SUPPLEMENTAL WEB-INFORMATION

METHODS

STUDY POPULATIONS

From the Second Northwick Park Heart Study (NPHS-II)[22] genotyping was conducted in 2676 Caucasian men of whom 103 developed a non-fatal MI after a median follow-up of 10.6 years. In a nested case-control genetic study from the West of Scotland Coronary Prevention trial (WOSCOPS),[23] which randomised 6595 male participants, initially free from clinically evident cardiovascular disease to either pravastatin or a placebo, 348 incident non-fatal MIs were identified and defined as cases and were matched by age and smoking status with 1103 controls drawn from the remainder of the cohort. In the Lower Extremity Arterial Disease Event Reduction (LEADER) trial[24] a randomised controlled trial of bezafibrate in men with lower extremity arterial disease, a total of 647 Caucasian men in the active treatment and 419 in the placebo arm were included. There were 43 incident non-fatal MIs during follow-up and these subjects were defined as cases. The Hypercoagulability and Impaired Fibrinolytic function MECHanisms predisposing to MI (HIFMECH)[25] study was a European multicentre case-control study of MI. For the present study a total of 491 cases and 517 controls were available for genotyping. The cross-sectional "Army-study" recruited Caucasian men from the Army Training Regiment, (Bassingbourn, UK) to evaluate the relationship between genetic factors and inflammatory response to exercise. DNA was extracted from 219 individuals for CRP genotyping and the measurement of CRP plasma concentration.[21] The UCL DiAbetes and Cardiovascular disease Study (UDACS) study[27] is a cross sectional case-control study to evaluate risk factors for coronary heart disease in subjects with diabetes mellitus. For the current study only Caucasian males (n=348) without coronary heart disease were included and contributed to first analysis. The Electron Beam Computerised Tomography (EBCT)[26] study was designed to compare coronary artery calcification and coronary risk factors in Caucasian Type-1 diabetic patients and non-diabetic participants.

Controls were a random sample of the general population (94 men and 107 women), stratified to have a similar age and gender distribution to the patients with diabetes. For the present study the male controls were included (n=72) and contributed to the first analysis.

LABORATORY ANALYSES

Plasma CRP concentrations for the NPHS-II (717 subjects) and LEADER studies were measured by use of commercial assays (R&D Systems). Inter-assay and intra-assay coefficients of variations were 6.2% and 1.9% respectively, with a detection limit of 0.1 mg/L. For additional 2221 subjects from the NPHS-II study an Enzyme Immunoassay (Kordia Life Sciences) was used to measured CRP concentrations. For the EBCT and HIFMECH studies CRP was measured with a highly sensitive in-house enzyme-linked immunosorbent assay with rabbit anti-human CRP (Dako, Copenhagen, Denmark) as a catching and tagging antibody with inter and intra-assay coefficients of variations of 4.7 and 3.8% and a limit of detection of 0.15mg/L. For the WOSCOPS study a validated in-house assay was used with a lower limit of detection of 0.1 mg/L. The intraassay and interassay coefficients of variation were 1.9% and 6.2%, respectively. In the Army-study, plasma CRP was measured on a BN Prospec (Dade Behring, Milton Keynes, UK). Inter-assay and intra-assay coefficients of variation were <4% and <2% respectively with a detection limit of 0.2 mg/L. In the UDACS study, CRP was measured using a highly sensitive ELISA assay (Dako A/S, Glostrup, Denmark). Inter-assay and intra-assay coefficients of variation were 8% and 10% respectively with a detection limit of 0.26 mg/L.

STATISTICAL ANALYSIS

Genotype and CRP concentration

We calculated the absolute geometric-WMD in CRP concentration by CRP genotype, using the following formula: geometric WMD= [(relative difference in CRP concentration between TT homozygotes and C-allele carriers × mean CRP concentration in C-allele carriers) – mean

CRP concentration in C-allele carriers]. In addition, to test whether the association between the CRP/+1444C>T polymorphism and CRP concentration could be confounded by other risk factors we compared the distribution of cardiovascular risk factors in groups defined by CRP genotype.

Derivation of the 95 percent confidence interval for the expected odds ratio

The 95% confidence interval for the expected OR for TT homozygous subjects with reference to C allele carriers was obtained by simulation. One million replications of the expected OR were obtained using the WMD in CRP by genotype and the OR from non-genetic observational studies with their corresponding standard errors, therefore the uncertainties surrounding the two associations, genotype-intermediate phenotype and intermediate phenotype-disease risk were take into account.[16] The values for the 2.5 and 97.5 percentiles of the simulated distribution were used as limits of the 95% confidence interval for the expected OR.[16] This expected OR was compared with the observed summary-OR obtained from the genetic studies, by means of an interaction test, as described in detail previously.[16,35]

Table I. Allele and genotype frequencies of CRP/+1444C>T polymorphism in the studies evaluated.

Study	Controls (number)	P value for H-W equilibrium	Cases (number)	P value for H-W equilibrium
NPHS-II				
+1444CC	1261	0.03	57	0.48
+1444CT	1047		41	
+1444TT	265		5	
T-allele frequency	0.30		0.24	
WOSCOPS				
+1444CC	511	0.88	175	0.17
+1444CT	481		136	
+1444TT	111		37	
T-allele frequency	0.32		0.30	
LEADER				
+1444CC	514	0.27	21	0.31
+1444CT	412		16	
+1444TT	97		6	
T-allele frequency	0.29		0.32	
HIFMECH				
+1444CC	254	0.27	246	0.28
+1444CT	224		196	
+1444TT	39		49	
T-allele frequency	0.29		0.30	
Army				
+1444CC	122	0.42	-	-
+1444CT	92		-	
+1444TT	13		-	
T-allele frequency	0.26		-	
UDACS				
+1444CC	167	0.12	-	-
+1444CT	139		-	
+1444TT	42		-	
T-allele frequency	0.32		-	
EBCT				
+1444CC	39	0.85	-	-
+1444CT	29		-	
+1444TT	6		-	
T-allele frequency	0.28		-	

-; Not applicable.