Mendelian Randomization Study of Interleukin-6 in Chronic Obstructive Pulmonary Disease

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Key Words

Chronic obstructive pulmonary disease, epidemiology,
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Abstract

Background: Cross-sectional studies have demonstrated that increased levels of interleukin-6 (IL6) are present in the airways and blood samples of patients with chronic obstructive pulmonary disease (COPD). **Objectives:** To investigate the association between IL6 and the risk of COPD using a Mendelian randomization approach. **Methods:** Eight common single-nucleotide polymorphisms (SNPs) in the region of the *IL6* gene were genotyped using both TaqMan and Illumina in the Rotterdam Study, a prospective population-based cohort study consisting of 7,983 participants aged 55 years or older, including 928 COPD patients. At baseline, blood was drawn in a random sample of 714 subjects to measure the IL6 plasma level. Analysis of variance, logistic regression, and Cox proportional hazard models – adjusted for age,

gender, pack years, and BMI – were used for analyses. **Results:** High levels of IL6 (>2.4 pg/ml, the highest tertile) were associated with a three-fold increased risk of developing COPD, in comparison to low levels (<1.4 pg/ml, the lowest tertile). The rs2056576 SNP was associated with a 10% increase in the risk of COPD per additional T allele. However, the association was no longer significant after adjustment. No association was found with other common SNPs in the *IL6* gene and COPD. **Conclusions:** Although increased IL6 plasma levels at baseline are associated with the risk of developing COPD during follow-up, there was no strong evidence for an association between common variation in the *IL6* gene and the risk of COPD.

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Introduction

Worldwide, chronic obstructive pulmonary disease (COPD) is a major and still increasing health problem. The disease is characterized by progressive airflow limitation driven by an abnormal inflammatory response of the airways to inhaled particles and fumes [1, 2]. Recently, COPD has been associated with elevated levels of several inflammatory markers in the systemic circulation, including interleukin-6 (IL6) and C-reactive protein (CRP) [3–7]. This may be a key link to various extrapulmonary effects which have been described in relation to COPD, such as cachexia, osteoporosis, skeletal muscle wasting, diabetes mellitus, cardiovascular disease, and depression [8–10].

IL6 is a powerful stimulant of the immune system and has been shown to be present in sputum and bronchoalveolar lavage fluid and in blood samples of stable patients with mild COPD after a hypoxic challenge and during COPD exacerbations [11–13]. Elevated plasma levels of IL6 also appear to correlate with disease severity [14]. IL6 is a protein with a helical structure; it is mainly secreted by monocytes and macrophages but can also be released in the airways by bronchial epithelial cells and fibroblasts [15]. IL6 is a multifunctional cytokine, originally identified as a B cell differentiation factor, with a central role in the induction of hepatically derived acute-phase proteins such as CRP [16–19]. In addition, IL6 also mediates the activation, growth, differentiation, and survival of T cells and has emerged as a key regulatory signal in the development of the new T-helper17 subset [18, 20].

The rs1800795 single nucleotide polymorphism (SNP) in the promoter region of the *IL6* gene influences plasma levels of IL6 and CRP [21, 22]. Although variation in this SNP has been associated with the risk and outcome of several inflammatory diseases, three studies could not find any association between this SNP and the risk of COPD [23–25]. In contrast, a recent report associated the rs1800795 SNP with a rapid decline of FEV₁ and susceptibility to COPD in smokers [26]. In addition, other variants in the *IL6* gene have been linked with the risk of COPD [24, 27].

In this Mendelian randomization study, we hypothesized that the common variation in the *IL6* gene that raises plasma levels of IL6 would modify the susceptibility of an individual to develop COPD, implicating that increased plasma levels of IL6 would be causally related to incident COPD. We tested these hypotheses in a large prospective population-based cohort with more than 15 years of follow-up.

Methods

Study Design and Setting

The present study is part of the Rotterdam Study, a population-based cohort study to assess the occurrence of and risk factors for chronic diseases in the elderly. The objectives and methods of the Rotterdam Study have been described elsewhere [28, 29]. In short, the Rotterdam Study cohort includes 7,983 participants aged ≥ 55 years, living in Ommoord, a well-defined suburb of the city of Rotterdam, The Netherlands. Almost all participants (99.8%) are of Caucasian descent. Baseline data were collected from 1989 until 1993. Participants were visited at home at the start of the study for a standardized interview on their health status. Following the start of the Rotterdam Study, each participant visited the research center every 2–3 years; during these visits, several measurements were performed. In addition, participants were continuously monitored for the onset of major events which occurred during follow-up through automated linkage with files from general practitioners. Trained research assistants collected information from medical records of the general practitioners, medical specialists (e.g. respiratory physicians), hospitals, and nursing homes. The medical ethics committee of the Erasmus Medical Center, Rotterdam, and the review board of The Netherlands Ministry of Health, Welfare, and Sports approved the study. Participants gave written informed consent and permission to retrieve information from the treating physicians.

Study Participants

Information about the COPD cohort definition and spirometry examination has been described previously [30] and is provided in online supplementary table 1 (for all suppl. material, see www.karger.com/doi/10.1159/000332336). In short, the diagnosis of COPD was classified as definite or probable. Definite COPD was defined by a moderate-to-severe obstructive spirometry (FEV₁/FVC < 0.7 and FEV₁ $< 80\%$ predicted) or as COPD diagnosed by a specialist in internal medicine (mainly respiratory physicians or internists with a subspeciality in respiratory medicine), based upon the combination of clinical history, physical examination, and spirometry. Probable COPD was defined by a mild obstructive spirometry (FEV₁/FVC < 0.7 and FEV₁ $\geq 80\%$ predicted) or as COPD diagnosed by a physician in another medical specialty (e.g. a general practitioner). For the risk analyses, participants with indication of asthma in their medical files or a spirometry report suggestive of restrictive respiratory disease (FEV₁/FVC ≥ 0.7 and FEV₁ $< 80\%$ predicted) were excluded.

The index date was defined as the date of diagnosis of COPD found in the medical reports or the date of a first prescription of COPD medication in patients with definite or probable COPD or the date of the obstructive lung function examination, whichever came first. Follow-up time was defined as the time period between cohort entry and the diagnosis of COPD, death, loss to follow-up, or the end of the study period on December 31, 2004, whichever came first.

Measurement of IL6 Plasma Levels and Common Variation in the IL6 Gene

Details on the methods for obtaining these measurements are provided in the online supplementary material. Plasma IL6 and high-sensitivity (hs)CRP levels were measured as previously described [3, 31, 32]. The rs1800795 (–174G/C) SNP was genotyped

Fig. 1. Cohort definition.

in 6,071 participants by using a TaqMan allelic discrimination assay (Applied Biosystems, Foster City, Calif., USA). In addition, seven common tagging SNPs within the *IL6* gene ± 10 kb and a minor allele frequency >0.10 (rs7801617, rs7805828, rs1880242, rs10499563, rs2056576, rs1554606, and rs10242595; chromosome 7, NCBI build) were extracted based on data from the Illumina Infinium II HumanHap550K BeadChip (version 3) in 5,639 participants.

Statistical Analyses

Demographic and clinical characteristics of the study population are expressed as medians and the interquartile ranges (IQR) for description in the text. The eight SNPs in the *IL6* gene were tested for Hardy-Weinberg equilibrium with χ^2 statistics.

First, we studied the association between the eight SNPs in the *IL6* gene and COPD. A logistic regression model – adjusted for age at baseline, gender, total number of pack-years, and BMI – was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between the different genotypes and COPD. Overall, additive genetic models in which persons homozygous for the common allele were used as the reference category were applied. All prevalent and incident COPD cases were used for analyses.

Second, hazard ratios (HR) and 95% CI for the association between plasma levels of IL6 and incident COPD were evaluated by use of Cox's proportional hazards analyses. Hereto, prevalent cases – subjects with a follow-up time of less than 3 years since the

index date – were excluded. The Cox models were adjusted for covariates that changed the point estimate by more than 10% or that were biologically plausible according to previous literature. An extensive description of the various covariates is presented in the online supplementary material. Interaction terms and subanalyses were introduced to explore for potential effect modification.

Differences in IL6 and hsCRP levels according to the *IL6* genotype were tested by means of analysis of variance (ANOVA). All participants with a blood sample were used for analyses. Because of the skewed absolute values of hsCRP and IL6 in our cohort, the natural logarithmic transformation of these markers was entered as a continuous variable in the models.

The difference between HR was interpreted by reference to 95% CI for each of the parameters of interest. $p < 0.05$ was considered as statistically significant. All statistical analyses were performed using PASW Statistics for Windows, version 18 (SPSS, Inc., Chicago, Ill., USA).

Results

Baseline Characteristics

Of the 7,983 participants in the Rotterdam Study, 7,081 donated a blood sample. Two hundred eighty-eight subjects were excluded because of a diagnosis of asthma in

Table 1. Baseline characteristics of the study population

Baseline characteristics	rs1800795 genotyped by Taqman			Seven SNPs genotyped by Illumina			Random sample of subjects with IL6 plasma levels		
	COPD	Controls	Overall	COPD	Controls	Overall	COPD	Controls	Overall
Participants	779	5,292	6,071	732	4,907	5,639	55	517	572
Median age at entry, years	67 (62–72)	69 (62–76)	68 (62–76)	67 (62–72)	69 (62–76)	68 (62–76)	66 (61–72)	70 (62–77)	69 (62–77)
Male	448 (58)	2,031 (38)	2,479 (41)	422 (58)	1,879 (38)	2,301 (41)	37 (67)	230 (45)	267 (47)
Smoking behavior									
Never-smoker	121 (16)	1,953 (37)	2,074 (34)	109 (15)	1,812 (37)	1,921 (34)	8 (15)	186 (36)	194 (34)
Current smoker	313 (40)	1,042 (20)	1,355 (22)	296 (40)	980 (20)	1,276 (23)	22 (40)	93 (18)	115 (20)
Former smoker	340 (44)	2,136 (40)	2,476 (41)	322 (44)	1,970 (40)	2,292 (41)	25 (46)	229 (44)	254 (44)
Missing	5 (1)	161 (3)	166 (3)	5 (1)	145 (3)	150 (3)	0 (0)	9 (2)	9 (2)
Median pack-years	26 (9–45)	4 (0–25)	6 (0–29)	26 (9–45)	4 (0–25)	7 (0–29)	30 (15–49)	6 (0–26)	9 (0–30)
Body mass index, kg/m ²	25 (23–28)	26 (24–28)	26 (24–28)	25 (23–28)	26 (24–28)	26 (24–28)	26 (23–28)	26 (24–29)	26 (24–28)
Diabetes	64 (8)	566 (11)	630 (10)	60 (8)	526 (11)	586 (10)	4 (7)	38 (7)	42 (7)
Cardiovascular comorbidity	246 (32)	1,625 (32)	1,871 (32)	226 (31)	1,501 (32)	1,727 (32)	14 (26)	142 (28)	156 (28)
Median IL6 plasma level, pg/ml	2.0 (1.4–3.5)	1.9 (1.2–3.1)	1.9 (1.2–3.1)	2.0 (1.3–3.3)	1.9 (1.2–3.0)	1.9 (1.2–3.0)	2.0 (1.3–3.6)	1.9 (1.2–2.9)	1.9 (1.2–3.0)

Categorical variables are expressed as numbers (percentages) unless otherwise specified. Values of continuous variables are expressed as medians (25–75 percentiles).

the medical files or a spirometry report suggestive of a restrictive syndrome. The rs1800795 SNP was successfully genotyped using the Taqman method in 6,071 participants, 779 of whom had COPD. Regarding the other seven SNPs, Illumina genotyping data were available in 5,639 participants, 732 of whom had COPD (fig. 1). With these data we cover 76, 84, and 92% of the common variation in the *IL6* gene as present in the Caucasian HapMap reference population at r^2 thresholds of 1, ≥ 0.7 , and ≥ 0.5 , respectively.

Among the participants who were randomly selected from the cohort for IL6 plasma level measurement, subjects with prevalent COPD or a follow-up time of less than 3 years were excluded from the analyses. In the remaining subgroup of 572 participants, 55 subjects developed COPD during a median follow-up time of 12 years (IQR 4) (fig. 1). Table 1 shows the baseline characteristics of the study population. Overall, the COPD cases were more often male and (current) smokers.

Association between Common Variants in the IL6 Gene and COPD

The rs1800795 TaqMan-genotyped SNP was in Hardy-Weinberg equilibrium ($\chi^2 = 1.79$; $p = 0.181$). The C allele of the rs1800795 SNP was positively associated with higher hsCRP levels (table 2). No association was found between the rs1800795 SNP and the total group of COPD patients (table 3).

All seven common SNPs genotyped on Illumina arrays were in Hardy-Weinberg equilibrium. None of the

seven SNPs were significantly associated with plasma IL6 levels. Three SNPs (rs10499563, rs1554606, and rs10242595) correlated significantly with plasma hsCRP levels (table 2). Only the rs2056576 SNP demonstrated an association with COPD (OR 1.1; 95% CI 1.01–1.27) (table 3); however, this was no longer significant after adjustment for age, gender, the total number of pack-years at the start of the study, and BMI. Prevalent diabetes or cardiovascular diseases did not substantially change the risk estimates. Additional subanalyses demonstrated that the increase in risk for this SNP was most pronounced in females (OR 1.2; 95% CI 1.02–1.43) and in never-smokers (OR 1.4; 95% CI 1.02–1.80) (data not shown).

Association between IL6 Plasma Levels and Incident COPD

The median IL6 plasma level was 2.0 pg/ml (IQR 2.3) in COPD cases and 1.9 (IQR 1.7) in control subjects (table 1). Female COPD cases had a significantly lower log mean IL6 plasma level (1.8 pg/ml) than male cases (2.7 pg/ml) ($p = 0.011$). When stratified according to smoking status, current smokers showed the highest log mean IL6 level (2.4 pg/ml) in comparison to participants who had never smoked (1.9 pg/ml; $p = 0.004$) or who were former smokers (2.0 pg/ml; $p = 0.011$).

An elevated plasma level of IL6 (>2.4 pg/ml, the highest tertile) was associated with a three-fold increased risk of developing definite COPD (95% CI 1.20–8.13; $p = 0.020$), in comparison to a low level of IL6 (<1.4 pg/ml, the lowest tertile) (table 4). The risk remained significant-

Table 2. Association between common SNPs in the *IL6* gene and plasma IL6 and serum hsCRP levels

<i>IL6</i> gene Db SNP No. ¹	Genotype	IL6 level, pg/ml			hsCRP level, mg/l		
		Number	Ln IL6	p value	Number	Ln CRP	p value
rs1800795	GG	238	0.72 (0.05)		2,151	0.56 (0.02)	
	GC	311	0.72 (0.04)	0.990	2,839	0.65 (0.02)	0.006
	CC	102	0.70 (0.07)	0.802	996	0.65 (0.03)	0.026
rs7801617	GG	466	0.71 (0.03)		4,479	0.62 (0.02)	
	GA	120	0.67 (0.07)	0.675	1,017	0.63 (0.03)	0.785
	AA	13	0.77 (0.17)	0.766	66	0.74 (0.13)	0.359
rs7805828	GG	191	0.64 (0.05)		1,754	0.64 (0.02)	
	GA	306	0.73 (0.04)	0.171	2,753	0.63 (0.02)	0.774
	AA	102	0.72 (0.08)	0.391	1,054	0.57 (0.03)	0.087
rs1880242	TT	172	0.73 (0.06)		1,648	0.59 (0.03)	
	TG	311	0.72 (0.04)	0.912	2,726	0.64 (0.02)	0.078
	GG	113	0.63 (0.07)	0.255	1,176	0.63 (0.03)	0.323
rs10499563	TT	330	0.68 (0.04)		3,263	0.65 (0.02)	
	TC	237	0.70 (0.04)	0.719	1,989	0.58 (0.02)	0.036
	CC	30	0.91 (0.16)	0.091	307	0.61 (0.06)	0.569
rs2056576	CC	281	0.69 (0.04)		2,473	0.62 (0.02)	
	CT	258	0.71 (0.04)	0.689	2,446	0.61 (0.02)	0.690
	TT	60	0.73 (0.11)	0.688	645	0.67 (0.04)	0.347
rs1554606	GG	197	0.69 (0.05)		1,828	0.56 (0.02)	
	GT	297	0.72 (0.04)	0.658	2,712	0.65 (0.02)	0.003
	TT	105	0.65(0.07)	0.589	1,018	0.65 (0.03)	0.031
rs10242595	GG	295	0.68 (0.04)		2,811	0.65 (0.02)	
	GA	241	0.74 (0.04)	0.348	2,280	0.60 (0.02)	0.061
	AA	63	0.62 (0.08)	0.556	472	0.53 (0.05)	0.017

ANOVA was used for analyses. Ln IL6 and Ln CRP values are means (standard error). p values (2-tailed) were calculated by a contrast test of the first genotype versus the second genotype and the first genotype versus the third genotype. Ln = Logarithmus naturalis. ¹ Seattle SNP database number.

ly increased when the analyses were adjusted (95% CI 1.11–8.76; $p = 0.031$). The increase in risk was less pronounced and no more significant in the total group of definite and probable COPD patients (HR 1.9; 95% CI 0.97–3.53; $p = 0.062$). Subanalyses suggested that the increase in risk was most pronounced in men and smokers; however, comparative numbers of women and never-smokers were low (data not shown). When analyses were restricted to participants with a study-acquired spirometry, the association between an elevated plasma level of IL6 and the risk of developing definite COPD remained significant (online suppl. table 1; HR 3.7; 95% CI 1.19–11.27; $p = 0.024$). Again, the increase in risk was less pronounced but still significant in the total group of definite and probable COPD participants (HR 2.7; 95% CI 1.22–6.06; $p = 0.014$).

Discussion

The results of this study demonstrated that increased IL6 plasma levels at baseline were associated with a higher risk of developing COPD during follow-up. This effect remained significant after adjustment for several confounders and despite the exclusion of incident cases in the first 3 years of follow-up. In contrast, we found no strong evidence for an association between various common variants in the *IL6* gene and the risk of COPD. Using the Mendelian randomization approach previously employed for the association with CRP [3, 6], we demonstrated that also elevated plasma IL6 is not causally related to COPD.

The rs1800795 SNP has previously been associated with the susceptibility to and the outcome of several acute

Table 3. Association between common SNPs in the *IL6* gene and COPD

<i>IL6</i> gene Db SNP No. ¹	Patients/cohort	Model 1			Model 2		
		OR	95% CI	p value	OR	95% CI	p value
rs1800795	779/6,071	1.0	0.87–1.08	0.602	1.0	0.88–1.10	0.779
rs7801617	731/5,637	1.0	0.87–1.24	0.678	1.1	0.89–1.29	0.460
rs7805828	732/5,637	1.1	0.95–1.18	0.339	1.0	0.91–1.15	0.694
rs1880242	732/5,625	0.9	0.84–1.05	0.267	1.0	0.85–1.07	0.425
rs10499563	731/5,634	0.9	0.82–1.07	0.334	0.9	0.81–1.06	0.246
rs2056576	732/5,639	1.1	1.01–1.27	0.037	1.1	0.99–1.25	0.076
rs1554606	731/5,633	1.0	0.89–1.11	0.925	1.0	0.91–1.14	0.752
rs10242595	732/5,638	1.1	0.93–1.19	0.404	1.0	0.92–1.17	0.560

Additive genetic models were used for analyses. Model 1 is not adjusted for confounders. Model 2 is adjusted for age, gender, the total number of pack-years at the start of the study, and BMI.

¹ Seattle SNP database number.

Table 4. Association between plasma IL6 levels and incident COPD

COPD definition	Patients/ cohort	IL6 plasma level, pg/ml	Model 1			Model 2		
			HR	95% CI	p value	HR	95% CI	p value
Definite COPD	6/182	<1.40	1.0	<i>Reference</i>		1.0	<i>Reference</i>	
	8/182	1.40–2.40	1.6	0.55–4.57	0.393	1.6	0.54–4.75	0.397
	14/181	>2.40	3.1	1.20–8.13	0.020	3.1	1.11–8.76	0.031
Definite and probable COPD	16/191	<1.39	1.0	<i>Reference</i>		1.0	<i>Reference</i>	
	17/191	1.39–2.40	1.3	0.65–2.55	0.469	1.2	0.61–2.51	0.563
	22/190	>2.40	1.9	0.97–3.53	0.062	1.6	0.80–3.29	0.178

Cox proportional hazard models were used for analyses. Model 1 is not adjusted for confounders. Model 2 is adjusted for age, gender, the total number of pack-years at the start of the study, and BMI.

and chronic inflammatory diseases, such as juvenile chronic polyarthritis, type I diabetes mellitus, atherosclerosis, and Alzheimer's disease [21, 33–36]. In COPD, patients carrying the *IL6* GG genotype of this SNP showed higher plasma levels of IL6 and higher pulmonary artery pressure than those carrying the CC or CG genotype [37, 38]. These results raise the possibility that individuals homozygous for the C allele may have a better prognosis in respiratory inflammatory diseases because of a less pronounced IL6-mediated acute-phase response. The results of our study demonstrate that the most frequently studied SNP in the promoter region of the *IL6* gene, rs1800795, did not influence the risk of COPD. Regarding other common variants in the *IL6* gene, the rs2056576 SNP demonstrated a small increase in the risk of COPD. However, the associations were no longer sig-

nificant after adjustment for age, gender, total number of pack-years, and BMI. In accordance with these results, we recently demonstrated in a murine model of COPD that – although IL6 levels were significantly increased after cigarette smoke exposure – the cigarette smoke-induced pulmonary and systemic manifestations were *IL6* independent [39].

We did not find an association between the eight investigated common variants in the *IL6* gene and the development of COPD, confirming preliminary evidence from earlier reports [23–25]. More recently, Smolonska et al. [40] and Castaldi et al. [41] both published a meta-analysis regarding genetic association studies of COPD using the candidate gene approach. Also in these meta-analysis, they could not demonstrate a significant effect of the –174 G/C *IL6* polymorphism on the risk of COPD.

In contrast, a recent study by He et al. [26] demonstrated that the rs1800795 SNP was associated with an accelerated decline in FEV₁ and susceptibility to COPD in smokers. In addition, Cordoba-Lanus et al. [24] did associate another SNP (rs1800796) with COPD and Yanbaeva et al. [27] showed that a haplotype of the *IL6* gene was associated with an increased risk of moderate-to-severe smoking-induced COPD. However, the design of these studies has several limitations. A first limitation is the small sample size of both the COPD and control groups, which may lead to spurious results and can compromise the power for genetic association analysis. Secondly, several studies had a cross-sectional design, which implicated difficulty to judge the sequence of cause and effect. Thirdly, the studies were not population based, which makes them prone to selection bias. Lastly, the studies of He et al. [26] and Yanbaeva et al. [27] only included smoking subjects.

There was no clear functional link between the different *IL6* gene variants and the plasma IL6 level although rs1800795 and three other SNPs (rs10499563, rs1554606 and rs10242595) correlated significantly with plasma hsCRP levels. IL6 plasma concentrations show considerable interindividual variability in acute and chronic inflammation. It has been speculated that these differences may be determined genetically by variations in the *IL6* gene, such as the rs1800795 polymorphism [16]. However, there is disagreement concerning the question of whether any genotype is associated with higher IL6 plasma concentrations [21, 42–44]. It is possible that the effect is dependent on interactions between several SNPs in the *IL6* gene and that one SNP is not an optimal marker in this respect. Nevertheless, there is agreement between our results and previous data by Vickers et al. [22] and a genome-wide association study of Okada et al. [45] which also show significant associations between SNPs in the promoter region of *IL6* and serum CRP levels.

The strengths of our study are the prospective, population-based cohort design and the large number of study subjects – almost 8,000 – with similar data collection procedures for every participant. In this large ongoing cohort study, more than 900 well-defined COPD cases were identified over a total follow-up time of 15 years (1989–2004), as previously described [30]. All data collection during follow-up of the cohort was performed in a prospective manner, without knowledge of future disease or research hypothesis, making selection and information bias unlikely. The prospective nature of this cohort study makes the results less prone to bias and made it possible to adjust analyses for established risk factors and confounders.

Possible limitations of the study are the limited sample size of the plasma IL6 measurements since they were only analyzed in a (random) subgroup of the total cohort, the absence of repeated measurements of the IL6 level, and the absence of a study-acquired spirometry and high resolution computed tomography of the chest of each participant. Therefore, we cannot completely exclude the presence of bronchiectasis and/or bronchial asthma, which could affect the blood IL6 levels of COPD participants and controls. The limited sample size of plasma IL6 measurements might explain the lack of association between the *IL6* genotypes and IL6 plasma levels in our study cohort. Nonetheless, we observed a significant association between hsCRP levels and the rs1800795 *IL6* genotype, as previously described [22]. Regarding the genotype data, there is a chance of missing associations with variants in low linkage disequilibrium with the SNPs. Furthermore, the results of this study are restricted to a European ancestry population since they are based on data from the HapMap CEU sample.

In conclusion, previous evidence has identified the presence of increased levels of IL6 in the plasma and airways of patients with COPD. However, it is not known whether IL6 was causally involved in the pathogenesis of COPD or was just an innocent bystander of the disease, related to smoking or other risk factors. The answer to this question became of clinical relevance with the recent development of tocilizumab, a humanized monoclonal IL6 receptor antagonist, which is already successfully used in refractory rheumatoid arthritis. In this Mendelian randomization study, we have demonstrated that elevated plasma levels of IL6 are significantly associated with an increased risk of incident COPD. In contrast, there was no strong evidence for an association between common variants in the *IL6* gene and the risk of developing COPD.

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