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GENETIC ASSOCIATION BETWEEN HUMAN CHITINASES AND LUNG FUNCTION IN COPD

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Abstract

Two primary chitinases have been identified in humans – acid mammalian chitinase (AMCase) and chitotriosidase (CHIT1). Mammalian chitinases have been observed to affect the host's immune response. The aim of this study was to test for association between genetic variation in the chitinases and phenotypes related to Chronic Obstructive Pulmonary Disease (COPD). Polymorphisms in the chitinase genes were selected based on previous associations with respiratory diseases. Polymorphisms that were associated with lung function level or rate of decline in the Lung Health Study (LHS) cohort were analyzed for association with COPD affection status in four other COPD case-control populations. Chitinase activity and protein levels were also related to genotypes. In the Caucasian LHS population, the baseline forced expiratory volume in one second (FEV_1) was significantly different between the AA and GG genotypic groups of the AMCase rs3818822 polymorphism. Subjects with the GG genotype had higher AMCase protein and chitinase activity compared with AA homozygotes. For CHIT1 rs2494303, a significant association was observed between rate of decline in FEV_1 and the different genotypes.

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In the African American LHS population, CHIT1 rs2494303 and AMCase G339T genotypes were associated with rate of decline in FEV_1. Although a significant effect of chitinase gene alleles was found on lung function level and decline in the LHS, we were unable to replicate the associations with COPD affection status in the other COPD study groups.

Keywords

Chronic Obstructive Pulmonary Disease; chitinase; polymorphism; lung function

INTRODUCTION

Chitin is the second most abundant polysaccharide found in nature and functions as a major structural component in fungi, crustaceans, and insects, but not in mammals. Chitinases are enzymes characterized by their ability to hydrolytically cleave chitin. In mammals, two primary chitinases have been identified – acid mammalian chitinase (AMCase) and chitotriosidase (CHIT1). In addition to their ability to cleave either inhaled or ingested exogenous chitin, mammalian chitinases have been observed to affect the host's immune response (Lee 2009). Recent studies have shown an association between the chitinase family of proteins and inflammatory lung diseases. For instance, high levels of AMCase in the lung were observed in a mouse model of asthma (Zhu 2004). CHIT1 levels were elevated in the bronchoalveolar lavage fluid of smokers with chronic obstructive pulmonary disease (COPD) (Létuvé 2010). A chitinase-like protein, commonly known as YKL-40, has also been discovered in mammals. Unlike AMCase and CHIT1, YKL-40 binds chitin polymers but lacks the active site residues necessary for cleavage. Nevertheless, genetic variants in the gene have been shown to influence levels of the protein and susceptibility to asthma (Chupp 2007; Ober 2008). In addition, elevated levels of this protein in the lung have been found in patients with COPD (Létuvé 2008). Moreover, studies show that chitin has the ability to recruit and activate immune cells that are involved in the development of COPD, suggesting an important role of chitinases and chitinase-like proteins in the disease (Lee 2008).

Although these studies suggest the involvement of chitinase and chitinase-like proteins in COPD, the underlying mechanism remains to be determined. It is possible that the presence of chitinase may result in changes to the immune response, which could lead to more frequent exacerbations and ultimately increased rate of decline in lung function. COPD is a lung disease characterized by airflow limitation that is not fully reversible. Smoking is a key causal factor in the disease, however, only a fraction of all smokers develop COPD, suggesting a genetic susceptibility (Khoury 1985). Therefore, the aim of this study was to test for association between genetic variation in the chitinases and phenotypes related to COPD. Polymorphisms in the genes encoding the chitinase and chitinase-like proteins were assayed to determine their association with lung function phenotypes in smokers. We hypothesized that these gene variants affect the baseline levels and rate of decline of lung function of smokers with mild-moderate COPD.

MATERIALS AND METHODS

Study participants

The participants in the primary analysis included in the present study were from the NHLBI-sponsored Lung Health Study (LHS) cohort previously described in detail (Connett 1993). Briefly, the participants were all smokers with evidence of lung function impairment. Lung function at the start of the study was measured as forced expiratory volume in 1 second (FEV_1) as a percentage of predicted value. The change in lung function, measured as change in FEV_1 per year over a five-year period, was the main outcome measure of the LHS. Of the

5887 total participants in the cohort, DNA samples and phenotypic data were available for 4344 subjects. Informed consent was obtained from all participants and this study received the approval of the Providence Health Care Research Ethics Board. Table 1 provides the characteristics of the LHS participants (Caucasians = 4123; African-Americans = 164; Other = 48). The single nucleotide polymorphisms (SNPs) that showed association with COPD in the LHS cohort were examined in four case-control cohorts, in which genome-wide association study data were available: (1) a case-control population from Norway (GenKOLS), (2) the National Emphysema Treatment Trial (NETT) cases and Normative Aging Study (NAS) controls, (3) the COPD cases and smoking controls of the ECLIPSE study (protocol number SCO104960) (Vestbo 2008), and (4) the first 1000 COPDGene study subjects (Table 2). Details of these populations have been previously described (Cho 2010; Cho 2011).

Gene variants

The SNPs in the chitinase and chitinase-like genes in this study were selected based on previous associations with respiratory diseases. Table 3 shows a brief description of the polymorphisms. SNP rs3818822 in the *CHIA* gene (which encodes for the AMCase protein) has been previously associated with asthma in a case-control study from Germany (Bierbaum 2005). Another SNP in the *CHIA* gene, G339T, has also been previously associated with asthma among African American subjects in several populations (Seibold 2009). A 24-bp duplication in exon 10 in the *CHIT1* gene results in aberrant splicing leading to the deletion of 87 nucleotides, producing an inactive form of the protein due to the lack of 29 amino acids. This polymorphism has been associated with reduced chitinase activity in the lungs of smokers (Seibold 2008). The *CHI3L1* gene encodes the YKL-40 protein. SNP rs4950928 in *CHI3L1* has been associated with asthma in both a Hutterite population and outbred populations as well as with serum levels of the protein (Ober 2008).

Tag SNP selection for the 24 bp duplication in *CHIT1*

To select a SNP that acts as a surrogate for the 24 bp duplication in the *CHIT1* gene, 44 Coriell samples (Coriell Institute for Medical Research, Camden, NJ, USA) were genotyped for the duplication. The 24 bp duplication in the *CHIT1* gene was assayed using electrophoresis on a 2 % agarose gel of an amplification product containing the duplication site. The region containing the duplication was amplified using a PCR cocktail that included 5 ng of genomic DNA, 0.2 μM primers, 0.2 mM dNTPs, 1.5 μL PCR buffer (Invitrogen), and 0.2 μL *Taq* DNA polymerase (5U/μL). PCR cycling conditions were as follows: 95°C for 15 min, 40 cycles of 95°C for 15 s, 60°C for 15 s, 72°C for 15 s, and a final extension at 72°C for 10 min. The results were compared with genotype data from SNPs within 100 kb of the duplication using the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov, accessed on February 18, 2010). Linkage disequilibrium was calculated using the CubeX algorithm (Gaunt 2007).

Genotyping

DNA from blood samples of the LHS subjects was whole genome amplified using the REPLI-g Mini Kit (Qiagen, Mississauga, ON, Canada) prior to genotyping. Genotyping was performed using commercially available TaqMan assays (Applied Biosystems, Foster City, CA, USA). The assays used are as follows: assay ID for rs3818822 = C_25984541_20; G339T = AHXOFUG; rs2494303 = C_424861_10; rs4950928 = C_27832042_10. For each assay, 5 ng of DNA was used for allelic discrimination.

Potential effect of missense polymorphism in the *CHIA* gene

To determine the potential effect of the missense polymorphism, rs3818822 (Gly102Arg), we used the SIFT and PolyPhen algorithms (http://sift.jcvi.org and http://genetics.bwh.harvard.edu/pph/), programs that predict the consequence of a polymorphism based on sequence homology and the physical properties of the amino acids.

AMCase protein levels

SDS-PAGE for AMCase in bronchoalveolar lavage (BAL) fluid had been previously performed (Seibold 2008) and using these data AMCase protein levels in BAL were examined between the GG (n = 18) and AG (n = 5) genotypes (subjects with AA genotype were not available).

Chitinase activity

To determine the effect of rs3818822 on chitinase activity, we used data from a chitinase activity assay that was previously performed (Seibold 2008). Chitinase activity levels were examined between the GG (n = 33) and AG/AA (AG = 9; AA = 1) genotypes.

Statistical analyses

Agreement of the genotype distributions with Hardy-Weinberg equilibrium in the LHS samples was assessed using a χ^2 goodness-of-fit analysis. The LHS subjects were then stratified into Caucasian and African American populations for further analyses (other ethnicities were excluded due to small sample sizes). The two outcomes used were mean baseline and rate of decline of post-bronchodilator FEV_1, expressed as a percent of predicted value. Statistical analyses were performed by multivariate linear regression analysis. Age, sex, and smoking history (pack-years) were adjusted for when performing association analyses to determine the influence of each gene variant on the outcomes. The *JMP 5.1* statistical software package (SAS Institute Inc., Cary, NC, USA) was used for analysis of the relationship between the genetic variants and the measures of lung function in the LHS. Analysis of the additive model in the LHS data was done using the SimHap package (Carter 2008). For the AMCase protein levels and chitinase activity analyses, an unpaired 2-tailed student's t-test with Welch correction was performed. A p-value < 0.05 was considered significant.

RESULTS

Analysis of Hardy-Weinberg equilibrium

All four SNPs were in Hardy-Weinberg equilibrium (rs3818822, p-value = 0.625; G339T, p-value = 0.663; rs2494303, p-value = 0.996; rs4950928, p-value = 0.995).

CHIA polymorphism rs3818822

Genotyping of rs3818822 was successful for 4025 LHS subjects (3879 Caucasians; 129 African Americans; 17 Hispanics excluded). In the Caucasian population, there was a significant decrease in baseline lung function associated with the AA genotype compared with the GG genotype (p = 0.0291). The AA genotype was associated with almost 3 % decrease in baseline FEV_1 compared with the GG + AG genotypes combined (p = 0.0285). No significant association was observed in the African American population, as shown in Table 4. Furthermore, no significant association was observed with rate of decline of FEV_1. An association study of this SNP was then performed in the four case-control populations; however no significant associations were observed (Table 5).

CHIA polymorphism G339T

Genotyping of G339T was successful for 3407 LHS subjects (3280 Caucasians; 114 African Americans; 13 Hispanics excluded). This variant was associated with the rate of decline of FEV_1 in African American subjects (p = 0.0021). As shown in Table 4, a slower rate of decline in lung function was associated with the GT genotype compared with the GG genotype (p = 0.0050). The TT genotype was associated with a significant increase in rate of decline in lung function compared to the GG genotype (p = 0.0005). This result remained significant (p = 0.008) after Bonferroni correction for multiple comparisons (4 SNPs and 4 outcomes). No significant association of the SNP was detected with baseline FEV_1 or rate of decline of FEV_1 in the Caucasian population.

CHIT1 polymorphism rs2494303

SNP rs2494303 was found to be in perfect linkage disequilibrium with the 24 bp duplication (r^2=1) and was chosen for further study. Subsequently, 281 LHS participants who had been previously genotyped for the duplication were genotyped for rs2494303 to validate the level of linkage disequilibrium with the duplication. We confirmed the strong linkage disequilibrium between the 24 bp duplication and rs2494303 (D' = 1.0, r^2 = 0.974) and this SNP was used to genotype the remaining LHS samples. Genotyping of rs2494303 was successful for 3802 subjects (3661 Caucasians; 126 African Americans; 15 Hispanics excluded). The variant was strongly associated with rate of decline of FEV_1 in the Caucasian population (p = 0.0083). In particular, a decrease in rate of lung function decline was associated with the AC genotype (heterozygous for duplication) compared to the CC genotype (p = 0.0021). Conversely, a faster rate of decline in lung function was associated with the AA genotype (homozygous for duplication) compared with the CC genotype, as shown in Table 4 (p = 0.0121). In the African American population, the AC genotype was associated with a faster rate of decline in lung function compared with the CC genotype (p = 0.0075). No significant associations were observed with baseline FEV_1 in either population. An association study of this SNP was then performed in the four case-control populations; however no significant associations with casecontrol status (Table 5) or FEV_1 levels (data not shown) were observed.

CHI3L1 polymorphism rs4950928

Genotyping of rs4950928 was successful for 3966 LHS subjects (3829 Caucasians; 121 African Americans; 16 Hispanics excluded). We did not detect any association of the rs4950928 polymorphism with baseline FEV_1 or rate of decline of FEV_1 in either population.

Potential effect of missense polymorphism rs3818822

The *CHIA* gene variant rs3818822 is a missense polymorphism that results in a glycine to arginine change. The SIFT and PolyPhen algorithms predicted that the glycine to arginine substitution may damage protein function due to its close contact with the functional site and the distinct changes in charge and hydrophobicity which could potentially affect folding of the protein.

AMCase protein levels

AMCase protein levels were examined between subjects with the GG and AG genotypes for rs3818822. It was determined that subjects with the GG genotype had higher BAL AMCase protein by over 2.4-fold compared with subjects who had the AG genotype, as shown in Figure 1A (p = 0.0209).

Chitinase activity

Chitinase activity was determined in subjects with GG and AG/AA genotypes for rs3818822. As shown in Figure 1B, it was found that subjects with the GG genotype had a higher BAL chitinase activity by over 4-fold compared with subjects who had the AG/AA genotypes ($p = 0.0475$).

DISCUSSION

CHIA codes for the human acid mammalian chitinase. In this study we showed that the *CHIA* gene variant rs3818822 was significantly associated with baseline FEV_1 in the LHS cohort. Specifically, smokers with the AA genotype had a lower baseline FEV_1 compared to smokers with GG genotype and GG + AG genotypes combined. Previously, this SNP has been associated with asthma in adults and children, with the G allele associated with protection against asthma (Bierbaum 2005). The rs3818822 gene variant is a missense polymorphism that results in an amino acid change, Gly102Arg. This variation is located in close proximity to the AMCase active site. However, the functional effect of rs3818822 has yet to be examined. Our hypothesis that the Gly102Arg substitution may affect the functionality of the AMCase protein is supported by *in silico* analysis using the SIFT and PolyPhen algorithms which predicted the polymorphism to be detrimental to the function of the protein. Furthermore, this study has found that the GG genotype was associated with higher AMCase protein levels and chitinase activity in BAL.

A previous study has documented that a splice variant of the AMCase protein is detectable in the lung; however, this variant is lacking exon 6, which contains the conserved active site residues required for enzymatic activity of the protein (Seibold 2008). Since rs3818822 is located near the active site, one can postulate that the Arg102 isoform may have a similar effect as the splice variant described in the previous study, i.e., result in a lack of catalytic activity of the protein. It is important to note, however, that the chitin-binding domain is located in exon 12, away from the active site. Thus, one can suggest that if the Arg102 isoform conserves its ability to bind chitin, the AMCase-chitin binding complex may act as a "chi-lectin" to modulate the immune response. It is possible that the complex stimulates pro-inflammatory responses, leading to lung function impairment in COPD. The idea of chitin as a potential immunological adjuvant has been explored in previous studies (Da Silva 2010). An alternative hypothesis is that the Gly102Arg change alters the tertiary structure of the protein and thus modifies chitin binding, despite being located far from the chitin-binding domain in the amino acid sequence. Further research is needed to differentiate between these hypotheses. Nonetheless, our present study shows a genetic association between an AMCase polymorphism and level of lung function in the LHS cohort.

The fact that the rs3818822 gene variant was associated with baseline FEV_1 but not rate of decline in FEV_1 suggests that AMCase may not contribute to the progression of lung function decline, but rather may have a role in lung development or early impairment of lung function. A comparison with a matched cohort of healthy subjects is required to complement this observation. The other *CHIA* gene variant examined was the G339T located in the fourth exon. This study illustrated that this SNP is associated with the rate of decline in FEV_1 in African Americans. The minor T allele of this SNP was found to be associated with protection against asthma among African American subjects (Seibold 2009). The presence of a single T allele was shown to be associated with a slower rate of decline in lung function compared with the GG genotype. The effect was not consistent in subjects with the TT genotype, however the sample size ($n = 5$) was low in this analysis.

CHIT1 encodes for the human chitinase, chitotriosidase. Unlike AMCase, this chitinase has previously been implicated in COPD. It was observed that CHIT1 is responsible for

chitinolytic activity in the lung and is elevated in the lungs of patients with COPD (Létuvé 2010). Furthermore, the 24-bp duplication allele results in a nonfunctional protein and is associated with a lack of CHIT1 activity (Seibold 2008). In this study we found an association between a genetic variant in *CHIT1* and the rate of change in lung function. Smokers in the LHS cohort who were homozygous for a SNP which is in linkage disequilibrium with the duplication had a faster rate of decline in FEV_1. This implies that smokers without a functional isoform of CHIT1 may develop a faster rate of decline of their lung function. Thus, the study suggests an active form CHIT1 may be protective against rapid disease progression in smokers who develop COPD. This duplication has been examined in coronary artery disease and asthma, however no association was found (Piras 2007).

CHI3L1 encodes for the chitinase-like protein, YKL-40. The rs4950928 gene variant was shown to be associated with reduced lung function in asthmatics, as well as with schizophrenia (Ober 2008; Zhao 2007); however, in this study we found no significant association with the level or rate of decline in lung function in smokers who had mild/moderate COPD. Since all of the participants in the LHS were smokers, it is possible that this strong environmental factor may have overwhelmed the effect of the rs4950928 polymorphism. This SNP is located in the core promoter of *CHI3L1*, within the binding site for the MYC and MAX transcription factors. The G allele is known to disrupt binding of these transcription factors, resulting in reduced transcription and lower mRNA levels, thus reducing levels of the YKL-40 protein (Zhao 2007).

Even though we were not able to replicate these associations with COPD affection status in four COPD case-control populations, we were able to demonstrate genetic association of chitinase polymorphisms with measures of baseline lung function and lung function decline in the COPD patients from the LHS cohort. One can suggest that the associations in the LHS represent false positive results; however a limited number of polymorphism were tested. The inconsistent results may be a result of the different demographics of the cohorts involved; for instance, the LHS participants were considerably younger than the subjects in the other cohorts. It is possible that the chitinase polymorphisms are risk factors early in the pathogenesis of COPD but their influence could be more difficult to detect in the later stages of the disease when the cumulative level of exposure to environmental factors (such as cigarette smoke) is larger. Another potential explanation for these results is that chitinase polymorphisms could influence lung function decline and/or severity in COPD subjects without influencing overall COPD susceptibility.

In the LHS, the associations of *CHIA* G339T with rate of decline of FEV_1 in the African Americans and *CHIT1* rs2494303 with rate of decline of FEV_1 in the Caucasians demonstrated that the values for heterozygotes were significantly lower (or greater) than those for both homozygous classes. As there is no obvious biological rationale for this observation, these data must be viewed with caution until replicated in independent data sets.

Chitin has been shown to act as an immunological adjuvant by stimulating the production of various cytokines and chemokines (Lee 2008). This suggests that chitinases such as CHIT1 may play a role in modulating the local and/or circulating concentration of chitins in the body and regulating the immune response to this common polysaccharide. Theoretically, when exogenous chitin from sources such as fungi or dust mites is present in the lungs, CHIT1 acts by cleaving chitin which subsequently could prevent chitin from stimulating immune responses. Therefore, one can speculate that without active CHIT1, an accumulation of chitin may be present in the lungs which could initiate an exaggerated pro-inflammatory response. This in turn could contribute to lung inflammation and ultimately to

the onset and progression of COPD. Prior studies that have shown associations with chitinases and lung function were among subjects with asthma. The differences observed in this study may be due to the different disease mechanisms between asthma and COPD. The method by which smoking causes the activation of chitinases is unclear. One hypothesis is that chitin particles are inhaled in tobacco smoke, due to fungal infection of the tobacco leaf (Verweij 2000) and this may initiate the action of chitinases in the lungs.

In this study we have demonstrated genetic associations between chitinase gene variants and lung function level and rate of decline in COPD patients from the LHS. In addition, a functional effect of the rs3818822 polymorphism on AMCase levels and activity was demonstrated. Although a significant effect of chitinase gene alleles was found in the LHS, we were not able to replicate the associations with COPD affection status in other COPD study populations. Therefore, we propose that chitinases may play a role in COPD disease risk in specific populations; however, more research is warranted to further clarify the precise function of chitinases in lung disease.

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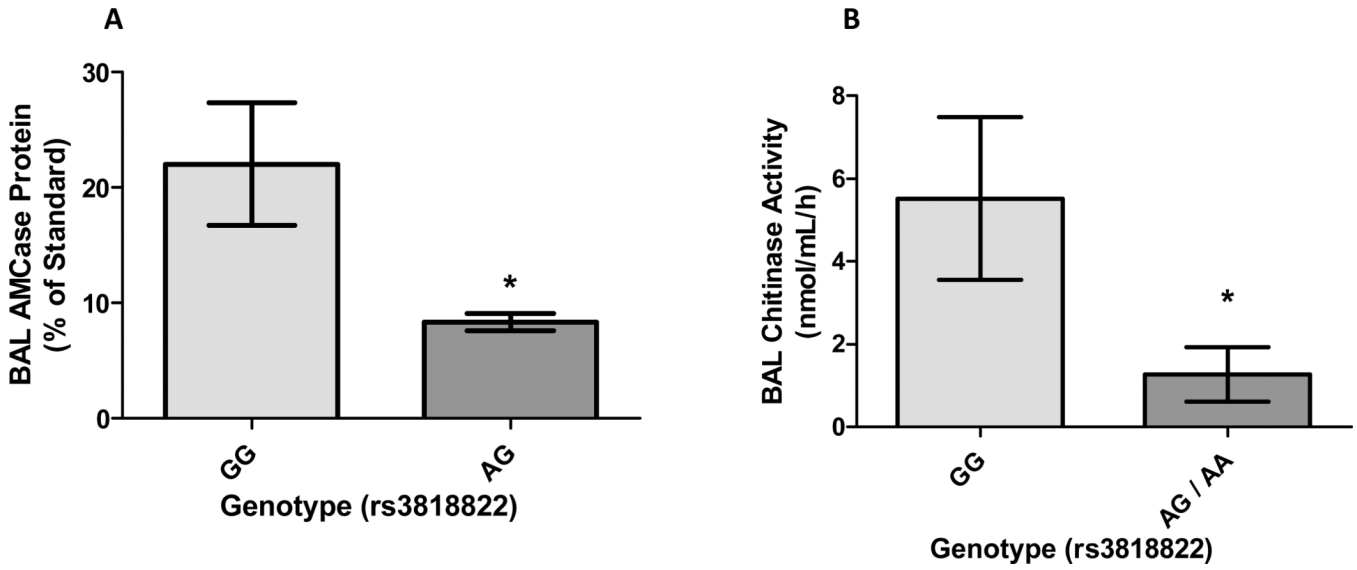

Figure 1.

(A) Effect of rs3818822 on bronchoalveolar lavage AMCase protein levels (± SEM) in subjects with the GG genotype (n = 18) and the AG genotype (n = 5); * $P < 0.05$ compared with the GG genotype. (B) Effect of rs3818822 on bronchoalveolar lavage chitinase activity (± SEM) in subjects with the GG genotype (n = 33) and the AG/AA genotypes (n = 10); * $P < 0.05$ compared with the GG genotype.

Table 1

Characteristics of the Lung Health Study participants

Characteristic	Male	Female	Total
Number of participants	2736	1608	4344
Age (mean ± SD), years	48.4 ± 6.9	48.7 ± 6.5	48.5 ± 6.8
Smoking history (mean ± SD), pack-years[a]	42.4 ± 19.3	35.9 ± 15.9	40.0 ± 18.4
Baseline FEV_1 post-bronchodilator (mean ± SD), % predicted[b]	78.63 ± 9.12	78.24 ± 9.11	78.48 ± 9.12
FEV_1 post-bronchodilator rate of decline (mean ± SD), % predicted/year[c]	−0.906 ± 1.75	−1.083 ± 1.92	−0.972 ± 1.81

[a] Number packs of cigarettes smoked per day × number of years of smoking.

[b] Lung function at the start of the study measured as forced expiratory volume in 1 second (FEV_1).

[c] Mean change in lung function per year over a five-year period measured as forced expiratory volume in 1 second (FEV_1).

Table 2

Characteristics of each case-control replication study group

Characteristic	COPDGene		ECLIPSE		NETT/NAS		GenKOLs	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Number of participants	499	501	1764	178	373	435	863	808
Age (mean ± SD), years	64.77 (8.12)	60.2 (8.66)	63.63 (7.10)	57.48 (9.44)	67.47 (5.78)	69.8 (7.49)	65.53 (10.03)	55.62 (9.71)
Smoking history (mean ± SD), pack-years[a]	54.76 (26.69)	38.87 (21.07)	50.29 (27.42)	32.11 (24.84)	66.43 (30.68)	40.66 (27.85)	31.98 (18.46)	19.66 (13.58)
FEV_1 (mean ± SD), % predicted[b]	48.73 (18.41)	97.98 (11.32)	47.63 (15.62)	107.83 (13.56)	28.12 (7.38)	99.97 (13.2)	50.63 (17.44)	94.91 (9.24)
Sex (% Male)	49.5%	50.1%	67.0%	57.9%	63.8%	100%	60.1%	50.1%

[a] Number packs of cigarettes smoked per day × number of years of smoking.

[b] Lung function measured as forced expiratory volume in 1 second (FEV_1).

Table 3

Description of the polymorphisms studied

Gene	Protein	SNP	Allele change	Location/amino acid change	Clinical Association
CHIA	Acid mammalian chitinase	rs3818822 G339T	G => A G => T	Gly102 => Arg Arg61 => Met	Associated with asthma (Bierbaum 2005; Seibold 2009)
CHIT1	Chitotriosidase	rs2494303	C => A	Intron	High linkage disequilibrium with 24-bp duplication that was associated with reduced chitinase activity in the lungs of smokers (Seibold 2008)
CHI3L1	YKL-40	rs4950928	C => G	5’ untranslated region	Associated with asthma (Ober 2008)

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Table 4

Genotype frequencies of chitinase polymorphisms in the LHS cohort and their associations with lung function

Caucasians										
Gene	**Polymorphism**	**Genotype**	**N**	**Baseline FEV_1[a]**	**P value**	**P value Additive model**	**N**	**Rate of FEV_1 Decline[b]**	**P value**	**P value Additive model**
CHIA	rs3818822	GG	3112 (80%)	78.64 ± 0.16	Reference	0.3124	3407 (82%)	−0.96 ± 0.03	Reference	0.6681
		AG	715 (18%)	78.61 ± 0.35	0.0521		709 (17%)	−1.01 ± 0.07	0.7526	
		AA	51 (1%)	76.01 ± 1.10	**0.0291**		49 (1%)	−0.94 ± 0.21	0.8993	
CHIA	G339T	GG	2493 (76%)	78.40 ± 0.18	Reference	0.8344	2448 (76%)	−0.97 ± 0.04	Reference	0.6766
		GT	728 (22%)	78.52 ± 0.34	0.7279		715 (22%)	−1.05 ± 0.07	0.2743	
		TT	58 (2%)	78.01 ± 1.16	0.8179		54 (2%)	−0.83 ± 0.24	0.4065	
CHIT1	rs2494303	CC	2328 (65%)	78.32 ± 0.18	Reference	0.0605	2328 (65%)	−1.02 ± 0.04	Reference	0.7693
		AC	1128 (31%)	78.99 ± 0.27	0.6554		1128 (31%)	−0.88 ± 0.05	**0.0021**	
		AA	132 (4%)	79.13 ± 0.82	0.5729		132 (4%)	−1.35 ± 0.16	**0.0121**	
CHI3L1	rs4950928	CC	2466 (64%)	78.52 ± 0.18	Reference	0.3671	2420 (64%)	−0.98 ± 0.04	Reference	0.6294
		CG	1216 (32%)	78.75 ± 0.25	0.9689		1193 (32%)	−0.95 ± 0.05	0.971	
		GG	145 (4%)	78.84 ± 0.76	0.67		144 (4%)	−0.94 ± 0.15	0.8293	
African Americans										
Gene	**Polymorphism**	**Genotype**	**N**	**Baseline FEV_1[a]**	**P value**	**P value Additive model**	**N**	**Rate of FEV_1 Decline[b]**	**P value**	**P value Additive model**
CHIA	rs3818822	GG	84 (65%)	76.87 ± 1.05	Reference	0.7405	83 (64%)	−1.25 ± 0.28	Reference	0.1490
		AG	43 (33%)	76.40 ± 1.27	0.8731		42 (33%)	−0.53 ± 0.39	0.1476	
		AA	2 (2%)	74.30 ± 4.90	Excluded		2 (2%)	−0.53 ± 0.55	Excluded	
CHIA	G339T	GG	85 (75%)	76.25 ± 1.00	Reference	0.5498	83 (74%)	−0.92 ± 0.24	Reference	**0.0348**
		GT	24 (21%)	76.31 ± 1.89	0.2096		24 (21%)	−0.71 ± 0.42	**0.005**	
		TT	5 (4%)	80.44 ± 3.73	0.169		5 (4%)	−4.89 ± 3.28	**0.0005**	
CHIT1	rs2494303	CC	108 (87%)	76.50 ± 0.87	Reference	0.7211	108 (87%)	−0.82 ± 0.21	Reference	**0.0222**
		AC	15 (12%)	78.18 ± 2.26	0.6923		15 (12%)	−2.57 ± 1.14	**0.0075**	
		AA	1 (1%)	62.3	Excluded		1 (1%)	−0.35	Excluded	
CHI3L1	rs4950928	CC	52 (43%)	75.92 ± 1.45	Reference	0.5775	52 (43%)	−0.93 ± 0.48	Reference	0.3990

		CG	52 (43%)	76.60 ± 1.23	0.8456		52 (43%)	−0.98 ± 0.21	0.6813
		GG	17 (14%)	77.54 ± 2.02	0.5864		16 (13%)	−1.54 ± 0.39	0.3694

[a] Lung function at the start of the study measured as forced expiratory volume in 1 second (FEV_1) % of predicted (± standard error).

[b] Mean change in lung function per year over a five-year period measured as FEV_1 % of predicted (± standard error).

Table 5

Genetic association of *CHIT1* and *CHIA* SNPs with COPD affection status in each COPD case-control study

Gene	Polymorphism	Genotype	COPDGene Effect (SE) P-value	ECLIPSE Effect (SE) P-value	NETT/NAS Effect (SE) P-value	GenKOLs Effect (SE) P-value	Meta-Analysis Effect (SE) P-value	Meta-Analysis Additive Model Effect (SE) P-value
CHIT1	rs2494303	CC	Reference	Reference	Reference	Reference	Reference	0.05 (0.06) 0.42
		AC	−0.16 (0.36) 0.67	0.13 (0.41) 0.74	0.54 (0.45) 0.22	−0.16 (0.30) 0.60	0.02 (0.18) 0.92	
		AA	0.06 (0.15) 0.69	0.00 (0.18) 0.99	0.08 (0.19) 0.69	0.15 (0.13) 0.25	0.08 (0.08) 0.28	
CHIA	rs3818822	GG	Reference	Reference	Reference	Reference	Reference	−0.07 (0.08) 0.41
		AG	−1.14 (0.70) 0.10	0.09 (0.77) 0.91	0.63 (0.73) 0.39	0.80 (0.83) 0.33	0.02 (0.38) 0.96	
		AA	−0.33 (0.17) 0.05	0.03 (0.03) 0.91	0.03 (0.23) 0.77	−0.01 (0.15) 0.94	−0.08 (0.09) 0.36	