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Functional single nucleotide polymorphisms of the *CCL5* gene and nonemphysematous phenotype in COPD patients

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ABSTRACT: It was previously reported that the gain-of-function -28 guanine allele of the promoter single nucleotide polymorphism (SNP; cytosine to guanine substitution of nucleotide -28 (-28C>G)) in the CC chemokine ligand 5 gene (*CCL5*) was associated with susceptibility to late-onset asthma in patients who developed asthma at age ⩾40 yrs. The clinical diagnosis of chronic obstructive pulmonary disease (COPD) includes emphysema and small airway disease, and upregulation of CCL5 has been described in the airways of patients with COPD. It was hypothesised that *CCL5* has a genetic impact upon the variable expression of emphysema in patients with COPD.

Patients with COPD were studied (n=267). All of the patients underwent pulmonary high-resolution computed tomography (CT), and visual scoring (CT score) was performed to determine emphysema severity. Three SNPs of *CCL5* were genotyped, including -403G>A, -28C>G and 375T>C.

A significant difference was found in CT score according to *CCL5* genotype; the -28G allele was inversely associated with CT score. When the analysis was confined to 180 patients with bronchial reversibility of <15%, even stronger evidence for this association was noted.

Functional single nucleotide polymorphisms in the CC chemokine ligand 5 gene were associated with milder emphysema. Together with previous findings, the present study may identify the CC chemokine ligand 5 gene as part of a common pathway in the pathogenesis of late-onset asthma and chronic obstructive pulmonary disease with milder emphysema.

KEYWORDS: Chronic obstructive pulmonary disease, emphysema, genetics, late-onset asthma, small airway disease

It was previously found that the cytosine (C) to guanine (G) substitution of nucleotide -28 (-28C>G) promoter polymorphism of the CC chemokine ligand 5 gene (*CCL5*), which has been associated with increased levels of mRNA and protein expression *in vitro* [1], was associated with susceptibility to late-onset asthma among patients who developed the disease after the age of 40 yrs [2]. In general, late-onset asthma is not strongly associated with specific allergen sensitisation. Rather, infections, including respiratory viruses, may be more likely to be involved in the pathophysiology of late-onset asthma through host response mechanisms [3]. Viral infections are associated with most exacerbations of asthma and chronic obstructive pulmonary disease (COPD) [4–8], and the most prominent aspect of the epithelial immune response towards viral respiratory infections consists of the production and release of CCL5 [9–12]. Indeed, exacerbation of mild COPD is associated with the upregulation of CCL5 in both the inflammatory and epithelial cells of the bronchial mucosa [11, 13].

The chronic airflow limitation associated with COPD is caused by a mixture of small airway disease and emphysema, the relative contributions of which vary from person to person [14]. These phenotypic variations of COPD may be influenced by several innate susceptibility factors to environmental stimuli, including tobacco smoking and viral respiratory infections. However, the

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relative importance of genetic factors in the pathogenesis of airway disease and emphysematous components of COPD is unknown. Given that accumulation of inflammatory immune cells and airway wall remodelling processes are common characteristics in the small airways of patients with asthma and COPD [15], a common genetic susceptibility may be present, with latent viral infections predisposing some patients to experience increased airway inflammation. *CCL5* may be involved in the pathogenesis of epithelial remodelling and chronic hyperreactivity in response to viral infections.

In the present study, using a well-characterised COPD cohort of Japanese subjects [16], the specific hypothesis that functional single nucleotide polymorphisms (SNPs) in the regulatory region of *CCL5* and their haplotypes have a genetic impact upon the variable expression of the emphysematous phenotype in patients with COPD was examined.

METHODS

Further details of all methods and procedures can be found in the supplementary material.

Study subjects

Among 274 patients with COPD recruited for the Hokkaido COPD cohort study [16], a total of 267 patients, for whom genetic samples were available, were examined in the present study. Study approval was obtained from governing ethics committees for each study centre, and all subjects provided written informed consent.

Lung computed tomography scans

Information regarding the computed tomography (CT) scanners and parameters assessed has been described previously [16]. The severity of emphysema was assessed visually by three independent pulmonologists according to the modified [16] scoring system of GODDARD *et al.* [17]; the pulmonologists were blinded to any clinical information regarding the patient. Six images in three slices were analysed in the lungs, including the aortic arch, the carina and 1–2 cm above the highest hemidiaphragm. Each image was scored on a scale of 0–4 (0: normal; 0.5: $\leqslant$5% affected; 1: $\leqslant$25% affected; 2: $\leqslant$50% affected; 3: $\leqslant$75% affected; 4: >75% affected); the mean score from six images was considered representative of the severity of emphysema in the lungs. When the three independent pulmonologists were split in their evaluation, the score assessed by the majority was used.

In order to confirm the accuracy and reliability of the present visual assessment, the severity of emphysema assessed visually was compared with that assessed by three-dimensional computerised analyses. The method of computerised assessment of emphysema for the whole lung has been described previously and is given in detail in the supplementary material. A strong correlation between the two methods of assessment was found (n=137; r=0.835; p<0.0001) [16].

Pulmonary function test

Spirometry was performed before and 30 min after bronchodilator (400 μg salbutamol) administration. Bronchial reversibility was expressed as the percentage change in forced expiratory volume in one second (FEV1) following salbutamol administration. The carbon monoxide diffusing capacity of the lung ($D_{L,CO}$) test was also performed, and $D_{L,CO}$/alveolar volume (V_A), adjusted for haemoglobin levels, was calculated.

Allele-specific PCR and detection of fluorescence-labelled PCR fragments

Three SNPs (-403G>A (National Center for Biotechnology Information SNP ID rs2107538), -28C>G (rs2280788) and 375T>C (rs2280789)) in the regulatory region were genotyped using an assay that combined kinetic (real-time quantitative) PCR with allele-specific amplification, as described previously [2].

Statistical analysis

The linkage disequilibrium between the three SNPs was analysed, and all of the SNPs were tested for conformation with Hardy–Weinberg expectations in patients with COPD using Haploview software, version 3.2 (Broad Institute, Cambridge, MA, USA) [18]. The genetic impact of the three regulatory SNPs on CT score was examined using a multivariate stepwise linear regression model. The model included sex, age, smoking status (current or ex-), cumulative smoking history in pack-years, body mass index, $D_{L,CO}/V_A$, FEV1 (percentage of the predicted value), levels of total serum immunoglobulin (Ig) E, and peripheral blood eosinophil counts. The genetic effects of the regulatory SNPs on CT score were also examined, using only the 180 patients whose bronchial reversibility was <15% (model 2), since the possibility might remain that the presence of bronchodilator reversibility indicates the presence of coexistent asthma, although patients with physician-diagnosed asthma were carefully excluded from the present study [16].

The association between the extent of emphysema as judged by a low attenuation volume (LAV), automatically assessed by three-dimensional CT, and the three SNPs was also examined (model 3; n=105).

The association between common haplotypes with a frequency of >1% and CT score was tested using global and haplotype-specific statistics using the Haplo.Score program (Mayo Clinic, Rochester, MN, USA) [19].

RESULTS

The clinical characteristics of the subjects are summarised in tables 1 and 2. The genotypic distributions of all of the three SNPs were in Hardy–Weinberg equilibrium. Among COPD-related phenotypes, including body mass index, FEV1 (% pred), bronchial reversibility and CT score, a significant difference was found in CT score for the -28C>G (p<0.05) and the -403G>A SNPs (p<0.05; table 2). On multiple linear regression analysis using 267 patients (model 1), the -28C>G *CCL5* SNP, but neither the -403G>A nor the 375T>C SNP, was significantly associated with CT score. The presence of the -28G allele was significantly associated with a lower CT score; mean$\pm$SD CT scores were 1.49$\pm$0.93, 1.15$\pm$0.87 and 0.93$\pm$0.69 for -28CC homozygotes, -28CG heterozygotes and -28GG homozygotes, respectively (p=0.00038; table 3; fig. 1E of the online supplementary material). In the subgroup analysis (model 2), the association between the -28G allele and a lower CT score was the most significant in the 180 patients who exhibited bronchial reversibility of <15% (p=0.00002; table 3). A linear dose–response relationship between genotype and phenotype was consistently found between CT score and

TABLE 1 Baseline characteristics of chronic obstructive pulmonary disease (COPD) patients

	All subjects	Bronchial reversibility <15%
Subjects n	267	180
Age yrs	69.6±8.1	68.9±8.3
Sex M/F n	251/16	168/12
BMI kg·m^{-2}	22.3±3.2	22.1±3.1
Current smoker %	27.3	30.6
Smoking history pack-yrs	56.0 (12–220)	54.5 (12–220)
FEV_1/FVC %	50.2±12.1	53.3±11.5
FEV_1 % pred	63.4±21.7	68.7±22.4
Bronchial reversibility# %	13.3±13.3	5.87±4.85
CT score	1.40±0.92	1.41±0.93
$D_{L,CO}/V_A$	63.2±24.2	62.3±24.0
Atopy %	24.1	24.9
Log [total serum IgE] IU·mL^{-1}	1.78±0.69	1.81±0.71
Log [eosinophils] cells ·mm^{-3}	2.20±0.33	2.18±0.32
-403G>A n		
GG	106	69
GA	122	82
AA	36	28
-28C>G n		
CC	196	133
CG	61	41
GG	6	3
375T>C n		
TT	117	75
TC	116	79
CC	34	26

Data are presented as mean±SD or median (range) unless otherwise indicated. It was not possible to determine cytosine (C) to guanine (G) substitution of nucleotide -28 (-28C>G) single nucleotide polymorphism (SNP) genotype in four COPD patients and -403G>A SNP genotype in three. M: male; F: female; BMI: body mass index; FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; % pred: % predicted; CT: computed tomography; $D_{L,CO}$: diffusing capacity of the lung for carbon monoxide; V_A: alveolar volume; Ig; immunoglobulin; A: adenine; T: thymidine; #: (post-bronchodilator FEV_1 – prebronchodilator FEV_1)/prebronchodilator FEV_1.

-28C>G genotype. Three independent variables, including smoking status (current or ex-), baseline FEV_1 (% pred) and $D_{L,CO}/V_A$, were consistently associated with CT score in both models, and other variables were excluded from the models. The adjusted R^2 associated with these fitting models (models 1 and 2) was 40 and 47%, respectively. This indicated that the models including smoking, baseline FEV_1 (% pred), $D_{L,CO}/V_A$ and the -28C>G genotype explain only 40–47% of the variance in CT score, and that unidentified factors other than those included in the present study also significantly influence the extent of emphysema in patients with COPD.

Using LAV, assessed by computerised analysis as an index of the severity of emphysema, the inverse association with the -28G allele remained significant (p=0.0015; fig. 1E of the online supplementary material) even though the number of patients in this analysis was limited (n=105). The data were also analysed by combining this group with that of the heterozygote because of the low frequency of the homozygous mutant genotype. When the homozygous wild-type was compared to the combined genotype (heterozygous plus homozygous mutant), a similar inverse association was found between emphysema score and the presence of the -28G allele in models 1 (p=0.00059), 2 (p=0.00005) and 3 (p=0.0049).

The three regulatory SNPs (-403G>A, -28C>G and 375T>C) were in significant linkage disequilibrium and were shown to be part of a single haplotype, with values of the normalised disequilibrium constant *D'* ranging 0.94–0.98. The four most common haplotypes constituted 95.9% of haplotypes in the 267 patients with COPD (table 4). The haplotype comprising three SNPs was significantly associated with CT score (global p-value=0.0023). The haplotype -403A/-28G/375C was most strongly associated with lower CT scores (p=0.0010), as judged by haplotype-specific scores on the basis of 10,000 simulations, whereas the -403G/-28C/375T haplotype was associated with higher CT scores (p=0.014; table 4). Confining the analysis to the 180 patients without bronchial reversibility strengthened the association between the haplotype and CT score (global p-value=0.00075); the -403A/-28G/375C haplotype was inversely associated with CT score (p=0.00015), and the -403G/-28C/375T haplotype was associated with CT score (p=0.0011). However, the association observed in the haplotype analysis was not stronger than that observed in the single-locus analysis using the -28C>G SNP.

DISCUSSION

COPD is a heterogeneous condition including emphysema and small airway disease. In the present study, the genetic effects of functional alleles in the *CCL5* regulatory region were investigated in a well-characterised cohort of 267 patients, and it was found that the gain-of-function allele was inversely associated with severity of emphysema in patients with COPD. Given that upregulation of CCL5 in the airways has been associated with exacerbation of COPD, and a significant association between the CCL5 -28G allele and late-onset asthma having been identified [2], it was possible to test whether or not the allele has a genetic effect on variable COPD phenotypes in a hypothesis-driven association study. This type of study is statistically more powerful than the typical association study that tests multiple genes with no *a priori* hypothesis. By investigating patients with asthma and COPD, a series of these studies may identify *CCL5* as a shared genetic risk factor for these chronic inflammatory airway diseases.

Three common SNPs of functional relevance have been identified in the regulatory region of *CCL5* (-403G>A, -28C>G and 375T>C); these three SNPs influence transcriptional activity *in vitro* and subsequent CCL5 expression in human cell lines [1, 20, 21]. These SNPs were associated with increased levels of CCL5 [2, 22], as well as increased blood eosinophil counts [23]. These SNPs have also been associated with several inflammatory immune diseases, including asthma, allergic rhinitis and atopic dermatitis [2, 20, 22, 24]. In the context of haplotypes involving these SNPs, the -28G/-403G haplotype has been associated with near-fatal asthma in Chinese children [23]. In addition, the -403A/-28G haplotype has been shown to be

TABLE 2 Patient characteristics by CC chemokine ligand 5 polymorphism

	-403G>A[#]			-28C>G[¶]			375T>C[+]		
	GG	GA	AA	CC	CG	GG	TT	TC	CC
Age yrs	69.5±8.09	69.9±8.08	68.2±8.40	69.4±7.96	70.7±8.60	66.0±7.52	69.8±7.95	69.7±8.23	68.6±8.36
Sex M/F n	104/2	113/9	31/5*	184/12	58/3	5/1	115/2	106/10	30/4*
BMI kg·m^{-2}	22.4±3.35	22.4±3.24	21.7±2.71	22.5±3.24	21.8±3.18	21.5±3.31	22.3±3.30	22.5±3.27	21.6±2.72
Current smoker %	26.4	26.2	33.3	27.0	26.2	66.7	27.4	25.9	32.4
Smoking history pack-yrs	57.0 (12.5–160.0)	55.0 (12.0–220.0)	56.0 (19.0–132.0)	57.0 (12.5–220.0)	50.0 (12.0–174.0)	76.5 (38.3–105.0)	57.0 (12.5–57.0)	55.0 (12.0–220.0)	59.9 (19.0–132.0)
FEV$_1$ % pred	54.6±19.6	58.4±25.5	63.3±19.1	57.5±23.3	58.0±21.0	61.0±76.9	55.1±19.0	58.5±26.2	62.1±18.9
Bronchial reversibility %	10.53 (-10.1–69.2)	10.22 (-2.99–60.0)	6.09 (-5.19–52.7)	9.52 (-10.1–69.2)	9.27 (-5.19–60.0)	11.21 (-0.64–25.9)	10.78 (-10.1–69.2)	9.71 (-2.99–60.0)	6.59 (-5.19–52.7)
***D*$_{L,CO}$/*V*$_A$**	61.9±24.2	64.2±25.1	64.1±22.4	65.0±24.3	58.8±23.2	61.0±24.6	61.2±24.1	65.3±24.7	63.1±22.5
CT score	1.53±0.94	1.38±0.89	1.17±0.90*	1.46±0.93	1.26±0.87	0.88±0.69*	1.51±0.92	1.36±0.92	1.20±0.90
Atopy %	25.0	22.1	30.6	26.0	18.0	33.3	25.2	21.6	29.4
Log [total serum IgE] IU·mL^{-1}	1.82±0.68	1.75±0.70	1.80±0.72	1.78±0.70	1.81±0.65	1.67±0.87	1.83±0.68	1.75±0.71	1.74±0.68
Log [eosinophils] cells·mm^{-3}	2.20±0.32	2.23±0.32	2.13±0.36	2.19±0.34	2.24±0.29	2.19±0.20	2.20±0.32	2.24±0.32	2.12±0.36
COPD stage n									
I	18	33	10	47	14	0	20	32	9
II	53	49	19	86	30	6	62	44	18
III	31	33	6	52	16	0	31	33	6
IV	4	7	1	11	1	0	4	7	1

Data are presented as mean±SD or median (range) unless otherwise indicated. -403G>A: guanine (G) to adenine (A) substitution of nucleotide -403; C: cytosine; T: thymidine; M: male; F: female; BMI: body mass index; FEV$_1$: forced expiratory volume in one second; % pred: % predicted; *D*$_{L,CO}$: diffusing capacity of the lung for carbon monoxide; *V*$_A$: alveolar volume; CT: computed tomography; Ig: immunoglobulin; COPD: chronic obstructive pulmonary disease. [#]: 264 subjects; [¶]: 263 subjects; [+]: 267 subjects. *: $p<0.05$ (one-way ANOVA or the Chi-squared test was used as appropriate).

associated with a slower rate of CD4+ T-cell depletion in HIV-1-infected Japanese subjects [1], and haplotypes that included 375C displayed a strong dominant association with rapid progression to AIDS among HIV-1-infected individuals in African-American, European-American and combined cohorts [21]. Therefore, the present authors believe that the genetic association observed in the present study is due to the functional consequences that these functional SNPs have on *CCL5* transcriptional activity, although the possibility that they act as markers of another important genetic abnormality without themselves being functionally relevant cannot be excluded. It is also interesting to note that the frequency of the -28G allele differs according to ethnicity; its frequency is ~15–20% in Asians, including Japanese, Chinese and Koreans, whereas it is very low (⩽2%) in Caucasians and African-Americans. Therefore, the genetic impact of the -28G allele observed in the present studies seems to be clinically important, especially in Asian populations.

In patients with COPD, the relative contributions of small airway disease and emphysema to the degree of airflow limitation vary [14]. Indeed, it has been shown that severity of emphysema varies widely, even in the same stage of COPD [16], and thus COPD patients with milder emphysema despite severe airflow limitation could be considered as having predominantly small airway disease. Within the context of the previous finding that the -28G allele was associated with late-onset asthma, the current observation of an inverse association between this allele and CT score in patients with COPD leads to a specific hypothesis that increased severity of small airway disease caused by a gain effect of the -28G allele may underlie the chronic inflammation and remodelling of the small airways of late-onset asthma and COPD with milder emphysema. Alternatively, a low attenuation area may reflect hyperlucency due to air trapping rather than emphysema itself, thus confounding the assumption that the CT score purely indicates the extent of emphysema [25]. However, high-resolution CT scans were performed at full inspiration; thus this latter possibility would be less likely than is the case when conventional CT scans are performed at expiration. In addition, if low attenuation areas reflected not only emphysema but also hyperlucency due to air trapping, a good correlation between CT score and airflow limitation would be expected; however, this was not the case in the present population [16].

An increased prevalence of viral infections, as well as the persistence of cells expressing viral proteins in patients with asthma [4, 5] or COPD [6–8], has been reported, suggesting that viral infections, such as human rhinovirus and respiratory syncytial virus (RSV), may play a critical role in the pathogenesis of airway inflammation and the subsequent deterioration in lung function in patients with asthma and COPD. Studies of respiratory secretions from individuals with

TABLE 3 Linear regression analyses of the functional CC chemokine ligand 5 polymorphisms with computed tomography (CT) score

	Subjects n	CT score	p-value
Model 1#			
-403GG	105	1.48±0.94	0.063
-403GA	121	1.39±0.89	
-403AA	36	1.24±0.90	
-28CC	196	1.49±0.93	0.00038
-28CG	61	1.15±0.87	
-28GG	6	0.93±0.69	
375TT	116	1.44±0.92	0.12
375TC	115	1.40±0.90	
375CC	34	1.24±0.92	
Model 2¶			
-403GG	69	1.57±0.94	0.006
-403GA	82	1.35±0.89	
-403AA	28	1.15±0.90	
-28CC	133	1.53±0.93	0.00002
-28CG	41	1.05±0.87	
-28GG	3	0.70±0.69	
375TT	75	1.54±0.92	0.009
375TC	79	1.36±0.90	
375CC	26	1.14±0.92	

Data are presented as mean±SD. Linear regression models were applied to test the association between the three polymorphisms and the CT score. Smoking status, diffusing capacity of the lung for carbon monoxide and percentage predicted prebronchodilator forced expiratory volume in one second were consistently associated with CT score in models 1 and 2. G: guanine; A: adenine; C: cytosine; T: thymidine. #: all subjects; ¶: bronchial reversibility <15%.

RSV bronchiolitis showed that CCL5 was highly expressed [26], suggesting a special role for this chemokine in antiviral defence. Interestingly, a genetic variant of the key receptor for CCL5, CC chemokine receptor 5, has been associated with the severity of bronchiolitis caused by RSV [27]. Viral infections are the most likely cause of CCL5 upregulation, and the epithelium of the small airways is a considerable source of CCL5. The presence of the gain-of-function allele as a common susceptibility factor to asthma and COPD with milder emphysema predisposes patients to greater expression of CCL5 in response to prolonged and repeated exogenous stimuli, including viral antigens, leading to amplified inflammation of the small airways.

In a mouse model with targeted disruption of *CCL5*, immunoregulatory and antiapoptotic effects of CCL5 have been suggested [28], which are distinct from those that have previously been identified in the setting of infection, such as the initiation of antiviral responses and airway inflammation *via* enhancement of inflammatory cell recruitment. The functional properties of CCL5, if any, in humans may provide an alternative explanation for the present findings, such as that the presence of the -28G allele predisposes an individual to some type of protection against the development of emphysema. However, the present authors believe that this possibility is less likely given that the -28G allele has also been associated with late-onset asthma and that increased small airway pathology is a common cardinal feature of asthma and COPD.

Although it is difficult to discriminate asthma from COPD in some older patients, the present authors believe that the findings of the present study were not the result of untoward inclusion of patients with late-onset asthma carrying the -28G allele. Among 267 patients with COPD, no correlation was found between CT score and levels of bronchial reversibility, levels of total serum IgE, peripheral blood eosinophil counts or

TABLE 4 Haplotypes comprising three CC chemokine ligand (CCL) 5 single nucleotide polymorphisms and computed tomography (CT) score

Patients	Nucleotide			Frequency	Haplotype-specific score	Empirical p-value
	-403	-28	375			
All subjects#	A	G	C	0.132	-3.218	0.00101
	A	C	T	0.017	-0.344	0.72
	A	C	C	0.210	1.021	0.31
	G	C	T	0.609	2.450	0.0143
Bronchial reversibility <15%¶	A	G	C	0.125	-3.746	0.00015
	A	C	T	0.017	-0.017	0.99
	A	C	C	0.235	-0.084	0.93
	G	C	T	0.594	3.234	0.0011

The frequencies of the haplotypes comprising the three *CCL5* polymorphisms were significantly associated with CT score based on p-values from 10,000 simulations of global score tests, as implemented in Haplo.Score [19]. The analyses were adjusted for smoking status, diffusing capacity of the lung for carbon monoxide and percentage predicted prebronchodilator forced expiratory volume in one second. The haplotype-specific score permits the evaluation of which haplotypes have the strongest association with a trait. It should be noted that the global score test does not give effect estimates, whereas negative haplotype-specific scores are associated with a protective effect and positive haplotype-specific scores are associated with an increased risk. #: global p=0.0023 (n=267); ¶: global p=0.00075 (n=180).

frequency of atopy, which makes it unlikely that inclusion of asthmatic patients occurred more often in a group with milder emphysema. In addition, the genetic association between the -28G allele and CT score became even stronger when the analysis was limited to patients who exhibited bronchial reversibility of <15% in order to reduce the risk of unknowingly including patients with asthma.

Although the $D_{L,CO}$ appears to be the best single physiological measure of emphysema severity, it was found that the -28C>G SNP was significantly associated only with visually assessed CT score and not with $D_{L,CO}/V_A$. Wide variations in CT score, even among patients who had the same $D_{L,CO}/V_A$, were noted (fig. 2E of the online supplementary material) despite the finding that $D_{L,CO}/V_A$ was significantly associated with CT score ($p<0.0001$). $D_{L,CO}$ are usually influenced by parenchymal destruction involving respiratory bronchioles, alveoli and the pulmonary capillary system, and are reduced in patients with emphysema because of the loss of alveolocapillary surface. However, $D_{L,CO}$ may be relatively insensitive to the loss of surface area for gas exchange when ventilation and perfusion remain well matched in the lung. $D_{L,CO}$ may also be influenced by several other factors, including the pathology of alveolar septa, inequality of blood/gas distribution in the lung and lung volume at the time of measurement, even if correction is made for V_A. Therefore, the present authors believe that the CT score indicate emphysema severity more specifically than $D_{L,CO}/V_A$ in the present study.

A visual scoring system was used to assess the extent of emphysema according to the modified [16] scoring system of GODDARD *et al.* [17]. Given that automatically calculated parameters, such as LAV, may be a more sensitive technique for the detection and quantification of pulmonary emphysema *in vivo*, visual assessment of emphysema is a limitation of the present study that could bias the results. However, when the severity of emphysema assessed by visual score was compared with that assessed by computerised analysis, a strong correlation was found between these two approaches (fig. 3E of the online supplementary material) [16]. In addition, subanalysis using automatically calculated emphysema scores confirmed the inverse association between the functional -28G allele and the severity of emphysema. In addition, computerised analysis may not be easy to obtain in many centres, and, therefore, may not be suitable for genetic studies of complex diseases such as COPD, especially since these studies usually require a large number of subjects in order to identify rather small effects of a gene or genes and because they also require replication studies in independent centres.

In conclusion, together with the previous finding of an association between the -28G allele and the development of late-onset asthma, the present study indicates that the CC chemokine ligand 5 gene may be involved in the common pathogenesis underlying late-onset asthma and chronic obstructive pulmonary disease with milder emphysema. The present findings led to speculation that specific components of the innate immune system may manifest an aberrant antiviral response as a basis for chronic inflammatory lung diseases, such as asthma and chronic obstructive pulmonary disease. Further studies of the disease phenotype presented in the current study are needed in order to improve understanding of the underlying pathophysiology and elucidate potential treatment modalities for the complex disease labelled chronic obstructive pulmonary disease.

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