

Genetic variation in chitinase 3-like 1 (*CHI3L1*) contributes to asthma severity and airway expression of YKL-40

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Background: Single nucleotide polymorphisms (SNPs) in the chitinase 3-like 1 (*CHI3L1*) promoter, the gene encoding YKL-40, are associated with circulating YKL-40 levels and asthma prevalence. However, the effects of gene polymorphisms on asthma severity and airway expression of YKL-40 have not been examined.

Objective: We sought to determine the effect of genetic variation in *CHI3L1* on asthma severity and YKL-40 expression in subjects from the Yale Center for Asthma and Airways Disease and the Severe Asthma Research Program.

Methods: SNPs spanning the *CHI3L1* gene were genotyped in 259 Yale Center for Asthma and Airways Disease and 919 Severe Asthma Research Program subjects. Association and haplotype analyses were conducted to identify effects on airflow obstruction, YKL-40 levels, and asthma severity.

Results: Fifteen SNPs in *CHI3L1* were associated with FEV₁, serum YKL-40 levels, or both. rs12141494 (intron 6) was the only SNP in subjects of European ancestry in both cohorts that was associated with serum YKL-40 levels and postbronchodilator FEV₁. Conditional analysis demonstrated that the effect on lung function was independent of the promoter SNP rs4950928, and haplotype analysis demonstrated that G alleles at rs12141494 and rs4950928 are associated with lower YKL-40 expression and higher FEV₁ percent predicted values. In asthmatic subjects the risk allele A at rs12141494 was

associated with severe asthma and higher YKL-40 expression in the airway ($P \leq .05$).

Conclusion: In contrast to the promoter SNP rs4950928, the intronic SNP rs12141494 in *CHI3L1* is associated with asthma severity, lung function, and YKL-40 expression in the blood and airway. These data suggest that SNP rs12141494 modulates YKL-40 expression in the airway and contributes to airway remodeling and asthma severity. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: Asthma, asthma severity, severe asthma, genetic association studies, genetic variation, *CHI3L1* protein, human, YKL-40 protein, human, airway remodeling

YKL-40, a chitinase-like protein, belongs to the chitinase and chitinase-like family of proteins. These evolutionarily conserved molecules interact with chitin, the second most abundant polysaccharide on earth. Human and mechanistic studies have demonstrated that chitinase 3-like 1 (*CHI3L1*)/YKL-40 plays a role in the pathobiology of asthma by modulating innate and adaptive immune and remodeling responses.¹ In the first human asthma studies we demonstrated that increased serum levels of YKL-40 correlate with asthma severity, airway remodeling, and increased thickness of the subepithelial basement membrane in subjects with asthma.² This work led to mechanistic studies demonstrating that bronchial epithelial cells exposed to YKL-40 generate higher levels of IL-8 and stimulate smooth muscle proliferation *in vitro*.³ An additional mechanism of YKL-40–increased bronchial smooth muscle proliferation involves the protease-activated receptor 2.⁴ Taken together, these studies established an important role for YKL-40 as a molecule uniquely juxtaposed between environmental exposure, inflammation, and the development of airway remodeling and severe asthma.

Genetic studies have also demonstrated that variation in *CHI3L1* contributes to the pathogenesis of asthma. A genome-wide association study of serum YKL-40 levels in a founder population of European descent by Ober et al⁵ identified an association between the rs4950928 promoter single nucleotide polymorphism (SNP) and circulating YKL-40 levels. This SNP was also associated with the risk of asthma and FEV₁ in 3 populations of European ancestry (EA) with mild asthma. Subsequent reports have shown an association with rs4950928 and asthma,⁶ although the risk allele was opposite of that reported by Ober et al.⁵ Additional publications have been conflicting with respect to the effect of genetic variation in the *CHI3L1* gene and asthma.^{7,8} To date, the effect of genetic variation in *CHI3L1* on asthma severity has not been examined.

To determine the effects of genetic variation in the *CHI3L1* gene on YKL-40 expression in the airway, airway remodeling, and asthma severity, we examined 2 cohorts of subjects with

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Abbreviations used

AA:	African Ancestry
ATS:	American Thoracic Society
CHI3L1:	Chitinase 3-like 1
EA:	European Ancestry
LD:	Linkage disequilibrium
SARP:	Severe Asthma Research Program
SNP:	Single nucleotide polymorphism
YCAAD:	Yale Center for Asthma and Airways Disease

asthma from the Yale Center for Asthma and Airways Disease (YCAAD) and the Severe Asthma Research Program (SARP). We hypothesized that genetic variation in *CHI3L1* is associated with persistent airflow obstruction, serum YKL-40 levels, asthma severity, and airway expression of YKL-40. To examine this hypothesis, we characterized the effect of SNPs in the *CHI3L1* gene on severe asthma traits, determined the interaction between SNPs using haplotype analysis, and correlated identified SNPs with airway expression of YKL-40. Ultimately, we identified a novel polymorphism in *CHI3L1* that is likely to contribute to airway remodeling and asthma severity through increased production of YKL-40 in the airway.

METHODS**Populations**

YCAAD. Study participants in the YCAAD cohort based in New Haven, Connecticut, underwent an extensive phenotypic characterization after institutional review board approval. Inclusion and exclusion criteria and the study protocol have been described previously.² Severe asthma was defined as outlined by the SARP clustering algorithm and was modified as follows: *severe asthma* included subjects with a baseline FEV₁ of less than 68% of predicted value (SARP clusters 4 and 5), whereas *nonsevere asthma* was defined by the presence of a baseline FEV₁ equal to or greater than 68% of predicted value (SARP clusters 1-3).⁹ For specific details related to definitions and specific study measurements, see the [Methods](#) section in this article's Online Repository at www.jacionline.org. A total of 259 subjects from this cohort were analyzed.

SARP. Subjects in the SARP cohort completed study visits by using established standard operating procedures, as previously described.⁹ The definition for severe asthma outlined above was also used in this cohort. Institutional review board approval at the SARP institutions was obtained for these studies. The characteristics of these subjects have been reported in previous publications.^{9,10} A total of 919 subjects from this cohort were analyzed.

Sputum induction

Subjects in the YCAAD cohort underwent sputum induction with inhaled hypertonic saline. Mucus plugs were removed by using a dissecting microscope and washed to remove squamous cell contamination. The cellular and aqueous compartments were separated, and cell counts, cell differentials, and viability were determined (Diff-Quik and trypan blue exclusion). Aliquots of supernatants were stored until processing. Samples with greater than 20% squamous cells were considered contaminated and were not processed further.

Measurement of serum and sputum YKL-40 levels

YKL-40 levels were measured in duplicate in serum and sputum specimens by using a commercially available ELISA kit for YKL-40 (Quidel, San Diego, Calif). The mean value of the 2 duplicates was used in the statistical analyses. Duplicate samples with coefficients of variation of greater than 20% were

reassayed. The mean coefficient of variation for all samples was 10%. The limit of detection is 5.4 ng/mL. Values of less than the limit of detection were defaulted to 0 ng/mL.

SNP genotyping

DNA was extracted from peripheral blood leukocytes by using standard protocols. The Sequenom MassARRAY system (Sequenom, San Diego, Calif) genotyping platform was used. Genotyping was performed according to the manufacturer's iPLEX application guide at the Center for Human Genomics at Wake Forest University Health Sciences and the Yale Keck DNA sequencing facility for SARP and YCAAD samples, respectively.¹¹ Resequencing of the *CHI3L1* gene was conducted on a subset of 6 subjects of EA in the YCAAD cohort, 3 with the CC genotype and 3 with GG genotype at the promoter SNP rs4950928 and with high and low YKL-40 levels, respectively, to identify rare and novel SNPs that could be associated with YKL-40 expression. Amplicon distribution was as follows: 13 kb upstream-5', 7.8 kb *CHI3L1* gene, 1.2 kb downstream-3', as previously described.¹² The identified SNPs were compared with SNPs genotyped in previous publications.^{5,6} These SNPs were analyzed with Haploview to identify tagging polymorphisms in the *CHI3L1* gene by using data from the HapMap project (version 3, release 27).¹³ A total of 17 SNPs spanning the *CHI3L1* gene identified through sequencing, previous publications,^{5,6} and haplotype tagging were genotyped in YCAAD (discovery). Simultaneously, 14 SNPs identified through haplotype tagging by using data from the HapMap project (version 3, release 27)¹³ were genotyped in SARP (validation). A total of 9 SNPs present in both data sets tagged all other SNPs reported in this study. Genotyping criteria for SNP quality included the removal of SNPs with low call rates (<80%), SNPs that violated Hardy-Weinberg equilibrium ($P < .05$) and had a minor allele frequency of less than 4%, and samples with low call rates (<98%). Pairwise marker linkage disequilibrium (LD) was estimated by using the Lewontin D' statistic and r^2 with Haploview.¹³

Statistical analysis

R software (R Foundation for Statistical Computing, Vienna, Austria) was used for data analysis. The R stats (version 3.0.1), SNPassoc (version 1.8-5), haplo.stats (version 1.6.3), and car (version 2.0-21) packages were implemented. Continuous variables were tested by using nonparametric tests, including the Kruskal-Wallis test. Categorical variables were analyzed with χ^2 tests. Results are reported as medians and interquartile ranges, unless otherwise specified. Hardy-Weinberg equilibrium calculation was performed to assess the distribution of the SNPs in each group by using Haploview.¹³ The SNPassoc package applies a generalized linear model to generate association P values. By using this statistical package, dominant, codominant, recessive, and additive models of association were tested for postbronchodilator FEV₁ percent predicted, which was used as a surrogate marker of airway remodeling, and serum YKL-40 levels on the discovery phase of the study. The additive association model was identified as the best fit based on the Akaike information criterion,¹⁴ and all reported association values were generated by using this model and adjusted for age and sex based on the effect these 2 features have on YKL-40 levels,¹⁵ pulmonary function,¹⁶ and the best fit based on the Akaike information criterion.¹⁴ In the discovery cohort (YCAAD) P values were adjusted for multiple testing with the Benjamini-Hochberg procedure (false discovery rate < 0.1)¹⁷ to balance power and the potential for false-positive results. Association values in the validation cohort (SARP) were adjusted for multiple testing with the Bonferroni method ($P < .05$) to provide a more stringent and reliable measure of true association. We conducted a conditional analysis on subjects of EA with the rs4950928 CC genotype to evaluate for independent associations on nonpromoter SNPs; all P values on the conditional analyses were adjusted with the Bonferroni method. Haplotypes were inferred by using the expectation maximization algorithm in Haploview.¹³ Haplotypes with a frequency of greater than 5% were considered in the analysis. A generalized linear model using the expectation maximization algorithm described by Excoffier and Slatkin¹⁸ was implemented in the haplotype analysis with the haplo.stats R package. Asthmatic and control subjects were considered in the discovery, validation,

TABLE I. Demographic characteristics

Subjects of EA, YCAAD	Control subjects (n = 39)	Subjects with nonsevere asthma (n = 137)	Subjects with severe asthma (n = 37)	P value
Age (y)	45 (31-57)	46 (36-56)	59 (50-68)	<.01
Female sex	27 (69%)	98 (72%)	23 (62%)	.52
Age of onset (y)	—	22 (5-40)	21 (4-45)	.46
BMI (kg/m ²)	25.5 (23.5-27.8)	26.3 (22.7-31.2)	29.3 (25.0-31.5)	.08
FEV ₁ PP before BD	99 (91-105)	89 (79-98)	53 (39-59)	<.01
FEV ₁ PP after BD	104 (96-110)	95 (84-106)	60 (45-70)	<.01
FENO (ppb)	22 (13-29)	26 (19-60)	40 (24-54)	.02
Total IgE (IU/mL)	20 (11-72)	95 (29-225)	129 (26-289)	.61
Serum YKL-40 (ng/mL)*	43 (31-67)	65 (37-105)	83 (37-110)	.01

Subjects of EA, SARP	Control subjects (n = 122)	Subjects with nonsevere asthma (n = 234)	Subjects with severe asthma (n = 328)	P value
Age (y)	27 (23-40)	31 (22-41)	41 (31-51)	<.01
Female sex	68 (66%)	168 (72%)	190 (58%)	.29
Age of onset (y)	—	10 (4-22)	12 (3-30)	.06
BMI (kg/m ²)	24.4 (21.7-28.4)	25.1 (22.1-29.3)	28.7 (24.4-34.0)	<.01
FEV ₁ PP before BD	98 (93-105)	89 (82-99)	60 (43-75)	<.01
FEV ₁ PP after BD	105 (97-110)	97 (89-107)	71 (36-86)	<.01
FENO (ppb)	14 (11-20)	26 (17-52)	29 (17-52)	.03
Total IgE (IU/mL)	35 (15-83)	117 (49-232)	115 (29-305)	.05
Serum YKL-40 (ng/mL)*	43 (33-53)	44 (32-60)	60 (41-101)	<.01

Subjects of AA, SARP and YCAAD	Control subjects (n = 22)	Subjects with nonsevere asthma (n = 132)	Subjects with severe asthma (n = 127)	P value
Age (y)	35 (29-40)	29 (22-40)	36 (22-45)	.79
Female sex	16 (73%)	88 (66%)	82 (65%)	.48
Age of onset (y)	—	8 (2-18)	4 (1-17)	.66
BMI (kg/m ²)	28.7 (25.8-34.7)	29.0 (24.7-36.6)	32.3 (23.5-37.5)	.50
FEV ₁ PP before BD	103 (96-110)	87 (80-96)	62 (52-77)	<.01
FEV ₁ PP after BD	108 (101-117)	98 (89-107)	80 (66-93)	<.01
FENO (ppb)	18 (16-28)	28 (15-55)	30 (18-44)	.63
Total IgE (IU/mL)	66 (39-162)	150 (47-376)	201 (69-569)	.02
Serum YKL-40 (ng/mL)*	41 (29-47)	39 (23-65)	60 (32-79)	.33

Values are expressed as numbers (percentages) or medians (interquartile ranges), as shown.

BD, Bronchodilator; BMI, body mass index; FENO, fraction of exhaled nitric oxide; PP, percent predicted.

*Numbers (percentages) with available measurements: subjects of EA, YCAAD = 213 (100%); subjects of EA, SARP = 143 (22%); subjects of AA, SARP and YCAAD = 92 (33%).

haplotype, and conditional analyses. The Levene test for homogeneity of variance on the 2 cohorts of asthmatic subjects was implemented with the car package. The analysis of asthma severity considered asthmatic subjects only.

RESULTS

Population characteristics and correlation of circulating YKL-40 levels with asthma severity and airway remodeling

Two populations of subjects of EA were evaluated separately to confirm that serum YKL-40 levels are associated with asthma severity and airway remodeling: discovery was conducted in YCAAD (n = 213; 174 asthmatic subjects vs 39 healthy control subjects), and validation was completed in SARP (n = 684; 562 asthmatic subjects vs 122 healthy control subjects). Because the 2 cohorts are predominantly of EA, subjects of African Ancestry (AA) from both cohorts were pooled together (n = 281; 259 asthmatic subjects vs 22 healthy control subjects). Baseline characteristics of all 3 groups are presented in Table I. Additional clinical characteristics of the YCAAD cohort are presented in Table E1 in this article's Online Repository at www.jacionline.org. Subjects of EA with asthma in YCAAD and SARP had lower FEV₁ percent predicted values compared

with healthy control subjects. These subjects also had higher fraction of exhaled nitric oxide values and higher serum IgE levels. In subjects of AA, no differences were seen in age, body mass index, or fraction of exhaled nitric oxide values between asthmatic and healthy control subjects. YKL-40 levels were evaluated in 213 subjects of EA in YCAAD, 143 subjects of EA in SARP, and 92 subjects of AA and were significantly greater in subjects of EA with severe asthma compared with those with nonsevere asthma and control subjects (Table I). YKL-40 levels were not associated with severe asthma in subjects of AA, despite similar median values compared with those seen in subjects of EA in SARP. This is the result of a lack of statistical power because of the small number of subjects of AA with available YKL-40 measurements. Additional characteristics of subjects of AA with asthma are presented on Table E2 in this article's Online Repository at www.jacionline.org. Postbronchodilator FEV₁ percent predicted values had a significant correlation with serum YKL-40 levels in subjects of EA in both YCAAD (Spearman rho = -0.19, P < .01) and SARP (Spearman rho = -0.24, P < .01). Concordant with higher serum YKL-40 levels in subjects with severe asthma, the postbronchodilator FEV₁ percent predicted value was significantly lower in both YCAAD and SARP. These findings indicate that the 2 EA cohorts have similar

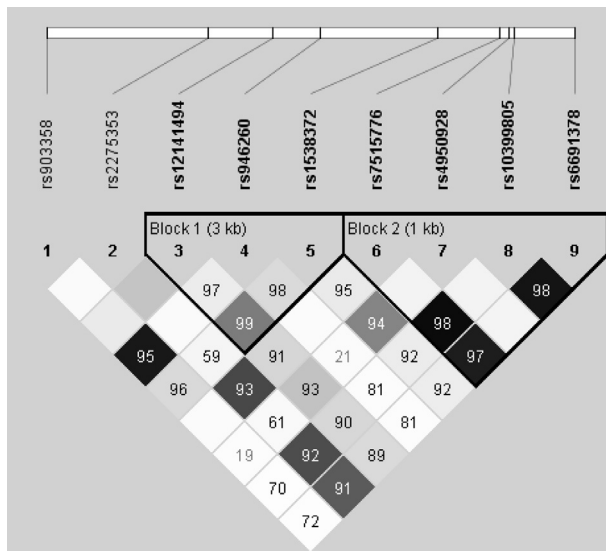


FIG 1. Haplotype map for the *CH13L1* gene in subjects of EA from YCAAD and SARP.

characteristics and confirm that serum YKL-40 levels are associated with irreversible airflow obstruction and severe asthma in subjects of EA.

***CH13L1* SNPs and LD structure**

To visualize the LD coefficients in the subjects of EA and subjects of AA, we generated haplotype maps using Haploview.¹³ Nine common SNPs in YCAAD and SARP were used to generate a haplotype map for subjects of EA (Fig 1) and for subjects of AA (see Fig E1, A, in this article's Online Repository at www.jacionline.org). This analysis revealed 2 haplotype blocks, one in the promoter region that includes SNPs in the 5' region of the gene and the promoter SNP rs4950928 and one including the intronic/exonic regions of the *CH13L1* gene. These haplotype blocks show common SNPs and a similar structure to those identified in the HapMap project in the Utah residents with Northern and Western European ancestry from the CEPH collection (CEU group; see Fig E1, B) and subjects of African ancestry in the Southwest United States (ASW group; see Fig E1, C), respectively. This indicates that the SNP distribution and LD patterns seen in our study populations were consistent with HapMap populations of similar ancestry.

Identification of *CH13L1* polymorphisms associated with serum YKL-40 levels and irreversible airflow obstruction

To identify polymorphisms in *CH13L1* that are associated with serum YKL-40 levels or postbronchodilator FEV₁ percent predicted values, subjects of EA in the YCAAD cohort (discovery) were analyzed by using an additive model and adjusted for multiple comparisons (false discovery rate < 0.10), as described in the Methods section (Table II summarizes the significant associations in the EA and AA groups). Consistent with previous reports,⁵ multiple SNPs were associated with serum YKL-40 levels in the discovery cohort, including rs4950928. In subjects of EA with the rs4950928 CC genotype, serum

YKL-40 levels were higher compared with levels in those with the GG genotype (72 vs 34 ng/mL, $P < .01$; see Fig E2, A, in this article's Online Repository at www.jacionline.org). In addition, 4 *CH13L1* SNPs (rs12141494, rs880633, rs884209, and rs6698204) were associated with postbronchodilator FEV₁ percent predicted values (Table II).

Validation of SNPs with an effect on serum YKL-40 levels and postbronchodilator FEV₁ percent predicted values that were identified in the discovery cohort was conducted in subjects of EA from the SARP cohort. These association values were adjusted for multiple comparisons (Bonferroni < 0.05), as described in the Methods section (Table II). This analysis identified multiple SNPs associated with serum YKL-40 levels or postbronchodilator FEV₁. Consistent with the findings in the discovery cohort, subjects of EA with the rs4950928 CC genotype have higher serum YKL-40 levels compared with those with the GG genotype (53 vs 16 ng/mL, $P < .01$; see Fig E2, B). Importantly, of the SNPs identified in the discovery cohort, only rs12141494 was significantly associated with both postbronchodilator FEV₁ percent predicted values ($P < .01$) and serum YKL-40 levels ($P < .01$) in the SARP cohort. Specifically, the rs12141494 A allele was associated with decreased postbronchodilator FEV₁ percent predicted values (effect size, -3.6 ; 95% CI, -5.6 to -1.6). No associations were seen between the promoter SNP rs4950928 and pulmonary function in any of the cohorts (Table II). In subjects of AA, only 1 SNP was associated with the quantitative traits evaluated, and therefore we restricted further analyses to subjects of EA. Table E3, in this article's Online Repository at www.jacionline.org, summarizes the association values for all SNPs tested in this study. Taken together, these analyses indicate that the nonpromoter SNP rs12141494 in intron 6 is strongly associated with persistent airflow obstruction and serum YKL-40 levels in subjects of EA.

Effect of *CH13L1* haplotypes on serum YKL-40 levels and lung function

A haplotype model was tested on all subjects of EA with asthma and healthy control subjects from the YCAAD and SARP cohorts to define potential interactions of *CH13L1* SNPs validated above on lung function and YKL-40 levels. Of the 9 SNPs genotyped in both cohorts, 5 were selected for this analysis based on their significant association with FEV₁ percent predicted values or serum YKL-40 levels in both the discovery and validation steps (Table II). Haplotype A, which is present in 18% of subjects of EA, involving the SNP interaction between the minor allele in the promoter SNP rs4950928 (G) and the (G) allele in rs12141494 was associated with improved postbronchodilator FEV₁ and lower YKL-40 levels (Table III). Haplotype B, which is present in 14% of the population, was associated with improved postbronchodilator FEV₁ involving the (G) allele in rs12141494. This analysis demonstrates that G alleles at both the promoter and the rs12141494 intronic SNP have a protective effect characterized by lower YKL-40 levels and higher postbronchodilator FEV₁ percent predicted values, demonstrating an interaction between promoter and nonpromoter SNPs in the *CH13L1* gene.

Identification of *CH13L1* SNPs associated with asthma severity

Subjects of EA with asthma from YCAAD and SARP were combined ($n = 735$) to determine the effect of *CH13L1* SNPs on

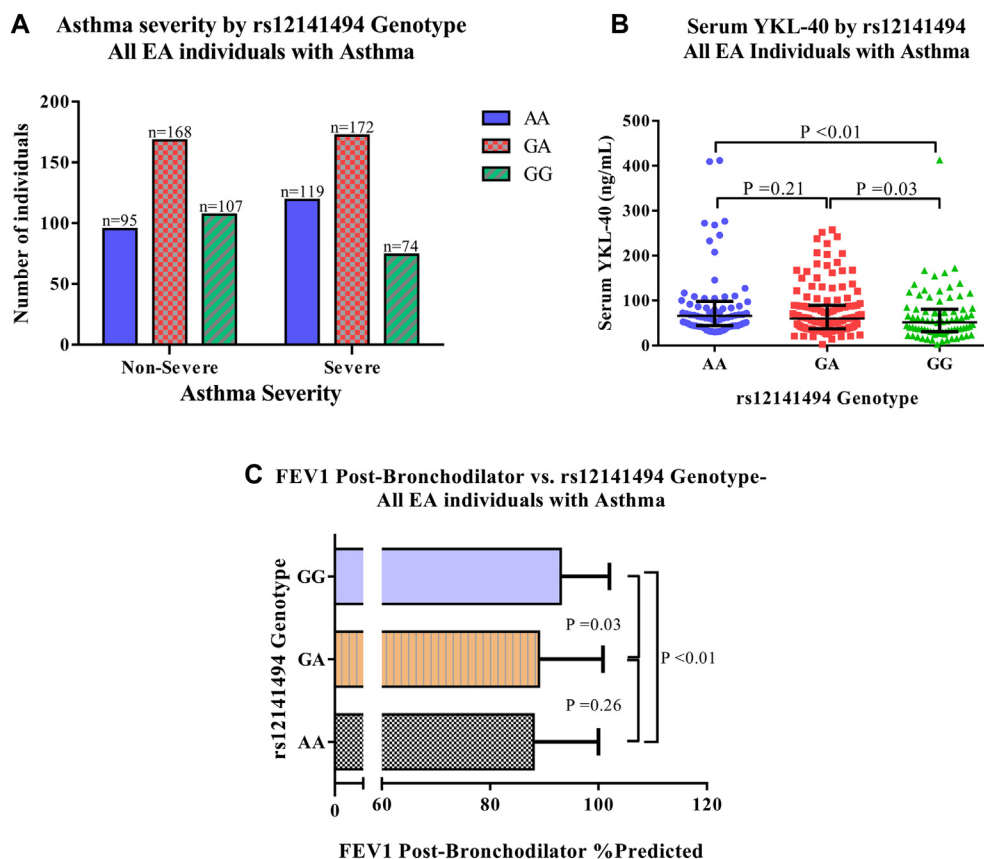


FIG 2. The A allele of rs12141494 in subjects of EA with asthma is associated with increased asthma severity (A), higher serum YKL-40 levels (B), and worse airflow obstruction (C).

asthma severity. The Levene test for homogeneity of variance was used before combining subjects from the 2 cohorts. Asthma severity and serum YKL-40 levels were included as independent variables. No differences in the variability of the 2 groups were seen. A linear regression analysis using an additive model of association was conducted in the combined group to identify their relationship with asthma severity (Table IV). The Hardy-Weinberg values for these SNPs were evaluated before implementing the association test to maintain the fundamental assumptions of equilibrium seen in the discovery and validation groups (Table IV). Of the SNPs evaluated, only rs12141494 was associated with asthma severity in subjects of EA (Bonferroni-adjusted $P = .04$). The G allele had a protective effect on severity (Fig 2, A) (odds ratio, 0.7; 95% CI, 0.6-0.9) and was associated with lower serum YKL-40 levels (Fig 2, B) (Bonferroni-adjusted $P = .04$; effect size, -14.3 ng/mL; 95% CI, -24.1 to -4.4 ng/mL) and higher postbronchodilator FEV₁ percent predicted values (Fig 2, C) (Bonferroni-adjusted $P < .01$; effect size, 3.8%; 95% CI, 1.9% to 5.7% of predicted value). This observation indicates that rs12141494 is strongly associated with the severe asthma phenotype, including SARP-defined severity, serum YKL-40 levels, and persistent airflow obstruction.

Effect of rs12141494 on YKL-40 expression in the airway

To determine the contribution of rs12141494 to airway expression of YKL-40, we quantified YKL-40 levels in sputum

supernatants of asthmatic subjects in the YCAAD cohort who underwent sputum induction, as described in the Methods section. Subjects of EA who were homozygous for the risk allele A had higher YKL-40 sputum levels compared with those in subjects homozygous for the G allele (23 ng/mL [0-59 ng/mL] vs 6 ng/mL [0-33 ng/mL], $P = .043$, Fig 3). The promoter SNP rs4950928 was not associated with sputum levels (data not shown). This observation shows that rs12141494 has an effect on YKL-40 expression in the airways of asthmatic subjects and is consistent with the findings that the rs12141494 G allele is associated with lower YKL-40 levels, milder asthma, and better lung function compared with those seen in subjects carrying the A allele.

In silico analysis of the functional genomic effect of intronic CH13L1 SNPs

An *in silico* analysis for the functional effect of rs12141494 was conducted with the Human Splicing Finder Version 2.4.1 (<http://www.umd.be/HSF/HSF.html>). The exon 6 *CH13L1* sequence was analyzed by selecting 1000 bp on the 5' and 3' flanks of exon 6 to include rs12141494. Mutation analysis of position 1344 from C to T (complementary to G and A) identified enhancement in the splice site strength according to the maximum entropy algorithm,¹⁹ suggesting a potential effect of rs12141494 on pre-mRNA processing. Analysis of *CH13L1* mRNA expression in the circulation of a subset of subjects in YCAAD demonstrates a significant genotype effect

TABLE II. Significant associations of genetic variation in *CHI3L1* with lung function and YKL-40 protein levels

Cohort	SNP	Position relative to TSS	Genomic coordinates*	Location	Minor allele	MAF	HW <i>P</i> value	Risk allele	FEV ₁ after BD (PP), <i>P</i> value	Serum YKL-40, <i>P</i> value
Discovery EA, YCAAD										
	rs884209	8462	201413912	3' UTR	A	0.44	.57	A	.085	.160
	rs12141494	4326	201418048	Intron 6	A	0.45	.44	A	.085	.170
	rs880633	2950	201419424	Exon5	C	0.45	.53	C	.085	.164
	rs1538372	1219	201421155	Intron 2	A	0.37	.08	G	.213	.031
	rs4950928	−131	201422505	Promoter	G	0.22	.85	C	.213	8.1×10^{-5}
	rs10399931	−329	201422703	5' UTR	C	0.26	.52	C	.136	1.1×10^{-4}
	rs10920579	−3221	201425595	5' UTR	A	0.21	.96	G	.221	6.7×10^{-5}
	rs6698204	−3432	201425806	5' UTR	G	0.44	.58	A	.085	.039
	rs4950929	−4375	201426749	5' UTR	G	0.21	.96	T	.221	6.7×10^{-5}
	rs946263	−9630	201432004	5' UTR	G	0.19	.95	A	.198	6.7×10^{-5}
	rs2486064	−13013	201435387	5' UTR	A	0.41	.38	G	.660	.030
Validation EA, SARP										
	rs2364572	9165	201413209	3' UTR	T	0.04	.42	T	.006	NA
	rs2275353	5678	201416696	Intron 7	A	0.18	1	G	.017	1
	rs12141494	4326	201418048	Intron 6	G	0.46	.75	A	.005	.001
	rs1538372	1219	201421155	Intron 2	A	0.31	1	G	1	.006
	rs4950928	−131	201422505	Promoter	G	0.20	.82	C	1	.011
	rs10399805	−247	201422621	5' UTR	A	0.14	.65	G	.035	1
	rs946261	−2122	201424496	5' UTR	G	0.38	.27	A	.011	.053
AA										
	rs6691378	−1371	201423745	5' UTR	A	0.27	.72	A	1	.006

For all the genotyped SNPs and phenotypic associations, see Table E2. All *P* values are adjusted for age and sex, followed by correction for multiple comparison. The false discovery rate threshold for the Discovery EA, YCAAD cohort is less than 0.1. The Bonferroni threshold for the Validation EA, SARP and AA cohorts is less than 0.05.

BD, Bronchodilator; HW, Hardy-Weinberg; MAF, minor allele frequency; NA, not applicable; PP, percent predicted; TSS, transcription start site; UTR, untranslated region.

*Genomic coordinates in chromosome 1 using the Human Genome NCBI Build 36.1 (March 2006).

TABLE III. Haplotype analysis in subjects of EA in YCAAD and SARP

Haplotype	Frequency	Phenotype	Effect size and direction	<i>P</i> value	rs2275353	rs12141494	rs1538372	rs4950928	rs10399805
A	18%	FEV ₁ PP after BD	4	<.01	G	G	A	G	G
A	20%	Serum YKL-40	−24	<.01	G	G	A	G	G
B	14%	FEV ₁ PP after BD	6	<.01	A	G	G	C	A
B	15%	Serum YKL-40	−0.6	.93	A	G	G	C	A
C	12%	FEV ₁ PP after BD	2	.19	G	G	A	C	G
C	13%	Serum YKL-40	−0.1	.99	G	G	A	C	G
Base	51%				G	A	G	C	G

TABLE IV. Significant SNP associations in subjects of EA with asthma

SNP	Location	Minor allele	MAF	HW <i>P</i> value	Risk allele	FEV ₁ PP after BD	Serum YKL-40	Asthma severity
rs12141494	Intron 6	G	0.47	.11	A	9.2×10^{-4}	0.043	0.040
rs1538372	Intron 2	A	0.31	.07	G	0.348	0.012	1
rs4950928	Promoter	G	0.20	.16	C	0.633	1.2×10^{-5}	1

concordant with that seen at the protein level in the airway (data not shown).

DISCUSSION

These studies are the first to examine the effect of genetic variation in *CHI3L1* on asthma severity and demonstrate that the intronic SNP rs12141494 is associated with disease severity, airflow obstruction, and YKL-40 levels in the serum. This effect is independent of the promoter SNP rs4950928, and rs12141494 was the only SNP found to be associated with airway expression

of the *CHI3L1* gene product YKL-40. Taken together, these findings suggest that rs12141494 contributes to asthma severity by altering airway expression of YKL-40 and contributes to airway remodeling, persistent airflow obstruction, and asthma severity.

Previous population studies of genetic variation in the *CHI3L1* gene and asthma have shown an association with the promoter SNP rs4950928, asthma prevalence, and YKL-40 expression; however, the risk allele reported in the studies has differed. A European population study⁶ identified the G allele in rs4950928 as the risk allele for atopic asthma in never smokers,

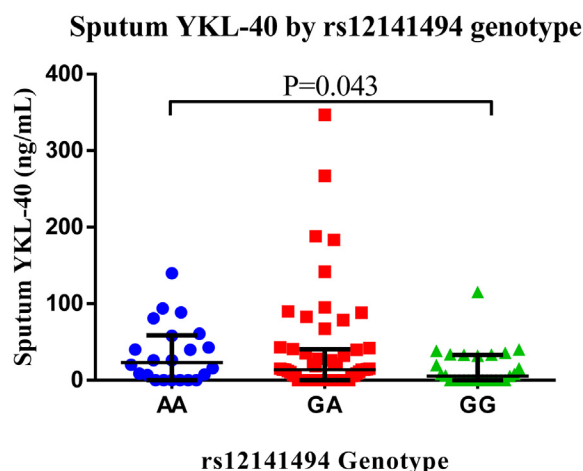


FIG 3. The rs12141494 AA genotype in subjects of EA with asthma is associated with higher YKL-40 sputum levels.

whereas the study by Ober et al⁵ reported that the C allele was associated with an increased risk of asthma and higher YKL-40 levels in the circulation. An additional publication has suggested that *CHI3L1* polymorphisms are not associated with asthma,⁷ and in Korean children other promoter polymorphisms are associated with atopy but not with asthma.⁸ In the current study we confirmed that the C allele at rs4950928 is associated with higher serum levels of YKL-40 in 2 cohorts of subjects of EA (see Fig E2). Our study was underpowered to detect associations in subjects of AA, and therefore additional studies are needed to evaluate these associations in these populations. Interestingly, we did not find that the rs4950928 polymorphism was associated with airflow obstruction or airway YKL-40 levels in either of the 2 populations of asthmatic subjects examined, which suggests that this polymorphism does not directly contribute to asthma severity.

Because serum YKL-40 levels were associated with severe asthma and lung function impairment, we suspected that other promoter or nonpromoter polymorphisms could contribute to severe asthma. This led us to discover that a novel intronic SNP, rs12141494, is associated with airway expression of YKL-40 and persistent airflow obstruction in subjects with severe asthma. This SNP was not examined in previous genetic studies of *CHI3L1* in asthmatic subjects and was identified by overlapping direct DNA sequencing data of the *CHI3L1* gene and HapMap data. Analysis of the *CHI3L1* LD structure shows that rs12141494 resides in intron 6 within one of 2 haplotype blocks separate from the promoter SNP rs4950928 (Fig 1). This SNP was associated with postbronchodilator FEV₁ percent predicted values and serum YKL-40 levels and, unlike rs4950928, was associated with YKL-40 levels in sputum. The observation that the additive model had the best fit in conjunction with the quantitative differences seen in these phenotypes suggests that there is a gene-dosage effect of the risk allele in these associations. The effect of this SNP on lung function persisted after controlling for the risk genotype (CC) at rs4950928 (see Table E4 in this article's Online Repository at www.jacionline.org) and evaluation of haplotypes, with the A allele associated with higher serum YKL-40 levels, lower lung function, and more severe asthma (Table III). Asthmatic subjects homozygous for the A allele of rs12141494 have

significantly higher YKL-40 sputum levels compared with homozygotes for the G allele, further supporting our finding that the rs12141494 genotype has a functional effect on the airway expression of YKL-40 (Fig 3). This suggests that rs12141494 is linked to severe asthma traits, whereas rs4950928 is not. rs12141494 might contribute to circulating YKL-40 levels through production of YKL-40 in the airway, and this effect might be enhanced by the presence of the rs4950938 C allele.

What are the possible functional effects of rs12141494 on the architectural changes characteristic of airway remodeling? The presence of higher YKL-40 levels in the airways of asthmatic subjects and the rs12141494 A allele suggests that increased expression of YKL-40 is the primary effect of this polymorphism. Bronchial epithelial cells exposed to YKL-40 have been shown to increase expression of IL-8, which, in turn, leads to proliferation and migration of bronchial smooth muscle cells *in vitro*.³ In addition, epithelial expression of YKL-40 correlates with bronchial smooth muscle mass and activation of the protease-activated receptor 2, AKT, extracellular-signal regulated kinase, and p38 pathways.⁴ Therefore higher local production of YKL-40 in the bronchial epithelium in subjects with the AA rs12141494 genotype might contribute to changes in airway structure, persistent airflow obstruction, and higher YKL-40 levels in the circulation. This is supported by results of haplotype analysis that reveal that the G alleles at both SNPs are associated with lower YKL-40 levels (Table III). In addition, *in silico* analysis suggests that the effect of the rs12141494 A allele on pre-mRNA processing enhances the strength of the intron 6 splice site, resulting in increased *CHI3L1* mRNA and YKL-40 protein levels. Analysis of mRNA expression in the circulation of a subset of subjects in YCAAD demonstrates a genotype effect concordant with that seen at the protein level in the airway.

In summary, these studies identify a novel SNP in *CHI3L1*, rs12141494, which is associated with persistent airflow obstruction, YKL-40 levels in the airway and blood, and severe asthma. The functional effect of the risk allele A might increase YKL-40 levels in the airway and circulation and might have synergistic effects in conjunction with the *CHI3L1* SNP rs4950928. We used a rigorous definition of asthma, 2 cohorts of asthmatic subjects who are representative of centers across the United States, and a single genotyping methodology to reduce bias to conduct these studies. Although future studies will determine the effect of rs12141494 on other ethnic groups and the cellular control of YKL-40 synthesis and expression, these observations add to the body of evidence demonstrating that genetic variation in *CHI3L1* and YKL-40 play important roles in the development of airway remodeling and severe asthma.

Key messages

- Intronic variation, rs12141494, in the *CHI3L1* gene encoding YKL-40 is associated with persistent airflow obstruction, airway remodeling, and severe asthma.
- Subjects with the AA risk genotype at rs12141494 have higher sputum YKL-40 levels, suggesting that its effect might be mediated through increased protein levels in the airway.

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METHODS

YCAAD cohort

Case definition for asthma was based on a physician's diagnosis of asthma. The diagnosis of asthma was validated by a pulmonary specialist in YCAAD. Subjects were excluded if they had a history of more than 10 pack years of smoking or active smoking, which was defined as smoking within the last year. Participants provided informed clinical consent, followed by clinical phenotyping, including a comprehensive questionnaire that includes data on asthma diagnosis, activity, medication requirements, environmental triggers, associated comorbidities, and health care use. Pulmonary function testing included "baseline" prebronchodilator spirometry and responsiveness to 2 to 8 puffs of short-acting β -agonists ("maximal" lung function). Atopy was assessed by means of skin prick testing and measurement of serum total IgE levels and blood eosinophil counts. Fraction of exhaled nitric oxide values were measured by using American Thoracic Society (ATS)-approved online devices at a constant flow rate, and induced sputum was collected in a subset of subjects for evaluation of inflammatory cells and protein markers. Severe asthma was defined as outlined by the SARP clustering algorithm and was modified as follows: severe asthma included subjects with a baseline FEV₁ of less than 68% of predicted value (SARP clusters 4 and 5), whereas nonsevere asthma was defined by the presence of a baseline FEV₁ equal to or greater than 68% of predicted value (SARP clusters 1-3).^{E1}

SARP cohort

Briefly, investigators recruited nonsmoking asthmatic subjects (<5 pack years of tobacco use) who met the ATS definition of severe asthma and an additional group of subjects with nonsevere asthma. After informed consent, clinical staff administered questionnaires that assessed demographic information, asthma symptoms and medication use, medical history, and health care use. Physiologic testing of lung function included "baseline"

prebronchodilator spirometry with withholding of appropriate medications, responsiveness to 2 to 8 puffs of short-acting β -agonists ("maximal" lung function), and bronchial hyperresponsiveness to methacholine in subjects with a baseline FEV₁ of greater than 55%. Atopy was assessed by means of skin prick testing and measurement of serum total IgE levels and blood eosinophil counts. Fraction of exhaled nitric oxide values were measured with ATS-approved online devices at a constant flow rate. Severe asthma was defined as outlined by the SARP clustering algorithm and was modified as follows: severe asthma included subjects with a baseline FEV₁ of less than 68% of predicted value (SARP clusters 4 and 5), whereas nonsevere asthma was defined by the presence of a baseline FEV₁ equal to or greater than 68% of predicted value (SARP clusters 1-3).^{E1}

RESULTS

Conditional analysis of the rs4950928 promoter C allele

Although LD analysis suggested that the rs4950928 and rs12141494s SNPs are not in significant LD, we conducted a conditional analysis of subjects of EA with the rs4950928 CC genotype to control for the associations affected by SNP-to-SNP interaction seen in the haplotype analysis. This analysis showed that in subjects of EA with the rs4950928 CC genotype ($n = 572$), rs12141494 was significantly associated with persistent airflow obstruction and serum YKL-40 levels (see [Table E4](#)).

REFERENCE

- E1. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;181:315-23.

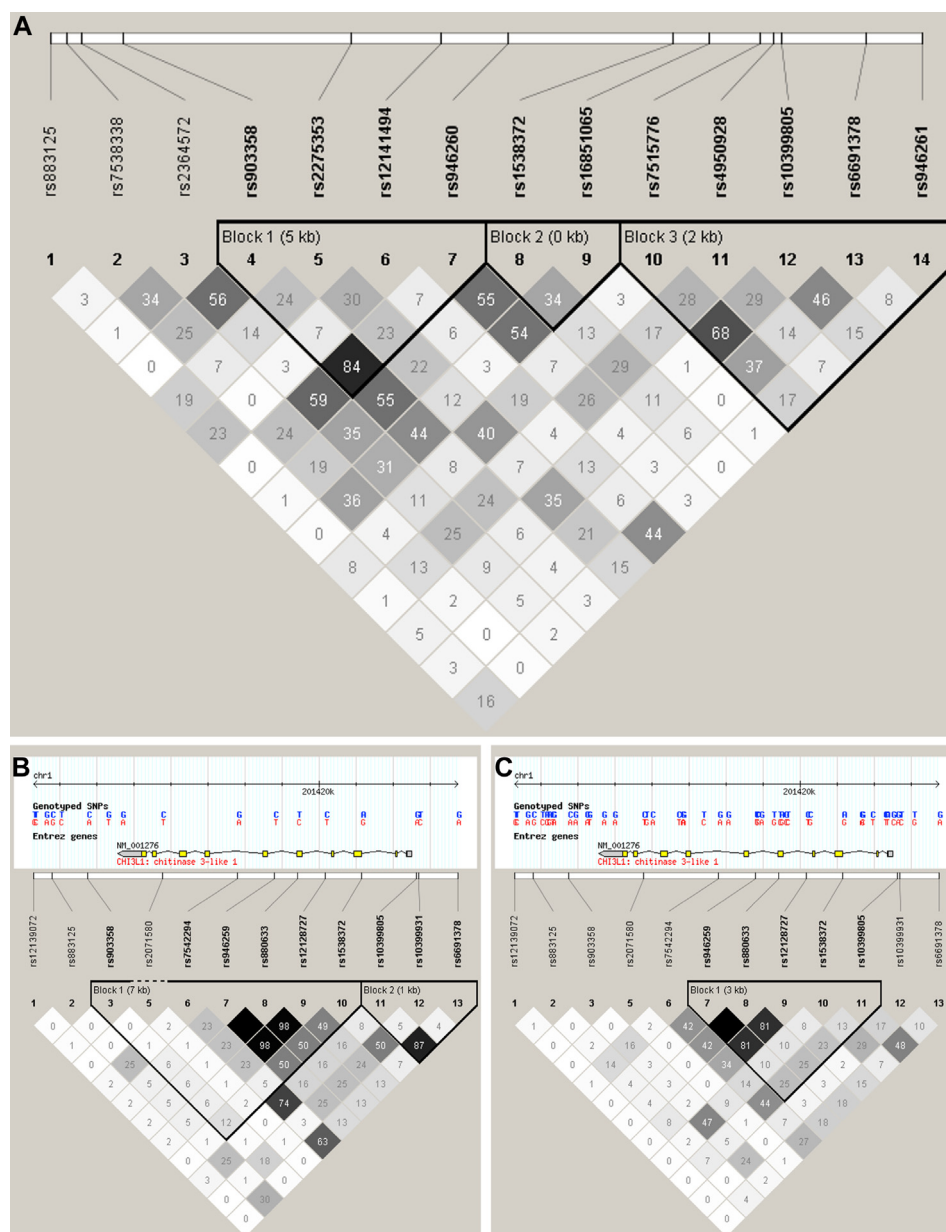


FIG E1. Haplotype maps for the *CHI3L1* gene in subjects of AA in YCAAD and SARP (**A**), Utah residents with Northern and Western European Ancestry from the CEPH collection HapMap (**B**), and African ancestry in the Southwest United States HapMap (**C**).

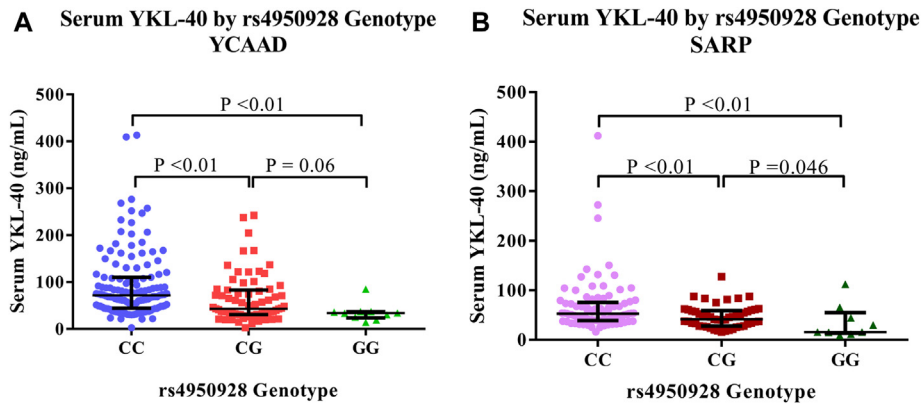


FIG E2. Variation in the promoter SNP rs4950928 in the *CHI3L1* gene and effect in YKL-40 protein levels in subjects of EA in YCAAD (**A**) and SARP (**B**).

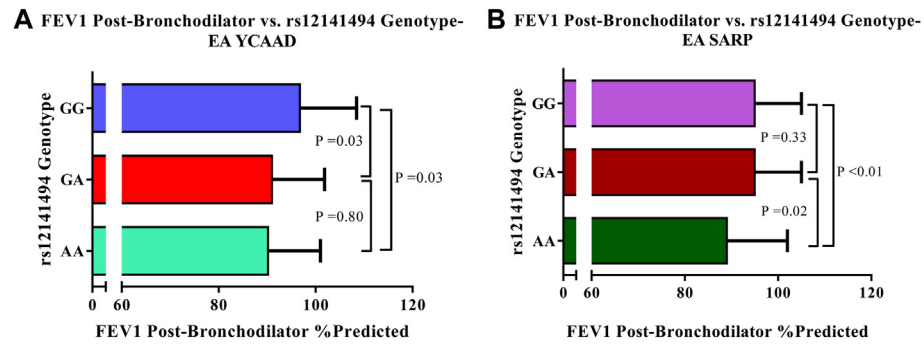


FIG E3. rs12141494 and airflow obstruction in subjects of EA in YCAAD (**A**) and SARP (**B**).

TABLE E1. Asthma-related health care use, symptom control, and comorbidities: YCAAD

	Subjects with nonsevere asthma (n = 166)	Subjects with severe asthma (n = 53)	<i>P</i> value
ACT score	15 (0-22)	12 (9-20)	.92
Hospitalizations in previous year (%)	22/166	12/53	.09
Emergency department visits in previous year (%)	53/166	23/53	.08
OCS pulse in previous year (%)	52/166	30/53	<.01
History of intubations (%)	16/166	13/53	<.01
Lifetime intensive care unit admission (%)	17/166	16/53	<.01
Sinus disease (%)	91/166	35/53	.15
Gastroesophageal reflux (%)	88/166	28/53	.98
Medication use			
No corticosteroids (%)	41/166	7/53	<.01
Inhaled corticosteroids (%)	125/166	46/53	<.01
Oral or systemic corticosteroids (%)	16/166	11/53	.03
Long-acting β -agonist (%)	96/166	41/53	.01
Leukotriene antagonist (%)	69/166	25/53	.48
Anticholinergics (%)*	21/166	10/53	.02
Omalizumab (%)	5/166	3/53	.37

Values are expressed as medians (interquartile ranges) or percentages.

ACT, Asthma Control Test; OCS, oral corticosteroid.

*Includes subjects using tiotropium or ipratropium.

TABLE E2. Distribution of subjects of AA with asthma per cohort

YCAAD	Subjects with nonsevere asthma (n = 29)	Subjects with severe asthma (n = 16)	P value
Age (y)	41 (31-50)	44 (34-52)	.34
Female sex	23 (79%)	10 (63%)	.23
Age of onset (y)	15 (3-30)	7 (1-14)	.14
BMI (kg/m ²)	34.4 (31.2-41.9)	33.8 (29.9-36.2)	.46
FEV ₁ PP before BD	90 (84-98)	52 (49-55)	<.01
FEV ₁ PP after BD	95 (86-107)	59 (54-70)	<.01
FENO (ppb)	24 (20-54)	33 (26-41)	.80
Total IgE (IU/mL)	136 (38-307)	209 (46-346)	.48
Serum YKL-40 (ng/mL)*	40 (28-91)	60 (35-81)	.35

SARP	Subjects with nonsevere asthma (n = 103)	Subjects with severe asthma (n = 111)	P value
Age (y)	28 (22-38)	34 (20-43)	.10
Female sex	65 (63%)	72 (65%)	.79
Age of onset (y)	6 (2-12)	4 (1-18)	.59
BMI (kg/m ²)	27.5 (23.9-33.7)	30.7 (22.5-37.5)	.25
FEV ₁ PP before BD	86 (79-95)	66 (53-79)	<.01
FEV ₁ PP after BD	99 (90-107)	81 (70-98)	<.01
FENO (ppb)	28 (13-58)	26 (16-45)	.61
Total IgE (IU/mL)	165 (54-420)	198 (77-737)	.12
Serum YKL-40 (ng/mL)*	34 (22-53)	60 (27-79)	.06

BMI, Body mass index; FENO, fraction of exhaled nitric oxide; PP, percent predicted.

*Numbers (percentages) of subjects with available measurements: YCAAD = 46 (100%); SARP = 46 (20%).

TABLE E3. Association of genetic variation in *CHI3L1* with airflow obstruction phenotypes and YKL-40 protein levels

Cohort	SNP	Position relative to TSS	Genomic coordinates	Location	Minor allele	MAF	HW <i>P</i> value	FEV ₁ after BD PP, <i>P</i> value	Serum YKL-40, <i>P</i> value
Discovery EA, YCAAD									
	rs903358	8612	201413762	3' UTR	G	0.10	1	.587	.940
	rs884209	8462	201413912	3' UTR	A	0.44	.57	.085	.160
	rs2275353	5543	201416831	Intron 7	A	0.20	1	.661	.166
	rs12141494	4326	201418048	Intron 6	A	0.45	.44	.085	.170
	rs946260	3431	201418943	Intron 5	G	0.08	1	.661	.605
	rs880633	2950	201419424	Exon5	C	0.45	.53	.085	.164
	rs1538372	1219	201421155	Intron 2	A	0.37	.08	.213	.031
	rs7515776	48	201422326	Intron 1	T	0.17	.70	.661	.160
	rs4950928	−131	201422505	Promoter	G	0.22	.85	.213	8.1×10^{-5}
	rs10399805	−247	201422621	5' UTR	A	0.18	.99	.661	.160
	rs10399931	−329	201422703	5' UTR	C	0.26	.52	.136	9.7×10^{-5}
	rs6691378	−1371	201423745	5' UTR	A	0.16	.99	.661	.149
	rs10920579	−3221	201425595	5' UTR	A	0.21	.96	.221	1.1×10^{-4}
	rs6698204	−3432	201425806	5' UTR	G	0.44	.58	.085	.039
	rs4950929	−4375	201426749	5' UTR	G	0.21	.96	.221	6.7×10^{-5}
	rs946263	−9630	201432004	5' UTR	G	0.19	.95	.198	6.7×10^{-5}
	rs2486064	−13013	201435387	5' UTR	A	0.41	.38	.661	.030
Validation EA, SARP									
	rs883125	9585	201412789	3' UTR	C	0.18	.86	1	1
	rs2364572	9165	201413209	3' UTR	T	0.04	.42	.006	NA
	rs903358	8612	201413762	3' UTR	G	0.06	.97	.462	1
	rs2275353	5678	201416696	Intron 7	A	0.18	1	.017	1
	rs12141494	4326	201418048	Intron 6	G	0.46	.75	.005	.001
	rs946260	3431	201418943	Intron 5	G	0.06	.99	.729	1
	rs1538372	1219	201421155	Intron 2	A	0.31	1	1	.006
	rs7515776	48	201422326	Intron 1	T	0.15	.69	.288	1
	rs4950928	−131	201422505	Promoter	G	0.20	.82	1	.011
	rs10399805	−247	201422621	5' UTR	A	0.14	.65	.035	1
	rs6691378	−1371	201423745	5' UTR	A	0.14	.89	.060	1
	rs946261	−2122	201424496	5' UTR	G	0.38	.27	.011	.053
AA									
	rs883125	9585	201412789	3' UTR	G	0.38	.52	1	1
	rs7538338	9365	201413009	3' UTR	G	0.16	.81	1	NA
	rs2364572	9165	201413209	3' UTR	T	0.17	.94	1	NA
	rs903358	8612	201413762	3' UTR	G	0.21	.61	1	1
	rs2275353	5543	201416831	Intron 7	G	0.49	.32	1	1
	rs12141494	4326	201418048	Intron 6	A	0.23	.74	1	1
	rs946260	3431	201418943	Intron 5	G	0.20	1	1	1
	rs1538372	1219	201421155	Intron 2	A	0.28	.83	1	1
	rs16851065	733	201421641	Intron 2	A	0.13	.90	1	NA
	rs7515776	48	201422326	Intron 1	T	0.46	.42	1	.070
	rs4950928	−131	201422505	Promoter	G	0.29	.22	1	1
	rs10399805	−247	201422621	5' UTR	A	0.42	.53	1	.165
	rs6691378	−1371	201423745	5' UTR	A	0.27	.72	1	.006
	rs946261	−2122	201424496	5' UTR	A	0.20	1	1	NA

BD, Bronchodilator; HW, Hardy-Weinberg; MAF, minor allele frequency; NA, not applicable; TSS, transcription start site; UTR, untranslated region.

TABLE E4. Significant associations in subjects of EA restricted to those with the CC genotype at rs4950928

SNP	FEV ₁ PP after BD	Serum YKL-40
rs903358	0.093	1
rs2275353	0.011	1
rs12141494	9.4×10^{-6}	0.025
rs946260	0.349	1
rs1538372	0.021	1.2×10^{-3}
rs7515776	0.096	0.362

BD, Bronchodilator.

TABLE E5. SNP associations in subjects of EA with asthma

SNP	Location	Minor allele	MAF	HW <i>P</i> value	FEV ₁ PP after BD, <i>P</i> value	Serum YKL-40, <i>P</i> value	Asthma severity, <i>P</i> value
rs903358	3' UTR	G	0.07	.86	.290	1	.289
rs2275353	Intron 7	A	0.17	.79	.060	1	.116
rs12141494	Intron 6	G	0.47	.11	9.2×10^{-4}	.043	.040
rs946260	Intron 5	G	0.07	.72		1	1
rs1538372	Intron 2	A	0.31	.07	.348	.012	1
rs7515776	Intron 1	T	0.15	.81	.234	1	.347
rs4950928	Promoter	G	0.20	.16	.633	1.2×10^{-5}	1
rs10399805	5' UTR	A	0.15	1	.054		.312
rs6691378	5' UTR	A	0.14	1	.080	1	1

BD, Bronchodilator; *HW*, Hardy-Weinberg; *MAF*, minor allele frequency; *UTR*, untranslated region.

TABLE E6. Unadjusted SNP associations in subjects of EA with asthma per cohort

SNP	Location	Minor allele	MAF	HW <i>P</i> value	FEV ₁ PP after BD, <i>P</i> value	Serum YKL-40, <i>P</i> value	Asthma severity, <i>P</i> value
YCAAD							
rs903358	3' UTR	G	0.101	1	.493	.944	.189
rs2275353	Intron 7	A	0.191	1	.444	.288	.847
rs12141494	Intron 6	A	0.468	.603	.087	.288	.516
rs946260	Intron 5	G	0.081	1	.793	.753	.362
rs1538372	Intron 2	A	0.355	.025	.403	.073	.401
rs7515776	Intron 1	T	0.171	.835	.230	.245	.979
rs4950928	Promoter	G	0.199	.410	.300	<.001	.273
rs10399805	5' UTR	A	0.174	.828	.303	.253	.643
rs6691378	5' UTR	A	0.163	.960	.326	.206	.543
SARP							
rs903358	3' UTR	G	0.061	.674	.057	.286	.027
rs2275353	Intron 7	A	0.167	.806	.009	.125	.009
rs12141494	Intron 6	G	0.452	.192	.004	<.001	.002
rs946260	Intron 5	G	0.06	.627	.109	.242	.118
rs1538372	Intron 2	A	0.3	.545	.166	.003	.129
rs7515776	Intron 1	T	0.143	1	.104	.857	.026
rs4950928	Promoter	G	0.196	.283	.309	.005	.867
rs10399805	5' UTR	A	0.138	.978	.018	.965	.023
rs6691378	5' UTR	A	0.134	1	.027	.962	.084

BD, Bronchodilator; HW, Hardy-Weinberg; MAF, minor allele frequency; UTR, untranslated region.