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Interaction between the C-260T polymorphism of the *CD14* gene and the plasma IL-6 concentration on the risk of myocardial infarction: the HIFMECH study

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Abstract

Experimental and clinical observations suggest that innate immunity plays a major role in the pathogenesis and progression of atherosclerosis. A common C-260T polymorphism in the promoter of the *CD14* gene, the trans-membrane receptor of lipopolysaccharides, has been inconsistently associated with coronary heart disease. Our objective was to evaluate the contribution of the CD14 polymorphism to the inflammatory response and to the risk of myocardial infarction (MI). We used an European case-control study, the HIFMECH study, comparing 533 men with MI and 575 sex- and age-matched controls. Associations between genotype and disease outcome, according to interleukin-6 (IL-6) and C-reactive protein (CRP) levels, were assessed using conditional logistic regression. The CD14/C-260T polymorphism was associated with plasma IL-6 levels, T/T subjects having higher plasma levels than C/C in cases but not in controls (mean $\pm$ S.D.: 2.04 ± 1.37 versus 1.70 ± 1.15, $p = 0.01$; 1.20 ± 0.75 versus 1.35 ± 0.88, $p = 0.31$, respectively). Overall, the CD14/C-260T polymorphism was not associated with the risk of MI. However, in individuals with IL-6 plasma levels in the highest tertile, T allele carriers had a higher risk of MI than C/C (OR: 1.85; CI 95 1.05–3.25). IL-6 increased the risk of MI in carriers of the T allele (OR for first versus third IL-6 tertile: 4.02; CI 95 2.24–7.21), but not in C/C (OR: 0.75; CI 95 0.32–1.74, $p = 0.004$ for interaction). The data indicate a role for CD14/C-260T in MI. The risk mediated by the polymorphism is highly dependent on IL-6 plasma levels.

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1. Introduction

Innate immunity is the early response of a host to infection that is characterised by a cascade of pro-inflammatory signaling events and the transcriptional activation of several immune-related genes [1]. Several molecules implicated in this response, such as C-reactive protein (CRP) and interleukin-6 (IL-6), have been considered as independent predictors of atherosclerosis and myocardial infarction (MI) [2]. CD14 plays a key role in innate immunity, as it is the receptor of lipopolysaccharides (LPS), a component of gram-negative bacteria, and is expressed mainly by mature monocytes, macrophages and activated neutrophil granulocytes [1]. Through the CD14 receptor, LPS induces inflammatory cytokines such as TNFα, IL-1, IL-6 and growth factors. Recently, a C-260T polymorphism has been described in the promoter of the *CD14* gene [3]. A potential functional role for this polymorphism has been suggested, as it alters an Sp1 transcription factor-binding site and modulates the activity of the promoter [4], with the T allele being associated with higher transcription. It has been shown that CD14/C-260T polymorphism is associated with circulating soluble CD14 (sCD14) levels and with the density of membranous CD14 in some [3,5,6] but not other studies [7,8].

Since the -260T allele results in higher levels of CD14 on the surface of monocytes, they would be expected to be more sensitive to activation than in C/C subjects, with higher secretion of inflammatory factors such as IL-6 and TNFα. The association between this polymorphism and the risk of coronary heart disease (CHD) is, however, contradictory. The -260T allele was associated with risk of MI in three case-control studies [5,7,8] and in a prospective study conducted in 429 subjects with CHD, the CD14 CC genotype was associated with incidence of new coronary occlusion [9]. By contrast, in a nested case control sample from the Physicians' Health Study [10] and in 3 case-control studies [6,11,12], no association between the CD14/C-260T polymorphism and CHD was observed.

Many genes are involved in immune function, indicating the vital survival advantage of such a complex system [13]. We, thus, hypothesised that such heterogeneity among studies could be due to interaction between the CD14/C-260T polymorphism and other variables involved in innate immunity on risk. We have used a European multicentre case-control study called the Hypercoagulability and Impaired Fibrinolytic Function MECHanisms Predisposing to Myocardial Infarction Study (HIFMECH). This has as its aim the identification of differences in risk markers for MI between subjects living in the North and the South of Europe.

2. Material and methods

2.1. Study subjects

Full details of the study design and recruitment criteria are presented elsewhere [14]. Male survivors of a first MI aged <60 years (excluding patients with familial hypercholesterolemia and insulin-dependent diabetes mellitus) and population-based individuals of the same age were recruited from the four centres: Stockholm, Sweden (STK); London, England (LDN); Marseilles, France (MRS); San Giovanni Retondo, Italy (SGR). Consecutive patients were invited to participate, along with randomly selected healthy men from the same catchment areas. In all, a total of 598 postinfarction patients and 653 controls were recruited into the study. Only 533 cases and 575 controls have been considered in any analysis as they correspond to individuals with partial or complete environmental information. Postinfarction patients were investigated 3–6 months after the acute event. Patients and control subjects were examined in parallel in the early morning after an overnight fast. Height and weight were recorded and the body mass index (BMI) was calculated as kg/m^2.

2.2. Genotyping for CD14/-260 C/T polymorphism and assay methods

Blood samples were obtained from the antecubital vein after an overnight fast, collected on citrate and EDTA and centrifuged at $2500 \times g$ for 30 min at 4 °C. Without delay, aliquots of platelet-poor plasma were transferred into plastic tubes, frozen on-site to −80 °C and subsequently sent on dry ice to core laboratories for centralised biochemical analyses. DNA was prepared in London by standard salting-out techniques [15]. Genotyping was performed by PCR-RFLP using restriction enzyme HaeIII. PCR was done in a 25 μl reaction volume, with 200 nM of each primer (sense: 5′-TAAGGCACTGAGGATCATCC and antisense: 5′-GGCTTCACACTTGTGAACTC), 200 μM of each dNTP, 75 ng of genomic DNA, 3.5 mM $MgCl_2$ and 0.25 U of Taq DNA polymerase. Conditions were: 40 cycles consisting of denaturation 94 °C, 30 s; annealing 60 °C, 45 s and elongation 72 °C 1 min, followed by a final extension step at 72 °C for 5 min. Eight microliters of the PCR product was digested using 2 U of HaeIII at 37 °C, and the digestion products were identified by migration on a 2% agarose gel stained with ethidium bromide. Digestion of the PCR product gives a 329 pb product for the T allele and 2 products of 170 and 159 pb, respectively, for the C allele.

Assay methods for lipids and insulin have been described [14]. Interleukin-6 was measured by a 2-site high sensitivity ELISA (R and D Systems, Oxon, UK). In SGR, problems with the shipment of a batch of samples led to data being unavailable for IL-6 concentrations in the first 100 cases and controls [17]. Cases and controls with IL-6 concentrations available did not significantly differ from those without with respect to the main cardiovascular risk factors.

C-reactive protein was determined with an in-house enzyme immunoassay, using rabbit anti-human antibodies [16]. Fibrinogen was determined by the Clauss thrombin-clotting method. Lipid parameters were assayed centrally in the Instituto di Ricovero e Cura a Carattere Scientifico at

the Casa Sollievo della Sofferenza hospital, San Giovanni Rotondo. Insulin, CRP and IL-6 were centrally measured in the Royal Free and University College Medical School (London), and fibrinogen in the Institute of Pharmacological Sciences (Milan).

2.3. Statistical methods

Statistical analysis was conducted using the package "Intercooled STATA" version 7.0 (College station, Texas). Departure from Hardy–Weinberg equilibrium in controls from each centre, was assessed using chi-squared tests. For a number of the variables, square root or log transformations were applied as a result of the non-normality of their distributions. The variables, which required log transformations, were systolic blood pressure, weight, body mass index, triglyceride, IL-6 and CRP, whereas fibrinogen required a square root transformation. The transformations were necessary to ensure that an assumption required for the statistical analysis was not invalidated. In order to present the results in more familiar form, for variables that were square root transformed, the means listed are the square of the mean of square root measurements and for log transformed variables, geometric means. Standard deviations for all transformed values given are approximate. Differences in continuous variables by CD14/C-260T genotype or by case control status were examined by analysis of variance (ANOVA), or analysis of covariance (ANCOVA) when adjustment was required, using transformed variables as appropriate. Differences in the frequency distribution of categorical variables by case-control status were examined by chi-squared tests. Associations between CD14 genotype and case control status were analysed by conditional logistic regression, hence, considering matching of cases to controls. Interactions between IL-6 and CD14/C-260T genotype, were considered via an interaction term in the model and hence represent deviations from multiplicative effects. IL-6 was considered both as a standardised continuous variable and as a categorical variable, relating to tertiles of IL-6, which were set in controls for each centre separately. The adjustment of continuous variables for centre was conducted using the 'adjust' command in STATA, which creates adjusted values in the linear regression setting. Examination of differences by genotype, after adjustment for centre differences, was conducted by computing estimates for each genotype group, while setting the values to the mean of all centres, i.e., the means are adjusted for centre and then presented by genotype.

3. Results

3.1. Baseline characteristics

The general characteristics of the HIFMECH study sample, divided on the basis of cases and controls are presented in Table 1. Cases had a higher BMI and were more likely to be current smokers, suffer from diabetes and have higher plasma triglyceride levels. Cholesterol concentrations and diastolic blood pressure were lower in cases than in controls but this was not unexpected since, following their MI, most cases were on lipid-lowering or antihypertensive medication. Cases showed significantly higher plasma concentrations of insulin, fibrinogen, IL-6 and CRP than controls. A total of 97.4% of the cases were treated with aspirin (all cases except those with contraindications or side effects), whereas 27.0% were on lipid-lowering treatment (26.7% in the North (STK + LDN) and 27.2% in the South (MRS + SGR)). B-blockers, calcium antagonists and ACE-inhibitors were taken by 60.2, 16.5 and 30.9% of the cases, respectively.

3.2. Association between CD14 genotypes and plasma concentrations of markers of inflammation

In the control groups, the distribution of CD14/C-260T genotypes was as expected from Hardy–Weinberg predic-

Table 1
General characteristics (mean ± S.D.) of the cases and controls studied[a]

Variable	Controls	Cases	p
Age (years)	51.5 (5.4); n = 563	51.9 (5.4); n = 525	0.18
BMI (kg/m^2)	26.1 (3.2); n = 564	27.1 (3.3); n = 521	<0.00005
Systolic blood pressure (mmHg)	127.9 (14.4); n = 562	127.9 (16.9); n = 515	0.99
Diastolic blood pressure (mmHg)	84.1 (8.5); n = 562	81.8 (10.2); n = 514	<0.00005
Smoking			
Current + Ex (%)	61.9; n = 349	82.3; n = 433	<0.00005
Never (%)	38.1; n = 215	17.7; n = 93	
Diabetes (type II) (%)	0; n = 0/558	11.3; n = 59/522	<0.00005
Insulin (pmol/L)	37.8 (23.9); n = 436	49.3 (34.3); n = 384	<0.00005
Proinsulin (pmol/L)	2.38 (1.67); n = 439	3.87 (3.40); n = 389	<0.00005
Cholesterol (mmol/L)	5.52 (0.97); n = 545	5.39 (1.18); n = 494	0.04
Triglyceride (mmol/L)	1.44 (0.61); n = 545	1.87 (0.77); n = 494	<0.00005
Interleukin-6 (pg/mL)	1.24 (0.78); n = 419	1.97 (1.34); n = 374	<0.00005
Fibrinogen (mg/dL)	340.2 (69.2); n = 525	371.3 (91.7); n = 487	<0.00005
C-reactive protein (mg/L)	1.44 (1.41); n = 238	2.38 (2.52); n = 490	<0.00005

[a] Based on the 526 cases and 564 controls with CD14 genotype.

tions (all $p > 0.1$). The frequency of the CD14/-260T allele was significantly different between centres in the control groups (Table 2). T allele carriers were more frequent in the South (MRS + SGR) than in the North (STK + LDN), this difference in allele frequency being observed in both cases and controls (79 versus 64%, $p < 0.01$; 79 versus 69%, $p < 0.05$; in controls and in cases, respectively).

As shown in Table 3, the CD14/C-260T polymorphism was associated with the plasma IL-6 concentration in cases ($p < 0.01$) but not in controls ($p = 0.31$), with carriers of the T allele having higher mean levels of IL-6. This difference remained after adjustment for environmental parameters known to influence IL-6 plasma levels such as age, smoking, systolic blood pressure, BMI, insulin, cholesterol and triglyceride plasma levels ($p < 0.01$). This difference was also present in non-smokers (1.61 versus 1.91 pg/mL for C/C and T/T, respectively; $p = 0.03$) and in individuals without diabetes (1.66 versus 1.93 pg/mL for C/C and T/T, respectively; $p = 0.02$). The effect of CD14/C-260T genotype on plasma IL-6 concentrations, observed in the entire patient group, was mainly accounted for by the patients taking lipid-lowering medication (Table 3). However, no significant interaction between lipid lowering drugs and CD14/C-260T genotype on IL-6 concentration was observed ($p = 0.44$). No differences in mean concentration of CRP or fibrinogen were observed according to CD14/C-260T genotypes in either cases or controls.

3.3. CD14/C-260T genotypes and the risk of MI

The genotype distribution of the CD14/C-260T polymorphism was not different between cases and controls, neither in the whole cohort ($p = 0.7$) nor in each centre separately (Table 2). To explore if genotype was modifying the relationship between inflammation risk factors and MI, three interactions were considered. No interaction between CRP and fibrinogen levels and the CD14/C-260T polymorphism were observed ($p = 0.3$ and 0.2, respectively). However, there was a significant interaction between CD14/C-260T genotype and plasma concentration of IL-6 on risk. IL-6 increased the risk of MI in carriers of the T allele (OR for 1-SD increase: 2.42; CI 95 1.91–3.04), but not in C/C subjects (OR: 1.35; CI 95 0.97–1.88, $p = 0.005$ for interaction). This interaction remained after adjustment for conventional cardiovascular risk factors (Table 4).

This interaction was also analysed after dividing the sample according to tertiles of IL-6. In individuals with plasma IL-6, concentrations in the highest tertile, T allele carriers had a higher risk of MI than C/C subjects (OR: 1.85; CI 95 1.05–3.25). In carriers of the T allele the risk of MI in individuals with IL-6 in the highest tertile was 6.37 compared with those in the lowest tertile (CI 95 3.78–10.83). In C/C subjects, the risk of MI in individuals with IL-6 in the highest tertile was 1.55 compared with those in the lowest tertile (CI 95 0.7–3.3). As shown in Fig. 1, the results after adjustment for conventional cardiovascular risk factors were not modified. In carriers of the T allele the risk of MI in individ-

Table 2
Genotype distribution and rare allele frequencies (95% CI) for CD14/C-260T in cases and controls (%)

Genotypes	Stockholm		London		North		Marseille		San Giovanni Rotondo		South		p^*
	Cases ($n = 179$)	Controls ($n = 176$)	Cases ($n = 54$)	Controls ($n = 70$)	Cases ($n = 233$)	Controls ($n = 246$)	Cases ($n = 99$)	Controls ($n = 121$)	Cases ($n = 194$)	Controls ($n = 197$)	Cases ($n = 293$)	Controls ($n = 318$)	
C-260T													
CC	60 (33)	65 (37)	12 (22)	24 (34)	72 (31)	89 (36)	20 (20)	29 (24)	42 (22)	39 (20)	62 (21)	68 (21)	0.004
CT	94 (53)	77 (44)	28 (52)	31 (44)	122 (52)	108 (44)	57 (58)	53 (44)	98 (51)	104 (53)	155 (53)	157 (49)	
TT	25 (14)	34 (19)	14 (26)	15 (22)	39 (17)	49 (20)	22 (22)	39 (32)	54 (28)	54 (28)	76 (26)	93 (29)	
T allele freq 95% CI	0.40 (0.35, 0.45)	0.40 (0.36, 0.46)	0.52 (0.42, 0.61)	0.44 (0.35, 0.52)	0.43 (0.38, 0.47)	0.42 (0.38, 0.46)	0.51 (0.44, 0.58)	0.54 (0.48, 0.60)	0.53 (0.48, 0.58)	0.54 (0.49, 0.59)	0.52 (0.48, 0.56)	0.54 (0.50, 0.58)	
p^{**}	0.20		0.34		0.18		0.11		0.88		0.61		
p^{***}	0.97		0.43		0.94		0.81		0.98		0.86		

* p for difference between centres in controls.
** p for difference in genotype distribution between cases and controls.
*** p for difference in T allele frequency between cases and controls.

Table 3
Mean concentrations (±S.E.) of markers of inflammation in patients and controls according to CD14/C-260T genotype (adjusted for centre)

Genotype	IL-6 (pg/mL)	CRP (mg/L)	Fibrinogen (mg/dL)
Controls			
CC	1.35 (0.88); $n = 121$	1.53 (1.48); $n = 145$	347.5 (69.9); $n = 146$
CT	1.24 (0.78); $n = 193$	1.43 (1.40); $n = 235$	339.5 (68.8); $n = 247$
TT	1.20 (0.75); $n = 105$	1.39 (1.37); $n = 129$	335.1 (68.4); $n = 132$
p	0.31	0.73	0.31
Cases			
All			
CC	1.70 (1.15); $n = 107$	2.10 (2.23); $n = 125$	368.5 (92.0); $n = 126$
CT	2.14 (1.47); $n = 194$	2.44 (2.58); $n = 256$	372.2 (91.9); $n = 256$
TT	2.04 (1.37); $n = 73$	2.54 (2.71); $n = 109$	371.0 (92.6); $n = 105$
p	0.01	0.32	0.93
No lipid lowering			
CC	1.80 (1.21); $n = 72$	2.38 (2.57); $n = 86$	373.7 (93.7); $n = 89$
CT	2.11 (1.41); $n = 133$	2.53 (2.72); $n = 179$	370.4 (92.7); $n = 180$
TT	2.07 (1.39); $n = 50$	2.73 (2.95); $n = 79$	370.0 (93.3); $n = 77$
p	0.27	0.72	0.96
Lipid lowering			
CC ($n = 34$)	1.37 (0.90); $n = 34$	1.56 (1.61); $n = 38$	355.7 (85.0); $n = 36$
CT ($n = 53$)	2.29 (1.47); $n = 53$	2.25 (2.30); $n = 69$	378.4 (86.4); $n = 68$
TT ($n = 24$)	2.06 (1.34); $n = 20$	2.22 (2.29); $n = 26$	375.2 (87.4); $n = 24$
p	0.003	0.19	0.44
p^*	0.44	0.65	0.44

* The *p*-value for interaction between lipid lowering drugs and CD14/C-260T polymorphism on plasma concentration of inflammation markers.

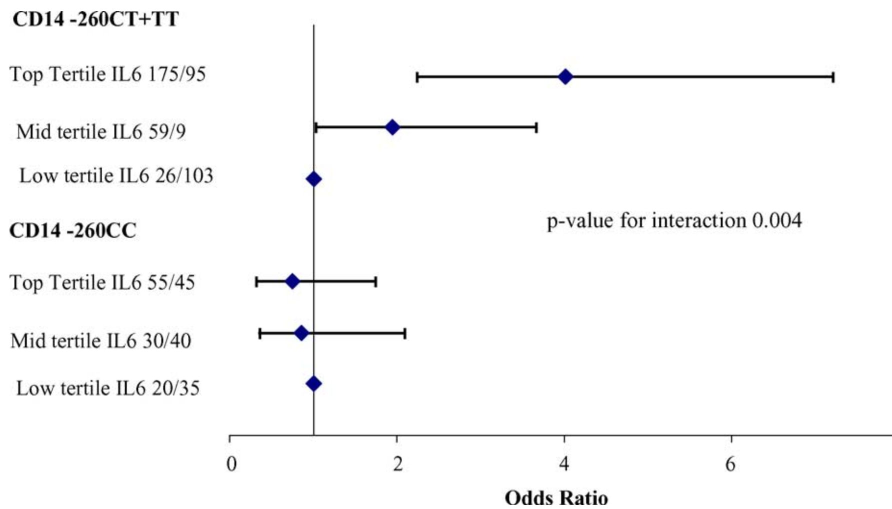

Fig. 1. Odds ratios for myocardial infarction for each CD14/C-260T genotype according to tertile of IL-6.

Table 4
Odds ratio (95% CI) for 1-SD increase in IL-6 plasma levels according to CD14/C-260T genotype after adjustment

CD14 C-260T	All	North	South
CC	0.93 (0.65, 1.34)	0.74 (0.45, 1.21)	1.17 (0.57, 2.40)
CT + TT	1.97 (1.55, 2.51)	1.65 (1.17, 2.33)	2.64 (1.68, 4.17)
p^*	0.0006	0.009	0.05

OR and 95% CI from stepwise reduced models initially considering adjustment for conventional cardiovascular risk factors: smoking, BMI, systolic blood pressure, insulin, triglycerides and cholesterol.

* The *p*-value for interaction between CD14/C-260T and IL-6 plasma levels on risk of MI.

uals with IL-6 in the highest tertile was 4.02 compared with those in the lowest tertile (CI 95 2.24–7.21). In C/C subjects, the risk of MI in individuals with IL-6 in the highest tertile was 0.75 compared with those in the lowest tertile (CI 95 0.32–1.74). This interaction was not altered after adjustment for other inflammation parameters such as CRP and fibrinogen ($p < 0.001$). This interaction was observed both in the North and the South (Table 4). However, the risk associated with higher IL-6 levels in individuals who carried the T allele was significantly higher in the South than in the North ($p = 0.01$ for interaction).

4. Discussion

The functional role of the CD14/C-260T polymorphism is still a matter of debate. Since the CD14/-260T allele results in higher levels of CD14 on the surface of monocytes, they would be expected to be more sensitive to activation than in C/C subjects. Since monocyte activation will lead to up-regulation and secretion of a number of inflammatory factors, including IL-6 [18], this would be seen as higher plasma IL-6 levels in CD14/-260T subjects. We observed such an association between the CD14/C-260T polymorphism and the IL-6 concentration in the HIFMECH study such that T allele carriers had higher IL-6 plasma levels. This effect would be predicted to be greater in subjects in an inflammatory situation such as those with CHD, explaining the fact that this effect was observed in cases but not in controls. In the study of Koenig et al. [6], no association was observed with plasma IL-6 levels, even in cases. This could be because the individuals studied had stable CHD, a situation less prone to inflammation and monocyte activation compared with acute events such as MI. It is known that the plasma IL-6 concentration is influenced by the polymorphism G-174C located in the promoter region of the *IL-6* gene, particularly in the inflammatory state [19,20]. We here provide evidence that plasma IL-6 levels are also controlled by the CD14/C-260T located in the *CD14* gene. The effect of this polymorphism is independent of the environmental factors that control IL-6 plasma levels such as age, smoking, BMI, glucose, insulin and lipids [21] as it remained significant after adjustment for these parameters. Moreover, the relation between plasma IL-6 concentration and the CD14/C-260T polymorphism was present in non-smokers and in individuals without diabetes. Interestingly, although there was no statistically significant genotype-by-treatment interaction, homozygotes for the C-allele appeared to normalise their IL-6 levels by lipid-lowering treatment (mean levels in non-treated 1.80 versus 1.37 pg/ml in treated) whereas carriers of the T-allele did not. This data must be interpreted with caution since it is based on a relatively small number of subjects, it is cross sectional, and precise data on the lipid-lowering drug and dose is not available. It implies that individuals with this genotype may be particularly sensitive to lipid lowering treatment (mostly statins in these subjects), but requires confirmation. A similar statin sensitivity in subjects with the IL-6/-174CC genotype has been reported [22].

The CD14/C-260T promoter polymorphism has been investigated as a risk factor for CHD in numerous studies, and inconsistent results have emerged from these analyses [5–12,23]. Despite the contribution on risk of MI by modification of plasma IL-6 levels, in the HIFMECH study, the CD14/C-260T polymorphism was not itself associated with a statistically significant effect on risk, in line with that has been observed in other studies [6,10–12]. However, the results of our study demonstrate a strong interaction between the CD14/C-260T polymorphism and plasma IL-6 levels on the risk of MI, an effect that was consistent in subjects from both the North and South of Europe. In individuals with IL-6 plasma levels in the highest tertile, T allele carriers had a higher risk of MI than C/C subjects (OR: 1.85; CI 95 1.05–3.25). IL-6 increased the risk of MI in carriers of the T allele (OR for first versus third IL-6 tertile: 4.02; CI 95 2.24–7.21), but not in C/C subjects (OR: 0.75; CI 95 0.32–1.74, $p = 0.004$ for interaction). The molecular mechanisms for this interaction on the risk of MI are not clearly understood. However, it seems not to be due to an overall effect of inflammation, as this interaction remained statistically significant after adjustment for CRP and fibrinogen, and there was also no significant evidence for interaction between CRP and fibrinogen levels and genotype on MI risk. IL-6 is not only a marker of inflammation, but participates itself in the immune response. It has been recently shown that IL-6, released from activated macrophages, acts as an amplifier during septic shock in a positive feedback loop, intensifying and disseminating the inflammatory response [18]. This effect could be due in part to a specific upregulation of CD14, as suggested by data showing that IL-6 increased CD14 expression by more than 10-fold in PMA-differentiated THP-1 cells [24]. It has been also suggested that one or more kinases activated by IL-6 may alter the phosphorylation state of the nuclear proteins binding to the Sp1 binding sites [25], thereby modulating its potency to stimulate CD14 transcription. Since the CD14/C-260T polymorphism is located at an Sp1 binding site that influences CD14 transcription [4], IL-6 may upregulate CD14 in an allele specific manner. This up-regulation in circulating monocytes, but probably more importantly in monocytes/macrophages, in the atherosclerotic plaque would cause inflammatory driven processes within the plaque that would destabilise the plaque [26] and increase risk of plaque rupture and thus of MI.

It is interesting to note that a North–South difference in allele frequencies was observed, with the T allele being more frequent in the South of Europe. The frequency of a number of CHD candidate gene variants has been reported to be different across Europe, most notably apoE [27] and to some extent these do contribute to the North–South CHD risk gradient. However, the higher frequency of the T allele in the South is contrary to this hypothesis, as is the higher risk associated with IL-6 levels in individuals who carried the T allele. Since CD14 has such an important role in innate immunity, this frequency difference is more likely to be due to selection pressure from infectious diseases, or simply chance events and population drift, than to selection via CHD.

One major concern of case-control studies such as this is the possibility of selection by mortality influencing the result. However, there is no pathophysiological reason to believe that carrying the C/C genotype increases the risk of coronary death. A second concern is the potential bias due to a continued acute phase inflammatory situation persisting even 3 months after the MI. This also appears unlikely to confound the interpretation since adjustment with other inflammatory markers such as CRP and fibrinogen did not alter the inter-

action between the CD14/C-260T genotype and IL-6 plasma levels on risk.

In conclusion, these results strengthen the probable functional role of the CD14/C-260T polymorphism. Moreover, they suggest a specific interaction between the CD14/C-260T polymorphism and plasma IL-6 levels on the risk of MI. This interaction provides a potential explanation to the discordance between studies on the relation between this polymorphism and the risk of vascular disease. In the studies where, by sample design, the cases have a low prevalence of high IL-6 concentration, the risk effect would be likely to be small. The data reinforce the importance of the inflammatory system in the development of CHD and support the role of anti-inflammatory therapies in its treatment.

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