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Chemokine Ligand 2 Genetic Variants, serum MCP-1 Levels and the Risk of Coronary Artery Disease

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Abstract

Objective—In humans, evidence about the association between levels of monocyte chemoattractant protein-1 (MCP-1), its coding gene chemokine (C-C motif) ligand 2 (*CCL2*) and risk of coronary artery disease (CAD) is contradictory.

Methods and Results—We performed a nested case-control study in the prospective EPIC-Norfolk cohort investigating the relation between *CCL2* single nucleotide polymorphisms (SNP)'s, MCP-1 concentrations and the risk for future CAD. Cases (n = 1138) were apparently healthy men and women aged 45-79 years who developed fatal or nonfatal CAD during a mean follow-up of 6 years. Controls (n=2237) were matched by age, sex, and enrollment time. Using linear regression analysis no association between *CCL2* SNPs and MCP-1 serum concentrations became apparent, nor did we find a significant association between MCP-1 serum levels and risk of future CAD. Finally, Cox regression analysis showed no significant association between *CCL2* SNPs and the future CAD risk. In addition we did not find any robust associations between the *CCL2* haplotypes and MCP-1 serum concentration or future CAD risk.

Conclusions—Our data do not support previous publications indicating that MCP-1 is involved in the pathogenesis of CAD.

Keywords

Atherosclerosis; Coronary Artery Disease; CCL2; MCP-1 and Single nucleotide polymorphism

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Introduction

Chemokines (chemotactic cytokines) are small heparin-binding proteins that direct the movement of circulating leukocytes towards sites of inflammation, such as injury or atherosclerotic plaque. One of the best characterized chemokines is monocyte chemoattractant protein 1 (MCP-1; in the systematic nomenclature the gene is know as chemokine ligand (C-C motif) 2; *CCL2*) [1]. *CCL2* lies on the long arm of chromosome 17. It has 3 exons extending over ≈ 2000 bp. The gene has both distal and proximal regulatory elements important for cytokine and constitutive activity, respectively. MCP-1 is a potent chemoattractant for monocytes, dendritic cells, memory T cells, and basophils[2, 3]. MCP-1 is present in macrophage-rich atherosclerotic plaques[4, 5], where its production in endothelial and smooth-muscle cells is induced by oxidized low-density lipoprotein (LDL) cholesterol. MCP-1 has thus emerged as a potential link between oxidized lipoproteins and the recruitment of monocytes to the arterial wall. Several lines of evidence suggest that MCP-1 is indeed involved in atherosclerosis.

To clarify the role of MCP-1 in the pathophysiology of CAD, we conducted an analysis of the associations among *CCL2* genetic variants, serum levels of MCP-1 and the risk of future CAD among apparently healthy men and women.

Materials and methods

Participants

For the present nested case-control study in the EPIC-Norfolk prospective cohort (for a description of the cohort, please see supplemental material), we identified apparently healthy individuals who developed fatal or nonfatal CAD during follow-up. Apparently healthy individuals were defined as study participants who did not report a history of heart attack or stroke at the baseline clinic visit. Controls were apparently healthy study participants who remained free of cardiovascular disease during follow-up. Controls were matched cases by sex, age (within 5 years), and date of visit (within 3 months). The average follow-up was 6 years.

Biochemical analyses

Non-fasting blood samples were taken by vein puncture into serum tubes. Blood samples were stored at minus 80° Celsius before analysis. Lipid levels and C-reactive protein (CRP) levels were measured as described previously[6]. Serum MCP-1 levels were determined by a multiplex assay using the Bioplex Suspension Array (Bio-Rad, Veenendaal, The Netherlands) as readout system. All samples above the 95th percentile were repeated. Intra-assay coefficient of variation (CV) was less than 3% whereas the inter-assay coefficient of variation was 3.2%. Samples were analyzed in random order to avoid systematic bias. Researchers and laboratory personnel had no access to identifiable information and could identify samples by number only.

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MCP-1 genotyping and haplotype analysis

We selected 7 common *CCL2* SNPs: −2835A>C (rs2857654), −2578A>G (rs1024611), −2136A>T (rs1024610), −1811A>G (rs3760399), −927G>C (rs3760396), +764C>G (rs2857657) and +3726T>C (rs2530797) spanning the gene based on previously published selection criteria[7]. The SNPs −2835, −2578, −2136 and −1811 are located on the distal regulatory region, whereas −927, +764 and +3726 are located on a promoter, intron 1 and 3 flanking region respectively. Positions of the 7 SNPs at the *CCL2* locus and LD structure are depicted in Supplemental Figure I. *CCL2* genotyping was performed on coded DNA samples by laboratory personnel blinded to clinical information. Genotyping was conducted by KBioscience (http://www.kbioscience.co.uk) using KASPar technology. Genotyping was carried out on an ABI 7900 system, using Assay by Design™ assays (Applied Biosystems, Foster City, CA, USA). Allelic discrimination was performed using FAM and VIC as fluorophore. PCR conditions were denaturation for 10 min at 95°C, followed by 40 cycles (30 sec 92°C, 45 sec 60°C). PCR assay mix was obtained from Applied Biosystems. Assays were considered successful if they met the following criteria: at least 75% for genotyping calls, a Hardy-Weinberg equilibrium with a P value >0.01 and a minor allele frequency > 5%. Haplotype block selection and estimations of the linkage disequilibrium were performed with the publicly available Haploview software package, version 4.2 (http://www.broadinstitute.org/mpg/haploview).

Power analysis

Using a logistic regression model, we calculated the power to detect statistically significant differences in CAD risk. With minor allele frequencies (MAF) ranging from 0.4 to 0.05, our study had 80% power to detect an odds ratio 1.3 to 1.65, respectively. Likewise, the study had 80% power to detect 10 to 4.25 pg/ml differences in MCP-1 levels assuming an overall standard deviation of 35 pg/ml and MAF ranging from 0.4 to 0.05. For both models, we assumed a (log)additive effect of the SNP and a corrected two-sided alpha of 0.0005. Calculations were carried out using Quanto (version 1.2,http://hydra.usc.edu/gxe/).

Statistical analysis

Baseline characteristics were compared between cases and controls with a mixed-effect model for continuous variables or conditional logistic regression for categorical variables. Because MCP-1, triglycerides and CRP levels had a skewed distribution, values were natural log-transformed before statistical analysis. The associations between MCP-1 quartiles and both cardiovascular risk factors and CAD risk were assessed. For this purpose, quartiles were based on the MCP-1 distribution among controls. The relations between *CCL2* genotype and MCP-1 levels were determined by a linear regression model. Multivariable-adjusted Cox regression analyses were conducted to examine the association between *CCL2* genotype and risk of CAD. We tested for interaction between sex and *CCL2* polymorphisms since sex-differences have been described previously for the association between MCP-1 and CAD risk[7]. Because we observed a statistically significant interaction between sex and one of the SNPs for CAD risk, we performed additional subgroup analyses for men and women separately. For all SNP statistical analyses with the seven typed SNPs we present uncorrected p-values and consider a multiple testing Bonferroni corrected p-

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value <0.0005 significant, otherwise a p-value <0.05 was considered statistically significant. Data were analyzed with SPSS version 16.0 (SPSS inc. Chicago, IL, USA), unless otherwise described.

Haplotype analysis

From the unphased SNP genotype data, haplotype frequencies and their association with MCP-1 concentrations and CAD risk were estimated using weighted linear or logistic regression, respectively[8, 9]. In short, haplotype effects and haplotype frequencies were jointly estimated using an expectation-maximization (EM) algorithm in which individual haplotypes were handled as missing data. In the first expectation (E) step, the initial probabilities were calculated using Bayes' theorem and estimated haplotype frequencies. In the following E steps, the posterior probabilities of haplotype pairs compatible with an individual's genotype were calculated based on the phenotype of the individual. In the maximization (M) steps, the haplotype effects were estimated using a weighted linear or logistic regression model, where the posterior probabilities functioned as weights. The E and M steps were alternated until convergence. Haplotype analyses were performed with R (GNU project. http://www.r-project.org/).

Results

MCP-1 serum levels, cardiovascular risk factors and risk of subsequent CAD

A complete dataset was available for 985 cases and 1778 matched controls. From these individuals, 793 cases were matched to two controls each, whereas 192 cases could be matched to one control only. Matching ensured that age and sex distributions were comparable between cases and controls. Table 1 shows the distribution of cardiovascular risk factors among cases and controls. As expected individuals who developed CAD during follow-up were more likely than controls to have cardiovascular risk factors. There was no significant difference between circulating MCP-1 level between cases and controls (Table 1). Serum MCP-1 levels were associated with waist circumference and triglycerides (Table 2). There were also weak but significant relationships with body mass index and systolic blood pressure.

Next, we examined the relationship between circulating MCP-1 levels and the risk of future CAD. We found no evidence for an association between serum MCP-1 levels and CAD risk (Table 3). Since we found a significant interaction for MCP-1-3725 with sex for CAD risk (p=0.004), we performed an additional sex-specific subgroup analyses showing no indication for an association between MCP-1 serum levels and CAD risk in men or women separately (see Supplemental Table I).

CCL2 genotype variants and circulating MCP-1 levels

Supplemental Table II displays characteristics for the CCL2 SNPs that were typed. Slight differences between the minor allele frequencies for cases and controls were found for *CCL2* −2835, −2578, −1811 and +764. All polymorphisms in the control population were in complete Hardy Weinberg equilibrium. The various cardiovascular risk factors were equally distributed among the seven different SNPs (see Supplemental Table I). Median levels of

MCP-1 serum concentration showed minor variations according to *CCL2* genotype, but no significant differences were found (Table 4.) Subgroup analyses for men and women separately showed no evidence for a significant association between *CCL2* genotype en MCP-1 serum levels (see Supplemental Table IV and V).

CCL2 genotype variants and risk of CAD

Although *CCL2* genotype variation was not associated with serum levels of MCP-1, genotype variations could still affect CAD risk via other mechanisms independent of circulating levels of MCP-1. We therefore assessed the association between *CCL2* genotype variants and the risk of CAD. Table 5 shows the association between the typed *CCL2* SNPs and risk of CAD. We did not find any robust associations with CAD risk for the specific *CCL2* genotype variants. A subgroup analysis among men showed significant associations with CAD risk for both *CCL2* −2835 (OR 1.28; 95% CI, 1.05 to 1.57; p=0.017 for CC vs. AA + AC) and *CCL2* −2578 (OR 1.26; 95% CI, 1.03 to 1.53; p=0.027 for AA vs. GG + GA). Adjustment for age, sex, body mass index, smoking status, systolic blood pressure, LDL-cholesterol, HDL-cholesterol, C-reactive protein and adjustment for the Framingham Risk Score did not influence these associations. In addition, among women only, *CCL2* +3726 was associated with CAD risk in a recessive model (OR 1.59; 95% CI 1.11 to 2.27; p-value 0.011), that was highly robust for multivariable correction and correction for the Framingham Risk Score. However, p-values did not reach significance beyond the multiple testing criterion of 0.0005 (see Supplemental Table VI and VII).

MCP-1 haplotype analysis

To better understand the associations among *CCL2* genetic variation, circulating MCP-1 concentrations and the risk for future CAD, we performed a haplotype-based analysis. The *CCL2* gene was encompassed in 1 haplotype block. We estimated six common haplotypes (H1 to H6) from the seven typed SNPs (see Supplemental Table VIII) that accounted for 99% of all possible *CCL2* haplotypes. Using the haplotype with the highest frequency in our study as reference, we did find a trend towards lower concentrations of MCP-1 for individuals with H4 (Ratio −1.81; 95% CI, −3.71 to −0.09; p=0.062) and a lower risk ratio for future CAD in individuals with H5 of 0.77 (95% CI, 0.61 to 0.98; p=0.030). These p-values did not reach significance above our predefined multiple testing criterion (Table 6). After finding a significant interaction between sex and the haplotype H5 for MCP-1 concentration, we performed subgroup analyses for men and women separately. We did not find any significant associations between *CCL2* haplotypes and MCP-1 serum levels or future risk of CAD (see Supplemental Table IX and X).

Discussion

In this large prospective case-control study we found no evidence for an association between MCP-1 serum levels and the risk of future CAD in apparently healthy men and women. In addition, no significant associations were found between *CCL2* genetic variants and either serum MCP-1 levels or the risk of future CAD. In addition, we found no robust evidence for any of these associations in a subsequent *CCL2* haplotype analysis.

Several studies have suggested that increased levels of MCP-1 are associated with atherosclerosis, myocardial infarction size, as well as with an increased risk of myocardial infarction, sudden death, coronary angioplasty and stent restenosis[10-14] while other studies could not confirm such an association[15]. Additionally, several studies have reported an association between the *CCL2* SNPs investigated in our analysis and MCP-1 serum levels. Increased levels of MCP-1 were found in individuals with the *CCL2*-2578G variant[16-18], while this could not be confirmed in other case-cohort studies[19, 20]. We found similar MCP-1 serum concentrations among *CCL2* −2578GG and −2578AA individuals. Likewise, in the community-based Framingham Heart Study Offspring Cohort, McDermott et al. demonstrated that the *CCL2*-2136 and the *CCL2*+764 polymorphisms were significantly associated with MCP-1 serum concentrations. Although we found a trend for *CCL2* −2136 and for *CCL2*+764, the p-values did not reach significance above our multiple testing criteria. In addition, three studies have reported associations between the *CCL2* −2578G allele and atherosclerosis[7, 21, 22]. We could not demonstrate an association for the *CCL2* −2578G allele with the future risk of CAD, but did find a non-significant trend for a higher risk of CAD among *CCL2* +3726CC individuals (OR 1.31, 95% CI 1.05 to 1.65; p=0.020).

To further clarify the role of *CCL2* genotype and the risk of future CAD, we performed a haplotype analysis estimating the effect of *CCL2* genotype combinations on MCP-1 serum concentrations and CAD risk. Haplotype frequencies were comparable to previously published studies showing associations between *CCL2* genotype combinations and MCP-1 serum levels[7]. We found no significant associations between *CCL2* haplotypes and MCP-1 serum levels or the risk for future CAD.

In contrast to other studies reporting evidence for MCP-1 in the pathogenesis of CAD, we could not confirm an association between MCP-1 and CAD risk in our cohort. To our best knowledge strong and consistent associations between a single *CCL2* SNP, MCP-1 serum level and the risk of future CAD have not been reported in large prospective studies. Despite the substantial amount of research into the role of MCP-1 in atherogenesis, there is little information with regard to the functionality of the MCP-1 protein affected by any of the known SNPs and this could explain inconsistent associations between *CCL2* genotype, MCP-1 serum levels and CAD. There are several other considerations that might explain the differences between our observations and previous publications. First, case ascertainment is an issue in the design of every prospective study, including this one. However, a validation study indicated that case ascertainment in our study was at least equivalent to that of other large prospective cohort studies[23]. Another possibility to explain our negative findings is an insufficient power to detect differences in MCP-1 serum levels or the risk of CAD. However, our power analysis showed that with the present sample size, the study has 80% power to detect an odds ratio of 1.3 for any of the typed SNPs. This is well below previously published ORs for the typed SNPs in previous publications, where ORs ranged between 1.5 and 2.6 and strong enough to detect clinically relevant differences[7]. This observation may point towards selective publication of positive findings in previous studies. Third, we present full and transparent data, in accordance with the current STREGA guidelines [24]. We present all tests performed for possible associations of a well-described, previously published large case-control study and specifically define or present the selection criteria and quality controls. Although this is not uncommon, several previous publications lack

crucial information to assess the reliability of the presented data and the underreporting of negative associations. Furthermore, many studies do not correct for multiple comparisons, which in our opinion should be taken into account when addressing associations between multiple SNPs and diseases, especially when sub-group analyses are performed. Furthermore, our results apply to Caucasians only and conclusions should not be compared with non-Caucasian populations, especially since *CCL2* genotype frequencies have been reported to vary substantially between various populations.

Conclusion

This large community-based prospective study among apparently healthy men and women does not support an association between common variants in the *CCL2* gene or MCP1 serum levels and the risk of future CAD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1
Baseline Characteristics of Study Participants

	Controls (n = 1,778)	Cases (n = 985)
Age, years	65.2 (64.8 to 65.6)	65.2 (64.6 to 65.7)
Women, n (%)	662 (37.2)	357 (36.2)
Body mass index, kg/m^2	26.2 (26.0 to 26.4)	27.0 (26.9 to 27.5)
Waist circumference, cm	91 (90 to 92)	94 (93 to 95)
Current smoker, n (%)	151 (8.5)	148 (15.0)
Diabetes mellitus, n (%)	29 (1.6)	62 (6.3)
Systolic blood pressure, mmHg	139 (138 to 140)	143.9 (143 to 145)
Diastolic blood pressure, mmHg	84 (83 to 84)	86 (85 to 87)
Total cholesterol, mmol/l	6.25 (6.19 to 6.30)	6.45 (6.37 to 6.53)
LDL-cholesterol, mmol/l	4.09 (4.04 to 4.14)	4.27 (4.20 to 4.35)
HDL-cholesterol, mmol/l	1.36 (1.34 to 1.38)	1.26 (1.23 to 1.28)
Triglycerides, mmol/l	1.60 (1.1 to 2.2)	1.80 (1.3 to 2.6)
C-reactive protein, mg/l	1.50 (0.7 to 3.1)	2.20 (1.0 to 5.0)
MCP-1, pg/ml	51.2 (38.3 to 66.8)	51.2 (38.2 to 69.7)

Data are presented as mean with the 95% confidence interval or numbers with the corresponding percentage. Triglyceride, CRP and MCP-1 concentrations are presented as median with the 25th to 75th percentile. LDL = low-density lipoprotein; HDL = high-density lipoprotein; MCP-1 = Monocyte chemoattractant protein-1.

Table 2
Distribution of CAD Risk Factors by MCP-1 Quartile

	MCP-1 serum concentration quartiles (pg/ml)				p*	R	P*
	1 (<38.3)	2 (38.3 to 51.2)	3 (51.2 to 66.8)	4 (>66.8)			
Participants, n	695	686	665	717	-	-	-
Age, years	65.2 (50.0 to 75.2)	65.1 (49.5 to 75.1	65.4 (50.6 to 75.4)	65.6 (51.2 to 75.4)	0.195	0.027	0.158
Women, n (%)	264 (38)	258 (38)	240 (36)	257 (36)	0.331	-	-
Body mass index, kg/m2	26.4 (21.4 to 32.8)	26.5 (21.2 to 32.9)	26.7 (21.3 to 33.3)	26.8 (21.7 to 33.4)	0.016	0.053	0.005
Waist circumference, cm	91 (71 to 110)	92 (73 to 111)	93 (74 to 112)	93 (75 to 113	<0.001	0.080	<0.001
Cigarette smoking, n (%)	64 (9)	74 (11)	73 (11)	88 (12)	0.073	-	-
Diabetes mellitus, n (%)	19 (3)	26 (4)	27 (4)	19 (3)	0.337	-	-
Systolic blood pressure, mmHg	139 (111 to 174)	140 (110 to 174)	141 (112 to 175)	142 (115 to 175)	0.017	0.045	0.018
Diastolic blood pressure, mmHg	83 (65 to 104)	84 (67 to 104)	85 (66 to 105)	85 (68 to 105)	0.079	0.041	0.033
Total cholesterol, mmol/l	6.30 (4.5 to 8.3)	6.3 (4.6 to 8.4)	6.3 (4.7 to 8.3)	6.4 (4.6 to 8.4)	0.151	0.021	0.268
LDL-cholesterol, mmol/l	4.2 (2.6 to 5.9)	4.1 (2.6 to 6.0)	4.2 (2.7 to 6.0)	4.2 (2.6 to 5.9)	0.550	0.007	0.707
HDL-cholesterol, mmol/l	1.33 (0.80 to 2.10)	1.35 (0.80 to 2.10)	1.34 (0.80 to 2.00)	1.31 (0.80 to 2.10)	0.267	−0.31	0.105
Triglycerides, mmol/l	1.6 (1.1 to 2.2)	1.7 (1.2 to 2.3)	1.7 (1.2 to 2.4)	1.8 (1.3 to 2.4)	<0.001	0.087	<0.001
C-reactive protein, mg/l	1.8 (0.8 to 3.7)	1.5 (0.7 to 3.9)	1.7 (0.8 to 3.9)	1.7 (0.8 to 3.6)	0.994	0.002	0.933

Data are presented as mean with the 95% confidence interval or number (percentage). Quartiles are based on values in control subjects.

Triglycerides and CRP are presented as median with the 25^{th} to 75^{th} percentile and log transformed for the p-value calculations. P = p-value for linearity between MCP-1 serum concentration quartiles and risk factor levels; R = Pearson or Spearmans correlation between log-transformed MCP-1 serum concentration and risk factors, and the corresponding p-value.

†(P). MCP-1 = Monocyte chemoattractant protein-1; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

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Table 3

Odds Ratios for Future CAD Events by MCP-1 Quartile and for MCP-1 as Continuous Variable

	MCP-1 quartiles						
	1	**2**	**3**	**4**	**P***	**Ln(MCP-1)†**	**P‡**
MCP-1 levels (pg/ml)	< 38.3	38.3 to 51.2	51.2 to 66.8	> 66.8	-	-	-
Total no. of patients	695	686	665	717	-	-	-
Cardiovascular events, n (%)	251 (36.1)	241 (35.1)	220 (33.1)	273 (33.1)	-	-	-
Model 1	1	0.95 (0.76 to 1.20)	0.86 (0.68 to 1.09)	1.08 (0.86 to 1.36)	0.246	1.05 (0.87 to 1.27)	0.624
Model 2	1	0.91 (0.71 to 1.16)	0.81 (0.63 to 1.05)	0.96 (0.75 to 1.24)	0.368	0.94 (0.76 to 1.15)	0.524
Model 3	1	0.89 (0.70 to 1.13)	0.79 (0.62 to 1.00)	0.96 (0.75 to 1.22)	0.209	0.96 (0.79 to 1.17)	0.658

Odds ratios and corresponding 95% confidence intervals calculated by conditional logistic regression, taking into account matching for age, gender, and enrollment time, per MCP-1 quartile. CRP, triglycerides and MCP-1 were log-transformed before analysis. Model 1: unadjusted. Model 2: Adjusted for body mass index, smoking status, systolic blood pressure, LDL-cholesterol, HDL-cholesterol and CRP. Model 3: adjustment for the FRS.

*P = p value for the association between MCP-1 quartiles and CAD risk.

†P = Odds ratios and corresponding 95% confidence intervals calculated by conditional logistic regression, taking into account matching for age, gender and enrollment time, for MCP-1 as continuous variable.

‡P = p value corresponding to Ln(MCP-1). MCP-1 = Monocyte chemoattractant protein-1; LDL = low-density lipoprotein; HDL = high-density lipoprotein; FRS = Framingham Risk Score.

Table 4
MCP-1 serum concentrations according to CCL2 polymorphisms of study participants

	MCP-1 serum concentration levels, pg/ml						
	Median	25th Lower percentile	75th Upper percentile	Beta coefficients (95% CI)	P*	p†	p‡
−2835 C/A				−1.27 (−3.21 to 0.67)	0.813	0.742	0.794
−2835 AA	50.68	37.85	70.59				
−2835 CA	50.88	38.06	66.49				
−2835 CC	51.64	38.82	67.57				
−2578 A/G				−1.27 (−1.50 to 1.41)	0.952	0.863	0.934
−2578 GG	49.96	37.53	70.63				
−2578 AG	50.90	38.09	66.49				
−2578 AA	51.91	38.98	67.60				
−2136 A/T				1.43 (−0.17 to 3.03)	0.080	0.083	0.075
−2136 AA	50.52	37.72	67.04				
−2136 AT	52.26	39.24	67.86				
−2136 TT	55.76	42.03	70.20				
−1811 A/G				1.33 (−1.90 to 4.56)	0.420	0.342	0.794
−1811 AG	50.27	36.62	66.12				
−1811 GG	51.09	38.26	67.57				
−927 G/C				0.82 (−0.76 to 2.40)	0.307	0.251	0.315
−927 GG	50.86	38.00	66.92				
−927 GC	50.76	39.1	68.39				
−927 CC	56.52	43.03	69.68				
+764 C/G				1.55 (−0.76 to 3.17)	0.062	0.068	0.058
+764 CC	50.54	37.76	66.97				
+764 CG	52.26	39.63	68.08				
+764 GG	56.05	42.12	70.11				
+3726 T/C				−0.36 (−1.69 to 0.98)	0.600	0.526	0.637
+3726 TT	51.43	38.18	69.00				
+3726 TC	50.39	38.28	66.75				
+3726 CC	51.35	38.22	65.88				

Beta coefficients adjusted for age and sex (95 % confidence interval) of MCP-1 serum concentration according to CCL2 polymorphisms with the corresponding p-values.

*P = unadjusted p value.

†P = p value adjusted for waist circumference, systolic blood pressure and triglycerides;

‡P = p value adjusted for the Framingham Risk Score.

Table 5
Odds Ratios for Future CAD Events by CCL2 polymorphism of study participants

			P*	p†	p‡
−2835 C/A					
AA (ref) – CA – CC	1.00 (0.72 to 1.39)	0.90 (0.65 to 1.24)	0.429	0.129	0.352
AA vs CA + CC	0.94 (0.69 to 1.29)		0.711	0.184	0.485
CC vs CA + AA	0.90 (0.76 to 1.06)		0.193	0.064	0.157
−2578 A/G					
GG (ref) – GA – AA	1.03 (0.75 to 1.42)	0.93 (0.68 to 1.27)	0.448	0.209	0.437
GG vs GA + AA	0.97 (0.71 to 1.32)		0.854	0.267	0.552
AA vs GA + GG	0.90 (0.77 to 1.01)		0.210	0.100	0.898
−2136 A/T					
AA (ref) – AT – TT	1.05 (0.88 to 1.25)	0.89 (0.59 to 1.33)	0.720	0.498	0.652
AA vs AT + TT	1.02 (0.86 to 1.21)		0.795	0.527	0.568
TT vs AT + AA	0.88 (0.58 to 1.31)		0.517	0.459	0.897
−1811 A/G					
AG vs GG	1.20 (0.90 to 1.60)		0.222	0.076	0.071
−927 G/C					
GG (ref) – GC – CC	0.92 (0.77 to 1.10)	0.87 (0.56 to 1.29)	0.549	0.559	0.499
GG vs GC + CC	0.91 (0.77 to 1.08)		0.288	0.934	0.309
CC vs GC + GG	0.90 (0.61 to 1.32)		0.575	0.332	0.338
+764 C/G					
CC (ref) – CG – GG	1.06 (0.89 to 1.26)	0.91 (0.60 to 1.40)	0.725	0.499	0.570
CC vs CG + GG	1.04 (0.88 to 1.23)		0.648	0.410	0.436
GG vs CG + CC	0.90 (0.59 to 1.37)		0.619	0.577	0.903
+3726 T/C					
TT (ref) – TC – CC	0.97 (0.81 to 1.16)	1.29 (1.01 to 1.66)	0.064	0.254	0.083
TT vs TC + CC	1.04 (0.88 to 1.23)		0.635	0.594	0.474
CC vs TC + TT	1.31 (1.05 to 1.65)		0.020	0.098	0.026

Odds ratios and the corresponding 95% confidence interval calculated by conditional logistic regression, taking into account matching for age, gender, and enrollment time per CCL2 polymorphism.

*P = Unadjusted p value.

†P = p value adjusted for body mass index, waist circumference, systolic blood pressure and triglycerides;

‡P = p value adjusted for the Framingham Risk Score.

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Table 6

Ratios for serum MCP-1 concentration and future CAD risk according to CCL2 haplotypes

Haplotype	MCP-1 serum ratio (95% CI)	P*	P†	P‡	CAD risk ratio (95% CI)	P*	P†	P‡
H1	Ref	-	-	-	Ref	-	-	-
H2	0.61 (−1.25 to 2.46)	0.521	0.510	0.536	0.90 (0.77 to 1.07)	0.228	0.201	0.157
H3	1.21 (−0.70 to 3.12)	0.213	0.245	0.212	0.95 (0.80 to 1.12)	0.527	0.419	0.620
H4	−1.81 (−3.71 to 0.09)	0.062	0.045	0.065	1.06 (0.90 to 1.25)	0.481	0.638	0.438
H5	−0.23 (−2.83 to 2.36)	0.859	0.810	0.842	0.77 (0.61 to 0.98)	0.030	0.015	0.023
H6	−1.21 (−4.62 to 2.20)	0.485	0.404	0.427	0.84 (0.62 to 1.13)	0.250	0.071	0.121

Age and sex corrected MCP-1 serum concentration and future CAD risk ratios with the corresponding 95 % confidence interval for the most six common CCL2 haplotypes. The haplotype with the highest frequency (H1) is used as reference haplotype.

*P = p value adjusted for age and sex.

†P = p value adjusted for age and sex, waist circumference, systolic blood pressure and triglycerides;

‡P = p value adjusted for the Framingham Risk Score.