)	31 (39%)
ICS treatment(s)	Budesonide	Budesonide	Fluticasone, salmeterol/ fluticasone combination	Triamcinolone	Budesonide*
Non-ICS treatment(s)	Placebo	Placebo	Montelukast	Placebo, salmeterol	Placebo*
Duration (wk)	207	207	57	30	60
N ICS group	164	90	92	17	45
N non-ICS group	224	98	50	39	35
FEVPPB (SD)	95 (14)	91 (14)	98 (12)	87 (15)	88 (13)
LNPC20B (SD)	0.048 (1.2)	0.058 (1.1)	-0.3 (1.3)	0.61 (0.57)	0.85 (0.64)
BDRB (SD)	11 (9.9)	11 (9.8)	8.4 (7.1)	Not available	9.8 (7.7)
SYMB (SD)	0.63 (0.45)	0.56 (0.43)	0.59 (0.35)	Not available	0.18 (0.28)

ACRN, Asthma Clinical Research Network; BDRB, bronchodilator percentage change at baseline; CARE, Childhood Asthma Research and Education; FEVPPB, FEV₁ percent predicted at baseline; LNPC20B, natural log PC₂₀ at baseline; N ICS group, subjects treated with ICS or a combination therapy including ICS; N non-ICS group, subjects treated with placebo or a non-ICS therapy; SYMB, average symptoms as recorded in daily diary card at baseline.

*IMPACT subjects had access to open-label budesonide as part of a symptom-based action plan and were subjected to a 10- to 14-day period of intense combined therapy that included ICSs and oral steroids at the end of the run-in and treatment phases of the study.

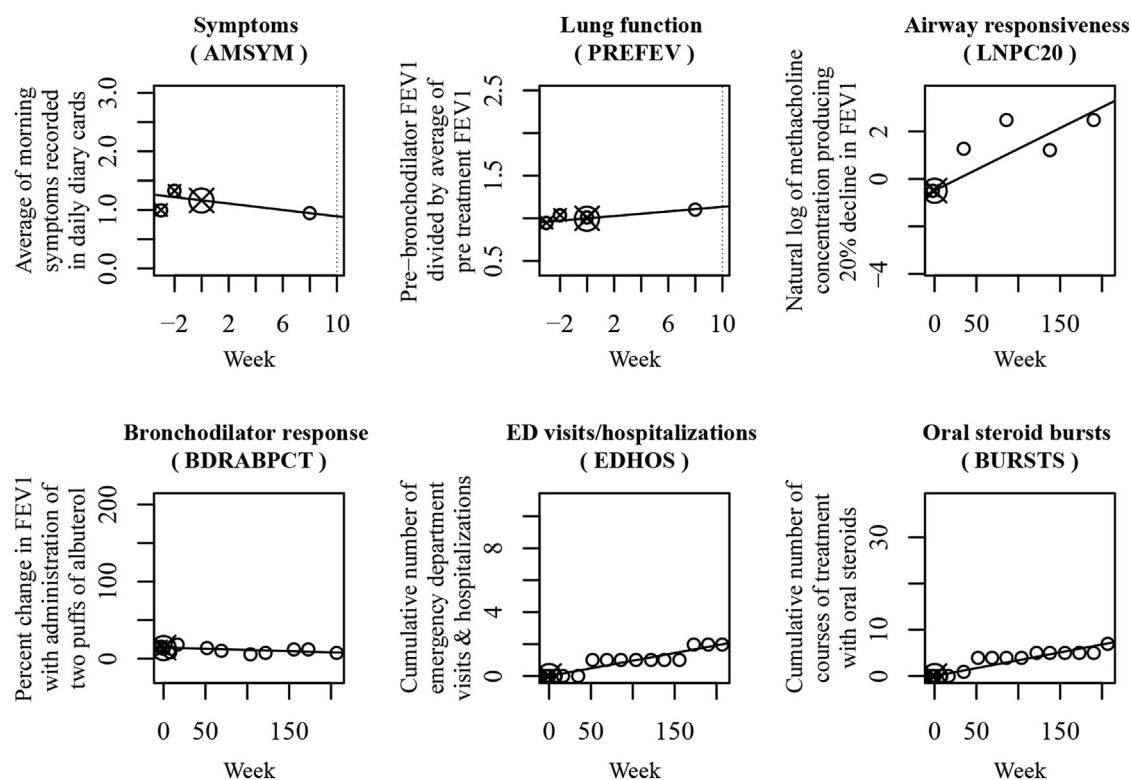


FIG 3. Clinical treatment response phenotypes for one CAMP study participant. We determined the value of each phenotype by performing linear regression of clinical observations versus time from start of treatment. We constrained the regression lines to pass through the average (target) of pretreatment values (small targets) at time 0. We interpreted the slopes of these lines as phenotypic values.

discarded subjects with missing phenotypes (see Table E1 in this article's Online Repository at www.jacionline.org) and performed PCA on 327 (ICS, 141; non-ICS, 186) subjects. PC1 accounted for 30% of the variance (see Table E2 in this article's Online Repository at www.jacionline.org).

Fig 4, A, shows PC1 and PC2 of ICS treatment response for subjects in the ICS treatment group (red triangle) and the non-ICS treatment group (black circles). Lines indicate the direction and strength with which each clinical phenotype contributed to PC1 and PC2. Increasing corticosteroid responsiveness, as

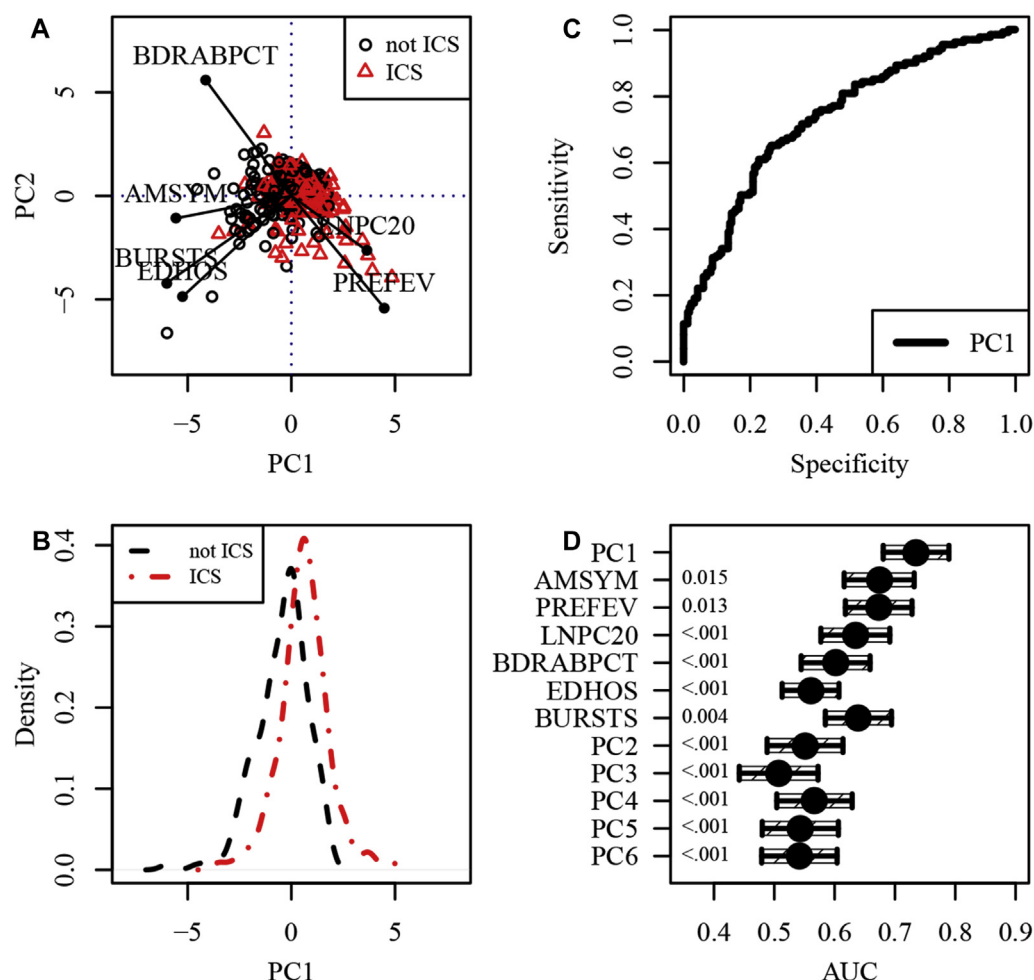


FIG 4. Learning the composite phenotype in the study population. **A**, PC1 and PC2 of ICS treatment response. **B**, The distribution of PC1 in treatment groups. **C**, PC1 ROC (AUC = 0.74). **D**, AUCs of PC1, clinical phenotypes, and other components, with 95% CIs (single-sided *P* values are shown along the left axis).

measured by PC1, was most closely aligned with decreasing symptoms (AMSYM phenotype) and also associated with decreasing bronchodilator response (BDRABPCT phenotype), decreasing cumulative ED visits/hospitalizations (EDHOS phenotype), decreasing cumulative oral corticosteroid bursts (BURSTS phenotype), increasing lung function (PREFEV phenotype), and decreasing airway responsiveness (increasing LNPC20 phenotype).

Fig 4, *B*, shows the distribution of the composite phenotype, PC1, in the ICS treatment group (red dashed dotted line) and the non-ICS treatment group (black dashed line). The distribution of PC1 in the treatment groups was reasonably normal.

Fig 4, *C*, shows the treatment effect receiver operating characteristic (ROC) curve for PC1. ROC analysis of PC1 determined a treatment effect AUC of 0.74, which significantly exceeded the AUC of all clinical phenotypes (Fig 4, *D*), which ranged from 0.56 for ED visits/hospitalizations ($P < .001$) to 0.67 for symptoms ($P = .015$) and significantly exceeded the AUC of all other PCs, which ranged from 0.51 for PC3 to 0.57 for PC4 (all $P < .001$). The PCA center, scale, and loading coefficients associated with PC1 (see Table E3 in this article's Online Repository at www.jacionline.org) were saved for use in the composite phenotype model. An analysis of the covariance of PC1 with sex and age

indicated no significant effects (see Tables E4 and E5 in this article's Online Repository at www.jacionline.org).

Replication populations

In replication populations missing clinical observations were generally minimal (see Table E1), with the following exceptions: the CAMP replication population was missing 23.9% of symptoms, and the SOCS population did not include symptoms or bronchodilator observations. We determined clinical phenotypes as described in the Methods section in this article's Online Repository, with the exception of the lung function window for IMPACT being increased to 18 weeks to include observations from the 15-week follow-up visit. Composite phenotypes were determined, as described above. The composite phenotype AUC was stable across the replication populations, ranging from 0.69 to 0.73, whereas the clinical measure phenotype AUCs were more variable, ranging from 0.46 to 0.81 (Fig 5). The composite phenotype AUCs significantly exceeded the AUCs of 14 of the 22 available clinical phenotypes (single-sided *P* values are shown along the left axes of Fig 5), exceeding airway responsiveness in 1 of 4, lung function in 2 of 4, symptoms in 2 of 3, bronchodilator response in 2 of 3, oral corticosteroid bursts in 3 of 4, and ED

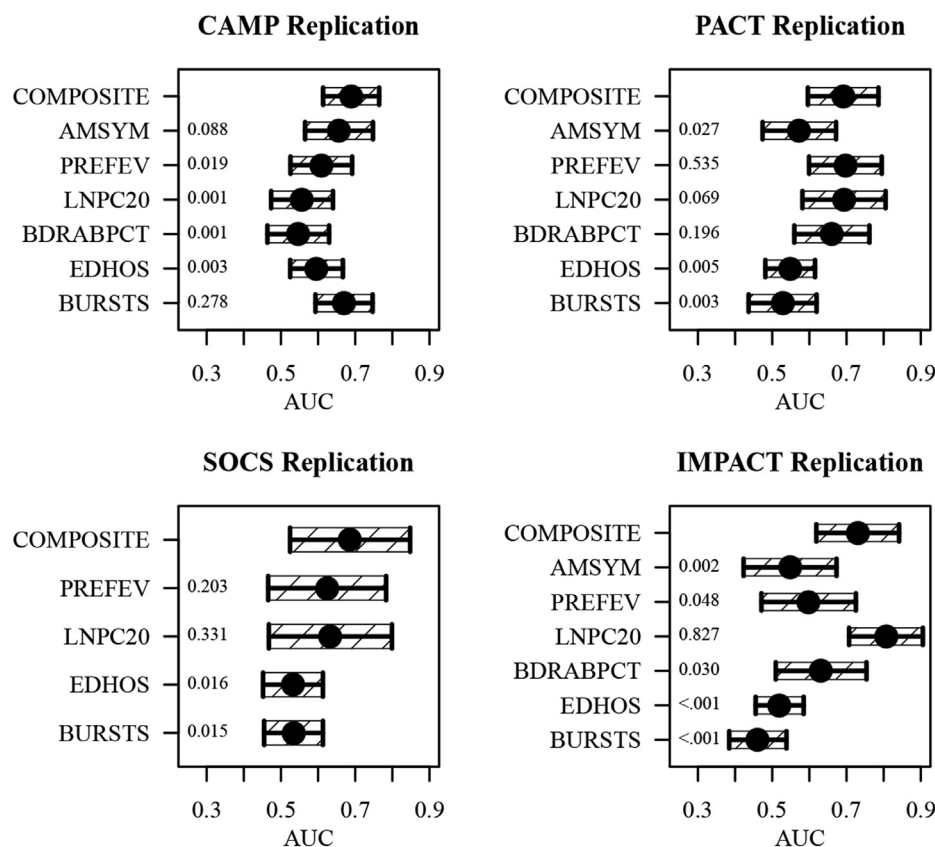


FIG 5. Composite phenotype performance in the replication populations. A comparison of the relative accuracy (AUC) with which the corticosteroid responsiveness endophenotype was measured by the composite (COMPOSITE), symptom (AMSYM), lung function (PREFEV), airway responsiveness (LNPC20), bronchodilator response (BDRABPCT), ED visit/hospitalization (EDHOS), and oral corticosteroid burst (BURSTS) phenotypes with 95% CI (single-sided *P* values are shown along the left axis).

visits/hospitalizations in 4 of 4 replication populations. Conversely, no clinical phenotype in any population measured the endophenotype with significantly higher relative accuracy.

In the IMPACT replication population the airway responsiveness AUC (0.81) exceeded the composite phenotype AUC (0.73), approaching statistical significance ($P = .18$). This might reflect outliers or confounding by IMPACT's somewhat atypical design (Table I). In the SOCS replication population, which contained the smallest ICS treatment group ($n = 17$) and was missing 100% of symptoms and bronchodilator observations, the composite phenotype (AUC = 0.69) demonstrated excellent robustness to missing data and small population size.

Sensitivity to missing clinical phenotypes

We created a pooled replication population as described earlier using all subjects with complete sets of clinical phenotypes found in the replication populations (see Table E6 in this article's Online Repository at www.jacionline.org). For all possible combinations of up to 0, 1, 2, 3, or 4 phenotypes missing, we determined the composite phenotype AUC and the single-sided *P* values (see Table E7 in this article's Online Repository at www.jacionline.org). The composite phenotype AUC was found to be significantly greater than the individual component clinical phenotype AUC in 144 of 186 cases, and in no case did a component clinical phenotype AUC exceed the composite phenotype AUC.

DISCUSSION

The composite phenotype measured the corticosteroid responsiveness endophenotype significantly more accurately than any of the 6 clinical phenotypes individually. This should allow the study of corticosteroid pharmacology with increased accuracy and reproducibility. As an example, the composite phenotype should enable asthma pharmacogenetic studies that possess more power for a given sample size or that require a smaller sample for a given power. The composite phenotype also captured virtually the entire corticosteroid responsiveness signal, as evidenced by the low AUCs of the other PCs. This result is consistent with the hypothesis of a single endophenotype modulating corticosteroid response in all 6 clinical phenotypes.

In the replication populations the composite phenotype demonstrated higher accuracy, stability, and robustness to missing data than any clinical phenotype alone. This suggests that the endophenotype is stable across the asthma populations explored in this study and that studies performed with the composite phenotype will replicate more readily.

Our primary goal in selecting replication populations was to create ICS and non-ICS groups on which to apply our methods. Other equivalence between the replication populations was not an objective or requirement and that is why we did not present *P* values in Table I. The consistent performance of the composite phenotype despite the variability between the replication populations is a strength of the study result.

In the pooled replication population with 0, 1, 2, 3, and 4 clinical phenotypes assumed missing, the composite phenotype performed as well as and usually significantly better than the individual clinical phenotypes. This suggests that there might be no penalty to using the composite phenotype in general and that it offers benefit when as few as 2 clinical phenotypes are available.

These results indicate that the addition of phenotypes can increase the AUC for the purpose of corticosteroid pharmacogenetic study design. Because, increases in AUC reduce sample size for a given power, such studies could be cost-optimized by trading off the costs of additional subjects and additional phenotypes.

The analysis of covariates indicated no significant sex or age effect in the CAMP study population subjects, who ranged in age from 5.2 to 13.2 years at randomization and were followed for 4 years. This suggests that the corticosteroid responsiveness endophenotype is stable from age 5 to 17 years and that an estimate of the endophenotype made at one point in childhood can reasonably be used later in childhood. This could be confirmed by an analysis of subjects tracked over a longer period of time and ideally into adulthood.

Our results have clinical relevance. The composite corticosteroid responsiveness model produced more accurate measurements of ICS response than individual phenotypes in white replication populations of children and adults with mild to moderately severe asthma and, for children, of multiple races. It performed robustly in child populations in whom budesonide or fluticasone was used and in adult populations in whom budesonide or triamcinolone was used. It reliably detected corticosteroid response in monotherapy or in combination with salmeterol. It accurately differentiated corticosteroid response from placebo and leukotriene receptor antagonist treatment control groups. Given that the composite model collapses multiple longitudinal clinical observations into an easily interpreted corticosteroid response metric and that it can be easily implemented in a simple computer program, it might have potential for use in the clinical setting, where it could allow the practitioner to more accurately estimate ICS response.

The idea of composite phenotypes is not new: composite indices have been used for 15 years in the assessment of therapeutic efficacy in clinical trials of rheumatoid arthritis.^{14,15} A number of composite score instruments of asthma control have also been proposed, which were generally focused on treatment responsiveness as a dichotomous outcome.¹⁶ However, the approach used here is novel in several ways.

First, we avoided the need for expert-based specification of model parameters or thresholds.

Second, we developed a continuous composite phenotype that is a linear combination of treatment response phenotypes determined algorithmically from clinical observations.

Third, we developed an unbiased phenotype by using an unsupervised dimensionality reduction approach to learn composite phenotype coefficients from the pattern of response in the study population.

Fourth, we assessed the relative accuracy with which different phenotypes measure the corticosteroid responsiveness endophenotype.

Finally, we arrived at a composite corticosteroid responsiveness phenotype with demonstrably higher accuracy. We did so using an approach that is free of subjective judgments other than the choice of study population, selection of clinical measures, and choice of modeling approach.

Recent asthma phenotype research has focused on disease subphenotypes.^{17,18} We expect that the composite phenotype can be applied to asthma subphenotype populations, with the possible exception of corticosteroid-resistant asthma.

Approximately 30% of asthmatic patients have been reported to not respond to ICS treatment.¹⁹ The study of such nonresponders could identify the cause of nonresponse and lead to new therapies. The composite phenotype could be used to characterize such subjects with higher accuracy. It could be used directly to study variability in corticosteroid response or, with appropriate thresholds, to dichotomize responders and nonresponders. We believe that the success of our composite phenotype approach derives from a study population with good power, a treatment effect that manifests in multiple clinical measures, and the presence of a primary drug responsiveness endophenotype. Our approach could be productively applied in other situations in which these conditions are met.

Other groups can apply the composite phenotype concept in 2 general ways. Asthma researchers can determine the value of the composite phenotype by determining clinical phenotypes using the regression method described and determining the composite phenotype by applying the model coefficients, as described. Researchers studying treatment response for other drugs or conditions could determine a custom composite phenotype for a new cohort by determining clinical phenotypes using the regression method described and determining a composite phenotype by applying PCA, as described.

Despite the strengths of this study, a few caveats deserve mention. Our study relied on clinical trial populations and hence might not be generalizable to asthmatic patients receiving corticosteroids in nontrial settings. Having actual glucocorticoid levels might have enhanced our endophenotype determination. Finally, we need to assess the endophenotype in genomic studies to assess its practical utility.

In conclusion, we developed a composite corticosteroid responsiveness phenotype model that measures the corticosteroid responsiveness endophenotype with higher accuracy and higher stability across populations than any single clinical measure phenotype. This new phenotype should allow the development of asthma pharmacologic models with increased power and reproducibility.

Key messages

- ICSs are the most commonly used controller therapies for asthma, producing treatment responses in 6 individual clinical phenotypes.
- We hypothesize that instead of being regulated by separate mechanisms, these responses are all regulated by a single corticosteroid responsiveness endophenotype.
- A composite phenotype combining all clinical phenotypes measures corticosteroid responsiveness with higher accuracy, stability across populations, and robustness to missing data than the individual clinical phenotypes.
- The composite phenotype offers the prospect for greater insight into corticosteroid response and resistance.

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METHODS

Clinical phenotypes

We selected 6 clinical measures that display statistically significant ICS treatment response in patients with mild-to-moderate asthma:

- *Symptoms*: the average of morning symptoms recorded in daily diary cards between visits (units: subject's reported symptom score in the range of 0-3);
- *Lung function*: prebronchodilator FEV₁ normalized by the average of baseline (pretreatment) observations (units: nondimensional ratio);
- *Airway responsiveness*: natural log PC₂₀ (units: natural log of milligram per milliliter);
- *Bronchodilator response*: relative percentage change in FEV₁ with administration of 2 puffs of albuterol (units: percentage);
- *ED visits/hospitalizations*: cumulative count of ED visit and hospitalization events from the start of treatment to the visit date (units: count); and
- *Oral corticosteroid bursts*: cumulative count of courses of treatment from the start of treatment to the visit date (units: count).

We determined values for clinical phenotypes based on these measures as follows. We performed simple linear regression of observations versus time in weeks from the start of treatment. For symptoms and lung function, we used observations up to and including the 10th week of observation because these phenotypes plateau within this observation window. For other clinical phenotypes, we used all available observations. We constrained the regression line to pass through the mean of baseline values at time zero or, for cumulative count variables, the origin. We interpreted the slope of this line as the value of the phenotype. The resulting clinical phenotype values are thus estimates of the rate of change per week in the respective clinical measures. If, for a given phenotype, pretreatment observations were not available or sufficient observations to perform regression were not available, we marked the phenotype as missing.

Endophenotype measurement accuracy

In clinical trials of ICSs, treatment response has typically been characterized by phenotypes based on the change in a clinical observation and treatment effect size reported as the difference in mean values between treatment groups. However, this approach is inappropriate for the current work, which requires a statistic that allows integration of treatment effects across disparate clinical measures. Several suitable treatment effect statistics are available, including Cohen *d*, relative risk, number needed to treat, and treatment effect AUC.^{E1} We chose to use treatment effect AUC, which is the AUC comparing responses to treatment and control.

A treatment effect AUC of 0.5 indicates no measurable effect, less than 0.5 indicates deleterious effects, and greater than 0.5 indicates benefit. AUC is also commonly used to evaluate diagnostic tests and predictive models,^{E2} where a perfect test or model produces an AUC of 1.0, and AUC values in the range of 0.75 to 0.95 are not uncommon. However, when measuring treatment effect, an AUC of 1.0 indicates the extremely unlikely result that every treated subject has a better outcome than every untreated subject. Therefore in practice treatment effect AUCs are lower than diagnostic test AUCs. For example, Cohen suggested a “small” treatment effect reference value (*d* = 0.2) is equivalent to an AUC of 0.56 and number needed to treat of

8.9, and a Cohen “large” treatment effect (*d* = 0.8) is equivalent to an AUC of 0.71 and a number needed to treat of 2.3 when conversions are made under the assumption of normal distributions with equal variance.^{E1}

Treatment effect AUC is usually interpreted as the probability that a randomly selected subject in the treatment group will have a better outcome than a randomly selected subject in the untreated group. When the treatment effect AUC of different phenotypes is calculated for a given population, it differs among the phenotypes. However, the actual outcome experienced by the subject is not changed by the choice of a different phenotype. Therefore we selected an interpretation of the treatment effect AUC based on the origin of the AUC in signal detection theory, which is more informative for our purposes. Under our assumption that the corticosteroid responsiveness endophenotype is latent in untreated subjects and active in treated subjects (Fig 1), we see that treatment effect AUC is the probability that the measured effect of the active endophenotype in a random treated subject exceeds the measured “background noise” of the asthma disease process in a random untreated subject. Thus, within our populations, a higher AUC indicates a phenotype that has more accurately measured the endophenotype's effect on the asthma disease process. Therefore we used the treatment effect AUC to evaluate the relative accuracy with which different phenotypes measure the endophenotype. We determined AUCs, AUC CIs, and single-sided *P* values for paired ROCs with 2000 stratified bootstrap replicates using the pROC package, version 1.7.2^{E3} and R version 3.1.0.^{E4}

Missing phenotypes

Table E1 shows missing phenotypes by population in percentages. Missing phenotypes were addressed as described in the text.

PCA results

Results were obtained from PCA on 6 clinical phenotypes in the CAMP study population performed as described in the text. Table E2 shows the importance of the PCs. Table E3 shows the resulting PCA center, scale, and PC1 loading values that we adopted for use in the composite phenotype model, as described in the text.

Analysis of covariates

We analyzed the covariates of PC1 by using sex and age, the variables typically considered as potential covariates in the study of asthma. Table E4 shows results for the CAMP study population, the training set used to determine PC1. Table E5 shows results for the ICS treatment group of the CAMP study population.

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TABLE E1. Missing clinical phenotypes (in percentages)

Population	CAMP study	CAMP replication	PACT replication	SOCS replication	IMPACT replication
Symptoms	14.9	23.9	0.0	100.0	1.2
Lung function	2.8	5.9	2.1	0.0	0.0
Airway responsiveness	0.5	1.1	31.7	3.6	6.2
Bronchodilator response	0.0	0.0	0.7	100.0	0.0
ED visits/hospitalizations	0.0	0.0	0.0	0.0	0.0
Oral steroid bursts	0.0	0.0	0.0	0.0	0.0

TABLE E2. PC importance

	PC1	PC2	PC3	PC4	PC5	PC6
SD	1.33	1.16	0.94	0.89	0.82	0.71
Proportion of Variance	0.30	0.22	0.15	0.13	0.11	0.08
Cumulative Proportion	0.30	0.52	0.67	0.80	0.92	1.00

TABLE E3. Composite corticosteroid responsiveness phenotype model coefficients

	Center	Scale	Loading
Symptoms	−1.38e-02	4.52e-02	−4.63e-01
Lung function	6.04e-03	1.40e-02	3.72e-01
Airway responsiveness	5.46e-03	8.28e-03	3.01e-01
Bronchodilator response	−1.25e-02	4.99e-02	−3.43e-01
ED visits/hospitalizations	4.00e-03	8.54e-03	−4.34e-01
Oral steroid bursts	2.18e-02	2.43e-02	−5.00e-01

TABLE E4. Covariates of PC1 in the CAMP study population

	Estimate	SE	t Value	P value
Intercept	0.31801	0.31389	1.01314	.31175
Treatment group	−1.04976	0.13784	−7.61576	.00000
Sex	0.05757	0.13929	0.41329	.67967
Baseline age	0.02796	0.03189	0.87691	.38118

TABLE E5. Covariates of PC1 in the CAMP ICS treatment group of the CAMP study population

	Estimate	SE	t Value	P value
Intercept	0.67946	0.47738	1.42329	.15691
Sex	0.04986	0.20991	0.23753	.81260
Baseline Age	−0.01268	0.04920	−0.25773	.79700

TABLE E6. Pooled replication population characteristics

Population	Pooled replication
Trial	CAMP, PACT, IMPACT
Network	CAMP, CARE, ACRN
No.	311
Race(s)	African American, white, Hispanic, other
Age (SD)	15 (12)
Sex (male)	175 (56%)
ICS treatment(s)	Budesonide, fluticasone, salmeterol/fluticasone combination*
Non-ICS treatment(s)	Placebo, montelukast*
Duration (wk)	207
N ICS group	168
N non-ICS group	143
FEVPPB (SD)	92 (13)
LNPC20B (SD)	0.04 (1.1)
BDRB (SD)	10 (8.9)
SYMB (SD)	0.48 (0.41)

ACRN, Asthma Clinical Research Network; BDRB, bronchodilator percentage change at baseline; CARE, Childhood Asthma Research and Education; FEVPPB, FEV₁ percent predicted at baseline; LNPC20B, natural log PC₂₀ at baseline; N ICS group, subjects treated with ICS or a combination therapy including ICS; N non-ICS group, subjects treated with placebo or a non-ICS therapy; SYMB, average am symptoms as recorded in daily diary card at baseline.

*IMPACT subjects had access to open-label budesonide as part of a symptom-based action plan and were subjected to a 10- to 14-day period of intense combined therapy that included ICSs and oral steroids at the end of the run-in and treatment phases of the study.

TABLE E7. Sensitivity study *P* values

Composite AUC	Symptoms	Lung function	Airway responsiveness	Bronchodilator response	ED visits/ hospitalizations	Oral steroid bursts
0.746	<.001*	.006*	.019*	.001*	<.001*	<.001*
0.733	<.001*	.013*	.046*	.002*	<.001*	
0.749	<.001*	.003*	.015*	<.001*		<.001*
0.742	<.001*	.009*	.025*		<.001*	<.001*
0.716	.001*	.050		.013*	<.001*	<.001*
0.730	<.001*		.073	.004*	<.001*	<.001*
0.745		.005*	.017*	<.001*	<.001*	<.001*
0.733	<.001*	.010*	.042*	.001*		
0.729	<.001*	.016*	.047*		<.001*	
0.747	<.001*	.005*	.013*			<.001*
0.706	.004*	.074		.027*	<.001*	
0.723	<.001*	.028*		.004*		<.001*
0.687	.007*	.202			<.001*	.003*
0.714	.004*		.147	.014*	<.001*	
0.730	<.001*		.053	.003*		<.001*
0.725	<.001*		.084		<.001*	<.001*
0.693	.012*			.083	<.001*	.004*
0.730		.013*	.040*	<.001*	<.001*	
0.747		.004*	.010*	<.001*		<.001*
0.735		.008*	.016*		<.001*	<.001*
0.701		.096		.021*	<.001*	.002*
0.726			.063	.002*	<.001*	<.001*
0.726	<.001*	.014*	.044*			
0.702	.005*	.084		.028*		
0.673	.010*	.305			.001*	
0.691	.001*	.158				.002*
0.712	.003*		.140	.011*		
0.710	.001*		.155		<.001*	
0.726	<.001*		.065			<.001*
0.675	.045*			.198	.002*	
0.696	.008*			.059		.004*
0.656	.090				.003*	.032*
0.720		.026*	.075	.001*		
0.724		.012*	.018*		<.001*	
0.740		.003*	.003*			<.001*
0.686		.181		.035*	.001*	
0.705		.073		.005*		.002*
0.680		.219			<.001*	.001*
0.709			.153	.006*	<.001*	
0.728			.049*	.001*		<.001*
0.712			.087		<.001*	<.001*
0.674				.174	.001*	.015*
0.657	.027*	.469				
0.699	.002*		.233			
0.667	.069			.267		
0.643	.126				.016*	
0.657	.047*					.038*
0.719		.013*	.008*			
0.673		.280		.049*		
0.674		.214			<.001*	
0.686		.140				<.001*
0.695			.301	.015*		
0.695			.206		<.001*	
0.714			.048*			<.001*
0.657				.374	.009*	
0.678				.084		.015*
0.611					.112	.416
Phenotype AUC	0.624	0.654	0.680	0.652	0.584	0.609

Table entries indicate single-sided *P* values for composite AUC greater than clinical phenotype AUC. Blank entries indicate that the clinical phenotype was assumed to be missing. *Composite AUC*, Value of the composite phenotype; *Phenotype AUC*, value of individual clinical phenotype AUC.

**P* < .05 (statistical significance).