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Manuscript Title: Measuring the Corticosteroid Responsiveness Endophenotype in Asthma

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Clinical Phenotypes

We selected six clinical measures that display statistically significant ICS treatment response in 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 to 3)
- Lung function: Pre-bronchodilator forced expiratory volume in 1 second (FEV1) normalized by the average of baseline (pre treatment) observations (units: non-dimensional ratio).
- Airway responsiveness: Natural log of the provocative concentration of methacholine producing a 20% decline in FEV1 (PC20, units: natural log of milligram per milliliter).
- Bronchodilator response: Relative percent change in forced expiratory volume in 1 second (FEV1) with administration of two puffs of albuterol (units: percent).
- Emergency department (ED) visits/hospitalizations: Cumulative count of ED visit & hospitalization events from the start of treatment to the visit date (units: count).
- 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 tenth week of observation, since these phenotypes plateau within this observation window. For other clinical phenotypes, we used all available observations. We constrained the regression line to pass through, at time zero, the mean of baseline values, or, for cumulative count variables, zero. 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, pre-treatment observations were not available, or sufficient observations to perform regression were not available, we marked the phenotype missing.

Endophenotype Measurement Accuracy

In clinical trials of ICS, 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's d*, *relative risk*, *number needed to treat*, and *treatment effect AUC* [E1]. We chose to utilize treatment effect area under the receiver operating characteristic curve (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 - 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's suggested "small" treatment effect reference value, d = 0.2, is equivalent to AUC = 0.56 and number needed to treat (NNT) = 8.9, and Cohen's "large" treatment effect, d = 0.8, is equivalent to AUC = 0.71 and NNT = 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 has 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 subjects is not changed by the choice of a different phenotype. We therefore selected an interpretation of the treatment effect AUC, based on the origin of AUC in signal detection theory, that is more informative for our purposes: Under our assumption that the corticosteroid responsiveness endophenotype is latent in untreated subjects and active in treated subjects (Figure 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 treatment effect AUC to evaluate the relative accuracy with which different phenotypes measure the endophenotype. We determined AUCs, AUC confidence intervals, and single-sided p-values for paired receiver operating curves (ROC) 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 percent. Missing phenotypes were addressed as described in the text.

PCA Results

The results of PCA on six clinical phenotypes in the CAMP study population performed as described in the text. Table E2 shows the importance of the principal components. 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 using gender and age, the variables typically consided 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.

Sensitivity to Missing Clinical Phenotypes

References

[E1] Kraemer HC and Kupfer DJ. Size of treatment effects and their importance to clinical research and practice. Biol Psychiatry, 59(11):990-6, Jun 2006.

[E2] Zou KH, O'Malley AJ, and Mauri L. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation, 115(5):654-7, Feb 2007.

[E3] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez J, et al. proc: an open-source package for r and s+ to analyze and compare roc curves. BMC Bioinformatics, 12:77, 2011.

[E4] R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, 2014.

Table E1. Missing Clinical Phenotypes (Percent)

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. Principal Component Importance

	PC1	PC2	PC3	PC4	PC5	PC6
Standard deviation	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	Std. Error	t value	Pr(> | t |)
Intercept	0.31801	0.31389	1.01314	0.31175
Treatment Group	-1.04976	0.13784	-7.61576	0.00000
Gender	0.05757	0.13929	0.41329	0.67967
Baseline Age	0.02796	0.03189	0.87691	0.38118

Table E5. Covariates of PC1 in the CAMP ICS Treatment Group of the CAMP Study Population

	Estimate	Std. Error	t value	Pr(> | t |)
Intercept	0.67946	0.47738	1.42329	0.15691
Gender	0.04986	0.20991	0.23753	0.81260
Baseline Age	-0.01268	0.04920	-0.25773	0.79700

Table E6. Pooled replication population characteristics

Population	Pooled Replication
Trial	CAMP, PACT, IMPACT
Network	CAMP, CARE, ACRN
N	311
Race(s)	African American, Caucasian, Hispanic, Other
Age (SD)	15 (12)
Sex (male)	175 (56%)
ICS Treatment(s)	budesonide, fluticasone, salmeterol/fluticasone combination [*]
not ICS Treatment(s)	placebo, montelukast [*]
Duration (weeks)	207
N ICS Group	168
N not ICS Group	143
FEVPPB (SD)	92 (13)
LNPC20B (SD)	0.04 (1.1)
BDRB (SD)	10 (8.9)
SYMB (SD)	0.48 (0.41)

Definitions: CAMP = Childhood Asthma Management Program; PACT = Pediatric Asthma
Controller Trial; IMPACT = The Improving Asthma Control Trial; SOCS = Salmeterol Or
Corticosteroids Study; CARE = Childhood Asthma Research and Education; ACRN = Asthma
Clinical Research Network; N ICS Group = Subjects treated with ICS or a combination therapy
including ICS; N not ICS Group = Subjects treated with placebo, or a non-ICS therapy; SD =
standard deviation; FEVPPB = FEV1 percent predicted at baseline; LNPC20B = natural log

PC20 at baseline; BDRB = bronchodilator percent change at baseline; 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 ICS and oral steroids at the end of 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	* 0.006	* 0.019	* 0.001	* <.001	* <.001
0.733	* <.001	* 0.013	* 0.046	* 0.002	* <.001	
0.749	* <.001	* 0.003	* 0.015	* <.001		* <.001
0.742	* <.001	* 0.009	* 0.025		* <.001	* <.001
0.716	* 0.001	0.050		* 0.013	* <.001	* <.001
0.730	* <.001		0.073	* 0.004	* <.001	* <.001
0.745		* 0.005	* 0.017	* <.001	* <.001	* <.001
0.733	* <.001	* 0.010	* 0.042	* 0.001		
0.729	* <.001	* 0.016	* 0.047		* <.001	
0.747	* <.001	* 0.005	* 0.013			* <.001
0.706	* 0.004	0.074		* 0.027	* <.001	
0.723	* <.001	* 0.028		* 0.004		* <.001
0.687	* 0.007	0.202			* <.001	* 0.003
0.714	* 0.004		0.147	* 0.014	* <.001	
0.730	* <.001		0.053	* 0.003		* <.001
0.725	* <.001		0.084		* <.001	* <.001
0.693	* 0.012			0.083	* <.001	* 0.004
0.730		* 0.013	* 0.040	* <.001	* <.001	
0.747		* 0.004	* 0.010	* <.001		* <.001
0.735		* 0.008	* 0.016		* <.001	* <.001
0.701		0.096		* 0.021	* <.001	* 0.002

Composite AUC	Symptoms	Lung function	Airway respon- siveness	Bronchodilator response	ED visits/ Hospital- izations	Oral steroid bursts
0.726			0.063	* 0.002	* <.001	* <.001
0.726	* <.001	* 0.014	* 0.044			
0.702	* 0.005	0.084		* 0.028		
0.673	* 0.010	0.305			* 0.001	
0.691	* 0.001	0.158				* 0.002
0.712	* 0.003		0.140	* 0.011		
0.710	* 0.001		0.155		* <.001	
0.726	* <.001		0.065			* <.001
0.675	* 0.045			0.198	* 0.002	
0.696	* 0.008			0.059		* 0.004
0.656	0.090				* 0.003	* 0.032
0.720		* 0.026	0.075	* 0.001		
0.724		* 0.012	* 0.018		* <.001	
0.740		* 0.003	* 0.003			* <.001
0.686		0.181		* 0.035	* 0.001	
0.705		0.073		* 0.005		* 0.002
0.680		0.219			* <.001	* 0.001
0.709			0.153	* 0.006	* <.001	
0.728			* 0.049	* 0.001		* <.001
0.712			0.087		* <.001	* <.001
0.674				0.174	* 0.001	* 0.015
0.657	* 0.027	0.469				

Composite AUC	Symptoms	Lung function	Airway respon- siveness	Bronchodilator response	ED visits/ Hospital- izations	Oral steroid bursts
0.699	* 0.002		0.233			
0.667	0.069			0.267		
0.643	0.126				* 0.016	
0.657	* 0.047					* 0.038
0.719		* 0.013	* 0.008			
0.673		0.280		* 0.049		
0.674		0.214			* <.001	
0.686		0.140				* <.001
0.695			0.301	* 0.015		
0.695			0.206		* <.001	
0.714			* 0.048			* <.001
0.657				0.374	* 0.009	
0.678				0.084		* 0.015
0.611					0.112	0.416
Phenotype AUC	0.624	0.654	0.680	0.652	0.584	0.609

Definitions: Composite AUC (left column) = value of the composite phenotype. Table entry = single sided pvalue for composite AUC > clinical phenotype AUC; Blank entry = the clinical phenotype is assumed to be missing; Phenotype AUC (bottom row) = the value of individual clinical phenotype AUC; p-values less than or equal to .05 are flagged with a *;