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Original Article

Associations of interleukin-1 gene cluster polymorphisms with C-reactive protein concentration and lung function decline in smoking-induced chronic obstructive pulmonary disease

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Abstract: Objective: We reported association of haplotypes formed by *IL-1*b (*IL1B*)-511C/T (rs16944) and a variable number of tandem repeats (rs2234663) in intron 3 of *IL-1 receptor antagonist* (*IL1RN*) with rate of lung function decline in smoking-induced COPD. The aim of current study was to further investigate this association. Methods: We genotyped an additional 19 polymorphisms in *IL1* cluster (including *IL1A*, *IL1B* and *IL1RN*) in non-Hispanic whites who had the fastest (n = 268) and the slowest (n = 292) decline of FEV1% predicted in the same study. We also analyzed the association of all 21 polymorphisms with serum CRP levels. Results: None of 21 polymorphisms showed significant association with rate of decline of lung function or CRP levels after adjusting for multiple comparisons. Before adjusting for multiple comparisons, only *IL1RN*_19327 (rs315949) showed significant association with lung function decline (P = 0.03, additive model). The frequencies of genotypes containing the *IL1RN*_19327A allele were 71.9% and 62.2%, respectively in the fast and slow decline groups (P = 0.02, odds ratio = 1.6, 95% confidence interval = 1.1-2.3); the *IL1B*_5200 (rs1143633) and rs2234663 in *IL1RN* were associated with serum CRP levels (P=0.04 and 0.03, respectively). Conclusions: No single marker was significantly associated with either rate of lung function decline or serum CRP levels.

Keywords: Chronic obstructive pulmonary disease (COPD), forced expiratory volume in one second (FEV_1), genetic polymorphism, *IL1* gene cluster, lung function decline, C-reactive protein

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is recognized as a complex genetic disorder. Identification of the genetic determinates of COPD and/or lung function decline is likely to provide insights into the pathogenesis of COPD and to allow individualized treatment regimens. Unfortunately, many of the results from COPD genetic studies have not been reproduced. One reason for lack of consistency may be that only a few genetic markers in each gene were examined and therefore the genetic variation in the studied genes was not comprehensively evaluated.

The *interleukin-1* (*IL1*) gene cluster encodes three members of IL-1 family. The first two members IL-1A and IL-1B are proinflammatory cytokines that bind to the IL-1 receptor, whereas the third is the receptor antagonist (IL-1RN). IL-1RN is a soluble inhibitor of IL-1 agonists and acts by competitively binding to the cell surface receptor without triggering signal transduction, downregulating the immune response. The *IL1A* and *IL1B* genes are located at chromosome 2q14 in a cluster that also contains the *IL1RN* gene. *IL1A* and *IL1B* are spaced 40 kb apart, while *IL1RN* is 280 kb telomeric from *IL1B*.

The genes of the *IL1* cluster have been investigated as COPD candidates because of their

critical role in pulmonary inflammation. In mice, IL1A plays a central role in the initiation of smoke-induced neutrophilic inflammation, dendritic cell recruitment and activation [1, 2]. As a pro-inflammatory cytokine, IL-1 stimulates the liver to synthesize acute phase proteins including C-reactive protein (CRP), which is associated with lower level and rapid decline of lung function in COPD patients [3, 4]. Sputum IL1B was identified as the best marker for bacteria, virus, or a sputum eosinophilia related COPD exacerbations [5], a well-known risk factor of lung function decline [6].

Functional variants in the *IL1* gene cluster have been reported previously. The T allele of *IL1A*_-1613C/T (rs1800587) has been associated with increased *IL1A* expression both at mRNA and protein levels [7, 8]. Functional single nucleotide polymorphisms (SNPs) in *IL1B* have also been reported to influence transcription [9, 10]. The *IL1RN* gene has an 86 bp variable number tandem repeat (VNTR) in intron 3 (rs2234663). The number of repeats may have a functional significance because the repeated sequence contains possible binding sites for transcription factors [11]. Association of CRP levels and variants in the *IL1* gene cluster has been reported in diseases other than COPD [12-15].

We previously reported there was no association of a SNP in *IL1B* (*IL1B*_-511C/T) and the VNTR in intron 3 of the *IL1RN* gene with rate of lung function decline in smoking-induced COPD [16]. However, haplotype analysis showed a significant association [16]. Since our report there have been several studies of variants of the *IL1* gene cluster and COPD and/or lung function, although the data have not been consistent with regard to the associated genes or SNPs [17-20]. A recent meta-analysis suggested that subjects carrying the IL1B (-511) polymorphism T allele and subjects carrying the IL1B (-31) C variant were associated with an increased risk for developing COPD in East Asian population but not in all study populations [21]. The meta-analysis also suggested that there was an association between the IL1RN (VNTR) polymorphism and COPD risk [21]. The aim of the current study was to further investigate our previous association by genotyping additional SNPs in the same study subjects, and to explore the association of variants in the *IL1* gene cluster with CRP concentrations and with rate of decline of lung function.

Materials and methods

Study participants

We selected 287 Non-Hispanic whites (NHWs) who had the fastest and 308 NHWs who had no decline of FEV1 from 3,216 subjects who continued to smoke during the initial 5 years of follow up in the Lung Health Study, a multicenter randomized clinical trial to determine whether a program of smoking intervention could slow the rate of decline in pulmonary function over a 5-year follow-up period [22]. Informed consent was obtained from all participants and this investigation received the approval of the Providence Health Care Research Ethics Board. In order to attain approximately 300 participants for each group, arbitrary cut off points of FEV1 % predicted/year decrease ≥ 3.0% and increase ≥ 0.4% were used for rapid decliners and non-decliners, respectively.

Measurements of serum CRP concentrations

The concentrations of serum CRP were measured using highly sensitive enzyme-linked immunosorbent assay kits (Alpha Diagnostics, San Antonio, TX) by laboratory personnel who were unaware of the study participants' clinical outcomes. The median interassay coefficient of variation was 6.3%.

TagSNP selection and genotyping

TagSNPs were selected from complete resequencing data from the European American Descent populations of the SeattleSNPs Program for Genomic Applications (pga.gs.washington.edu) using the LDselect program [23]. LDselect parameter thresholds of $r^2 > 0.8$ and minor allele frequencies (MAFs) greater than 10% were used. Five, six and nine SNPs in the *IL1A*, *IL1B* and *IL1RN* genes were chosen and genotyped, respectively. Among these 20 SNPs, one SNP in *ILB* has been reported in our previous study [16].

Genotyping of the 20 selected SNPs was performed in 384 well plates in a total volume of 5 μl by the TaqMan 5' exonuclease assay using primers and probes supplied by Applied Biosystems (Applied Biosystems, Foster City,

Table 1. Distribution of demographic characteristics for the two study groups

	Fast Decliners (n = 280)	Non Decliners (n = 306)	*P* value
Men/Women	164/116	204/102	0.0428
Age (years)	49.50 (0.382)	47.6 (0.392)	0.0006
Smoking history (pack-yrs)*	43.0 (1.143)	38.4 (1.038)	0.0031
Body Mass Index (kg/m^2)	25.2 (0.226)	25.7 (0.216)	0.0754
ΔFEV_1/yr (% predicted pre)†	-4.14 (0.064)	1.08 (0.040)	< 0.0001
ΔFEV_1/yr (% predicted post)‡	-3.44 (0.077)	0.68 (0.053)	< 0.0001
Baseline FEV_1 (% predicted pre)§	72.68 (0.531)	75.68 (0.473)	< 0.0001
Baseline FEV_1 (% predicted post)‖	74.88 (0.547)	79.95 (0.456)	< 0.0001

Values are means ± SE for continuous data. *Number of packs of cigarettes smoked per day × number of years smoking. †Change in lung function over a 5 year period per year as % predicted FEV_1 pre bronchodilator. ‡Change in lung function over a 5 year period per year as % predicted FEV_1 post bronchodilator. §Lung function at the start of the Lung Health Study as measured by FEV_1 (%) predicted pre bronchodilator. ‖Lung function at the start of the Lung Health Study as measured by FEV_1 (%) predicted post bronchodilator.

CA). Twelve DNA samples with known genotypes from the CEPH panel were included as positive controls and 8 no template wells were included as negative controls in each plate. No discrepancies were detected in the 10% of samples that were randomly selected and genotyped in duplicate. One SNP in *IL1B* was also genotyped previously by a PCR-Restriction Fragment Length Polymorphism method [16] and the concordance rate with the TaqMan method was 99.6%.

Statistical analysis

Hardy-Weinberg equilibrium tests and linkage disequilibrium (LD) estimation were calculated using the genetics package for R (www.r-project.org). Multiple linear regression was performed for the complete data set to test for associations of SNPs with log-transformed CRP level.

Confounding factors included body mass index (BMI), age, gender, pack years of smoking, and smoking status. Multiple logistic regressions for the decline of lung function were performed to test for the association with SNPs; covariates included in the model were age, gender, pack years of smoking and recruitment centre.

Haplotype association was tested using Hapassoc, a contributed R package available at www.r-project.org. An additive effect of haplotype on phenotype was assumed. Haplotypes of each gene were considered separately. All covariates previously considered in the single SNP association linear regression models were included in the linear regression models testing for association of haplotypes with CRP expression levels or rate of decline of lung function.

Results

Characteristics of the study participants

In the total of 595 participants, there was no DNA available for the current study for 35 subjects; genotyping success rates were 94.1% to 98.5% for the 20 SNPs genotyped by the TaqMan method in 560 participants who had DNA available. The VNTR polymorphism in *IL1RN* was successfully genotyped previously using a traditional gel based PCR method on 569 out of 595 participants [16]. There were no significant demographic differences (age, gender, pack-years) between those with and without genotypic data in the rapid and non-decliner groups. The demographic characteristics for the 586 participants who had at least one genotype result are shown in **Table 1**.

There were significant differences in several potential confounding factors between rapid and non-decline groups. Therefore, the associations of genotypes/haplotypes with CRP expression and lung function were analyzed by logistic regression to adjust for these factors.

Hardy-Weinberg disequilibrium and LD pattern

The positions of the genotyped tagSNPs within the *IL1A*, *IL1B* and *IL1RN* genes are shown in **Table 2** and **Figure 1**. All the SNPs, with the exception of the *IL1RN*_102572, were in Hardy-Weinberg equilibrium. *IL1RN*_102572 was not in Hardy-Weinberg equilibrium (*P* = 0.004).

The LD patterns of the 20 tagSNPs in our study participants are shown in **Figure 2**. In general, SNPs in *IL1A* and *ILB* were in high LD, as were SNPs in *IL1RN*. SNPs in *IL1A* and *IL1B* were in low LD with SNPs in *IL1RN*, which is to be

Table 2. Associations of SNPs in *IL1A*, *IL1B* and *IL1RN* with rate of decline of lung function

Gene	SNP ID	Allele	Bin to which SNP represents*	Position in RefSequence†	Position in gene	Minor allele frequency‡		*P* value§
						Fast decliners	Slow decliners	
IL1A	rs3783515	T/C	1	2264	promoter	0.420	0.387	0.371
	rs3783521	C/T	2	4521	promoter	0.294	0.293	0.795
	rs2071374	A/C	3	10747	intron 4	0.292	0.247	0.115
	rs17561	G/T	4	10876	exon 5	0.285	0.311	0.371
	rs3783546	G/C	5	13270	intron 6	0.288	0.298	0.891
IL1B	rs1143625	A/G	1	302	promoter	0.277	0.259	0.742
	rs16944	C/T	2	794	promoter	0.336	0.320	0.895
	rs3917354	T/*	3	2766	intron 3	0.199	0.206	0.667
	rs1143633	G/A	4	5200	intron 4	0.343	0.363	0.648
	rs1143634	C/T	5	5277	exon 5	0.246	0.232	0.688
	rs1143637	G/A	6	6334	intron 5	0.250	0.233	0.621
IL1RN	rs4251961	T/C	1	1018	promoter	0.395	0.358	0.278
	rs2234678	A/G	2	2116	promoter	0.270	0.279	0.756
	rs315919	G/T	3	2765	intron 1	0.392	0.407	0.568
	rs315934	T/C	4	10257	intron 1	0.169	0.199	0.281
	rs3087263	G/A	5	12319	intron 2	0.113	0.092	0.133
	VNTR	A1/A2/A3	2‖	14659	Intron3	0.270	0.274	0.777
	rs380092	A/T	6	15453	intron 4	0.296	0.318	0.527
	rs579543	G/A	7	16184	intron 4	0.267	0.292	0.249
	rs315953	C/T	8	16556	intron 4	0.245	0.286	0.091
	rs315949	G/A	9	19327	intergenic	0.449	0.384	0.033

*The bins are derived from LDselect using SeattleSNP genotype data of a European population. SNPs in the same bin have high LD ($r^2 > 0.8$) and therefore provide similar information. †RefSequence of *IL1A* is AF536338, RefSequence of *IL1B* is AY137079 and RefSequence of *IL1RN* is AY196903. ‡In the case of the *IL1RN*_VNTR, the frequency of the A2 allele is listed in the table. §*P* values are for the additive genetic model adjusted for age, gender, pack-years and research center. ‖No genotype information was available from SeattleSNPs PGA. However, from the LD pattern of our genotypic data, it has strong LD with SNPs in bin 2.

expected since *IL1A* and *IL1B* are separated by only 40 kb, while *IL1RN* is 280 kb away from *IL1B*. Although we selected tagSNPs based on the criteria of $r^2 > 0.8$, high LD ($r^2 > 0.8$) exists between the following SNPs: *IL1A*_4521 with *IL1A*_13270, *IL1B*_302 with *IL1B*_794, *IL1B*_2766 with *IL1B*_5277 and *IL1B*_6334, *IL1RN*_1018 with *IL1RN*_19327, *IL1RN*_2116 with *IL1RN*_16184, *IL1RN*_15453 with *IL1RN*_16556.

Associations of SNPs and haplotypes in genes of the IL1 cluster with rate of decline of lung function

Before adjusting for multiple comparisons, only *IL1RN*_19327 (rs315949) showed significant association with lung function decline after adjustment for confounding factors (*P* = 0.03, additive model) (**Table 2**). The frequencies of genotypes containing the *IL1RN*_19327 A allele were 71.9% and 62.2%, respectively in fast and slow decline groups (*P* = 0.02, odds ratio = 1.6, 95% confidence interval = 1.1-2.3). However, none of 21 polymorphisms showed significant association with rate of decline of lung function after adjusting for multiple comparisons (21 comparisons).

No significant association was found for haplotypes of the three *IL1* cluster genes with rate of decline of lung function (**Table 3**).

Associations of SNPs and haplotypes in genes of the IL1 cluster with serum CRP concentrations

The associations of SNPs and haplotypes in genes of the *IL1* cluster with serum CRP concentrations were analyzed in all subjects by

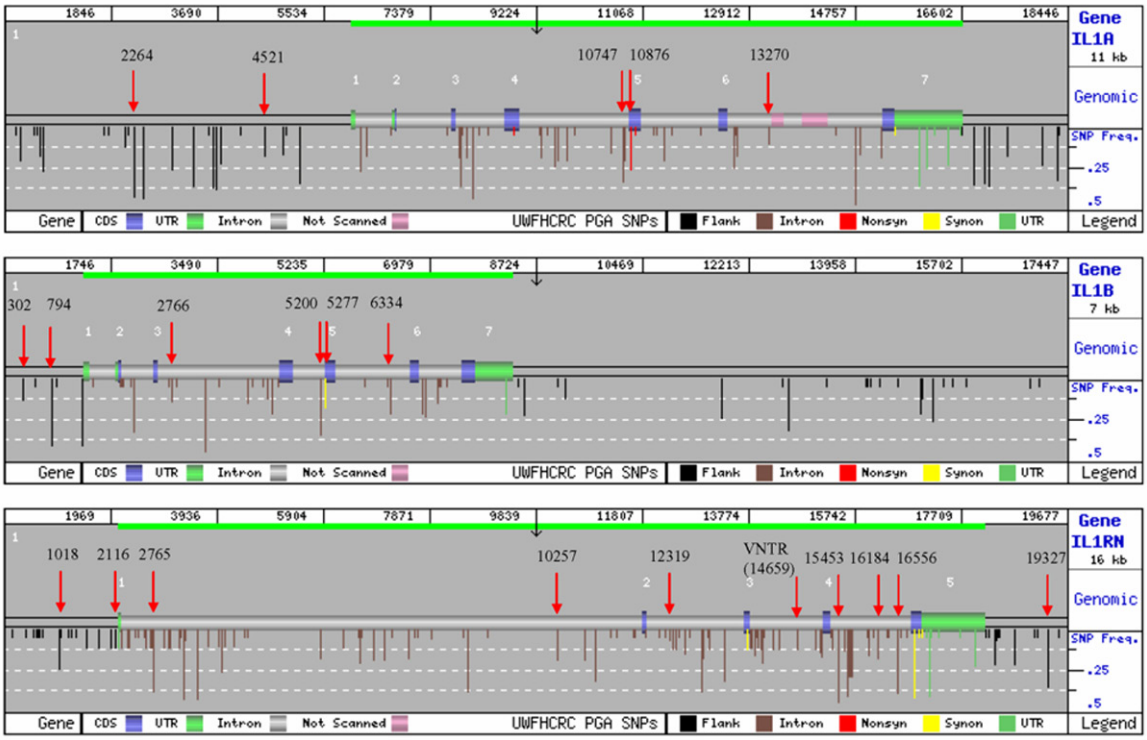

Figure 1. Position of genotyped tagSNPs within the *IL1A*, *IL1B* and *IL1RN* genes. GeneSNPs images of *IL1A*, *IL1B* and *IL1RN* from SeattleSNPs with locations of genotyped SNPs in current study indicated by red arrows. Coding regions of exons are shown in blue, and the UTR is shown in green. Polymorphic positions are indicated with ticks below the axis. Nonsyn = nonsynonymous; Synon = synonymous; CDS-coding sequence.

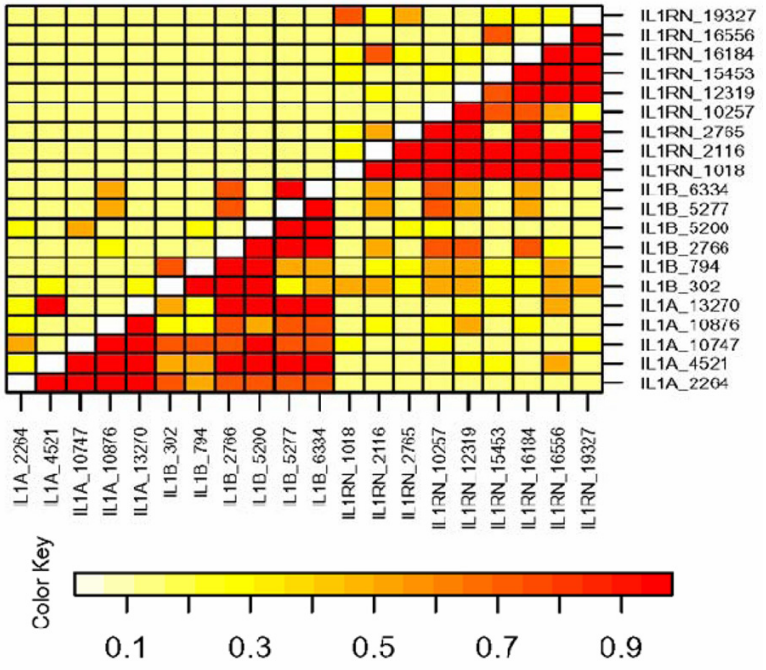

Figure 2. Pairwise measurement of linkage disequilibrium for the 20 genotyped tagSNPs of the *IL1* gene cluster. The color intensity denotes the r^2 or D' value with the r^2 presented in the upper left of the diagram and the D' in the lower right of the diagram.

multiple linear regressions adjusted for BMI, age, gender, and pack-years of smoking (**Table 4**). The *IL1B*_5200 SNP (rs1143633) in *IL1B* and the VNTR polymorphism in *IL1RN* were associated with log serum CRP levels (P = 0.04 and 0.03, respectively) before adjusting for multiple comparisons. After adjustment for multiple comparisons (21 comparisons), neither of the two associations remained significant. No significant association was found for haplotypes of the *IL1* gene cluster with serum CRP levels (data not shown).

Discussion

The rationale for this study was to follow-up our previous discovery of associations between haplotypes of *IL1B* and

Table 3. *IL1A*, *IL1B* and *IL1RN* haplotypes and decline of lung function

Gene	Haplotype	Haplotype frequency		*P* value*	*P* value† (Wald test)
		Fast decliners	Slow decliners		
IL1A	hTCATG	0.289	0.314		
	hTTAGC	0.289	0.296	0.737	0.368
	hCCCGG	0.289	0.247	0.152	
	hCCAGG	0.130	0.141	0.881	
	Other pooled	0.000	0.002	-	
IL1B	hACIACG	0.317	0.347		
	hGTIGCG	0.220	0.225	0.884	0.282
	hACDGTA	0.182	0.200	0.654	
	hACIGCG	0.116	0.110	0.646	
	hATIGCG	0.059	0.061	0.810	
	Other pooled	0.107	0.057	-	
IL1RN	hCAGTGAGCA	0.374	0.330		
	hTGTTGAACG	0.148	0.171	0.148	0.551
	hTATTGTGTG	0.120	0.129	0.337	
	hTAGCGTGTG	0.108	0.137	0.173	
	hTGTTAAACG	0.092	0.081	0.898	
	Other pooled	0.157	0.152	-	

*Comparisons of one haplotype against all other haplotypes between the two groups, adjusted for age, gender, pack-years and research center. †Global test of all haplotypes between the two groups.

Table 4. *IL1* gene cluster genotype associations with CRP concentrations

SNPs	Genotypes	N	B (95% CI)	*P* value*
*IL1B*_5200	GG+AG	477		
(rs1143633)	AA	72	-0.298 (-0.579, -0.017)	0.038
*IL1RN*_VNTR	A1A1	274		
(rs2234663)	A1A2	223	0.009 (-0.207, 0.225)	0.935
	A1A3	21	0.385 (-0.170, 0.941)	0.175
	A2A2	44	-0.101 (-0.491, 0.290)	0.614
	A2A3	7	1.027 (0.113, 1.941)	0.028

*Adjusted for age, sex, BMI and smoking. Only associations with $P < 0.05$ are listed in the table.

IL1RN and rate of lung function decline in smoking-induced COPD [16]. We performed more extensive genotyping to capture the genetic variation in *IL1A*, *IL1B* and *IL1RN* to see which variants related to the rate of decline of FEV_1 and serum CRP concentrations using the same nested case-control sample from the LHS. No associations were found for *IL1* gene cluster variants and either the rate of decline of FEV_1 or serum CRP concentrations after adjustment for multiple comparisons although moderate associations were found for *IL1RN* variants with rate of decline of lung function and for *IL1RN* and *IL1B* variants with serum levels of CRP before correcting for multiple comparisons.

In order to compare our studies with the seven published studies involving associations of variants in the *IL1* gene cluster with lung function and/or COPD, we have summarized these results in **Table 5**. In a recent study, Shukla et al. showed that in a subgroup analysis, Genotype IL1RN*2/IL1RN*2 was protected from COPD in male subjects, but IL1RN*1/IL1RN*2 was risk factor for females COPD [20]. Hegab and associates reported that, in a single SNP analysis, *IL1B* -511C/T (rs16944), -31T/C (rs1143627), and +3954C/T (rs1143634) polymorphisms were not associated with COPD [17]. However, haplotypes of *IL1B* -31T/C and +3954C/T were significantly associated with COPD in an Egyptian population but not in a Japanese population [17]. Asada et al. reported that the T allele of the -511 SNP of *IL1B* had a significant protective role against COPD in a Japanese population [18], which was not consistent with other studies performed in Japanese individuals [24] and India subjects [20]. Josyula et al. demonstrated that the annual rate of FEV_1 decline was greater in firefighters with the CT or TT genotypes of *IL1RN* 2108C/T than in those subjects with the CC genotype in a mixed population (non-Hispanic whites were the majority of participants) [19]. Recently, Issac MS, et al. reported that IL-1RN*1/*1 was associated with higher forced expiratory flow (FEF) 25-75% when compared with *1/*2 [25]. Haplotype IL-1RN *1/IL-1β T was associated with higher FEF25-75% predicted and FEV1/

Table 5. Association studies of *IL1* gene cluster variants with rate of decline of lung function or COPD

Study and population	Phenotype and sample size	SNP or haplotype	Bin to which SNP belongs*	Association
Current study Caucasian (USA and Canada)	FEV_1 decline 287 FEV_1 fast decliners 308 FEV_1 non-decliners	*IL1RN*_19327 (rs315949)	9 of *IL1RN*	Minor allele (A allele) was associated with fast decline of FEV1, P = 0.03 before adjusting for multiple comparisons.
Issac MS, et al. (2014) [25] Egyptian	COPD 63 patients 25 asymptomatic smokers 26 healthy controls	*IL1RN*_VNTR	2 of *IL1RN*	IL-1RN*1/*1 was associated with higher forced expiratory flow (FEF) 25-75% when compared with *1/*2 (P = 0.013)
		*IL1B*_-511 (rs16944)	2 of IL1B	Haplotype IL-1RN *1/L-1β T associated with higher FEF 25-75% predicted and FEV_1/FVC when compared with IL-1RN *2/IL-1β T (P = 0.005 and 0.048, respectively)
Shukla et al. (2012) [20] India	COPD 204 patients 208 control	*IL1RN*_VNTR	2 of IL1RN	No association was found in primary analysis IL1RNA2/IL1RA2 was found protective for male COPD, P = 0.017; IL1RNA1/IL1RNA2 was associated with females COPD, P = 0.011
		*IL1B*_-511C/T	2 of IL1B	No association was found
Our previous study (2001) [16]	FEV_1 decline	Haplotypes of *IL1B*_-511C/T, (rs16944) and *IL1RN*_VNTR	2 of *IL1B* and 2 of *IL1RN*	Global test P = 0.0005
Josyula et al. (2007) [19] USA	Rate of FEV_1 decline in 67 firefighters	*IL1RN*_2108 (in strong LD with the *IL1RN*_VNTR, is *IL1RN*_2116 in the reference sequence)	2 of *IL1RN*	Genotypes with minor allele (T allele) were associated with fast decline of FEV_1, p = 0.05 before adjusting for multiple comparisons
Hegab et al. (2005) [17] Japanese/Egyptian	COPD (Japanese) 88 patients 61 control COPD (Egyptian) 106 patients 72 control	*IL1B*_-31 (rs1143627)	2 of *IL1B*	No association was found
		*IL1B*_-511 (rs16944)	2 of *IL1B*	No association was found
		*IL1B*_F105F (rs1143634): is *IL1B*_5277 in the reference sequence	5 of *IL1B*	No association was found
		Haplotypes of *IL1B*_-511C/T and *IL1B*_3954 (*IL1B*_5277 in the reference sequence)	Haplotypes of bin 1 and 5 of *IL1B*	No association was found in Japanese, *P* = 0.0037 for Egyptians
Asada et al. (2005) [18] Japanese	COPD 85 patients 68 control	*IL1B*_-511 (rs16944)	2 of *IL1B*	The minor allele (T allele) was protective from COPD, *P* = 0.02 before adjusting for multiple comparisons
		*IL1B*_-31 (rs1143627) is *IL1B*_1274 in reference sequence	2 of *IL1B*	No association was found
Ishii et al. (2000) [24] Japanese	COPD 53 patients 65 control	*IL1B*_-511 (rs16944)	2 of *IL1B*	No association was found
		*IL1B*_F105F (rs1143634): is *IL1B*_5277 in the reference sequence	5 of *IL1B*	No association was found

Note: The bins are derived from LDselect and are numbered as in **Table 2**. SNPs in the same bin have high LD and therefore provide similar genetic information.

FVC when compared with IL-1RN *2/IL-1β T [25]. It should be noted that in previous studies all positive results were not adjusted for multiple comparisons or not results from primary analysis, and the negative studies did not have enough power to rule out true associations due to small sample sizes. Our relatively large sample size yields adequate power to detect moderate genetic effects especially with variants that have MAFs of more than 0.20 (18 out of 21 polymorphisms studied in the current study have MAF > 0.20) [26, 27]. Several possible reasons for our failure to identify the single most significant variants from previous significant haplotype associations include: 1) polymorphisms of the *IL1* gene cluster do not play

Table 6. Association studies of the IL1 gene cluster variants with serum CRP levels

Study and population	Subjects, sample size, genes and SNPs	Associated SNPs or haplotypes	Bin to which associated SNPs belong*	Association
Current study Caucasian (USA and Canada)	287 FEV_1 fast decliners 308 FEV_1 non-decliners 5 *IL1A* variants 6 *IL1B* variants 10 *IL1RN* variants	*IL1B*_5200 (rs1143633)	4 of *IL1B*	Homozygous minor allele (A allele) associated with lower CRP levels, *P* = 0.04 before adjusting for multiple comparisons
		*IL1RN*_VNTR	2 of *IL1RN*	Genotype A2A3 associated with higher CRP level, *P* = 0.03 before adjusting for multiple comparisons
Berger et al. [15] (2001) USA	454 individuals underwent angiography *IL1A*_4845 *IL1B*_3954, -511 *IL1RN*_2018	*IL1B*_3954 (*IL1B*_5277 in reference sequence)	5 of *IL1B*	The minor allele (T allele) was associated with higher CRP level, *P* = 0.001 before adjusting for multiple comparisons
Eklund et al. [14] (2003) Finland	338 blood donors *IL1A*_-899 *IL1B*_-511, 3954 *IL1RN*_VNTR	*IL1B*_3954 (*IL1B*_5277 in reference sequence)	5 of *IL1B*	The minor allele (T allele) was associated with lower CRP level, *P* = 0.038 before adjusting for multiple comparisons
Latkovskis et al. [12] (2004) Latvia	160 subjects with coronary artery disease *IL1B*_3954, -511 *IL1RN*_VNTR	*IL1B*_3954 (*IL1B*_5277 in reference sequence	5 of *IL1B*	The minor allele (T allele) was associated with higher CRP level, *P* = 0.014 before adjusting for multiple comparisons
		*IL1RN*_VNTR	2 of *IL1RN*	The allele 2 was associated with lower CRP level, *P* = 0.048 before adjusting for multiple comparisons
Lin et al. [30] (2007) Chinese	390 subjects with coronary artery disease *IL1B*_3954, -511, -31 *IL1RN*_VNTR	*IL1B*_-511 (rs16944)	2 of *IL1B*	Genotypes with minor allele (T allele) were associated with lower CRP, *P* = 0.06 before adjusting for multiple comparisons
Kikuchi et al. [34] (2007) Japanese	489 health checkup examinees IL1B_-31 (rs1143627)			No association was found
Reiner et al. [31] (2008) USA	1627 subjects from the Coronary Artery Risk Development in Young Adults study 1208 subjects from the Carotid Lesion Epidemiology and Risk Study 4310 subjects from the Cardiovascular Health Study 121 SNPs in 16 genes including 7 *IL1RN* SNPs	All 7 SNPs global test	7 SNPs	*P* = 0.01 after adjusted for multiple comparisons
		*IL1RN*_1018 in reference sequence	1 of *IL1RN*	The minor allele (C allele) was associated with higher CRP level (*P* < 0.0001) in pooled analysis
		*IL1RN*_13888 (r2232354)	Is not included in the bins of **Table 2**	Minor allele (G allele) was associated with lower CRP level (*P* = 0.06) in pooled analysis
		*IL1RN*_15132 (rs432014) C allele in complete LD with allele 2 of *IL1RN*_VNTR	2 of *IL1RN*	Minor allele (C allele) was associated with lower CRP level (*P* = 0.06) in pooled analysis
		*IL1RN*_15453 (rs380092)	6 of *IL1RN*	Minor allele (T allele) was associated with lower CRP level (*P* = 0.008) in pooled analysis

Note: The bins are derived from LDselect are numbered as in **Table 2**. SNPs in the same bin have high LD and therefore provide similar genetic information.

an important role in lung function change in COPD patients; 2) polymorphisms of the *IL1* gene cluster do play an important role in lung function change in COPD patients but the causal variant(s) have not been examined in the current study. Since we only studied polymorphisms with MAF ≥ 10%, we cannot rule out the possibility that variants with lower MAFs may play important roles for lung function change. 3) the causal allele(s) may be contained within several different haplotypes, which may only be identifiable by deeper resequencing of those subjects with risk haplotypes.

It has been reported that increased CRP concentrations were associated with low lung function levels in healthy individuals [28, 29] and/or lung function decline in smoking-induced COPD [4, 28, 29]; and that variants in the *IL1* gene cluster may influence CRP levels. To increase the power of our association study, CRP concentration was used as an intermediate pheno-

type. In order to compare our results with other published studies, associations of variants of the *IL1* gene cluster with CRP levels are summarized in **Table 6**. Significant associations have been found mainly with variants of *IL1A* and *IL1RN*. In general, in small to moderate sample size studies including ours, the associations were significant only before correcting for multiple comparisons; in addition the associations were not consistent, e.g. the *IL1B*_3954T allele (*IL1B*_5277T in the reference sequence AY137079) was reported to be associated with higher CRP level [12, 15], lower CRP level [14] or not associated [30]. There are relatively few studies of variants of *IL1RN* with regard to CRP level, but Latkovskis et al. reported that *IL1RN*_VNTR allele 2 was associated with lower CRP level [12]. Reiner and colleagues reported that several *IL1RN* tagSNPs were associated with serum CRP in a total of over 7,000 subjects consisting of three independent studies and the associations were not affected after adjusting for SNPs that are not in high LD with each other [31]. The order of significant associations from strong to weak was *IL1RN*_1018 (rs4251961), *IL1RN*_19327 (rs315919), *IL1RN*_13888 (rs22323540), *IL1RN*_VNTR [31]. Although we did not find significant associations of *IL1RN*_VNTR allele 2 with CRP level, we did find that subjects who were homozygous for allele 2 had the lowest CRP levels, which can be used to explain homozygous for allele 2 was a protective factor for male subjects. We could not confirm our result that subjects with the A2A3 genotype had the highest CRP levels due to the lack of this genotype in other studies.

Among many factors that could explain the inconsistent associations of *IL1RN* variants with CRP levels, a small effect size is an important consideration. Reiner et al. recently reported a two stage whole genome association study and candidate gene study on CRP levels on 6015 subjects from three studies; they found that *IL1RN* was only the 13th most important among 17 genes that were associated with CRP levels [32]. The most significant gene was *CRP*, however, *CRP* SNPs only accounted for 1.4% of the total variation in CRP concentrations in a previous study [33].

In our study, before adjusting for multiple comparisons, the two variants (*IL1B*_5200 and the *IL1RN*_VNTR) that were found to be associated with CRP levels were not associated with decline of lung function; the variant (the *IL1RN*_19327) that we found associated with decline of lung function was not associated with CRP levels in our study. We reasoned that this may be due to multiple SNPs contributing to CRP levels as Reiner et al. demonstrated [32] and that variants of *IL1* gene cluster only contribute to a very small portion of total variation in CRP concentrations. The fact that *IL1B*_19327 was significantly associated with CRP level [31] and the fact that *IL1B*_19327 is in high LD with *IL1B*_1018, which had the most significant association with CRP levels in the study of Reiner et al. [31], support our finding that there was an association of *IL1B*_19327 with lung function. However, even if this association is true it cannot explain the haplotype association we found previously [16].

In summary, in the current study we were unable to identify the causal allele(s) that contribute to the haplotype association we previously reported [16]. However, if the haplotype association we found previously is replicated in other studies, deeper resequencing of individuals with risk haplotypes is warranted in order to identify the causal allele(s)

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Disclosure of conflict of interest

None.

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