Associations of *IL6* polymorphisms with lung function decline and COPD

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ABSTRACT

Background: Interleukin-6 (IL6) is a pleiotropic pro-inflammatory and immunomodulatory cytokine which probably plays an important role in the pathogenesis of chronic obstructive pulmonary disease (COPD). There is a functional single nucleotide polymorphism (SNP), -174G/C, in the promoter region of *IL6*. It was hypothesised that *IL6* SNPs influence susceptibility for impaired lung function and COPD in smokers.

Methods: Seven and five SNPs in *IL6* were genotyped in two nested case-control samples derived from the Lung Health Study (LHS) based on phenotypes of rate of decline of forced expiratory volume in 1 s (FEV_1) over 5 years and baseline FEV_1 at the beginning of the LHS. Serum IL6 concentrations were measured for all subjects. A partially overlapping panel of nine *IL6* SNPs was genotyped in 389 cases of COPD from the National Emphysema Treatment Trial (NETT) and 420 controls from the Normative Aging Study (NAS).

Results: In the LHS, three *IL6* SNPs were associated with decline in FEV_1 ($0.023 \leqslant p \leqslant 0.041$ in additive models). Among them, the *IL6*_-174C allele was associated with a rapid decline in lung function. The association was more significant in a genotype-based analysis ($p = 0.006$). In the NETT-NAS study, *IL6*_-174G/C and four other *IL6* SNPs, all of which are in linkage disequilibrium with *IL6*_-174G/C, were associated with susceptibility to COPD ($0.01 \leqslant p \leqslant 0.04$ in additive genetic models).

Conclusion: The results suggest that the *IL6*_-174G/C SNP is associated with a rapid decline in FEV_1 and susceptibility to COPD in smokers.

Interleukin 6 (IL6) is a pleiotropic pro-inflammatory and immunomodulatory cytokine secreted by airway epithelial cells, alveolar macrophages, adipocytes and myocytes as well as other tissues and cells.[1 2] The potential importance of IL6 in the pathogenesis of chronic obstructive pulmonary disease (COPD) is suggested by studies showing that high levels of serum or sputum IL6 are associated with impaired lung function or a faster decline in lung function.[1 2] IL6 has been related to skeletal muscle weakness in COPD,[3] as well as to exacerbations[4] and pulmonary infections[5] in patients with COPD. In addition, overexpression of IL6 in the murine lung resulted in airway inflammation and emphysema-like airspace enlargement.[6] Furthermore, IL6 is an important mediator of the acute phase response and can upregulate C-reactive protein (CRP) at the transcriptional level.[7] CRP has been associated with lung function levels in healthy individuals and/or lung function decline in smoking-induced COPD.[8 9] Taken together, these data support *IL6* as an appealing candidate gene for smoking-induced lung function impairment and COPD.

The *IL6* gene is located on chromosome 7p21. Previous studies have identified a functional single nucleotide polymorphism (SNP), -174G/C, in the promoter region of *IL6*.[10] Before initiation of the current study, a small study reported no association of an *IL6* SNP with COPD.[11] Recently, another group showed that the IL6_-572C allele was associated with COPD.[12] Large well-designed studies with carefully defined COPD phenotypes are required to unravel the exact role of *IL6* genetic variants in the pathogenesis of COPD.

We investigated smokers with mild to moderate airflow obstruction who were participants in the Lung Health Study (LHS) cohort and hypothesised that there would be significant associations between SNPs and haplotypes in *IL6* with the rate of decline and/or the level of lung function, and that these associations would be mediated by influencing IL6 serum concentrations. The LHS cohort provides an excellent opportunity to explore associations between gene polymorphisms and haplotypes with percentage predicted forced expiratory volume in 1 s (FEV_1),[13 14] as well as the rate of decline in FEV_1.[15 16] To validate novel associations between *IL6* SNPs with lung function phenotypes, replication of results was sought in cases of COPD from the National Emphysema Treatment Trial (NETT) with participants from the Normative Aging Study (NAS) serving as controls.[17 18]

METHODS

Study participants

LHS participants

A total of 1488 subjects were selected from approximately 4800 LHS subjects for whom DNA and serum were available. The selection generated two nested case-control studies based on the extremes of rate of decline in lung function and baseline lung function. In the decline of lung function study we selected the 266 and 293 non-Hispanic white subjects with the fastest and slowest rate of decline of lung function, respectively, during the 5-year follow-up period (arbitrary cut-off points of $\geqslant$3.0% predicted decrease/year and $\geqslant$0.4% predicted increase/year in FEV_1 were used for rapid decliners and non-decliners, respectively). The rationale to select nested case-control studies with the indicated sample sizes is that (1) this approach has the advantage of

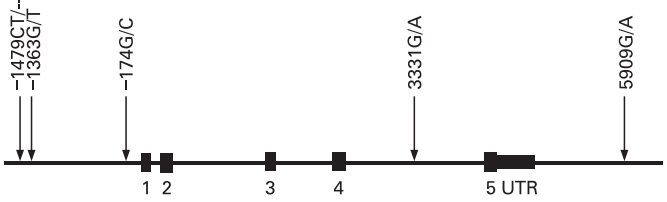

Figure 1 The *IL6* gene structure and position of single nucleotide polymorphisms genotyped in subjects in the Lung Health Study. Numbered regions represent exons. A, adenine; C, cytokine; G, guanine; T, thymidine; UTR, untranslated region.

reducing cost while keeping satisfactory statistical efficiency when compared with the full cohort approach;[19 20] (2) the Common Disease/Common Variants hypothesis (CD/CV) was suggested one decade ago which states that disease susceptibility alleles of common diseases will be present at high frequencies;[21] and (3) this sample size has relatively adequate power to detect common genetic risk variants as shown in our previous power analyses.[22] The baseline lung function study consisted of the 532 and 527 participants who had the highest and lowest baseline percentage predicted FEV_1, respectively (arbitrary cut-off points of $\geqslant$88.9% and $\leqslant$67.0% predicted were used for the high and low baseline groups, respectively). One hundred and thirty participants overlapped between the two sets of nested cases and controls owing to the fact that subjects in the rate of decline study group had baseline lung function within one of the categories for baseline lung function.

NETT-NAS participants

We selected 389 non-Hispanic white subjects who were enrolled in the NETT Genetics Ancillary Study. The control group was composed of 420 participants with normal spirometry from the NAS, a longitudinal study over the past four decades of healthy adult men that was initiated by the Boston Veterans Administration. More information on the participants is included in the online supplement.

TagSNP selection and genotyping methods

In the LHS, five tagSNPs were chosen from the SeattleSNPs database using the LDSelect program based on a relatively stringent linkage disequilibrium (LD) threshold of $r^2 \geqslant 0.8$ and minor allele frequency cut-off of 10%. An additional two SNPs selected for the NETT-NAS study were subsequently chosen for genotyping in the decline of lung function study in order to make the two studies more comparable. The nomenclature for the polymorphisms used in the study is summarised in table E1 in the online supplement. SNP genotyping was performed using the TaqMan method (Applied Biosystems, Foster City, California, USA) for five tagSNPs and the Illumina Bead Array System Illumina Inc, San Diego, California, USA for the additional two SNPs. The positions of the selected and successfully genotyped five tagSNPs are shown in fig 1.

In the NETT-NAS, the same criteria were used to select six LD-tagging *IL6* SNPs and three additional *IL6* SNPs were also selected for genotyping. The SNPs were genotyped on an Illumina BeadStation 500G System Illumina Inc using the GoldenGate assay technology (Illumina Golden Gate Assay, San Diego, California, USA). SNP selection criteria are shown in more detail in the online supplement.

Measurements of serum IL6 concentration in LHS participants

After collection the blood samples were separated into their various components and shipped to the LHS data coordinating centre on dry ice and kept at −70°C until use. The serum samples were thawed once for IL6 measurements. The concentrations of IL6 were measured using a highly sensitive chemiluminescent multiplexed sandwich immunoassay (SearchLight Proteome Array System, Rockford, Illinois, USA).

Statistical analysis

In the LHS, Hardy-Weinberg equilibrium tests and LD estimations were calculated using the genetics package for R (www.r-project.org). Multiple logistic regressions for rate of decline and baseline lung function were performed to test for the association with *IL6* SNPs and with IL6 serum levels. Confounding factors included body mass index, age, gender, pack years of smoking and smoking status. Multiple linear regression was performed for the complete data set to test for association of *IL6* SNPs with log IL6 serum levels. Haplotype analysis was done using the R hapassoc package. In the NETT-NAS, similar analyses were performed with SAS Genetics (Cary, North Carolina, USA). The statistical analysis is described in more detail in the online supplement.

RESULTS

Characteristics of study participants

In the total of 1488 participants from the LHS, genotyping success rates were 96.4–98.6% for the five studied *IL6* tagSNPs in all subjects and 97.9% for the additional two SNPs in the rate of decline study. The demographic characteristics are shown in table 1.

There were significant differences in several potential confounding factors such as age, gender, pack years of smoking and smoking status between study groups. Multiple regressions were therefore performed to adjust for relevant confounding factors.

In the total of 809 participants in the NETT-NAS, the genotype call rate for *IL6*_615A/G (rs2069832) was 85%; for all other SNPs the call rates were $\geqslant$97%. The demographic characteristics for the study groups are shown in table 2.

Linkage disequilibrium (LD) pattern, Hardy-Weinberg disequilibrium and performance of tagSNPs

The LD pattern of the five *IL6* tagSNPs in the full set of 1488 LHS study participants is shown in fig 2A. The r^2 values ranged from 0.04 to 0.89. It is worth noting that the r^2 values between *IL6*_-1479 (rs2069825) and *IL6*_-174 (rs1800795), as well as *IL6*_3331 (rs2069845) and *IL6*_-174, were greater than 0.86, which indicates that it is necessary to genotype only one of these three SNPs. The LD patterns of the low and high lung function subgroups were similar to that of all subjects (data not shown), as were those of the fast-declining and non-declining subgroups; the LD pattern of all seven SNPs genotyped in fast-declining and non-declining subgroups are shown in fig 2B. All the studied SNPs were in Hardy-Weinberg equilibrium. More information on performance of tagSNPs is included in the online supplement.

Associations of SNPs and haplotypes in the *IL6* gene with rate of decline and baseline FEV_1

Three of seven *IL6* SNPs were associated with FEV_1 decline ($0.023 \leqslant p \leqslant 0.041$ in additive genetic models; table 3). The well-known functional SNP *IL6*_-174G/C (rs1800795) was among

Table 1 Distribution of demographic characteristics for all subjects and those in the two nested case-control study groups in the Lung Health Study (LHS)

		Rate of decline study			Baseline lung function study		
	All participants (N = 1488)	Fast decliners (n = 266)	Non-decliners (n = 293)	p Value	High function (n = 532)	Low function (n = 527)	p Value
Gender (M/F)	948/540	158/108	197/96	N/A	352/180	325/202	N/A
Age (years)	48.41 (0.18)	49.47 (0.40)	47.48 (0.40)	<0.001	46.21 (0.77)	50.76 (0.26)	<0.001
Smoking history (pack-years)*	40.41 (0.48)	43.23 (1.18)	38.35 (1.06)	0.002	35.33 (0.13)	45.24 (0.81)	<0.001
Smoking status during 5-year follow-up†							
Continuing smokers	979	266	293	N/A	264	286	N/A
Intermittent quitters	315	N/A	N/A	N/A	157	158	N/A
Sustained quitters	194	N/A	N/A	N/A	111	83	N/A
BMI (kg/m^2)	25.49 (0.10)	25.22 (0.25)	25.77 (0.21)	0.091	25.39 (0.15)	25.57 (0.18)	0.455
ΔFEV_1/year (% predicted pre-BD)‡	−0.98 (0.05)	−4.13 (0.07)	1.09 (0.04)	<0.001	−0.55 (0.07)	−1.22 (0.08)	<0.001
ΔFEV_1/year (% predicted post-BD)§	−0.85 (0.04)	−3.44 (0.08)	0.70 (0.05)	<0.001	−0.75 (0.06)	−0.74 (0.08)	0.949
Baseline FEV_1 (% predicted pre-BD)¶	74.15 (0.30)	72.68 (0.54)	75.51 (0.47)	<0.001	86.49 (0.13)	61.07 (0.18)	<0.001
Baseline FEV_1 (% predicted post-BD)**	77.57 (0.34)	75.00 (0.56)	79.80 (0.47)	<0.001	91.81 (0.10)	62.57 (0.14)	<0.001
Median (IQR) IL6 (pg/ml)	2.60 (1.80–4.20)	2.75 (2.00–4.03)	2.70 (1.70–4.50)	0.184	2.50 (1.70–3.90)	2.70 (1.85–4.55)	0.12

Values are mean (SEM) for continuous data.
BD, bronchodilator; BMI, body mass index; FEV_1, forced expiratory volume in 1 s; IL6, interleukin 6.
*Number of packs of cigarettes smoked per day × number of years smoking.
†Continuing smokers: participants who reported smoking at each annual visit. Sustained quitters: participants who were validated by salivary cotinine or exhaled carbon monoxide levels as abstinent at every annual visit. Intermittent quitters: participants who were not sustained quitters or continuing smokers.
‡Change in lung function over a 5-year period per year as pre-bronchodilator percentage predicted FEV_1.
§Change in lung function over a 5-year period per year as post-bronchodilator percentage predicted FEV_1.
¶Lung function at the start of the Lung Health Study as measured by pre-bronchodilator FEV_1 percentage predicted.
**Lung function at the start of the Lung Health Study as measured by post-bronchodilator FEV_1 percentage predicted.

them. The frequency of the *IL6*_-174C allele was significantly higher in the group with rapid decline of FEV_1 than in the non-declining group (45.2% vs 39.6%, odds ratio (OR) 1.30, 95% CI 1.01 to 1.66, p = 0.041). The association was more significant in the genotype-based analysis (p = 0.006), with six out of seven SNPs reaching a significance level of p<0.05 (table 3).

It is worthwhile to note that the most significant association was found for the *IL6*_-1479CT in/del (rs2069825): the *IL6*_-1479CT deletion was associated with a rapid decline in FEV_1 (p<0.001, table 3). Three other *IL6 SNPs* were not significantly associated in the additive model but were significant in the genotype-based analysis (table 3). The *IL6*_-1479 CT in/del and another two associated *IL6* SNPs were in high LD with *IL6*_-174G/C. Interestingly, the *IL6*_5909G/A and *IL6*_1754C/G, which were not in high LD with *IL6*_-174G/C and not in high LD with each other ($r^2 = 0.52$), were also significantly associated with decline in lung function (table 3). No association was found for *IL6* haplotypes with rate of decline in FEV_1; *IL6* SNPs and haplotypes were not associated with the baseline level of FEV_1 (data not shown).

Table 2 Distribution of demographic characteristics for NETT COPD cases and NAS controls

Demographic characteristics	NETT (n = 389)	NAS (n = 420)	p Value
Age (years)	67 (6)	68 (9)	0.9
Pack-years	66 (30)	39 (27)	<0.001
FEV_1 (% predicted, post-BD)*	28 (7)	92 (11)	
Median (IQR) modified BODE score†	5 (3)	NA	
Gender (% male)	64%	100%	

Values are mean (SD) unless otherwise stated.
BD, bronchodilator; FEV_1, forced expiratory volume in 1 s; IQR, interquartile range; NAS, Normative Aging Study; NETT, National Emphysema Treatment Trial.
*FEV_1 percentage predicted values for the NETT and NAS are based on the prediction equations of Crapo *et al*;[36] however, the NAS-1988 standards were used in the selection of the control group.
†Modified BODE score incorporates the University of San Diego Shortness of Breath Questionnaire.

Associations of *IL6* SNPs and haplotypes with serum IL6 concentrations

The associations of *IL6* SNPs with serum IL6 concentrations were analysed in all subjects in the LHS for five tagSNPs and in subjects in the rate of decline study for two additional SNPs by linear regressions adjusted for BMI, age, gender, pack years of smoking and smoking status (table 4). No significant association was found for *IL6* SNPs with IL6 concentrations. *IL6* haplotypes were also not associated with IL6 concentrations (data not shown).

Associations of serum IL6 concentrations with rate of decline and baseline FEV_1

As shown in table 1, there were no significant differences in IL6 concentrations between the rapid decline and non-decline groups or between high and low FEV_1 groups.

Replication of novel *IL6* associations in NETT-NAS participants

In the LHS, *IL6* SNPs were significantly associated with rate of decline of FEV_1 in patients with mild COPD. Since rapid decline of lung function in smokers is the likely method of development of COPD, we reasoned that the same SNPs would be associated with advanced COPD. To test this we used a case-control sample that has been very useful in revealing genes associated

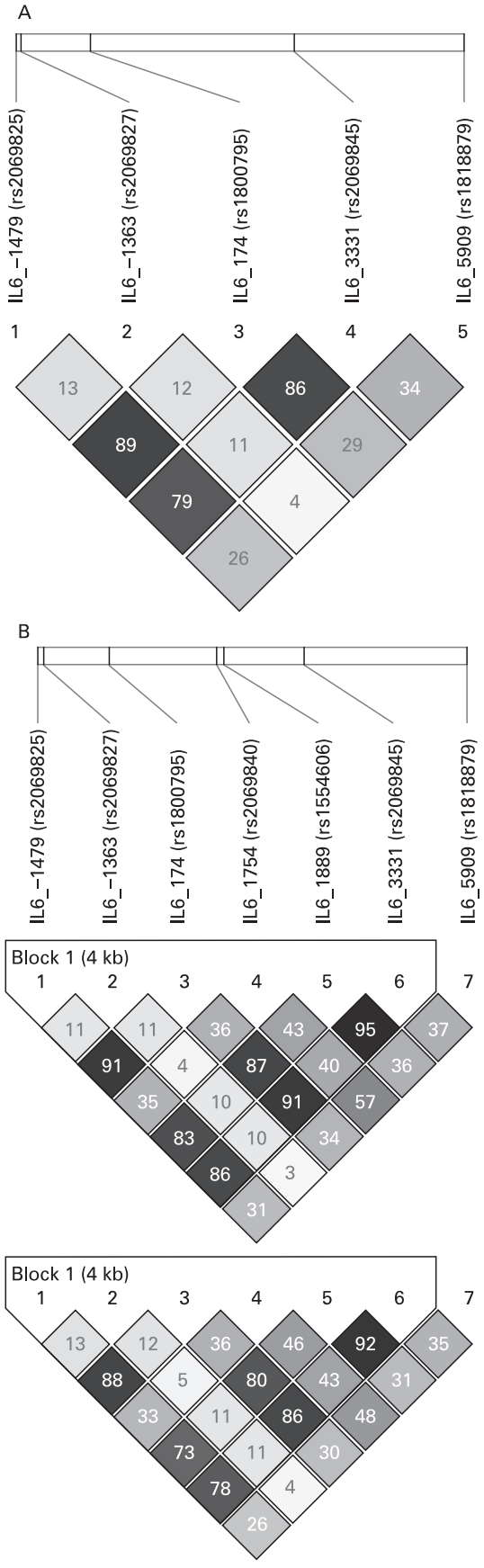

Figure 2 Linkage disequilibrium (LD) of single nucleotide polymorphisms (SNPs) of *IL6* in Lung Health Study subjects using HAPLOVIEW. The LD between any two SNPs is listed in the cross cell. The darker the colour, the higher the LD between any two SNPs. (A) All subjects. (B) Top: fast-declining group; bottom: slow-declining group.

with COPD.[17 18] In the NETT-NAS study, cases had advanced COPD requiring lung volume reduction surgery and controls were derived from a population of smokers who have not developed COPD. The *IL6*_-174G/C and another four *IL6* SNPs, which had high LD with *IL6*_-174G/C, were associated with susceptibility to COPD ($0.01 \leqslant p \leqslant 0.04$ in additive genetic models). The *IL6*_-174C allele was associated with susceptibility to COPD (OR 1.3, 95% CI 1.1 to 1.7, $p = 0.01$ in an additive genetic model). The frequency of the *IL6*_-174C allele was significantly higher in the NETT group than in the NAS group (42% vs 36%). The association was also significant in genotype-based analysis ($p = 0.03$, table 3).

DISCUSSION

Only three studies have been published on associations of *IL6* SNPs with COPD. Seifart *et al* reported that there was no association of *IL6*_-174 with COPD,[11] Broekhuizen *et al* did not find an association between *IL6*_−174 and a cachexia phenotype in subjects with COPD[23] and Córdoba-Lanús *et al* recently reported that *IL6*_-572 but not *IL6*_-174 was associated with COPD.[12] All three studies have relatively small sample sizes. The associations of *IL6* SNPs with decline in FEV_1 in the current study are novel and are the most significant findings among all the studies we have published using the LHS cohort.[13–16 24–27] To strengthen our initial finding in the LHS, we incorporated an association study of *IL6* SNPs with COPD in the NETT-NAS. All SNPs that were genotyped and in high LD with the *IL6*_-174G/C showed significant or borderline association with rapid decline of lung function in the LHS and with COPD in the NETT-NAS. We believe that the strength of the associations, the concordant results with several SNPs in high LD with the *IL6*_-174G/C SNP, the available previous functional data on *IL6*_-174G/C, the replication in a second population and the biological plausibility for association provide strong evidence that this is a true association.

We examined the association of *IL6* SNPs with IL6 serum levels as well as relationships between IL6 serum levels and lung function decline. We did not find any associations. We also found that adjusting the associations between *IL6* SNPs and lung function for serum CRP levels in the LHS had no effect on the strength of the associations (data not shown). Therefore, we did not find evidence that the associations we report were mediated through an influence on production of IL6 or CRP.

Studies that have examined the effects of *IL6* SNPs on IL6 mRNA and protein expression have led to conflicting results. The first reporter gene study demonstrated that a construct containing the -174G allele had higher reporter gene expression in HeLa cells, both under basal conditions and after LPS or IL1 stimulation.[10] However, a second reporter gene study showed that a construct containing -174C had higher IL1-induced expression in HeLa cells than that of the -174G construct, although the difference did not reach statistical significance.[28] By comparison of the two different cell types, the authors concluded that there is a cell type-specific regulation of IL6 expression.[28] Nine of the most recently published studies of *IL6* SNPs with circulating IL6 concentrations are summarised in table E2 in the online supplement. A recent meta-analysis of 5659 subjects from 17 studies concluded that the -174 IL6 SNP was not associated with circulating IL6 levels.[29] There are several explanations for the lack of consistent associations. First, the *IL6*_-174G/C polymorphism might not be a strong determinant of serum IL6 levels. Second, the serum half-life of IL6 is short. Serum IL6 levels show marked diurnal variability.[30] The blood samples for IL6 measurement in most studies, including our

Table 3 Associations of SNPs in *IL6* with rate of decline of FEV_1 in the LHS and association with COPD in the NETT-NAS

			LD (r^2 with rs1800795)		Rate of FEV_1 decline study in the LHS				COPD case-control study in the NETT-NAS			
Bin	SNP ID	SNP in gene	LHS	NETT-NAS	Fast decline MAF %	Non-decline MAF %	p Value‡ genotype based	p Value‡ additive	NETT MAF %	NAS MAF %	p Value§ genotype-based	p Value§ additive
1	rs1800797†	-598G/A	NA	0.93					0.41	0.35	0.06	0.02
1	rs1800795*†	-174G/C	–	–	0.45	0.40	0.006	0.041	0.42	0.36	0.03	0.01
1	rs2069832†	615A/G	NA	0.98					0.42	0.37	0.10	0.09
1	rs1474348†	1090G/C	NA	0.97					0.41	0.36	0.10	0.04
1	rs1474347†	1306G/T	NA	0.98					0.42	0.36	0.06	0.03
2	rs1554606*†	1889G/T	0.84	0.87	0.47	0.43	0.011	0.103	0.44	0.38	0.02	0.01
2	rs2069845*	3331G/A	0.89	NA	0.47	0.42	0.007	0.078				
3	rs2069825*	-1479CT/–	0.90	NA	0.44	0.37	<0.001	0.023				
4	rs2069840*†	1754C/G	0.36	0.33	0.32	0.37	0.005	0.064	0.35	0.36	0.30	0.70
5	rs1818879*	5909G/A	0.32	NA	0.29	0.34	0.012	0.035				
6	rs2069827*†	-1363G/T	0.12	0.14	0.08	0.08	0.786¶	0.669	0.09	0.09	0.90	0.40
Other	rs2069849†	4338C/T	NA	0.02					0.03	0.02	0.20	0.20

COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; LD, linkage disequilibrium; LHS, Lung Health Study; MAF, minor allele frequency; NAS, Normative Aging Study; NETT, National Emphysema Treatment Trial; SNP, single nucleotide polymorphism.
*Genotyped in the LHS.
†Genotyped in the NETT-NAS.
‡Adjustment for confounding factors such as age, gender, pack-years of smoking and research centre.
§As the NAS controls were uniformly male smokers with normal lung function, the models were adjusted for age and pack-years.
¶The p values were from a dominant genetic model because the minor allele frequency of this SNP was very low.

own, were not taken at a specific time of the day. A third explanation is that the SNPs studied may not be the actual functional SNPs. Recently, Samuel and colleagues have identified a novel *IL6* transcriptional regulatory region (−5307 to −5202) much farther from the transcription initiation site than *IL6*_-174.[31] This report coupled with more recent identification of a novel functional SNP, *IL6*_-6331T/C (rs10499563), with the T allele preferentially binding to Oct-1 transcription factor and producing higher reporter gene expression, provides evidence that additional functional SNPs do exist in *IL6*.[32] However, since IL6_-6331T/C is in low LD with IL6_-174, our finding is not likely to be explained by these new functional data.

If *IL6* SNPs are not related to IL6 levels, then what is the basis for their association with FEV_1 decline and COPD? One possible explanation is that the association is truly driven via local pulmonary IL6 expression or that it is driven by serum IL6 levels but that the variability and lability of serum IL6 levels obscures this relationship; FEV_1 may reflect the average

Table 4 Association of serum concentrations of interleukin 6 (IL6) and *IL6* genotypes (linear regression*)

		IL6 level (ln IL6 (pg/ml))		
SNP	Genotype	N	Coefficient (SE)	p Value
	II	514		
*IL6*_-1479	ID	681	−0.011 (0.046)	
(rs2069825)	DD	229	−0.080 (0.063)	0.424†
*IL6*_-1363	GG	1185		
(rs2069827)	GT+TT	234	0.000 (0.057)	0.997†
	GG	483		
*IL6*_-174	GC	691	0.013 (0.047)	
(rs1800795)	CC	255	−0.056 (0.061)	0.486†
	CC	234		
*IL6*_1754	CG	234	0.000 (0.048)	
(rs2069840)	GG	72	−0.030 (0.064)	0.820‡
	GG	169		
*IL6*_1889	GT	261	0.013 (0.044)	
(rs1554606)	TT	110	−0.057 (0.054)	0.542‡
	AA	431		
*IL6*_3331	AG	689	0.015 (0.049)	
(rs2069845)	GG	293	−0.020 (0.060)	0.819†
	AA	685		
*IL6*_5909	AG	593	0.012 (0.044)	
(rs1818879)	GG	137	−0.025 (0.074)	0.877†

SNP, single nucleotide polymorphism.
*p Values were from genotype-based analysis (co-dominant genetic models) except for *IL6*_-1363 where p values were from a dominant genetic model because the minor allele frequency for this SNP was very low.
†Adjusted for body mass index, age, gender, pack years of smoking and smoking status.
‡Adjusted for body mass index, age, gender and pack years of smoking.

IL6 levels and thus the degree of lung inflammation over the years of the study. In addition, the SNPs could influence IL6 levels and thus lung inflammation during exacerbations but not the constitutive levels during stable periods. IL6 is a pleiotropic cytokine which also modulates expression of many other genes.[33] It may be that it is the effect of the *IL6* variants on these genes that is the underlying mechanism for the associations we observed.

How can we explain the observation that *IL6* SNPs were not associated with baseline FEV_1 in the LHS but were associated with the presence of COPD in the NETT-NAS study? The mean age of the LHS participants was 48 years compared with 68 years for the participants in the NETT-NAS study. Baseline FEV_1 at age 48 is influenced both by maximal attained FEV_1 at about 25 years of age and by the rate of decline in lung function after the age of 25.[34] However, the relative contribution of rate of decline in lung function will be much greater by age 68 than at age 48. Thus, FEV_1 at age 68 in the NETT-NAS participants is likely to largely reflect the rate of decline of lung function during their long smoking history, whereas there is likely to be a weaker relationship between FEV_1 decline and baseline lung function at age 48.

Compared with previous studies, the strengths of this study include larger sample size and good power. This sample size has adequate power to detect common genetic risk variants as shown in our previous power analyses; for example, it has 80% power to detect a relative risk of 2.0 when the frequency of the risk factor is 10% or above.[22]

There are several potential limitations of this study. First, population stratification could have led to false positive results. However, it has been reported that significant false positive associations are unlikely to arise from population stratification in the non-Hispanic white population, especially in well-designed, moderately-sized, case-control studies such as ours.[35] In addition, there was no significant evidence of population stratification in the NETT-NAS cases and controls.[17] Second, false positive results might have arisen from multiple comparisons. However, the consistent results in the NETT-NAS replication study make false positive results unlikely. Third, we have not identified the causal SNP for the associations. The identification of a novel functional SNP *IL6*_-6331T/C (rs10499563), which has low LD with IL6_-174G/C (rs1800795) with r^2 of 0.169 in the CEU HapMap database, indicates that the control of IL6 transcription is likely to be complex.[32] We cannot exclude the possibility that SNPs other than the *IL6*_-174G/C are also causal SNPs. Finally, serum IL6 levels were measured at year 5 of the LHS, so it may not be appropriate to link IL6 levels at year 5 with the baseline FEV_1 as well as the rate of decline of FEV_1 during 5-year follow-up.

In summary, we report associations of *IL6* variants with rate of decline of lung function and with smoking-induced COPD.

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Lung alert

Vitamin D levels are inversely proportional to the "common cold"

The role of vitamin D in bone metabolism and associated pathology is well established. This paper is the first high-powered population study to demonstrate an inverse relationship between vitamin D levels and recent upper respiratory tract infection (URTI). Vitamin D levels were measured in 18 883 participants in the Third National Health and Nutrition Examination Survey in the USA and they were asked whether they had symptoms suggestive of an URTI in the preceding few days. The study was adjusted for diversity in age, sex, race, season, location, body mass index, smoking, asthma and chronic obstructive pulmonary disease (COPD); 24% of those with a vitamin D level <10 ng/ml had a recent URTI compared with 21% in those with levels of 10–<30 ng/ml and 17% in those with vitamin D levels >30 ng/ml. Perhaps the most important finding was that patients with asthma had an odds ratio of 5.67 of recent URTI with vitamin D levels <10 ng/ml compared with those with vitamin D levels >30 ng/ml, and for COPD the odds ratio was 2.26.

The most important application of this research may be in those with asthma or COPD in whom an URTI may lead to lower respiratory tract infections or more life-threatening complications such as pneumonia. One of the limitations of this paper is that it is not clear whether vitamin D is a surrogate of poor nutrition in the context of chronic lung disease, and therefore susceptibility to URTI is due to that rather than pure vitamin D deficiency. More work is needed in the basic science of vitamin D and immunity which can then be translated to clinical trials.

► Ginde AA, Mansbrach JM, Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. *Arch Intern Med* 2009;**169**:384–90.

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