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Systemic inflammation, genetic susceptibility and lung function

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ABSTRACT

Local inflammation in airway diseases is well recognized, but less in known about the association between low-grade systemic inflammatory processes and lung function. Our aim is to assess the association between inflammatory markers and lung function taking into account polymorphisms in genes coding for inflammatory markers.

In 134 post-myocardial infarction patients six repeated measurements of C-reactive protein (CRP), interleukin 6 (IL-6) and fibrinogen in peripheral blood were assayed using high-sensitivity tests (n=741). Spirometry was conducted at baseline. Genotyping of single nucleotide polymorphisms (SNPs) was performed in genes coding for the inflammatory markers.

CRP and IL6 levels were negatively associated with FEV_1, FVC and FEF_{25-75}. *CRP* gene (both the polymorphism rs1205 and the haplotype 2 in this gene) showed a protective association with FEV_1 and FEF_{25-75}, and, to a lesser extent, with FVC. Rs1205 and haplotype 2 were both negatively associated with CRP levels in peripheral blood. Analysis with instrumental variables also showed a protective effect between these CRP gene polymorphisms and lung function (p=0.05).

Results are very suggestive that heritability of lung function is at least partly controlled by the *CRP* gene. Applying a Mendelian randomization approach, the study supports a causal association between low-grade general inflammation and airway diseases.

The local inflammatory process in the pathogenesis of COPD is well recognized (1). Less in known about the low-grade systemic inflammatory process in COPD (2). Many studies in the general population have reported higher levels of CRP and fibrinogen in peripheral blood of individuals with impaired lung function (3-9) and in patients suffering from COPD (10,129). Such associations may lead to the suggestion that pharmaceutical agents that lower CRP and fibrinogen levels could lower risk of COPD. However, reverse causality cannot be excluded given that most studies conducted to date are cross-sectional, and that a recent longitudinal study did not find an effect of CRP on decline in lung function over nine years (9). This study contradicts a previous longitudinal study showing that fibrinogen levels five years before moderately predicted lung function (13). This latter finding might suggest that low-grade systemic inflammation could be primary to the inflammatory process in the airways, parenchyma and pulmonary vasculature. However, these proteins were also associated with several factors that could confound the observed association, such as the occurrence of cardiovascular diseases. An approach to avoiding residual confounding and reverse causation is the use of Mendelian randomization (14). In this approach genotypes associated with acute phase proteins are directly related with the outcome. Very few studies have explored associations between genetic predisposition for an exaggerated inflammatory response and risk of deterioration in pulmonary function (15). Our aim is to assess the association between inflammatory markers and lung function taking into account polymorphisms in genes coding for these inflammatory markers.

METHODS

In a multicentre study in myocardial infarction (MI) survivors (the AIRGENE study) we collected repeated samples for measuring inflammatory markers; in one of the centres, Rome (Italy), lung function was measured. In total 134 post-MI patients were recruited 2.7 years, on average, after the last MI. Six repeated clinical examinations were scheduled--one every 4 weeks--providing a total of 741 blood samples. Patients with

chronic inflammatory diseases were excluded. Each clinical visit was scheduled on the same time and day of the week. If patients suffered from acute infections examinations were postponed. At each visit, an EDTA-plasma sample was collected and centrally assayed for CRP, fibrinogen, and IL-6 using a nephelometry (BN-II, Dade-Behring) and a high-sensitivity colorimetric sandwich ELISA (R&D Systems, Wiesbaden, Germany).. Baseline questionnaires, and a shorter instrument at each follow-up visit, were administered by interview. Blood pressure, height, and weight, N-terminal pro-B-type natriuretic peptide (NT-proBNP), cholesterol and glycosylated haemoglobin were measured at baseline. All partners received approval of the study protocol by their local human subjects committees. Informed consent was obtained from all patients at the first clinical visit after a detailed description of the study protocol.

Lung function was measured at baseline following the ATS-ERS standard procedure (16). Forced Vital Capacity (FVC), forced expiratory volume of the first second (FEV_1) and forced mid-expiratory flow (FEF_{25-75}) were selected as the outcome measurements. The last was chosen in order to have a measurement of bronchial obstruction more sensitive and independent from the expiratory effort than FEV_1.

Genotyping of 36 polymorphisms was performed in the genes for C-reactive protein (*CRP*), interleukin-6 (*IL6*) and fibrinogen (*FG*α, *FG*β and *FG*γ) (17). For the SNP selection, the SeattleSNPs Program for Genomic Applications database (http://pga.gs.washington.edu/) was used. SNPs were chosen on the basis of positional and functional aspects to enhance the chance of detecting associations. PCR primers were designed by Sequenom's MassArrayAssayDesign program, and then aligned to the gene cluster in order to check for accuracy and to eliminate false results from genotyping repeat regions (http://genome.ucsc.edu/cgi-bin/hgBlat). Genotyping was performed by using the MassARRAY system (Sequenom, San Diego, USA) as described previously (18). To reduce the problem of multiple comparisons, we assessed haplotypes.

Haplotypes have been reconstructed within blocks of high linkage disequilibrium using expectation-maximization algorithm and following the reading direction of the gene (18). Each SNP was tested for departures from Hardy-Weinberg-equilibrium (HWE) by means of a chi-square test or Fisher's exact test depending on allele frequency ($p>0.05$). None of these SNPs showed deviations from HWE.

Linear regression models were applied to evaluate the associations between FEV_1, FVC and FEF_{25-75} (as the outcomes) and inflammatory marker levels and SNPs, after adjusting for confounders including smoking, age, gender, height, and body mass index (BMI). Variables such as NT-proBNP, cholesterol and glycosylated haemoglobin were also treated as confounders. For each subject, the mean of the repeated measures of inflammatory markers was calculated. Levels of inflammatory markers were treated both continuously (after log transformation) and categorized in tertiles to assess the linearity of relationships. The common homozygote of each SNP was used as a reference group. Instrumental variables methods (19) were applied to obtain estimates of the unconfounded association between CRP and lung function, using the genetic variables as "instruments" or predictors of CRP levels in system of two simultaneous equations. This method takes into account the association between genotypes and CRP levels as well as the relationship between CRP levels and lung function, and has been previously applied to the Mendelian randomization approach (14, 20). Mendelian randomization assumes that the inheritance of common polymorphisms in genes associated with circulating levels of acute-phase proteins should be randomly allocated in maternal and paternal alleles at the time of gamete formation, according to the Mendel's second law, and therefore the association obtained under instrumental variables could not be confounded. Comparison of linear regression and instrumental variable regression results was done with the Durbin-Wu-Hausman test (19).

RESULTS

Lung function values as well as subject's characteristics and inflammatory markers are reported in table 1. CRP was negatively associated with all three lung function indicators, both using the inflammatory markers in tertiles and as continuous variables (table 2). IL6 was also associated with FEV_1 and FVC, with significant associations with the highest tertile or using a continuous variable. These associations were not confounded by smoking characteristics, or by variables related with MI severity (data not shown). Associations with fibrinogen were not statistically significant.

Table 1. Description of lung function, subject's characteristics and inflammatory markers. (N=134)

		Males (N=116)				Females (N=18)			
		Mean	Std. Dev.	Min	Max	Mean	Std. Dev.	Min	Max
FEV1	(l.)	2.95	(0.71)	[1.31 -	4.96]	2.21	(0.67)	[0.85 -	3.55]
FVC	(l.)	3.93	(0.78)	[2.11 -	5.82]	2.82	(0.74)	[1.23 -	4.27]
FEF 25-75	(l.)	2.53	(1.20)	[0.44 -	6.89]	2.15	(1.11)	[0.49 -	4.25]
Age	(years)	63.03	(8.69)	[39 -	79]	61.39	(11.62)	[42 -	80]
Height	(cm)	170.47	(6.92)	[154 -	190]	156.78	(8.00)	[145 -	172]
Weight	(kg)	79.65	(12.51)	[47 -	113]	66.78	(16.57)	[42 -	106]
BMI	(kg/m2)	27.37	(3.76)	[15 -	39]	27.15	(6.14)	[15 -	39]
Pack-years		1.19	(1.34)	[0.0 -	8.6]	0.66	(0.78)	[0.0 -	2.9]
Time since quitting smoking		N	%			N	%		
Current		11	9.5%			1	5.6%		
Ex <5 years		49	42.2%			9	50.0%		
Ex >5 years		27	23.3%			2	11.1%		
Never		29	25.0%			6	33.3%		
		Median		Min	Max	Median		Min	Max
		(N=647)				(N=94)			
Fibrinogen	(g/l)	3.03		[1.94 -	5.18]	3.75		[2.44 -	4.54]
CRP	(mg/l)	1.57		[0.16 -	15.33]	1.76		[0.37 -	11.46]
IL-6	(pg/ml)	2.25		[0.95 -	61.37]	3.19		[1.04 -	6.42]

Table 2. Change in ml of lung function measures per each increase in tertiles of inflammatory markers,
and with doubling‡ of inflammatory markers concentrations.

	FEV1 (ml)		FVC (ml)		FEF 25-75 (ml)	
	coef†	(95% CI)	coef†	(95% CI)	coef†	(95% CI)
Fibrinogen (g/l)						
< 2.9	0	(ref)	0	(ref)	0	(ref)
2.9-3.5	53.1	(-158.6 , 264.8)	52.1	(-180.5 , 284.6)	-21.5	(-422.2 , 379.2)
>3.5	-44.5	(-278.0 , 188.9)	-127.0	(-383.5 , 129.5)	1.7	(-440.2 , 443.6)
Log(Fibrinogen)‡	*-305.3*	*(-778.3 , 167.8)*	*-434.9*	*(-955.3 , 85.5)*	*-317.3*	*(-1213.9 , 579.3)*
CRP (mg/l)						
<1	0	(ref)	0	(ref)	0	(ref)
1-2.6	-166.6	(-365.3 , 32.1)	-331.9	(-549.1 , -114.7)	98.0	(-280.4 , 476.3)
>2.6	-344.3	(-563.4 , -125.3)	-378.0	(-617.5 , -138.5)	-423.9	(-841.1 , -6.7)
Log(CRP)‡	*-162.4*	*(-256.2 , -68.6)*	*-165.8*	*(-270.1 , -61.4)*	*-237.4*	*(-417.7 , -57.2)*
IL-6 (pg/ml)						
<1.8	0	(ref)	0	(ref)	0	(ref)
1.8-2.8	7.6	(-199.7 , 214.9)	31.8	(-197.5 , 261.0)	-73.3	(-469.4 , 322.7)
>2.8	-261.6	(-498.5 , -24.7)	-269.2	(-531.1 , -7.2)	-415.5	(-868.0 , 37.1)
Log(IL-6)‡	*-184.1*	*(-357.7 , -10.4)*	*-213.9*	*(-405.4 , -22.5)*	*-189.5*	*(-521.1 , 142.1)*

† Coefficient and 95% confidence interval, adjusted for age, sex, height, BMI, time since quitting smoking and pack-years.

Among polymorphisms in *CRP* and *IL6* genes, only SNPs in the *CRP* gene showed an association with lung function measures. The homozygote rare alleles of SNPs rs1205 and rs1800947 in the *CRP* gene were associated with better lung function (Table 3), rs1205 with a $p<0.01$ while the association for rs1800947 was not statistically significant.

Table 3. Change in ml of lung function measures for SNP's from *CRP* gene.

		Freq	**FEV1 (ml)**		**FVC (ml)**		**FEF 25-75 (ml)**	
	Allele	(%)	coef†	(95% CI)	coef†	(95% CI)	coef†	(95% CI)
CRP								
rs1205	CC	51.13	0	(ref)	0	(ref)	0	(ref)
	TC	39.85	119.3	(-52.5 , 291.0)	76.6	(-112.3 , 265.4)	197.7	(-137.6 , 533.0)
	TT	9.02	404.2	(109.4 , 699.1)	368.8	(44.5 , 693.1)	699.6	(123.9 , 1275.2)
rs1130864	AA	45.11	0	(ref)	0	(ref)	0	(ref)
	AG	42.86	-138.8	(-319.5 , 41.9)	-71.3	(-268.2 , 125.7)	-299.9	(-651.0 , 51.2)
	GG	12.03	-125.7	(-403.4 , 152.1)	-86.0	(-388.7 , 216.8)	-262.3	(-802.0 , 277.5)
rs1417938	AA	45.11	0	(ref)	0	(ref)	0	(ref)
	TA	42.11	-140.3	(-321.8 , 41.2)	-71.5	(-270.1 , 127.1)	-301.4	(-653.4 , 50.7)
	TT	12.78	-149.5	(-421.6 , 122.6)	-125.3	(-423.0 , 172.4)	-271.4	(-799.2 , 256.4)
rs3093068	GG	85.82	0	(ref)	0	(ref)	0	(ref)
	GC+CC	14.18	37.5	(-206.4 , 281.4)	58.1	(-206.8 , 323.0)	-2.5	(-476.0 , 471.1)
rs1800947	CC	92.54	0	(ref)	0	(ref)	0	(ref)
	GC	7.46	101.6	(-212.8 , 416.0)	28.0	(-314.1 , 370.2)	405.0	(-202.1 , 1012.1)
rs2794521	CC	48.51	0	(ref)	0	(ref)	0	(ref)
	TC	44.03	17.8	(-156.0 , 191.5)	28.4	(-161.0 , 217.8)	-8.1	(-347.2 , 331.1)
	TT	7.46	-279.5	(-605.8 , 46.9)	-257.8	(-613.4 , 97.9)	-422.8	(-1059.9 , 214.2)
rs3091244	GG	34.59	0	(ref)	0	(ref)	0	(ref)
	GA+GT	47.37	-61.8	(-250.9 , 127.3)	1.8	(-204.3 , 207.9)	-224.6	(-591.7 , 142.4)
	AA+AT+TT	18.05	-184.5	(-435.7 , 66.8)	-138.0	(-411.8 , 135.8)	-334.0	(-821.6 , 153.6)

† Coefficient and 95% confidence interval, adjusted for age, sex, height, BMI.

Consistently with the analysis of SNPs, haplotypes 2 and 5 in *CRP* gene, tagged by the rs1205 and rs1800947 SNPs, were associated with greater levels of lung function markers (table 4). The association with haplotype 2 was statistically significant for FEV_1 and FEF_{25-75} ($p<0.02$). No association was seen for haplotypes in the *IL-6* gene.

Table 4. Change in ml of lung function measures for haplotypes of *CRP* gene.

	Freq	**FEV1 (ml)**		**FVC (ml)**		**FEF 25-75 (ml)**	
	%	coef†	(95% CI)	coef†	(95% CI)	coef†	(95% CI)
CRP‡							
TAACCG	32.95	0	(ref)	0	(ref)	0	(ref)
TGTCTG	24.62	182.3	(32.4 , 332.3)	145.5	(-19.5 , 310.5)	316.3	(23.8 , 608.9)
CGTCCG	29.17	26.6	(-129.8 , 183.1)	15.5	(-156.7 , 187.6)	57.3	(-247.9 , 362.6)
TTTCCC	7.58	70.3	(-168.4 , 309.1)	64.2	(-198.5 , 326.9)	123.0	(-342.8 , 588.8)
TGTGTG	3.41	166.8	(-180.0 , 513.6)	80.0	(-301.7 , 461.6)	515.4	(-161.1 , 1192.0)
Rare*	2.27	238.9	(-174.8 , 652.6)	276.6	(-178.7 , 731.8)	232.5	(-574.6 , 1039.7)

† Coefficient and 95% confidence interval, adjusted for age, height, sex, BMI.
‡Order of SNP's tagging the haplotype: rs2794521 rs3091244 rs1417938 rs1800947 rs1205 rs3093068
* haplotypes with frequencies less 1%

Larger reductions of lung function levels in relation to CRP levels were observed after applying an instrumental variable regression approach based on SNP rs1205 and haplotypes 2 and 5 in the *CRP* gene (table 5) than when using linear regression (table 2), although the confidence intervals were larger and the p-values at the limits of statistical significance (Table 5). The generally low p-values of the test comparing the two sets of results suggests the models using instrumental variables are more suitable for these data than ordinary linear regression.

Table 5. Change of lung function measures (in ml.) with doubling of CRP levels using as Instrumental Variables the SNP rs1205 from *CRP* gene or the *CRP* Haplotype 2 and 5.

	CRP (Instrumental Variables)					
	RS 1205			Haplotype 2 - 5		
	coef† (ml)	95% CI	p*	coef† (ml)	95% CI	p*
FEV1	-750.5	(-1566.3 , 65.4)	0.028	-666.1	(-1391.5 , 59.3)	0.051
FVC	-628.0	(-1402.8 , 146.8)	0.108	-509.3	(-1186.9 , 168.3)	0.211
FEF 25-75	-1283.5	(-2792.7 , 225.7)	0.052	-1240.3	(-2664.5 , 184.0)	0.053

† Change (in ml) with doubling the CRP level.
* p-value for test of equality between ordinal linear regression and instrumental variables regression.

DISCUSSION

CRP and IL6 peripheral levels were found to be associated with lung function. We found *CRP* gene (the polymorphism rs1205 and the haplotype 2) was associated with better lung function. Rs1205 has been previously associated with lower levels of CRP in several studies (21-23). Rs1205 was strongly associated with lower baseline levels of CRP in the AIRGENE study including all individuals from the six centres (24). We also found better lung function in individuals with haplotype 2 of this gene. Haplotype 2 was related with lower levels of CRP in two previous studies (21, 25). Results of this study suggest that heritability of lung function as well as of baseline CRP levels is at least partly controlled by the CRP gene. Moreover, we found similar associations between rs1205 and haplotype 2 and lung function after using instrumental variables which, according to the Mendelian randomization approach, supports a primary association between low-grade general inflammation and airway diseases. The *IL6* gene was not associated with lung function, which might be explained by the local origin of IL6. Finally, we did not find an association between fibrinogen levels and lung function, and accordingly we did not find any association between FGα, *FG*β and *FG*γ genes and lung function.

An association between genotype and lung function provides evidence of a primary role of systemic inflammation in airway diseases based on the use of the Mendelian randomization approach (14). Mendelian randomization has been used to discard causal relationships between acute-phase protein genes and metabolic syndrome (14), blood pressure (20) or a cardiac event (26). In the present study, by contrast, an association between genotype and lung function was observed, suggesting a primary effect of low grade inflammation on lung function. A limitation however, is the small size of our study, as reflected in the precision of the estimation of the associations between the SNP rs1205 and CRP in our sample ($F = 5$, $p = 0.01$) in comparison with the greater precision when all subjects in the AIRGENE study were included ($p=10^{-8}$). It is important to know that the association between rs1205 and CRP is homogeneous across the six centres in the AIRGENE study. However, the lack of power when using data from the single center with available lung function measures might have biased our estimations using instrumental variables (19). Nevertheless, the fact that no association was observed between lung function and the SNPs unrelated to CRP levels (table 3), that after stratifying by smoking the association between CRP levels and lung function was stronger among never smokers (p for interaction=0.01), and the lack of any association between the rs1205 and the confounding variables ($p>0.7$) supports a potential causal association. The frequency of the rs1205 allele varied largely in different countries. In our study around 9% of subjects had the homozygote rare allele and around 24% had haplotype 2.

We observed a stronger effect on FEF_{25-75} than on FVC or FEV_1. This could be due to the small size of our study and random variation. However, given the internal consistency of our results and the higher sensitivity of FEF_{25-75} in detecting the bronchial obstruction at an early stage of the obstructive disease, we think that this result could be explained by an early effect of genetic predisposition on the peripheral airways.

As stated above a limitation in the present study is the small sample size that limits the possibility of finding significant associations with other SNPs in any of the genes examined. This could explain a lack of statistically significant associations with SNP

rs1800947 in the *CRP* gene, as suggested by the haplotype analysis. The origin of the study population--survivors of myocardial infarction--might also explain the lack of associations with fibrinogen, given that almost all patients were undergoing statin treatment. In addition, the likelihood of surviving a myocardial infarction may be dependent on respiratory comorbidity and also on the genetic predisposition which could affect the generalizability of the present results. However, the long time elapsed since the last MI implies that individuals should have returned to baseline levels of CRP when they had blood drawn for the present study. In addition, adjustment for variables related with MI severity and treatment did not confound the associations with lung function (data not shown). Moreover, the basis of the Mendelian randomization approach lies in the fact that genetic variants will not generally be liable to confounding by behavioural, socioeconomic, and physiological factors; will not be influenced by the onset of disease or by the tendency for individuals with disease to differentially report exposure history; and will not generally be influenced by factors determining how participants are selected into a study, either as a case or a control (26). In any case, the present study should be replicated in a general population.

The strength of the present study is the genotyping of the genes corresponding to inflammatory markers and the examination of their relation to lung function given the lack of studies to have assessed these genes previously (15). Another strength of the present study is the repetition of blood measures in all participants, which reduced the inter-subject variability common to these biomarkers. A recent paper on the effect of taking only a single measurement in a study of biomarkers has shown that for CRP using a single measurement could modify the regression coefficient by 40% (24). Finally, potential limitations of the Mendelian randomization apprach such as linkage disequilibrium, population stratification, and pleiotropy (26) do not affect our polimorphisms.

Overall, the present paper strengthens the idea that systemic low-grade inflammation has a primary effect on lung function. However, due to the small size of the present study and

the population origin, larger studies in general population involving genes in systematic inflammatory process are required to replicate present findings.

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References:

1. Pawels R, Rabe K. Burden and clinical features of chronic obstructive pulmonary disease (COPD. Lancet 2004; 364:613-20.
2. Sin DD, Man SF, Commentary: Fuelling the fire-systemic inflammation and development of lung disease in the general community. *Int J Epi 2006;35:1008-10.*
3. Dahl M, Tybjaerg-Hansen A, Vestbo J, Lange P, Nordestgaard BG. Elevated plasma fibrinogen associated with reduced pulmonary function and increased risk of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med 2001;164:1008-11.*
4. Engstrom G, Lind P, Hedblad B, Wollmer P, Stavenow L, Janzon L, Lindgarde F.. Lung function and cardiovascular risk: relationship with inflammation-sensitive plasma proteins. *Circulation 2002;106:2555-60.*
5. Mannino DM, Ford ES, Redd SC. Obstructive and restrictive lung disease and markers of inflammation: data from the Third National Health and Nutrition Examination. *Am J Med 2003;114:758-62*
6. Gan WQ, Man SF, Sin DD. The interactions between cigarette smoking and reduced lung function on systemic inflammation. *Chest 2005;127:558-64.*
7. Kony S, Zureik M, Driss F, Neurkirch C, Leynaert B, Neurkirch F. Association of bronchial hyperresponsiveness and lung function with C-reactive protein (CRP): a population based study. Thorax 2004; 59:892-6.
8. Aronson D, Roterman I, Yigla M, Kerner A, Avizohar O, Sella R, Bartha P,Levy Y, Markiewicz W. Inverse association between pulmonary function and C-reactive protein in apparently healthy subjects. Am J Respir Crit Care Med. 2006;174:626-32.
9. Fogarty AW, Britton JR, Jones S, Lewis SA, McKeever T. A prospective study of systemic inflammation and decline in lung function in a general population. Thorax. 2007 Jan 24; [Epub ahead of print]
10. Sin DD, Man SF. Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease. *Circulation 2003;107:1514-19.*
11. Gan WQ, Man SF, Senthilselvan A, Sin DD. Association between chronic obstructive pulmonary disease and systemic inflammation: a systematic review and a meta-analysis. Thorax 2004 Jul; 59(7):574-80.

12. VM Pinto-Plata, H Müllerova, JF Toso, M Feudjo-Tepie, JB Soriano, RS Vessey, BR Celli. C-reactive protein in patients with COPD, control smokers and non-smokers. Thorax 2006; 61:23-28.
13. Thyagarajan B, Jacobs DR, Apostol GG, Smith LJ, Lewis CE, Williams D. Plasma fibrinogen and lung function: the CARDIA Study. *Int J Epi 2006;35:1001-8*.
14. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day INM, Palmer L, Hattersley AT, Ebrahim S, Lowe GDO, Rumley A, Smith GD. C-reactive protein and its role in metabolic syndrome:mendelian randomisation study. *The Lancet, 2005;366:1954-59.*
15. Yanbaeva DG, Dentener MA, Creutzberg EC, Wouters EF. Systemic inflammation in COPD: is genetic susceptibility a key factor? COPD. 2006;3:51-61..
16. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. Standardisation of spirometry. Eur Respir J. 2005;26:319-38.
17. Peters A, Schneider A, Greven S, Bellander T, Forastiere F, Ibald-Mulli A, Illig T, Jacquemin B, Katsouyanni K, Koenig W, Lanki T, Pekkanen J, Pershangen G, Picciotto S, Rueckerl R, Schaffrath Rosario A, Stefanadis C, Sunyer J, AIRGENE Study Group. Air Pollution and inflammatory response in myocardial infarction survivors: gene-environment-interactions in a high-risk group. Study design of the AIRGENE study. (in press).
18. Weidinger 2005: Weidinger S, Klopp N, Wagenpfeil S, Rummler L, Schedel M, Kabesch M, Schafer T, Darsow U, Jakob T, Behrendt H, Wichmann HE, Ring J, Illig T. Association of a STAT 6 haplotype with elevated serum IgE levels in a population based cohort of white adults. *J Med Genet*. 2004;41:658-663.
19. Martens EP, Pestman WR, de Boer A, Belitser SV, Klungel OH. Instrumental variables. Apllications and limitations. Epidemiology 2006;17:260-7.
20. Davey Smith GD, Lawlor DA, Harbord R, Timpson N, Rumley A, Lowe GDO, Day INM, Ebrahim S. Association of C-reactive protein with blood pressure and hypertension. Life course confounding and mendelian randomization tests of causality. *Arteriocler Throm Vasc Biol 2005;25(5):1051-6*.
21. Kardys I, de Maat MP, Uitterlinden AG, Hofman A, Witteman JC. C-reactive protein gene haplotypes and risk of coronary heart disease: the Rotterdam Study. *Eur Heart J*. 2006;27:1331-1337.
22. Russell AI, Graham DSC, Shepherd C, Roberton CA, Whittaker J, Meeks J, Powell RJ, Isenberg DA, Walport MJ, Vyse TJ. Polymorphism at the C-reactive protein locus influences gene expression and predisposes to systemic lupus erythematosus. *Human Molecular Genetics*. 2004;13:137-147.
23. Miller DT, Zee RY, Suk DJ, Kozlowski P, Chasman DI, Lazarus R, Cook NR, Ridker PM, Kwiatkowski DJ. Association of common CRP gene variants with CRP levels and cardiovascular events. *Ann Hum Genet*. 2005;69:623-638

24. Kolz M, Koening W, Müller M, Andreani M, Greven S, Illig T, et al. Eddect of DNA variants involved in inflammatory pathways on mean plasma levels and variability of C-reactive protein in myocardial infaction survivors. (under review).
25. Block G, Dietrich M, Norkus E, Jensen C, Benowitz NL, Morrow JD, Hudes M, Packer L. Intraindividual variability of plasma antioxidants, markers of oxidative stress, C-reactive protein, cotinine, and other biomarkers. Epidemiology 2006;17:404-12.
26. Davey Smith G, Ebrahim S. What can mendelian randomisation tell us about modifiable behavioural and environmental exposures?"BMJ 2005 330:1076-9.