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The CRP Genotype, Serum Levels and Lung Function in Men: The Caerphilly Prospective Study

Short Title: CRP genotype and lung Function

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Abstract

Background Systemic C-reactive protein (CRP) has been associated with impaired lung function. A causal relationship would increase the value of CRP as both a diagnostic and therapeutic tool. We assessed the association between lung function parameters, circulating CRP and *CRP* polymorphisms using Mendelian Randomisation in efforts to attribute causality to known associations.

Methods Spirometric parameters of FEV₁ and FVC were determined in 2173 men participating in the Caerphilly Prospective Study. Lung function measures on 1021 participants were available at follow up (16.8 mean yrs later). Serum CRP level was measured at baseline and three *CRP* polymorphisms were analysed. Haplotype analysis was performed.

Results Serum CRP levels at baseline were inversely associated with contemporaneous FEV₁ and FVC as well as at follow-up ($p < 0.001$) even after adjustment for conventional confounders. Serum CRP was associated with FEV₁ decline, $p = 0.04$. All three *CRP* polymorphisms (rs1800947, rs1130864, rs1205) predicted serum CRP; however there were no clear associations of the polymorphisms or haplotypes with lung function or with lung function decline.

Conclusion Serum CRP was associated with lung function cross-sectionally however *CRP* polymorphisms were not associated with lung function or decline, suggesting that the CRP-lung function relationship is either due to reverse causality, unmeasured confounding factor or only has modest causal effect.

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Introduction

Circulating C-reactive protein (CRP), an acute phase protein has been used widely in studies evaluating associations between low-grade systemic inflammation and chronic respiratory disease. Several studies have demonstrated the association of CRP to impaired lung function, expressed as forced expiratory volume in first second (FEV₁) in healthy subjects [1,2] and additionally with greater decline in lung function, (1) although there are conflicting results [2]. The association of CRP with lung function has been shown to be independent of confounders such as smoking. Systemic inflammation has also been implicated in chronic obstructive pulmonary disease (COPD), which is associated with a lower FEV₁ and higher serum CRP [3,4].

Low-grade systemic inflammation has also been implicated in cardiovascular morbidity and mortality and hence an inflammatory link has been postulated in the observed relationship between lower lung function and more cardiovascular events [5-7] CRP is a central target for predictive and possibly therapeutic purposes in both COPD and cardiovascular disease. Indeed, some studies have suggested there may be benefits from pharmacological lowering of serum CRP levels in at-risk populations [8]. However reverse causality or confounders may explain the associations [9,10].

In this study we hypothesised that a causal role for CRP in reducing lung function could be identified by the method of Mendelian Randomisation: the *CRP* gene contains single nucleotide polymorphisms (SNPs), which are known to determine baseline serum CRP levels. If higher serum CRP levels reduce lung function, then a *CRP* polymorphism that was associated with higher levels of serum CRP should also lead to impaired lung function [11]. By Mendelian laws of inheritance, alleles are inherited independently of each other and randomly at a population level tend not to be associated with confounding factors [12]. Furthermore, disease processes do not influence germline genetic variants, so reverse causation whereby disease processes influence apparent exposures, rather than vice versa, will also be avoided [12]. Our study would thereby mimic a randomised trial [13]. Sunyer *et al* employed this strategy in their small cross-sectional study of post-MI survivors in Italy [14]. Similarly, two studies demonstrate conflicting results for an association of *CRP* polymorphism with development or presence of COPD [15,16].

Here we examine the association between three previously well-studied *CRP* polymorphisms (rs1130864, 1800947, rs1205) and haplotypes, circulating CRP levels and the continuous variables of lung function of FEV₁ and FVC and with lung function decline. These data allow us to explore whether the existing association between serum CRP and lung function is explained by residual confounding, reverse causation or implicates a genuine role for inflammation on adverse lung function.

Methods

Subjects

The Caerphilly Prospective Study is a population-based male cohort study of all men aged between 45 to 59 years who were resident in the small South Wales town of Caerphilly[17]. The initial examination (phase 1) took place between 1979 and 1983 where 2512 of the 2818 eligible men (89%) were recruited. An additional 447 patients were recruited at phase 2. Men were re-invited for further follow-up approximately every five years. The last follow-up (phase 5) occurred between 2002 and 2004.

Subjects gave written informed consent and the study had the approval of the local research ethics committee. At commencement, a detailed questionnaire was administered and review of their personal and medical history was collected. Items included their occupational social class (grouped as non-manual/manual), any established diagnoses of ischaemic heart disease, diabetes mellitus or hypertension, and their smoking habit. Smoking status was categorised according to never, ex, pipe and cigar smokers, or current cigarette smokers (1-15, 15+ cigarettes/day).

At phase 5 smoking status was re-established by classification into never, ex and current smokers.

Clinic measures

Height in bare feet was measured using a Holtain stadiometer and weight in light clothes using standardised scales. From this, body mass index (BMI) was calculated – kg/m^2 as a measure of obesity.

Spirometry

Spirometry at phase 2 was performed using the McDermott spirometer in a standing position. The FEV₁, forced vital capacity (FVC) and their ratio (FEV₁/FVC) were calculated. An acceptable result was noted if the two valid, best traces of the 3 performed had less than 100mls variability in FEV₁. At Phase 5, spirometry was performed using the Vitalograph spirometer (Vitalograph, Bucks, UK) with the same strict criteria and methodology. All measurements were taken pre-bronchodilator.

Venous blood samples

At phase 2, subjects attended an early morning clinic having fasted overnight and a blood sample was taken with minimal venous stasis. CRP was measured in a citrated sample, which had been centrifuged within one hour and stored at -70°C until analysed using a high sensitivity nephelometric assay (Dade-Behring, Germany). Intra- and inter-assay cv's for CRP were 4.7% and 8.3% respectively. The samples were analysed in 2000. Values are reproducible, stable over time and methods highly-standardised [18].

Genotyping

Genetic variants of the CRP gene on chromosome 1q21-q23 were identified on blood samples taken at phase 4. Three SNPs were used in the analysis; rs1130864 (G-A base change in the 3' untranslated region, AF449713 position 3014), rs1800947 (C-G base change at codon 188, AF449713 position 2667), and rs1205 (C-T base change in the 3' flanking/untranslated region, AF449713 position 3872). These SNPs were chosen on the basis of available evidence showing their replicable and robust association with circulating CRP levels [19-21]. Genotyping was undertaken by Kbiosciences (Hertfordshire, UK), which designed and used assays based on its proprietary competitive allele-specific polymerase chain reaction system method (www.kbioscience.co.uk). All assays were validated before use with a standard 96-well validation plate used by Kbiosciences. All variants were checked for adherence to Hardy-Weinberg equilibrium to avoid gross genotyping error, and a small number of internal validations and non-DNA test controls were used to validate genotyping after assay. Hardy-Weinberg equilibrium was tested at each SNP locus on a contingency table of observed-versus-predicted genotypic frequencies using an exact test. Our allelic frequencies at rs1800947 represent the base on the antisense strand, and C and G frequencies are therefore reversed in our analyses.

Statistical analysis

Statistical analysis was conducted in Stata version 10. Circulating CRP was \log_{10} transformed as it was not normally distributed. We have expressed results as back-transformed geometric means for serum CRP levels. All analyses involving lung function were adjusted for age (at specific phase) and height. While examining the effects of adjusting for more curvilinear models with different age

variables, we found that this did not improve the model significantly; hence the linear model for the age variable was used as the best simplest fit.

For the analyses, smoking status was grouped into never-(non)smokers, ex-smokers at phase 2, smokers at phase 2 and smokers at both phases 2 and 5. Subjects with a heterozygous state for rs1800947 (ie.C:G) were grouped with the homozygous genotype (G:G) due to low numbers (6 (phase 2) and 4 (phase 5) subjects had the G:G genotype). All 3 polymorphisms were found to be in Hardy-Weinberg equilibrium.

We used linear regression models to assess the association between CRP polymorphisms, serum CRP level and lung function tests. We then introduced various confounders in the model to assess any change in the association between serum CRP levels and lung function tests (BMI, smoking status, presence of ischaemic heart disease, diabetes or hypertension).

Haplotypes were constructed using the genetic data analysis program SIMHAP (<http://www.genepi.com.au>). SIMHAP uses current estimation–maximization-based methods for the estimation of haplotypes from unphased genotype data [22]. The current implementation of SIMHAP uses the statistical computing package R (www.r-project.org) to resolve haplotypes and provide their posterior probabilities. After the construction of haplotypes, SIMHAP is also able to examine relationships between common CRP haplotypes and circulating CRP concentration. All possible haplotype configurations are resolved for each individual within the program itself, and the posterior probability of each configuration is calculated. Association analyses within the generalized linear model framework then uses simulation to correctly deal with the uncertainty around imputed haplotypes. In the case of plasma CRP concentration, mean values by common CRP haplotype were derived from the regression coefficients and CIs estimated by SIMHAP. For these analyses, all common haplotypes were run in the model assuming additive effect of each haplotype and setting a baseline haplotype as the most common in this sample (1000 iterations were used).

Results

In phase 2 there were 2398 participants, of which 2173 individuals had valid lung function measures. By the time the men were rescreened for phase 5, only 1225 men were seen for whom we obtained valid lung function data on 1021 (83%). For the men not seen, 667 had died, 86 had moved out of the area, 125 could not be traced and 295 refused to participate. Lung function at phase 5 was measured on average 16.8 years later (interquartile range 16 to 18 years). Basic participant characteristics as well as baseline data on non-participants are shown in Table 1.

There were differences in the mean values or percentages of participants in phase 2 who have follow-up lung function measures at phase 5 compared to participants who have been lost to follow-up, Table 1. These findings are largely consistent with the healthy survivor effect: participants who have been lost to follow-up (ie. likely deceased) are more likely to have had worse lung function, been heavier smokers and suffered from concomitant cardiovascular disease or diabetes.

Table 2 shows the relationship between alleles of the *CRP* polymorphism rs1800947 and serum CRP along with potential confounders. The geometric mean ratio (GMR) for rs1800947 was 0.72, 95% CI 0.61 to 0.84 $p < 0.001$. We present data for this *CRP* polymorphism because it showed the strongest effects on serum CRP levels. The effects of rs1130864 and rs1205 polymorphisms on serum CRP were as follows: GMR for rs1130864 GMR=1.1, 95% CI 1.00 to 1.21, $p=0.04$; for rs1205 GMR=0.87, 95% CI 0.79 to 0.95, $p=0.002$. No association was found between confounding variables and the polymorphisms except the rs1205 where the heterozygotes had a reduced IHD risk of $p=0.01$ compared to either the wild or mutant homozygous, which is likely to represent a chance finding.

Phase 2 circulating CRP showed predictive value for both phase 2 and 5 lung function measures (p values all ≤ 0.02), Table 3. A one log-unit increase in Phase 2 CRP level is associated with reduced FEV₁ and FVC ($\beta = -0.13$ and -0.14 respectively), and reduced FEV₁/FVC ratio ($\beta = -0.007$) in phase 2.

Repeat analysis adjusting for potential confounders (BMI, smoking, presence of diabetes, ischaemic heart disease and hypertension, occupational status) had only minor effects on the β -coefficients, with significant relationship maintained, except for FEV₁/FVC ratio at phase 5 ($p=0.104$); hence the association with serum CRP could not be abolished by conventional confounders. The FEV₁ and FEV₁/FVC decline, but not FVC, between phase 2 and 5 was associated with Phase 2 serum CRP, but was attenuated in the fully adjusted model.

Having found associations between lung function with serum CRP, we then explored lung function in relation to the *CRP* genotype, Table 4. In phase 2 and 5, the *CRP* polymorphisms were not associated with lung function measures. Looking at lung function decline, however, there were weak associations with FVC decline. The rare rs1205 genotype (T:T) is associated with lower CRP levels, but was associated with an increased decline in FVC ($\beta = 8.8$, 95% CI 0.89 to 16.79, $p=0.03$).

The haplotype frequency was as previously published [23]. There were 4 common haplotypes with a frequency $>5\%$. Inclusion of the haplotype into the lung function model (either phase 2 or phase 5 or the longitudinal change) was not superior to excluding them (all p values for likelihood ratio test were all greater than a p value of 0.1). The lack of haplotype supporting data for the weak effect of polymorphism rs1205 on FVC decline reinforces the borderline p value and perhaps reflects multiple p values.

Discussion

This study demonstrates a robust association between *CRP* polymorphisms and serum CRP level but in this large male cohort found no clear association between the *CRP* polymorphisms and lung function at either phase 2 or 5 or longitudinally, which was supported further by the haplotype analysis [21,24]. We have confirmed the inverse association between serum CRP and lung function parameters as previously described [1,25,26], which is not attenuated by adjustment for confounders such as cardiovascular disease, smoking and BMI. Additionally, in the longitudinal analysis, circulating CRP at phase 2 was predictive of decline in FEV₁ over 16+ years.

Genetic polymorphisms are useful tools in assessing causality and associations between established risk factors and chronic disease states [10,27]. Their random acquisition by Mendelian Inheritance laws equates to a randomised controlled trial, and relationships are not affected by confounders [13]. Van Durme *et al.* failed to demonstrate an association of CRP haplotype and polymorphisms with a risk of developing COPD, although, here, the definition of COPD appears to be either physician based or according to spirometric evaluation alone, with “probable COPD” potentially encompassing a wider range of diagnosis and not dependent necessarily on spirometry [16]. In parallel, spirometric definitions of COPD according to the method protocol presented could have encompassed other non-COPD airways obstruction as smoking history, diurnal variation and atopic features did not appear to be a requisite. Our work is consistent with this report, but we have extended into the natural history of lung function deterioration, the intermediary of developing disease such as COPD or another chronic respiratory disease.

In the small cross sectional study on a limited number of post-MI patients recruited into the Italian AIRGENE study, Sunyer *et al* explored polymorphisms within the *CRP* and other inflammatory marker genes, noting that the rs1205 *CRP* polymorphism and one haplotype were associated with better lung function, the rare rs1205 polymorphism being associated with lower circulating CRP levels [14]. However, by the nature of the study, the participants all had a cardiovascular history and were pre-selected by being the “survivor” subset of myocardial infarction. In our study, the rate of decline was clearly only possible in those that had survived the follow-up period but was strengthened by the cross-sectional data (at 2 time-points) and was not reliant on every subject surviving a major cardiovascular insult. Meanwhile, Yanbaeva *et al.* detected an increased allele frequency between patients with COPD and controls for the CRP SNP rs1800947, although its significance after adjusting for age, sex and tobacco consumption was lost [15]. The haplotype inclusion was consistent with chance and interestingly, the biggest difference in haplotype frequency was the H2 (24.2% in COPD patients and 30.3% in the controls), yet H2 showed only a modest effect on CRP, $p=0.34$. They did detect however a more clear association of a 4 fold increased COPD risk with *IL-6* polymorphism yet this was associated with a lower CRP level.

The inverse association of circulating CRP with lung function measures may thus indicate that reverse causality is the link - with impaired lung function causing the elevated circulating CRP. In patients with COPD, there is an apparent persisting inflammatory state with further elevation in inflammatory mediators at times of exacerbations [3,28]. In addition, serum inflammatory status has been associated with lung function in apparently healthy subjects, even never smokers[28]. However, in patients with mild to moderate COPD, there was no parallel in inflammatory mediators in the sputum and serum [29]. Similarly, in healthy subjects, there was no set systemic response in subjects when exposed to inhalational lipopolysaccharide [30]. Both of these studies suggest the systemic inflammation is not simply overspill from the airways component.

The elevated serum CRP in patients with lower lung function may be mediated through other pathways for eg. *IL-6* is an upstream mediator of CRP production. In deed, Yanbaeva *et al.* found a much stronger relationship of *IL-6* polymorphism with a diagnosis of COPD than with the *CRP* polymorphisms however this polymorphism was actually related to lower circulating CRP levels (14). He *et al.* have recently reported SNP's to *IL-6* associated with lung function decline and susceptibility to COPD in smokers,[31] however this requires replication. Alternatively, an, as yet,

unknown common mediator may be the key. In a similar manner, whilst serum CRP has been associated with atherosclerosis and with the metabolic syndrome, *CRP* genotypes have failed to be associated with arterial stiffness, carotid-intima thickness, metabolic syndrome or coronary heart disease [10,23,32-34]

Our study has several strengths by being a large community based study of unselected male population. It examined the associations both cross-sectionally and with longitudinal measures of lung function decline over a long period of time. The latter is a much more sensitive phenotypic measure as cross-sectional measures cannot differentiate between developmental and degenerative trajectories over the life course [35]. For lung function decline, there was likely a healthy survivor effect but the lack of association of genotype with lung function at either cross sectional analysis strengthens the lack of association with decline. Our sample size cannot rule out moderate sized effects given our confidence intervals. However to avoid the problems of publication bias, it is important that such results are which can then be included in a meta-analysis to explore small or modest effect sizes. Unfortunately, from the data collected, smoking pack years could not be calculated. It must be borne in mind, that the Caerphilly Prospective study is limited to Welsh men only and therefore may not be generalisable to women. Although similar aetiology is likely, verification is required. Similarly it is not generalisable to other geographical areas as there are differences. Lung function measures were pre-bronchodilator and thus may include asthmatics. However, our aim *a priori* was not to diagnose disease states but to explore the lung function as a continuous variable and over time. Mendelian randomisation provides an opportunity to test causality. In general, limitations of this approach include confounding by polymorphisms in linkage disequilibrium with the polymorphism being studied, pleiotropy of the specific polymorphism, compensation during development of the effect of the polymorphism or if there was not a suitable functional polymorphism [12]. However, for this paper, we had recognised functional polymorphisms and we measured potential confounders in order to overcome these.

In conclusion, we have demonstrated that CRP is inversely associated with lung function parameters of FEV₁ and FVC. However, the *CRP* genotypes are not associated with lung function and for the first time we demonstrate they are not associated with lung function decline. This implies there may be no causal association though we cannot rule out small effect estimates. We believe that the association between CRP and lung function is more likely to reflect reverse causation or an unknown common mediator.

Author Contribution: YBS and JG were responsible for attaining the funding for Phase 5; YBS, JG and GDS sit on the steering committee for Phase 5 Caerphilly Prospective Study; CEB, JRC and YBS developed the hypothesis; CEB, WS, NT and YBS analysed the data; CEB wrote the first draft; all authors contributed to the interpretation of the data and writing the paper.

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References

- (1) Shaaban, R., Kony, S., Driss, F., Leynaert, B., Soussan, D., Pin I., Neukirch, F. and Zureik, M. (2006) Change in C-reactive protein levels and FEV1 decline: a longitudinal population-based study. *Respir Med*; **100**:2112-2120.
- (2) Fogarty, A.W., Jones, S., Britton, J.R., Lewis, S.A. and McKeever, T.M. (2007) Systemic inflammation and decline in lung function in a general population: a prospective study. *Thorax*; **62**:515-520.
- (3) Gan, W.Q., Man, S.F., Senthilselvan, A. and Sin, D.D. (2004) Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. *Thorax*; **59**:574-580.
- (4) Eid, A.A., Ionescu, A.A., Nixon, L.S., Lewis-Jenkins, V., Matthews, S.B., Griffiths, T.L. and Shale, D.J. (2001) Inflammatory response and body composition in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*; **164**:1414-1418
- (5) Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P. and Hennekens, C.H. (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* ; **336**:973-979.
- (6) Mendall, M.A., Strachan, D.P., Butland, B.K., Ballam, L., Morris, J., Sweetnam, P.M. and Elwood P.C. (2000) C-reactive protein: relation to total mortality, cardiovascular mortality and cardiovascular risk factors in men. *Eur Heart J*; **21**:1584-1590
- (7) Sin, D.D., Wu, L. and Man, S.F. (2005) The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. *Chest*; **127**:1952-1959
- (8) Ridker, P.M., Danielson, E., Fonseca, F.A., Genest, J., Gotto, A.M., Jr, Kastelein, J.J., Koenig, W., Libby, P., Lorenzatti, A.J., MacFadyen, J.G., Nordestgaard, B.G., Shepherd, J., Willerson, J.T. and Glynn, R.J.; JUPITER Study Group. (2008) Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med*; **359**:2195-2207
- (9) Zacho, J., Tybjaerg-Hansen, A., Skov Jensen, J., Grande, P., Sillesen, H. and Nordestgaard, B.G. (2008) Genetically Elevated C-Reactive Protein and Ischemic Vascular Disease. *New Eng J Med*; **359**:1897-1908
- (10) Timpson, N.J., Lawlor, D.A., Harbord, R.M., Gaunt, T.R., Day, I.N., Palmer, L.J., Hattersley, A.T., Ebrahim, S., Lowe, G.D., Rumley, A. and Davey Smith, G. (2005) C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. *Lancet*; **366**:1954-9
- (11) Carlson, C.S., Aldred, S.F., Lee, P.K., Tracy, R.P., Schwartz, S.M., Rieder, M., Liu, K., Williams, O.D., Iribarren, C., Lewis, E.C., Fornage, M., Boerwinkle, E., Gross, M., Jaquish, C., Nickerson, D.A., Myers, R.M., Siscovick, D.S. and Reiner, A.P. (2005) Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet*; **77**(1):64-77
- (12) Davey Smith, G. and Ebrahim, S. (2003) 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*; **32**(1):1-22

- (13) Davey Smith, G. And Ebrahim, S. (2005) What can Mendelian randomisation tell us about modifiable behavioural and environmental exposures. *BMJ*; **330**:1076-9.
- (14) Sunyer, J., Pistelli, R., Plana, E., Andreani, M., Baldari, F., Kolz, M., Koenig, W., Pekkanen, J., Peters, A. and Forastiere, F. (2008) Systemic inflammation, genetic susceptibility and lung function. *Eur Respir J* ;**32**:92-97
- (15) Yanbaeva, D.G., Dentener, M.A., Spruit, M.A., Houwing-Duistermaat, J.J., Kotz, D., Passos, V.L. and Wouters E.F. (2009) IL6 and CRP haplotypes are associated with COPD risk and systemic inflammation: a case-control study. *BMC Med Genet*; **10**:23.
- (16) van Durme, Y.M., Verhamme, K.M., Aarnoudse, A.J., Van Pottelberge, G.R., Hofman, A., Witteman, J.C., Joos, G.F., Brusselle, G.G. and Stricker, B.H. (2009) C-reactive protein levels, haplotypes, and the risk of incident chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*; **179**:375-382.
- (17) The Caerphilly and Speedwell Collaborative Group. (1984) Caerphilly and Speedwell collaborative heart disease studies. *J Epidemiol Community Health* 1984; **38**:259-262.
- (18) Emerging Risk Factors Collaboration, Kaptoge, S., Di Angelantonio, E., Lowe, G., Pepys, M.B., Thompson, S.G., Collins, R. and Danesh, J. (2010) C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. *Lancet*; **375**(9709):132-40
- (19) Russell, A.I., Cunninghame Graham, D.S., Shepherd, C., Robertson, C.A., Whittaker, J, Meeks, J., Powell, R.J., Isenberg, D.A., Walport, M.J. and Vyse, T.J. (2004) Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. *Hum Mol Genet* ; **13**(1):137-147.
- (20) Suk, H.J., Ridker, P.M., Cook, N.R. and Zee, R.Y. (2005) Relation of polymorphism within the C-reactive protein gene and plasma CRP levels. *Atherosclerosis*; **178**(1):139-145.
- (21) Zee, R.Y. and Ridker, P.M. (2002) Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. *Atherosclerosis*; **162**(1):217-219
- (22) Excoffier, L. and Slatkin, M. (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. *Mol Biol Evol*; **12**(5):921-927.
- (23) Schumacher, W., Cockcroft, J., Timpson, N.J., McEniery, C.M., Gallacher, J., Rumley, A., Lowe, G., Smith, G.D., Wilkinson, I.B. and Ben-Shlomo, Y. (2009) Association between C-reactive protein genotype, circulating levels, and aortic pulse wave velocity. *Hypertension*; **53**(2):150-157.
- (24) Hage, F.G. and Szalai, A.J. (2007) C-reactive protein gene polymorphisms, C-reactive protein blood levels, and cardiovascular disease risk. *J Am Coll Cardiol*; **50**(12):1115-1122.
- (25) Kony, S., Zureik, M., Driss, F., Neukirch, C., Leynaert, B. and Neukirch, F. (2004) Association of bronchial hyperresponsiveness and lung function with C-reactive protein (CRP): a population based study. *Thorax*; **59**(10):892-896.
- (26) Aronson, D., Roterman, I., Yigla, M., Kerner, A., Avizohar, O., Sella, R., Bartha, P., Levy, Y. and Markiewicz, W. (2006) Inverse association between pulmonary function and C-reactive protein in apparently healthy subjects. *Am J Respir Crit Care Med*; **174**(6):626-632.

- (27) Davey Smith, G., Lawlor, D.A., Harbord, R., Timpson, N., Rumley, A., Lowe, G.D., Day, I.N. and Ebrahim, S. (2005) Association of C-reactive protein with blood pressure and hypertension: life course confounding and mendelian randomization tests of causality. *Arterioscler Thromb Vasc Biol*; **25**(5):1051-1056.
- (28) Wedzicha, J.A., Seemungal, T.A., MacCallum, P.K., Paul, E.A., Donaldson, G.C., Bhowmik, A., Jeffries, D.J. and Meade, T.W. (2000) Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. *Thromb Haemost*; **84**(2):210-215.
- (29) Vernooij, J.H., Kucukaycan, M., Jacobs, J.A., Chavannes, N.H., Buurman, W.A., Dentener, M.A. and Wouters, E.F. (2002) Local and systemic inflammation in patients with chronic obstructive pulmonary disease: soluble tumor necrosis factor receptors are increased in sputum. *Am J Respir Crit Care Med*; **166**(9):1218-1224.
- (30) Michel, O., Dentener, M., Corazza, F., Buurman, W. and Rylander, R. (2001) Healthy subjects express differences in clinical responses to inhaled lipopolysaccharide that are related with inflammation and with atopy. *J Allergy Clin Immunol*; **107**(5):797-804.
- (31) He, J.Q., Foreman, M.G., Shumansky, K., Zhang, X., Akhbar, L., Sin, D.D., Man, S.F., DeMeo, D.L., Litonjua, A.A., Silverman, E.K., Connett, J.E., Anthonisen, N.R., Wise, R.A., Paré, P.D. and Sandford, A.J. (2009) Associations of IL6 polymorphisms with lung function decline and COPD. *Thorax*; **64**: 698-704
- (32) Lange, L.A., Carlson, C.S., Hindorf, L.A., Lange, E.M., Walston, J., Durda, J.P., Cushman, M., Bis, J.C., Zeng, D., Lin, D., Kuller, L.H., Nickerson, D.A., Psaty, B.M., Tracy, R.P. and Reiner, A.P. (2006) Association of polymorphisms in the CRP gene with circulating C-reactive protein levels and cardiovascular events. *JAMA*; **296**(22):2703-2711.
- (33) Schnabel, R., Larson, M.G., Dupuis, J., Lunetta, K.L., Lipinska, I., Meigs, J.B., Yin, X., Rong, J., Vita, J.A., Newton-Cheh, C., Levy, D., Keaney, J.F. Jr, Vasan, R.S., Mitchell, G.F. and Benjamin, E.J. (2008) Relations of inflammatory biomarkers and common genetic variants with arterial stiffness and wave reflection. *Hypertension*; **51**(6):1651-1657.
- (34) Elliott, P., Chambers, J.C., Zhang, W., Clarke, R., Hopewell, J.C., Peden, J.F., Erdmann, J., Braund, P., Engert, J.C., Bennett, D., Coin, L., Ashby, D., Tzoulaki, I., Brown, I.J., Mt-Isa, S., McCarthy, M.I., Peltonen, L., Freimer, N.B., Farrall, M., Ruukonen, A., Hamsten, A., Lim, N., Froguel, P., Waterworth, D.M., Vollenweider, P., Waeber, G., Jarvelin, M.R., Mooser, V., Scott, J., Hall, A.S., Schunkert, H., Anand, S.S., Collins, R., Samani, N.J., Watkins, H., Kooner, J.S. (2009) Genetic Loci Associated With C-Reactive Protein Levels and Risk of Coronary Heart Disease. *JAMA*; **302**:37-48
- (35) Ben-Shlomo, Y. and Kuh, D. (2002) A life course approach to chronic disease epidemiology: conceptual models, empirical challenges and interdisciplinary perspectives. *Int J Epidemiol*; **31**: 285-293

Table 1. Baseline Characteristics of subjects at Phases 2 and 5

	<i>Participants with phase 5 data</i>		<i>Participants without phase 5 data</i>		
	N	Mean or percent (SD/IQR)	N	Mean or percent (SD/IQR)	P
<u>Phase 2</u>					
Age (yrs)	1022	56.0 (4.4)	1302	57.6 (4.5)	<0.001
Height (m)	1013	1.72 (0.06)	1283	1.71 (0.06)	<0.001
CRP (mg/L) *	707	1.45 (0.73, 2.78)	825	2.01 (0.99, 4.22)	<0.001
FEV ₁ (litres)	958	3.01 (0.7)	1215	2.63 (0.7)	<0.001
FVC (litres)	1022	3.81 (0.7)	1302	3.50 (0.8)	<0.001
FEV ₁ /FVC ratio	958	0.79 (0.1)	1215	0.75 (0.1)	<0.001
BMI (kg/m ²)	1012	26.6 (3.4)	1279	26.3 (3.8)	0.02
Hypertension (%)	204	20.0	340	26.1	<0.001
Diabetes mellitus (%)	23	2.6	65	5.0	0.001
Ischaemic heart disease (%)	256	25.1	456	35.0	<0.001
Smoking status (%)					
Never-smoker	238	23.4	181	13.9	
Ex-smoker	415	40.8	462	35.5	
1-14 cigs/day or cigar/pipe	220	21.6	369	28.4	
15+ cigs/day	145	14.2	288	22.2	<0.001
<u>Phase 5</u>					
Age (yrs)	1022	72.8 (4.2)			
CRP (mg/L) *	699	2.80 (1.47, 5.58)			
FEV ₁ (litres)	1021	2.26 (0.6)			
FVC (litres)	1020	3.31 (0.8)			
FEV ₁ /FVC ratio	1016	0.68 (0.1)			
Smoking status (%)					
Never-smoker	283	30.2			
Ex-smoker	491	52.5			
1-14 cigs/day or cigar/pipe	107	11.4			
15+ cigs/day	55	5.9			
<u>Lung function decline between phase 2 and 5</u>					
FEV ₁ change (mls/yr)	957	-44.0 (21.7)			
FVC change (mls/yr)	1020	-29.8 (31.7)			
FEV ₁ /FVC change (%/yr)	952	-0.64 (0.55)			

Abbreviations : Standard deviation (SD) ; Interquartile range (IQR) ; Forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC); C-reactive protein (CRP); body mass index (BMI)

* geometric mean for log-transformed variable

Table 2. Mean values and coefficients for each allele of SNP 1800947

	C:C	C:G / G:G	Difference (β -coeff or %)	95% CI	P
	n=1164 (85.7%)	n=195 (14.3%)			
<i>Phase 2</i>					
Age (yrs)	56.8	56.5	-0.29	-0.96 , 0.38	0.40
Height (mtr)	1.71	1.72	0.003	-0.007 , 0.01	0.55
CRP (mg/L) *	1.72	1.23	0.72*	0.61 , 0.84	<0.001
BMI (kg/m ²)	26.6	26.5	-0.11	-0.67 , 0.44	0.69
Smoking status %					
Never	15.6	3.5			
Ex-smoker	34.3	4.9			
Cigar, pipe or 1-14/day	21.5	3.6			
15+/day	14.2	2.4	-0.06	-0.21 , 0.09	0.41
Ischaemic heart disease %	86.6	13.4	-0.02	-0.09 , 0.05	0.51
Hypertension %	84.8	15.2	0.01	-0.05 , 0.08	0.64
Diabetes Mellitus %	90.5	9.5	-0.01	-0.04 , 0.01	0.37
Non-manual %	84.2	15.8			
Manual %	86.4	13.6			0.29

Abbreviations : C-reactive protein (CRP); body mass index (BMI); confidence interval (CI); significance (P); β regression coefficient

* geometric mean ratios for log-transformed variables

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Table 3. Association between lung function and phase 2 serum (log)CRP

	Age and height adjusted				Fully adjusted model*			
	N	β	95 % CI	P	N	B	95 % CI	P
<i>Phase 2</i>								
FEV ₁	1418	-0.13	-0.16 , -0.10	<0.001	1408	-0.09	-0.13 , -0.06	<0.001
FVC	1516	-0.14	-0.17 , -0.10	<0.001	1506	-0.09	-0.12 , -0.05	<0.001
FEV ₁ /FVC	1418	-0.007	-0.01 , 0.002	0.005	1408	-0.01	-0.01 , 0.002	0.004
<i>Phase 5</i>								
FEV ₁	723	-0.12	-0.16 , -0.07	<0.001	721	-0.07	-0.12 , -0.03	0.002
FVC	721	-0.13	-0.18 , -0.08	<0.001	719	-0.08	-0.13 , -0.02	0.003
FEV ₁ /FVC	718	-0.01	-0.02 , 0.001	0.02	716	-0.01	-0.01 , 0.001	0.104
<i>Decline</i>								
FEV ₁	657	-1.98	-3.72 , -0.24	0.03	655	-1.89	-3.72 , -0.07	0.04
FVC	698	-1.70	-4.15 , 0.74	0.17	696	-1.64	-4.23 , 0.95	0.22
FEV ₁ /FVC	653	-0.05	-0.10 , -0.01	0.03	651	-0.04	-0.08 , 0.01	0.12

* Model adjusted for BMI, smoking, and presence of diabetes, ischaemic heart disease, hypertension and social class

Abbreviations significance (P); confidence interval (CI); Forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC);

All analyses adjusted for age and height (age at respective phase)

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Table 4. Associations between CRP polymorphisms and lung function parameters

		FEV ₁					FVC				
		N	β	95% CI	P	N	β	95% CI	P		
Phase 2											
<i>rs1800947</i>	CG/GG vs. CC	1224	0.01	-0.08 , 0.11	0.82	1305	0.008	-0.09 , 0.11	0.88		
<i>rs1130864</i>	AG vs. GG	1221	-0.02	-0.09 , 0.05	0.54	1302	0.01	-0.07 , 0.08	0.80		
	AA vs. GG		-0.003	-0.11 , 0.10	0.95		0.02	-0.09 , 0.14	0.72		
	P for trend				0.75				0.70		
<i>rs1205</i>	TC vs. CC	1221	0.04	-0.04 , 0.11	0.33	1301	0.01	-0.06 , 0.09	0.75		
	TT vs. CC		-0.04	-0.15 , 0.07	0.45		-0.09	-0.20 , 0.03	0.13		
	P for trend				0.90				0.31		
Phase 5											
<i>rs1800947</i>	CG/GG vs. CC	762	0.08	-0.04 , 0.20	0.21	763	0.1	-0.03 , 0.25	0.11		
<i>rs1130864</i>	AG vs. GG	763	-0.01	-0.1 , 0.08	0.80	764	-0.006	-0.1 , 0.09	0.90		
	AA vs. GG		0.1	-0.03 , 0.24	0.13		0.1	-0.05 , 0.26	0.18		
	P for trend				0.31				0.34		
<i>rs1205</i>	TC vs. CC	761	0.03	-0.06 , 0.12	0.49	761	0.04	-0.07 , 0.14	0.49		
	TT vs. CC		-0.04	-0.18 , 0.09	0.52		-0.02	-0.17 , 0.14	0.84		
	P for trend				0.86				0.88		
Lung function decline											
<i>rs1800947</i>	CG/GG vs. CC	511	-0.5	-5.71 , 4.65	0.84	544	4.3	-3.03 , 11.56	0.25		
<i>rs1130864</i>	AG vs. GG	515	-1.03	-4.72 , 2.65	0.58	547	-2.73	-7.96 , 2.51	0.31		
	AA vs. GG		2.83	-3.05 , 8.72	0.35		0.57	-7.76 , 8.90	0.89		
	P for trend				0.67				0.69		
<i>rs1205</i>	TC vs. CC	512	-0.9	-4.62 , 2.72	0.61	544	1.37	-3.89 , 6.63	0.61		
	TT vs. CC		1.05	-4.53 , 6.63	0.71		8.8	0.89 , 16.79	0.03		
	P for trend				0.96				0.06		

N=Number, CI= confidence interval, β= regression coefficient, P = significance

Abbreviations: Forced expiratory volume in 1 second (FEV₁); forced vital capacity (FVC); regression coefficient (β); confidence interval (CI); significance (P), number (N)

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