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GENETICS

Association of CHI3L1 in African-Americans with Prior History of Asthma Exacerbations and Stress

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Rationale. Asthma exacerbations are influenced by multiple factors including environmental exposures, psychosocial interactions, and genetic variations. *Aim.* To better understand the correlation between clinical, physiologic, genetic, and psychological dimensions in asthma phenotypes and exacerbations. *Methods.* Supervised cluster analysis of a previously conducted clinical trial of asthma was used to identify subpopulations with differing exacerbation rates in an African-American study population ($n = 475$). The clusters were characterized by their clinical characteristics and genetic variations. The genetic analysis ($n = 322$) compared subgroups across 40 different polymorphisms of 10 genes associated with asthma exacerbations. *Results.* Four clusters were identified with varying annualized rates of exacerbations. Cluster 1 ($n = 272$) was represented by subjects with a mean age of 25 years and 52% females. In contrast, cluster 4, most divergent from cluster 1, was represented by subjects with the highest rate of asthma exacerbations (1.18 events per year), was mostly female (>80%), with a mean body mass index of 34, and was distinguished by the report of stress and emotions as the cause for prior exacerbations. Lower lung function and increased rescue medication use was also reported in cluster 4. Additionally, genetic analysis revealed a significant difference in distribution of genotypes among the four clusters for rs4950928, a single nucleotide polymorphism (SNP) located in the promoter region of the *CHI3L1*, the chitinase 3-like 1 gene encoding YKL-40. *Conclusions.* African-Americans who reported stress and emotions as a primary historical cause of exacerbations had the highest annualized rate of exacerbation. Further, a significant correlation with the genotypes in *CHI3L1*/YKL-40 was observed in the context of stress and asthma severity.

Keywords African-Americans, asthma exacerbations, cluster analysis, stress

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INTRODUCTION

Asthma exacerbations are influenced by multiple and diverse interacting factors, which include environmental and psychosocial exposures, complex traits, and genetic variations. African-Americans bear a disproportionate burden of asthma, have a greater frequency of urgent care visits, more hospitalizations than Caucasians, and have a higher death rate due to asthma (1–3). The reasons for these disparities are not yet completely understood.

One key and common psychosocial exposure in patients with asthma is stress. Stress has been associated with the activation of the sympathetic and adrenomedullary system and the hypothalamic–pituitary–adrenocortical (HPA) axis. Chronic stress is generally thought of as the chronic load of day-to-day stressors. Researchers have long been recognized that stress may protect the body, but when more chronic, can also damage it (4). Chronic psychological stress may cause a counter-regulatory response in stimulated lymphocytes with consequent downregulation of the expression and/or function of glucocorticoid receptors leading to functional steroid resistance (5, 6). There is also evidence that psychosocial stress directly provokes proinflammatory cytokine production in the absence of infection or injury. For example, norepinephrine-dependent adrenergic stimulation can activate transcription factor nuclear factor-kappa B (NF-κB), an intracellular signaling molecule that activates gene expression of several inflammatory mediators. Activation of NF-κB represents a downstream effector for the neuroendocrine response to stressful psychosocial events and links the changes in the activity of the neuroendocrine axis to the cellular response (7).

To understand the role of interacting factors in asthma, including stress, data from clinical studies can be examined using cluster analysis, where similar or dissimilar patterns within a dataset are partitioned based on cluster algorithms, and the "clustering" can be accomplished using an unsupervised or supervised method. Unsupervised cluster algorithms do not use predefined class labels or examples that would indicate grouping properties in the dataset (8). In contrast, supervised clustering uses prior knowledge and constraints to facilitate and guide the grouping of the data in a relevant and useful pattern. We examined an asthma clinical study dataset with the specific objective of identifying clusters of data associated with varying exacerbation rates in an African-American study population with asthma. The utility of supervised cluster analysis in characterizing asthma exacerbations was recently reported in patients seen in the primary care (9). We used a similar approach here to maximize the difference of variables associated with the exacerbations. The resultant grouping allowed for classification of data subsets with diverse baseline characteristics (e.g., triggers associated with exacerbations, peak flow, and

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medical history) and identified patterns that were associated with varying rates of exacerbation. In addition, 40 different polymorphisms of 10 genes reported to be associated with asthma were used to explore any associations between the polymorphisms and the identified clusters.

Material and Methods

Patient Population

This cluster analysis represents a post hoc analysis of a clinical trial dataset (SFA103153, NCT 00102765) that examined the exacerbation rate in a population of African-Americans with asthma. The study randomized 475 African-American subjects with asthma to a double-blind treatment (fluticasone propionate/salmeterol 100/50 mcg, 239 subjects; fluticasone propionate 100 mcg, 236 subjects). The primary outcome of the study was the annual exacerbation rate per subject. Exacerbation was defined as worsening of asthma that required any of the following: oral steroids, hospitalization, unscheduled urgent care, $\geq$30% decrease in forced expiratory volume in 1 second (FEV_1) from baseline, or $\geq$30% decrease in morning peak expiratory flow (AM PEF) on any two consecutive days. A full report of the clinical trial is reported elsewhere (10). The study protocol and informed consent forms were reviewed and approved by the institutional review board (IRB) for each participating site. Written informed consent was obtained from all subjects prior to any study-specific procedures. For subjects <18 years of age, written informed consent was obtained from a parent and/or legal guardian.

Genetic Markers

The genetic analysis ($n = 322$) compared the subgroups for variations across 40 different polymorphisms of 10 genes associated with asthma exacerbations (Table 1). The following single nucleotide polymorphisms (SNPs) were evaluated: *ADRB2* (13 SNPs), *ALOX5* (2 SNPs), *ABCC1* (1 SNP), *CH13L1* (2 SNPs), *FCER2* (4 SNPs), *IL4* (3 SNPs), *IL4R* (9 SNPs), *IL13* (3 SNPs), *LTA4H* (1 SNP), and *LTC4S* (1 SNP). Germline DNA was extracted from peripheral blood (QiAmp DNA Blood Kit; Qiagen, Valencia, CA, USA). Genotyping for SPN analysis was carried with KASPar assays by KBiosciences (Hertforshire, UK). In addition, genotypes from selected SNPs were assayed with the Illumina HumanOmni 1-Quad platform (Expression Analysis, Durham, NC).

Statistical Methods

Descriptive statistics were used to present demographic and medical history data (e.g., duration of asthma, prior asthma medication, cause of prior asthma exacerbations, etc.). Features that were relevant to the asthma population (e.g., body mass index (BMI), duration of asthma, relatives with asthma, baseline lung function parameters, baseline asthma symptoms and albuterol use, and prior history of exacerbations, etc.) were identified and included in the cluster selection process.

A recursive partitioning approach was used to identify from the overall population clusters that maximized the difference among subjects with exacerbations. The minimum size of each cluster or terminal node was set to equal at least 10% of the data, to avoid very small clusters. The binary tree was pruned to minimize the cross-validation error (11, 12). Prior to this supervised cluster analysis, correlation among baseline covariates was examined using Pearson's correlation coefficients, where redundant variables were removed from the analysis. Variable importance was determined using a modified random forest technique, where important variables were selected.

Univariate differences of the baseline characteristics including the SNP variables were also determined among the identified clusters. A dominant model was used to test for differences on genotype distribution between cluster 1, cluster 2, and cluster 3 versus cluster 4 (reference group). All tests were two-sided and p-values $\leq$.05 were considered statistically significant. Rate ratios and 95% confidence intervals calculated using linear contrast were used to estimate the relative exacerbation rates for each cluster using cluster 1 as the reference group. The analyses were performed using R version 2.12.2 (The R Foundation for Statistical Computing) and SAS version 9.2 (SAS Institute, Inc, Cary, NC, USA).

Results

This analysis identified a group of baseline (prior to study treatment) characteristics that were associated with individuals who had a high rate of exacerbations during the 52-week double-blind treatment period. The demographic characteristics used to describe each cluster are listed in Table 1. Of the 475 subjects included in the study

Table 1.—Genetic markers, phenotypical, and environmental characteristics among African-American subjects with asthma exacerbations.

Genetic		Phenotypic	Environmental
Family history of asthma		Age	Stress
Family history of allergy, or eczema		Body mass index	Smoking
Relative with rhinitis		Duration of asthma	Allergens
ADRB2 (13 SNPs)	IL4 (3 SNPs)	Gender	Viral infection
ALOX5 (2 SNPs)	IL4R (9 SNPs)	FEV_1/AM PEF	Cold air
ABCC1 (2 SNPs)	IL13 (3 SNPs)	Asthma symptoms	Air pollution
CHI3L1 (2 SNPs)	LTA4H (1 SNP)	Medical history	
FCER2 (4 SNPs)	LTC4S (1 SNP)	Rescue medication usage	

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population, a subset of 322 subjects (68%) contributed DNA for the genetic analysis. There were no obvious differences between these two populations.

The first characteristic used to dichotomize and maximally differentiate the clusters was baseline AM PEF (see Figure 1). The recursive partitioning identified an AM PEF = 272 L/min as the cutoff (<272 vs. ≥272) that resulted in the lowest *p*-value associated with the exacerbation rate ratio of the two resultant clusters. When the analysis process was repeated, the node including subjects with an AM PEF ≥ 272 L/min was further differentiated by age (≤42.5 vs. >42.5 years). With respect to subjects with lower baseline AM PEF (<272 L/min), they were further differentiated according to whether they reported stress/emotions as a potential cause of past exacerbations. In summary, cluster 1 was defined as subjects with a baseline AM PEF ≥ 272 L/min and age ≤ 42.5 years, cluster 2 as subjects with a baseline AM PEF ≥ 272 L/min and age >42.5 years, cluster 3 as subjects with a baseline AM PEF < 272 L/min and did not report stress/emotions as a potential cause of past exacerbations, and cluster 4 as subjects with a baseline AM PEF < 272 L/min as well as reporting stress/emotions as a potential cause of past exacerbations (Figure 1). A summary of the most distinguishing features of each cluster is as follows.

Cluster 1

The largest of the four clusters, this cluster included 272 subjects with a mean age of 25 years and a balanced representation of males (47.8%) and females (52.2%). Subjects were also overweight (mean BMI = 27.63), with approximately 40% considered obese (BMI > 30). Relative to the other clusters, subjects had the shortest duration of disease (14 years), the highest baseline AM PEF (mean AM PEF = 353.4 L/min), the fewest number of albuterol puffs used per day ($n = 1.71$), and the lowest baseline 24-h asthma symptom score. More than two-thirds of subjects reported allergy, exercise, respiratory infection, and tobacco smoke as potential causes of their prior exacerbations (Table 2). Along with subjects in cluster 4, those in cluster 1 were more likely to report having a relative with eczema, food allergy, pet allergy, and rhinitis compared with subjects in clusters 2 and 3. The most commonly reported comorbidities were nervous system, skin, and subcutaneous tissue disorders.

Cluster 2

The second largest of the four clusters ($n = 91$), subjects in this cluster were also the oldest with a mean age of 47 years. In this cluster, more females (65.9%) were represented, and the mean BMI and the proportion considered obese were much higher compared with cluster 1 (Table 1). The mean duration of disease was longest in this cluster compared with others. Allergy, tobacco smoke, and withholding or reducing asthma medication were the reasons reported as potential causes of their prior exacerbations in more than two-thirds of the subjects (Table 2). Approximately 50% of subjects were reported having a relative with asthma or rhinitis, while the most frequently reported comorbidities included musculoskeletal and connective tissue, cardiac, reproductive system, and gastrointestinal disorders.

Cluster 3

This cluster included 57 subjects with a mean age of 34 years. Approximately 80% were female and nearly 40% had a BMI > 30. The mean duration of disease was 16 years and the mean FEV_1 at baseline was 74.2% of predicted. Subjects reported the highest baseline albuterol use (2.42 puffs per day) compared with the other clusters. Allergy and tobacco smoke were reported by more than two-thirds of subjects as potential causes for their prior exacerbations. Of note, no subjects reported stress/emotions as a potential cause of prior exacerbations as part of their baseline characteristics. Nearly, one-third of subjects in cluster 3 were reported having a history of musculoskeletal and connective tissue disorders and skin and subcutaneous tissue disorders.

Cluster 4

This cluster included 55 subjects with a mean age of 36 years and predominantly females (82%). The mean duration of disease was 20 years and more than half had a BMI consistent with obesity (mean BMI 34). Subjects had a mean predictive FEV_1 at baseline (76% predicted), the lowest baseline AM PEF (237.3 L/min), and reported requiring 2.23 puffs per day of albuterol (Table 2). All subjects (100%) reported at baseline stress/emotions to be associated with their prior exacerbations as well as an increased prevalence of psychological and nutritional issues relative to the other clusters. A higher proportion of relatives with atopy and environmental triggers associated with history of exacerbations was also reported in this cluster compared with the other clusters.

Genetic Assessment

Following the identification of the four clusters, we examined the variability of 40 SNPs in 10 genes. The analysis showed only one SNP (rs4950928) in the chitinase 3-like 1 (*CHI3L1*) gene to be significantly different across the four subgroups ($p = .0255$). Cluster 4 was used as the reference group using a dominant model. When cluster 3 was compared to cluster 4, the data showed a genotype versus stress association ($p = .023$) (see Table 3). The rs4950928 SNP (–131C→G) is a SNP located in the promoter region of *CHI3L1* gene encoding YKL-40.

Discussion

The majority of the morbidity and mortality associated with asthma is linked with exacerbations creating a need for a more precise characterization of patients at risk for asthma exacerbations. The overall objective of this cluster analysis was to identify specific phenotypes or endotypes linked to exacerbations based on baseline medical history, including the cause or triggers of previous exacerbations, personal characteristics, and genetic markers in African-

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TABLE 2.—Description of baseline characteristics by clusters.

Characteristic	Cluster 1 ($n = 272$)	Cluster 2 ($n = 91$)	Cluster 3 ($n = 57$)	Cluster 4 ($n = 55$)	*P-value
Demographics					
Mean age (years)	25.0 (16.0–34.0)	47.0 (44.0–52.0)	34.0 (17.0–46.0)	36.0 (24.0–48.0)	<.0001
Male	130 (47.8%)	31 (34.1%)	11 (19.3%)	10 (18.2%)	<.0001
Body mass index (BMI)	27.63 (22.95–33.60)	31.17 (25.96–37.41)	27.70 (23.47–34.43)	33.99 (24.71–41.95)	.001
Rhinitis	249 (91.5%)	77 (84.6%)	48 (84.2%)	50 (90.9%)	.1576
Double-blind compliance	98.39 (92.75–100.4)	98.39 (95.10–100.6)	98.39 (93.07–100.8)	97.55 (92.03–99.45)	.231
Asthma exacerbation rate (events per year)	0.28	0.63	0.72	1.8	–
Duration of asthma	14.00 (8.00–22.00)	21.00 (10.00–42.00)	16.00 (12.00–30.00)	20.00 (10.00–36.00)	<.0001
Patient reported triggers associated to prior exacerbation					
Air pollution	137 (50.4%)	49 (53.8%)	34 (59.6%)	36 (65.5%)	.1661
Allergy	216 (79.4%)	68 (74.7%)	39 (68.4%)	46 (83.6%)	.1813
Cold air/cold weather	169 (62.1%)	57 (62.6%)	31 (54.4%)	39 (70.9%)	.3527
Exercise	184 (67.6%)	52 (57.1%)	35 (61.4%)	33 (60.0%)	.2641
Respiratory infection	189 (69.5%)	54 (59.3%)	33 (57.9%)	46 (83.6%)	.0067
Stress/emotions	109 (40.1%)	40 (44.0%)	0 (0.0%)	55 (100.0%)	<.0001
Tobacco smoke	181 (66.5%)	60 (65.9%)	41 (71.9%)	46 (83.6%)	.0748
Withholding or reducing asthma medication	167 (61.4%)	60 (65.9%)	34 (59.6%)	40 (72.7%)	.3722
Environmental smoke	67 (24.6%)	19 (20.9%)	11 (19.3%)	18 (32.7%)	.3207
Exposure to pets	83 (30.5%)	27 (29.7%)	9 (15.8%)	13 (23.6%)	.1255
Family history of allergic conditions					
Asthma	182 (66.9%)	46 (50.5%)	37 (64.9%)	41 (74.5%)	.0126
Rhinitis	178 (65.4%)	45 (49.5%)	29 (50.9%)	33 (60.0%)	.0228
Eczema	54 (19.9%)	12 (13.2%)	5 (8.8%)	13 (23.6%)	.0861
Food allergy	47 (17.3%)	12 (13.2%)	2 (3.5%)	13 (23.6%)	.0184
Pet allergy	64 (23.5%)	12 (13.2%)	2 (3.5%)	11 (20.0%)	.0021
Baseline markers of lung function and asthma control					
FEV_1 at baseline (L)	2.58 (2.27–2.96)	2.10 (1.86–2.53)	1.95 (1.70–2.29)	2.07 (1.81–2.35)	<.0001
FEV_1 at baseline (percent predicted)	81.78 (76.00–89.09)	80.50 (73.55–88.32)	74.18 (68.16–80.77)	75.91 (68.04–83.67)	<.0001
Baseline AM PEF (L/min)	353.37 (317.4–398.4)	324.71 (300.4–378.6)	243.14 (208.3–259.3)	237.29 (203.1–258.4)	<.0001
Baseline albuterol use (number of puffs)	1.71 (1.14–3.00)	1.86 (0.57–3.14)	2.43 (2.00–3.43)	2.23 (1.14–4.00)	.0029
Baseline 24-h asthma symptom score	2.00 (1.29–2.71)	2.14 (1.57–2.86)	2.29 (1.57–3.00)	2.14 (1.14–2.86)	.166
Baseline medical history and clinical conditions					
Blood and lymphatic system disorders	12 (4.4%)	7 (7.7%)	2 (3.5%)	8 (14.5%)	.026
Cardiac disorders	25 (9.2%)	32 (35.2%)	8 (14.0%)	15 (27.3%)	<.0001
Ear and labyrinth disorders	18 (6.6%)	6 (6.6%)	2 (3.5%)	3 (5.5%)	.8318
Endocrine disorders	14 (5.1%)	11 (12.1%)	3 (5.3%)	5 (9.1%)	.1258
Eye disorders	27 (9.9%)	12 (13.2%)	2 (3.5%)	7 (12.7%)	.2501
Gastrointestinal disorders	44 (16.2%)	28 (30.8%)	11 (19.3%)	16 (29.1%)	.0099
Immune system disorders	30 (11.0%)	14 (15.4%)	7 (12.3%)	8 (14.5%)	.6943
Metabolism and nutrition disorders	11 (4.0%)	12 (13.2%)	4 (7.0%)	10 (18.2%)	.0006
Musculoskeletal and connective tissue disorders	59 (21.7%)	38 (41.8%)	18 (31.6%)	17 (30.9%)	.0022
Nervous system	79 (29.0%)	21 (23.1%)	14 (24.6%)	15 (27.3%)	.6939
Psychiatric disorders	23 (8.5%)	4 (4.4%)	2 (3.5%)	5 (9.1%)	.3639
Reproductive system and breast disorders	44 (16.2%)	30 (33.0%)	10 (17.5%)	21 (38.2%)	.0001
Skin and subcutaneous tissue disorders	72 (26.5%)	18 (19.8%)	18 (31.6%)	16 (29.1%)	.3847
Vascular disorders	6 (2.2%)	7 (7.7%)	7 (12.3%)	2 (3.6%)	.0042
Infections	10 (3.7%)	6 (6.6%)	4 (7.0%)	3 (5.5%)	.5646

Note: *P-values represent comparison across the different clusters.

TABLE 3.—Description of SNP (rs4950928) in *CHI3L1* gene among African-American subjects with asthma exacerbations.

SNP	Level	Overall	Cluster 1	Cluster 2	Cluster 3	Cluster 4
rs4950928	C,C	166 (51.6%)	90 (49.5%)	31 (48.4%)	27 (69.2%)	18 (48.6%)
	C,G +G,G	156 (48.5%)	92 (50.5%)	33 (51.6%)	12 (30.8%)	19 (51.4%)
[a]*P-value			0.277	0.307	0.023	ref

Note: [a]Comparison of genotype distribution between cluster 1, cluster 2, cluster 3 versus cluster4 (reference group)

Americans. Stress and related psychological correlates (e.g., depression) may influence asthma morbidity indirectly by affecting how patients perceive and manage their asthma. Patients with poor psychological adjustment may have difficulty in accurately appraising asthma symptoms and detecting deterioration (13). This is particularly important in the more severe asthma population or those with more frequent exacerbations. Moreover, an individual's perceptions, expectations, and interpretations of health may be altered by stress, thus affecting the sensitivity of

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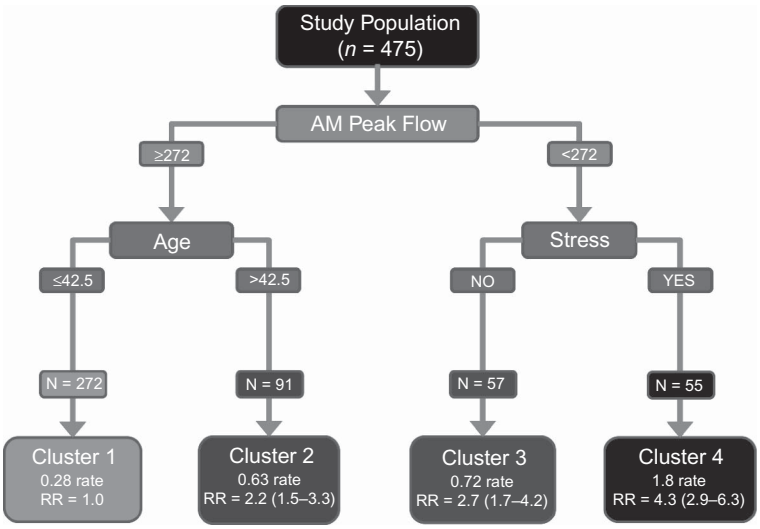


FIGURE 1.—Classification tree for African-American subjects with asthma exacerbations, clustered by baseline characteristics. The percent rate of exacerbations for each cluster is presented in the rectangles. Rate ratio (RR) is in relation to cluster 1 (reference).

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other subjective outcome measures (i.e., asthma-related quality of life). Additionally, stress may affect adherence to medications and self-management strategies, resulting in worsened asthma control (13, 14).

One hypothesis linking stress, neuroendocrine disruption (i.e., HPA axis), and immune function considers a glucocorticoid resistance model. We found that African-Americans who reported stress and emotions as a primary historical cause of exacerbations had the highest annualized rate of exacerbation during a randomized clinical trial. These data support the concept that stress alters airway inflammation through neuronal and hormonal modification of immune stasis. Results from this analysis further support this association as we identified a significant correlation with genotypes in *CHI3L1*, a gene which has been associated with asthma severity (15, 16).

The examination of the 40 SNPs in 10 genes revealed that only one SNP in the *CHI3L1* promoter region was statistically significantly represented across the four clusters. *CHI3L1* encodes a glycoprotein, YKL-40, which is secreted by synovial fibroblasts, macrophages, neutrophils, endothelial-vascular smooth muscle cells, granulocytes, and chondrocytes (17). YKL-40 expression is under the control of NF-κB (18). It is regulated at the acute protein phase, and its levels are significantly elevated in conditions such as rheumatic diseases (19) and peripheral artery disease (20). Two functional variants of the promoter region, rs4950928 (aka *CHI3L1*-1) and rs10399931 (aka *CHI3L1*-2), have been reported to be associated with schizophrenia and sarcoidosis (21, 22). One function of *CHI3L1* is to act as a cellular survival factor in response to an adverse environment. Various types of physiologic stress, such as inflammation, hypoxia, and nutrient deprivation, have been shown to induce high expression of *CHI3L1* (17). A genome-wide association study of serum YKL-40 levels in 632 Hutterites identified a polymorphism (–131C→G; rs4950928) in the promoter of the *CHI3L1* gene as significantly associated with elevated YKL-40 levels and the asthma-related traits: asthma ($p = .047$), bronchial hyperreactivity (BHR) ($p = .002$), and lung function ($p = .046$–$.002$) (16). Chupp et al. (15) reported serum YKL-40 levels in three cohorts of patients with asthma. In all the three cohorts, the serum YKL-40 levels correlated with the asthma severity and lower FEV_1. Patients with elevated levels of YKL-40 had significantly more frequent rescue medication use, oral corticosteroid use, and a greater rate of hospitalization than those with lower levels. In one of the cohorts, elevated levels of YKL-40 were correlated ($p = .003$) with airway remodeling (measured as the thickness of the subepithelial basement membrane). YKL-40 showed to increase bronchial smooth muscle proliferation and migration *in vitro*, and epithelial cell expression was correlated with bronchial smooth muscle mass in asthma (23). In our analysis, the same SNP (rs4950928) was disproportionately represented among subjects in cluster 4 compared with those in the other clusters. Specifically, when cluster 3 was compared to cluster 4, the data suggested a genotype versus stress association ($p = .023$). Of interest, all the subjects in cluster 4 reported stress associated with prior exacerbations. In contrast, none of the subjects in cluster 3 reported stress associated with their exacerbations.

Previous reports have highlighted the association between psychological disorders and asthma morbidity, although the exact mechanism is still yet to be defined. Studies have linked psychological morbidity to poor control of asthma (14, 24). Consistent with these findings, our analysis differentiated cluster 4 from cluster 3 based on the self-report of stress/emotions as a potential cause of prior exacerbations. One explanation for this association is that patients predisposed to stress may have been more likely to have poorly controlled disease and, therefore, at greater risk of experiencing an exacerbation. A possible mechanism driving this association could be the diminished sensitivity to the anti-inflammatory effects of glucocorticosteroids. This cellular response may be mediated through downregulation of the glucocorticoid receptor. Polymorphisms in the

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gene coding for factors involved in the feedback mechanisms in the glucocorticoid response to stress such as corticotrophin-releasing hormone (*CRHR1* and *CRHR2* genes), tumor necrosis factor (TNF), and other cytokine loci have been reported (6). Variants of the glucocorticoid receptor gene may contribute to interindividual variability in HPA axis activity and glucocorticoid sensitivity in response to stress. In this study, we did not evaluate the genetic contribution of these genes, but a recently published study (25) explored the potential *T* gene–NR3C1 (glucocorticoid receptor) interactions. Although not conclusive, the results indicate that the *T* gene is coexpressed with three other genes (NRIP1, FOXA2, and TTPA) that directly interact or are predicted to interact with NR3C1. *T* gene variants were associated with lung function in response to inhaled corticosteroids (25).

We identified in this population of moderate asthma in four clusters using recursive partitioning that maximally differentiated baseline characteristics and rate of exacerbation. Subjects in cluster 4 had a mean age of 36 years with long duration of asthma disease (20 years) as well as the highest rate of exacerbations during the 12-month trial. Not surprisingly, disease severity was also associated with subjects in cluster 4. For example, subjects had a low-baseline FEV_1 (76% predicted) and the lowest baseline AM PEF (237.3 L/min) and reported requiring more than two puffs per day of rescue medication. With respect to BMI, they had the highest BMI relative to the other clusters and also had the highest proportion of relatives with atopy. Additionally, all subjects in this subgroup had reported stress and/or emotions as a potential cause for their prior exacerbations.

Lower baseline pulmonary function was associated with a higher exacerbation rate and greater frequency of rescue medication use (clusters 3 and 4). This is consistent with the previous findings reported by Moore et al., where the combination of prebronchodilator and postbronchodilator measurements of FEV_1 was best differentiated in patients of the mildest and the most severe groups (26).

Some limitations should be considered when interpreting these findings. The current study included only moderate asthma and did not include mild or severe disease. The study was limited to African-Americans of which a subset (68%) submitted samples for genetic testing and as such the findings may not be generalizable to other populations. In addition, bias related to the quality of respondent recall must be considered when evaluating these results, since the information regarding exacerbations and triggers was self-reported through patient's reported history and patient's completed questionnaires.

Conclusions

African-Americans with asthma, who participated in an asthma clinical trial and reported stress and emotions as a primary cause of prior exacerbations, also had the highest annualized rate of exacerbations. A significant correlation with *CHI3L1*, a gene that encodes YKL-40, was observed in subjects when compared with history of stress. This further supports the hypothesis that YKL-40 may be a biomarker that correlates with asthma severity or insensitivity to steroids.

Declaration of Interest

All authors were employees of GlaxoSmithKline at the time of the study. Dr. Anderson is currently an employee of the University of North Carolina.

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