Circulation Research

ORIGINAL RESEARCH

Circulating Monocyte Chemoattractant Protein-1 and Risk of Stroke

Meta-Analysis of Population-Based Studies Involving 17 180 Individuals

Marios K. Georgakis, Rainer Malik, Harry Björkbacka, Tiberiu Alexandru Pana, Serkalem Demissie, Colby Ayers, Mohamed A. Elhadad, Myriam Fornage, Alexa S. Beiser, Emelia J. Benjamin, Matthijs S. Boekholdt, Gunnar Engström, Christian Herder, Ron C. Hoogeveen, Wolfgang Koenig, Olle Melander, Marju Orho-Melander, Alexandru Schiopu, Martin Söderholm, Nick Wareham, Christie M. Ballantyne, Annette Peters, Sudha Seshadri, Phyo K. Myint, Jan Nilsson, James A. de Lemos, Martin Dichgans

RATIONALE: Proinflammatory cytokines have been identified as potential targets for lowering vascular risk. Experimental evidence and Mendelian randomization suggest a role of MCP-1 (monocyte chemoattractant protein-1) in atherosclerosis and stroke. However, data from large-scale observational studies are lacking.

OBJECTIVE: To determine whether circulating levels of MCP-1 are associated with risk of incident stroke in the general population.

METHODS AND RESULTS: We used previously unpublished data on 17 180 stroke-free individuals (mean age, 56.7±8.1 years; 48.8% men) from 6 population-based prospective cohort studies and explored associations between baseline circulating MCP-1 levels and risk of any stroke, ischemic stroke, and hemorrhagic stroke during a mean follow-up interval of 16.3 years (280 522 person-years at risk; 1435 incident stroke events). We applied Cox proportional-hazards models and pooled hazard ratios (HRs) using random-effects meta-analyses. After adjustments for age, sex, race, and vascular risk factors, higher MCP-1 levels were associated with increased risk of any stroke (HR per 1-SD increment in ln-transformed MCP-1, 1.07; 95% CI, 1.01–1.14). Focusing on stroke subtypes, we found a significant association between baseline MCP-1 levels and higher risk of ischemic stroke (HR, 1.11 [1.02–1.21]) but not hemorrhagic stroke (HR, 1.02 [0.82–1.29]). The results followed a dose-response pattern with a higher risk of ischemic stroke among individuals in the upper quartiles of MCP-1 levels as compared with the first quartile (HRs, second quartile: 1.19 [1.00–1.42]; third quartile: 1.35 [1.14–1.59]; fourth quartile: 1.38 [1.07–1.77]). There was no indication for heterogeneity across studies, and in a subsample of 4 studies (12 516 individuals), the risk estimates were stable after additional adjustments for circulating levels of IL (interleukin)-6 and high-sensitivity CRP (C-reactive protein).

CONCLUSIONS: Higher circulating levels of MCP-1 are associated with increased long-term risk of stroke. Our findings along with genetic and experimental evidence suggest that MCP-1 signaling might represent a therapeutic target to lower stroke risk.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: atherosclerosis ■ cerebrovascular disorders ■ chemokine CCL2 ■ inflammation ■ stroke

In This Issue, see p 725 | Meet the First Author, see p 726

Stroke is the leading cause of adult disability and the second most common cause of death worldwide.[1,2] Inflammatory mechanisms contribute to the pathogenesis of stroke, most notably to large artery atherosclerotic stroke,[3,4] but the specific proinflammatory factors mediating stroke risk are largely elusive. Discordant results from the CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study)[5–8] and CIRT (Cardiovascular

Circulation Research is available at www.ahajournals.org/journal/res

Novelty and Significance

What Is Known?

- Inflammatory mechanisms contribute to the pathogenesis of vascular disease, and inflammatory cytokines have been identified as potential therapeutic targets for lowering vascular risk.
- Using genetic data, we recently showed in Mendelian randomization that lifetime higher MCP-1 (monocyte chemoattractant protein-1) levels are associated with a higher risk of ischemic stroke.
- Preclinical studies in animal models of experimental atherosclerosis further suggest a critical role of MCP-1 in the initiation and propagation of atherosclerosis.

What New Information Does This Article Contribute?

- We performed a meta-analysis of 6 population-based cohort studies involving 17 000 stroke-free individuals who were followed up for 16 years.
- After adjustment for traditional vascular risk factors, higher baseline MCP-1 levels were associated with a higher risk of any stroke and ischemic stroke but not hemorrhagic stroke over follow-up.
- On top of experimental and genetic data, our findings provide additional evidence supporting MCP-1 signaling as a promising target for lowering stroke risk.

In view of recent findings suggesting the efficacy of anti-inflammatory approaches in lowering vascular risk, there is a need for identification of specific inflammatory mediators that show promise as potential therapeutic targets. Experimental and genetic evidence suggests MCP-1—a chemokine involved in monocyte recruitment—to play a critical role in atherosclerosis and stroke. Here, we aimed to amplify this concept by exploring in a meta-analysis of 6 previously unpublished cohort studies whether MCP-1 levels are associated with risk of stroke. Following up 17 000 stroke-free individuals for a mean of 16 years, we found baseline MCP-1 levels to be associated with a higher risk of any stroke, independently of traditional vascular risk factors. Across stroke subtypes, there was a significant association of MCP-1 levels with the risk of ischemic stroke but not hemorrhagic stroke. Adjustments for IL-6 (interleukin-6) and CRP (C-reactive protein) levels did not attenuate these associations, thus indicating that MCP-1 signaling might contribute to stroke risk independently of the well-established IL-6–CRP axis. Along with genetic and experimental data, our findings provide triangulation of evidence suggesting MCP-1 as a causal risk factor for stroke and MCP-1 signaling as a potential therapeutic target.

Nonstandard Abbreviations and Acronyms

ARIC	Atherosclerosis Risk in Communities
CAD	coronary artery disease
CANTOS	Canakinumab Anti-Inflammatory Thrombosis Outcomes Study
CCL2	CC-chemokine ligand 2
CRP	C-reactive protein
DHS	Dallas Heart Study
eGFR	estimated glomerular filtration rate
EPIC-Norfolk	Norfolk Arm of the European Prospective Investigation of Cancer
FHS	Framingham Heart Study
HbA1c	glycosylated hemoglobin type A1C
HDL	high-density lipoprotein
HR	hazard ratio
hsCRP	high-sensitivity C-reactive protein
IL	interleukin
KORA	Kooperative Gesundheitsforschung in der Region Augsburg
LDL	low-density lipoprotein
MCP-1	monocyte chemoattractant protein-1
MDCS	Malmö Diet and Cancer Study
MONICA	Monitoring of Trends and Determinants in Cardiovascular Disease Communities

Inflammation Reduction Trial)[6] randomized controlled trials emphasize the importance of targeting specific mediators and pathways for lowering vascular risk.[5–8] Treatment with an anti−IL (interleukin)-1β monoclonal antibody reduced the levels of IL-6 and high-sensitivity CRP [C-reactive protein] (hsCRP) leading to a reduction in the combined primary end point of nonfatal myocardial infarction, nonfatal stroke, or cardiovascular death independent of LDL (low-density lipoprotein) cholesterol levels,[5] whereas treatment with low-dose methotrexate neither reduced cardiovascular event rates nor the levels of IL-1β, IL-6, and hsCRP.[6]

In a Mendelian randomization study on circulating levels of 41 cytokines and growth factors, we recently found genetic predisposition to higher levels of the CC-chemokine MCP-1 (monocyte chemoattractant protein-1; also known as CCL2 [CC-chemokine ligand 2]) to be associated with increased risk of stroke, ischemic stroke, coronary artery disease (CAD), and myocardial infarction.[9] MCP-1 recruits monocytes to the subendothelial space of the atherogenic arterial wall,[10–12] and studies in experimental models of atherosclerosis suggest that targeting MCP-1 or its receptor CCR2 (C-C chemokine receptor type 2) limits plaque size, plaque progression, and plaque destabilization.[13–17] These findings define the MCP-1/CCR2 axis as a potential additional target for reducing residual inflammatory risk in vascular disease. However, data on MCP-1 and vascular risk in humans remain scarce.

Among patients with acute coronary syndromes in the OPUS-TIMI 16 (Orbofiban in Patients With Unstable Coronary Syndromes by the Thrombolysis in Myocardial Infarction Study Group)[18] and A-to-Z trial,[19] high circulating MCP-1 levels were associated with a significantly increased risk of death or myocardial infarction during follow-up, independently of baseline variables including hsCRP levels. In population-based studies, higher MCP-1 levels were associated with subclinical atherosclerosis and incident CAD during follow-up.[20,21] In contrast, the relationship between circulating MCP-1 levels and incident stroke remains unknown as does the relationship between MCP-1, IL-6, and CRP in mediating vascular risk.

Here, leveraging data from 6 population-based prospective cohort studies encompassing 17 180 stroke-free individuals with long-term follow-up, we set out to (1) determine the association between circulating MCP-1 levels at baseline and risk of incident stroke, (2) explore associations of MCP-1 levels with risk of major stroke subtypes (incident ischemic and hemorrhagic stroke), and (3) assess whether any association with stroke risk is independent of the IL-6/CRP axis by adjusting for the circulating levels of IL-6 and hsCRP.

METHODS

This study is based on summary statistics produced by the studies included in the systematic review. The main individual-study results are provided in the Online Data Supplement. All summary data that support the findings of this study are further available from the corresponding author on reasonable request. For accessing individual-level data of the included studies the readers should contact the authors representing the respective studies and follow the required processes.

Systematic Review

We systematically searched PubMed from inception through March 15, 2019, for population-based prospective cohort studies exploring associations between circulating MCP-1 levels and the risk of incident vascular outcomes including CAD, myocardial infarction, fatal or nonfatal stroke, and peripheral artery disease. The reference lists of the identified studies were further hand searched. The detailed search strategy is available in the Online Appendix. We subsequently contacted the corresponding authors of the selected studies inquiring about their interest to contribute data for the current meta-analysis examining the association between circulating MCP-1 levels and risk of incident stroke. Investigators of the following 6 studies agreed to participate, and the following studies were thus included in the current meta-analysis: the ARIC study (Atherosclerosis Risk in Communities),[20] DHS (Dallas Heart Study),[21] the EPIC-Norfolk study (Norfolk Arm of the European Prospective Investigation of Cancer),[22] the Offspring Cohort of FHS (Framingham Heart Study),[23] the MONICA (Monitoring of Trends and Determinants in Cardiovascular Disease) subcohort of the KORA study (Kooperative Gesundheitsforschung in der Region Augsburg),[24] and the cardiovascular subcohort of MDCS (Malmö Diet and Cancer Study).[25] With the exception of the FHS Offspring study, which had previously published part of the data included in this

analysis (96 versus 172 incident events),[23] none of the studies previously published data on the association between circulating MCP-1 levels and risk of incident stroke. The flowchart describing the study selection is depicted in Online Figure I.

Study Populations, MCP-1 Level Measurements, and Assessment of Stroke Outcomes

The study design, population characteristics, methods used for quantifying circulating MCP-1 levels, stroke outcome definitions, and assessments in individual cohorts are detailed in Online Table I. In brief, all studies were population-based prospective cohorts, and participants included in the current analyses were selected from these cohorts based on the availability of MCP-1 measurements at baseline. Circulating MCP-1 levels were measured in serum or plasma samples drawn during the baseline assessments. Because incident stroke was the primary outcome of the current study, all participants with a history of stroke at baseline assessments (prevalent cases) were excluded from subsequent analyses. Stroke occurrence was assessed at follow-up visits during mean intervals of 11 to 23 years based on self-reported information and validation from medical records of the participants. In addition to information on any stroke, all studies further provided information on the major stroke subtypes (ischemic versus hemorrhagic stroke).

Quality Assessment

Study quality was assessed using the cohort subscale of the Newcastle-Ottawa scale.[26] The criteria for awarding quality points were the following: a general population sample (representativeness of exposed cohort), selection of patients for inclusion independently of MCP-1 levels (selection of the nonexposed cohort), measurement of MCP-1 levels in the serum or plasma based on a validated assay (ascertainment of exposure), exclusion of patients with prevalent stroke at baseline (outcome not present at the start of study), adjustments for age and sex, as well as for conventional vascular risk factors (comparability items), assessment of stroke outcomes blindly to MCP-1 levels with validation based on medical records (assessment of outcome), a follow-up interval >5 years (follow-up duration), and a completion of follow-up rate of >90% (adequacy of follow-up cohorts).

Statistical Analysis

A predefined analysis protocol was circulated to investigators of each of the cohort studies requesting summary results for meta-analysis. MCP-1 levels were ln-transformed in all studies for normalization. We did not consider absolute MCP-1 values because of marked differences in mean MCP-1 level values between studies, probably related to different assays used for MCP-1 quantification (Table). We first examined descriptive associations between MCP-1 levels and conventional vascular risk factors. We pooled study-specific Z scores reflecting differences of MCP-1 levels from the overall mean of each study with random-effects models across the risk factor categories and statistically examined associations using meta-regression.

To examine associations between baseline MCP-1 levels and incident stroke, Cox proportional-hazards models were fit in each study. MCP-1 levels were included in the models as either a continuous variable (1-SD increment in ln-transformed MCP-1 levels) or categorized in 4 quartiles (first quartile as reference category) to also assess for potential

Table. Descriptive Baseline Characteristics of the 6 Included Population-Based Prospective Cohort Studies

Cohort	ARIC	DHS	EPIC-Norfolk	FHS Offspring	MONICA/KORA	MDCS-CV
Geographic setting (baseline assessment)	The United States (1986–1989)	The United States (2000–2002)	United Kingdom (1993–1997)	The United States (1998–2001)	Germany (1984–2002)	Sweden (1991–1994)
Individuals included in the analysis, n	1234	2931	3182	3069	2055	4709
Follow-up, y	23.0 [13.2–27.8]	11.0 (1.7)	16.8 (6.4)	13.8 (3.7)	15.7 (6.4)	19.5 (4.9)
Incident stroke events, n	153	64	503	172	116	427
Incident ischemic stroke events, n	141	42	458	141	99	352
Incident hemorrhagic stroke events, n	12	9	76	22	17	69
Fatal stroke events, n	10	6	132	26	22	30
Age, y	56.9 (5.3)	44.0 (10.0)	65.3 (7.8)	61.6 (9.4)	52.4 (10.3)	57.5 (4.9)
Male sex, n (%)	738 (59.8)	1254 (42.8)	2009 (63.1)	1421 (46.3)	1093 (53.2)	1873 (39.8)
Hypertension, n (%)	417 (33.9)	944 (32.7)	2029 (63.8)	1378 (44.9)	877 (42.7)	2958 (62.8)
SBP, mm Hg	125 (20)	124 (19)	141 (18)	127 (19)	133 (19)	141 (19)
DBP, mm Hg	74 (12)	78 (10)	85 (11)	74 (10)	82 (11)	87 (9)
Diabetes mellitus, n (%)	156 (12.6)	296 (10.1)	623 (19.6)	379 (12.3)	103 (5.0)	183 (3.9)
Hypercholesterolemia, n (%)	760 (61.6)	377 (12.9)	414 (13.0)	1615 (52.6)	1251 (57.4)	2918 (62.8)
LDL cholesterol levels, mg/dL	142.8 (39.9)	107.4 (35.3)	160.1 (39.4)	119.9 (32.7)	148.5 (2.4)	161.3 (37.9)
HDL cholesterol levels, mg/dL	49.6 (16.5)	50.0 (14.6)	51.8 (15.1)	53.9 (16.7)	56.0 (17.0)	53.8 (14.3)
BMI, kg/m²	27.4 (5.1)	29.7 (7.0)	26.6 (3.6)	28.1 (5.3)	27.2 (4.1)	25.6 (3.9)
Smoking status, n (%)						
Never smokers	461 (37.3)	1639 (55.9)	1201 (10.3)	1077 (35.1)	947 (46.1)	1916 (40.1)
Ex-smokers	397 (32.2)	496 (16.9)	1652 (51.9)	1604 (52.3)	591 (28.8)	1777 (37.8)
Current smokers	376 (30.5)	796 (27.2)	329 (37.7)	388 (12.6)	517 (25.1)	1010 (21.5)
eGFR, mL/min per 1.73 m²	100.0 (16.6)	99.5 (23.7)	74.5 (24.9)	83.3 (16.5)	87.9 (17.4)	76.9 (15.3)
Coronary artery disease, n (%)	68 (5.5)	79 (2.7)	0 (0)	265 (8.6)	46 (2.2)	78 (1.7)
Atrial fibrillation, n (%)	1 (0.1)	35 (1.2)	NA	119 (3.9)	NA	34 (0.7)
Heart failure, n (%)	53 (4.3)	83 (2.8)	0 (0)	31 (1.0)	119 (5.7)	2 (0.04)
hsCRP levels, mg/L	2.4 [1.3–5.3]	2.8 [1.2–6.8]	2.0 [1.0–3.8]	2.2 [1.0–5.1]	1.4 [0.7–3.3]	1.3 [0.7-2.7]
Sample used for MCP-1 assessment	Plasma	Plasma	Serum	Serum	Serum	Plasma
MCP-1 levels, pg/mL	398.9 [348.4–467.1]	166.5 [122.9–224.4]	51.5 [38.8–68.1]	313.4 [253.9–382.3]	298.0 [127.6–323.8]	2.52 [2.22–2.82]*

The numbers correspond to n (%) for categorical variables and to mean (SD) or median [25th–75th percentile] for continuous variables. ARIC indicates Atherosclerosis Risk in Communities Study; BMI, body mass index; DBP, diastolic blood pressure; DHS, Dallas Heart Study; eGFR, estimated glomerular filtration rate; EPIC-Norfolk, European Prospective Investigation of Cancer, Norfolk; FHS Offspring, Framingham Heart Study–Offspring Cohort; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; KORA, Kooperative Gesundheitsforschung in der Region Augsburg; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; MDCS-CV, Malmö Diet and Cancer Study–Cardiovascular Subcohort; MONICA, Monitoring of Trends and Determinants in Cardiovascular Disease; NA, not available; PEA, proximity extension assay; and SBP, systolic blood pressure.

*The used assay in MDCS did not provide MCP-1 measurements as absolute values but as relative expression levels obtained by PEA.

nonlinear associations. We applied 3 models with different levels of adjustment: model 1 was adjusted for age, sex, and race; model 2 was additionally adjusted for conventional vascular risk factors (hypertension, diabetes mellitus, hypercholesterolemia, body mass index, smoking [current versus noncurrent], estimated glomerular filtration rate [eGFR], CAD, atrial fibrillation, and heart failure); and model 3 was further adjusted for circulating hsCRP levels on top of these variables. Model 2 was predefined as our main model for analyses. In these models, we defined hypertension as a history of physician-diagnosed hypertension, systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or use of ≥1 antihypertensive medications.[27] We defined diabetes mellitus as a history of physician-diagnosed diabetes mellitus, HbA1c (glycosylated hemoglobin type A1C) ≥6.5%, fasting glucose ≥126 mg/

dL, random glucose levels ≥200 mg/dL, or use of glucose-lowering medications.[28] Hypercholesterolemia was defined as LDL cholesterol levels ≥130 mg/dL, total cholesterol levels ≥200 mg/dL (if LDL cholesterol was not available) or use of lipid-lowering drugs,[29] and chronic kidney disease as eGFR <60 mL/min per 1.73 m².[30] In an alternative model (alternative model 2), we directly adjusted for the components of these definitions instead of the binary variables: thus, instead of hypertension, diabetes mellitus, hypercholesterolemia, and chronic kidney disease, we included systolic blood pressure (as a continuous variable), use of antihypertensive medications, fasting glucose levels (as continuous), use of glucose-lowering medications, LDL cholesterol levels (as continuous), administration of lipid-lowering medications, and eGFR (as continuous).

The purpose of the main models was to explore MCP-1 as a potentially causal risk factor for stroke and not to evaluate the predictive values of its levels. In subsequent models, we aimed to explore whether the association between MCP-1 levels and risk of stroke is independent of the IL-6/CRP pathway that was recently shown to provide an efficient drug target for reducing vascular risk.[31] To indirectly examine this, we applied additional adjustments for circulating IL-6 and hsCRP levels. In one model, we included IL-6 on top of age, sex, race, and vascular risk factors, and in a subsequent model, we included both IL-6 and hsCRP levels. We did this because CRP is a downstream effector of IL-6 but also comprises a more general marker of inflammation, and thus the alternative adjustments provide different levels of information regarding the involved inflammatory pathways. Data for IL-6 circulating levels were not available in ARIC and the EPIC-Norfolk. Thus, these cohorts were not included in these analyses.

Analyses were conducted separately for any stroke, ischemic stroke, and hemorrhagic stroke. DHS was excluded from the analysis for hemorrhagic stroke, where MCP-1 was examined in quartiles, because of the low numbers of incident events across the quartile categories of MCP-1 levels. The hazard ratios (HRs) and the 95% CIs derived from each study were pooled with random-effects (DerSimonian-Laird) meta-analyses to allow for heterogeneity across studies related to the different baseline characteristics and the different methods of MCP-1 assessment. Heterogeneity across studies was assessed with the I^2 and the Cochran Q statistic ($I^2 > 50\%$ and $P < 0.10$ were considered statistically significant).

To examine whether the pooled risk estimates were driven by any individual study, we also applied sensitivity analyses by pooling the risk estimates across studies after excluding one study at a time. To explore potential interactions between MCP-1 levels and known cardiovascular risk factors, we performed meta-regression analyses examining how the prevalence of cardiovascular risk factors or the mean or median values of biomarkers were associated with the risk estimates for stroke in each study. We further performed subgroup analyses by sex, presence of hypertension, presence of diabetes mellitus, and body mass index levels (<30 versus ≥30 kg/m^2). Differences in the effect sizes across the subgroup categories were examined by assessing heterogeneity ($I^2 > 50\%$ and $P < 0.10$ were considered statistically significant). Finally, we performed separate analyses for fatal and nonfatal stroke (fatal stroke defined as death occurring within 30 days after the stroke event).

Statistical significance was set at a 2-sided $P < 0.05$ for the main analysis for any stroke. For the subsequent analysis for stroke subtypes, we corrected for multiple comparisons based on the Bonferroni method ($P < 0.05/2$ stroke subtypes, 0.025). Finally, we corrected for multiple comparisons in the descriptive analyses exploring the correlations between MCP-1 levels and baseline variables (threshold for statistical significance at $P < 0.05/12$ variables, 0.004). All analyses were conducted with SAS (v9.4) and Stata (v13.0).

RESULTS

Following a systematic review and contact with the lead investigators, 6 population-based prospective cohort studies contributed previously unpublished data for this meta-analysis. All studies scored high in quality as they fulfilled the full set of Newcastle-Ottawa scale criteria (Online Table II). The baseline characteristics of each study are presented in the Table. In total, 17 180 individuals (mean age, 56.7±8.1 years; 48.8% men), who were stroke-free at baseline, were followed for a mean interval of 16.3 years (range of mean follow-up, 11−23 years) with 280 522 person-years at risk. A total of 1435 incident stroke cases were diagnosed during follow-up, which were classified as ischemic in 1233 cases and as hemorrhagic in 205 cases. Two hundred twenty-six (15.7%) incident stroke events were fatal. Median MCP-1 levels differed between studies possibly reflecting differences in the methods used for MCP-1 quantification (Online Table I). Figure 1 displays associations of standardized MCP-1 levels with conventional vascular risk factors in the pooled sample. We found the following baseline factors to be associated with higher circulating MCP-1 levels: older age, male sex, higher systolic blood pressure, presence of diabetes mellitus, higher LDL cholesterol levels, higher HDL (high-density lipoprotein) cholesterol levels, higher body mass index, current smoking, lower eGFR, history of CAD, higher hsCRP levels, and higher IL-6 levels.

In the pooled analysis, we found higher MCP-1 levels at baseline to be associated with an increased risk of any stroke both in a model adjusted for age, sex, and race (model 1: HR per 1-SD increment in ln-transformed MCP-1, 1.10; 95% CI, 1.01−1.19; $P=0.02$) and in the main model further adjusted for vascular risk factors (model 2: HR, 1.07; 95% CI, 1.01−1.14; $P=0.03$; Figure 2; Online Table III). In analyses comparing MCP-1 quartiles, we found the association between MCP-1 levels and risk of stroke to follow a dose-response pattern with a higher risk among individuals in the upper quartiles of circulating MCP-1 levels as compared with the first quartile (HRs from model 2: second quartile, 1.16 [95% CI, 0.99−1.36; $P=0.07$]; third quartile, 1.31 [95% CI, 1.12−1.53; $P=0.001$]; fourth quartile, 1.33 [95% CI, 1.05−1.68; $P=0.008$]). The results were further stable in a model additionally adjusting for circulating hsCRP levels (model 3 in Figure 2 and Online Table III).

We next examined the associations of circulating MCP-1 levels at baseline with stroke subtypes (Figure 3; Online Tables IV and V) and found significant associations of higher MCP-1 levels at baseline with the risk of ischemic stroke (HR per 1-SD increment in ln-MCP-1 from model 2, 1.11; 95% CI, 1.02−1.21; $P=0.009$) but not with hemorrhagic stroke (model: HR, 1.02; 95% CI, 0.82−1.29; $P=0.83$). MCP-1 levels in the second, third, and fourth quartiles, as compared to the first, were associated with a higher risk for ischemic stroke after adjusting for age, sex, race, and vascular risk factors (HRs from model 2: second quartile, 1.19 [95% CI, 1.00−1.42; $P=0.05$]; third quartile, 1.35 [95% CI, 1.14−1.59; $P<0.001$]; fourth quartile, 1.38 [95% CI, 1.07−1.77; $P=0.008$]). The results
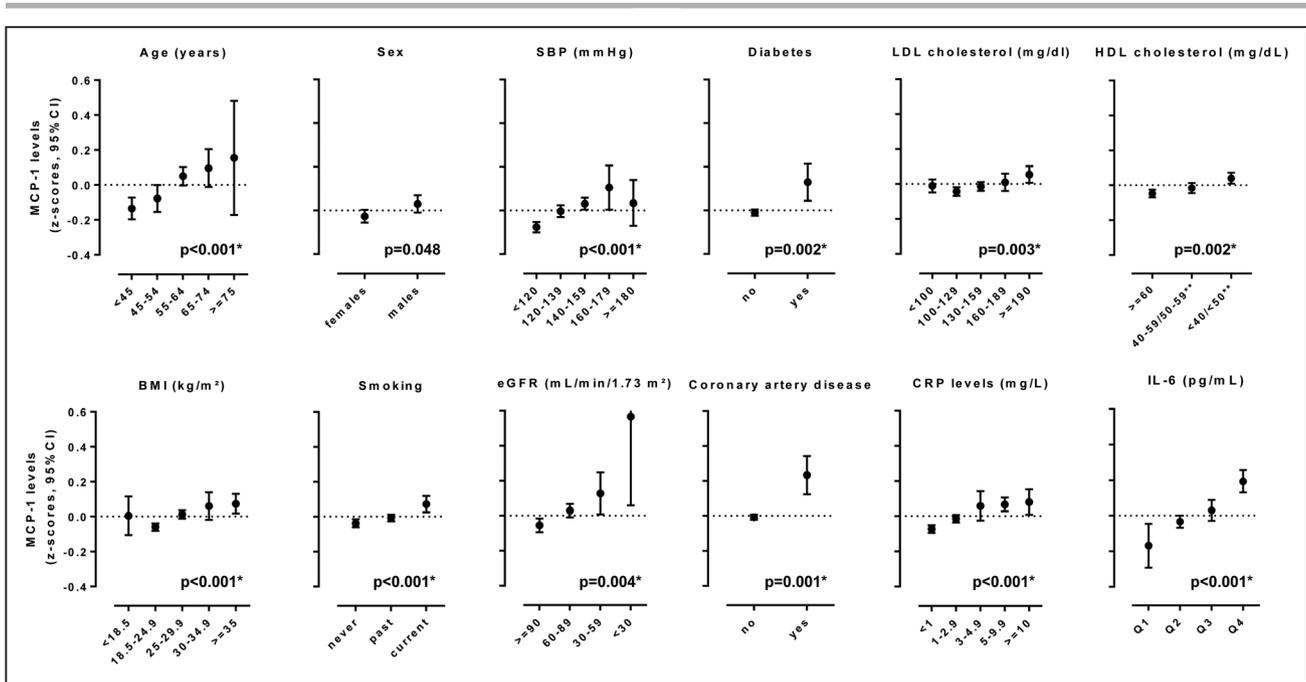
Figure 1. Cross-sectional associations between baseline circulating MCP-1 (monocyte chemoattractant protein-1) levels, demographic factors, conventional vascular risk factors, and inflammatory biomarkers.
Shown are the results from the pooled sample consisting of 6 population-based studies. Z score for circulating MCP-1 levels correspond to differences from the mean value of each study. P values are derived from meta-regression. BMI indicates body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; IL, interleukin; LDL, low-density lipoprotein; and SBP, systolic blood pressure. *Statistically significant results (after correction for multiple comparisons, statistical significance was set at $P < 0.05/12 = 0.004$). **<40 and 40 to 59 mg/dL for men, <50 and 50 to 59 mg/dL for women.

were highly consistent in the model additionally adjusting for circulating hsCRP levels on top of the vascular risk factors (model 3 in Figure 3 and Online Table IV).

Study-specific risk estimates are depicted in Online Figures II through IV. There was no evidence of heterogeneity in any of the analyses ($I^2 < 50\%$ and Cochran Q-derived $P > 0.10$), except for moderate heterogeneity in the analysis of the upper fourth MCP-1 quartile for any stroke and ischemic stroke ($I^2 = 49.8\%$, $P = 0.08$ and $I^2 = 46.1\%$, $P = 0.10$, respectively). The results were similar for both fatal and nonfatal stroke ($I^2 = 0\%$ for between-subgroup comparisons), although the CIs
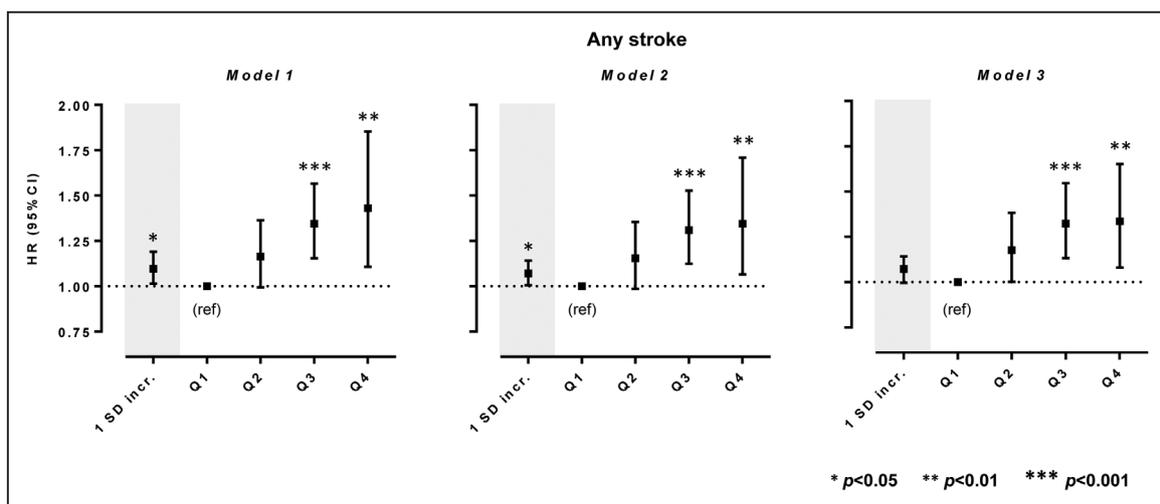
Figure 2. Associations between baseline circulating MCP-1 (monocyte chemoattractant protein-1) levels and risk of any stroke.
Shown are the results from random-effects meta-analyses of the pooled sample consisting of 6 population-based studies. Model 1 is adjusted for age, sex, and race. Model 2 is adjusted for age, sex, race, and vascular risk factors including body mass index (1 kg/m² increment [incr.]), smoking (current vs noncurrent), estimated glomerular filtration rate (1 mL/min per 1.73 m² incr.), history of coronary artery disease, diabetes mellitus, hypercholesterolemia, hypertension, atrial fibrillation, and heart failure at baseline. Model 3 is additionally adjusted for circulating high-sensitivity CRP (C-reactive protein) levels. Analyses for 1-SD incr. correspond to ln-transformed MCP-1 levels. HR indicates hazard ratio.
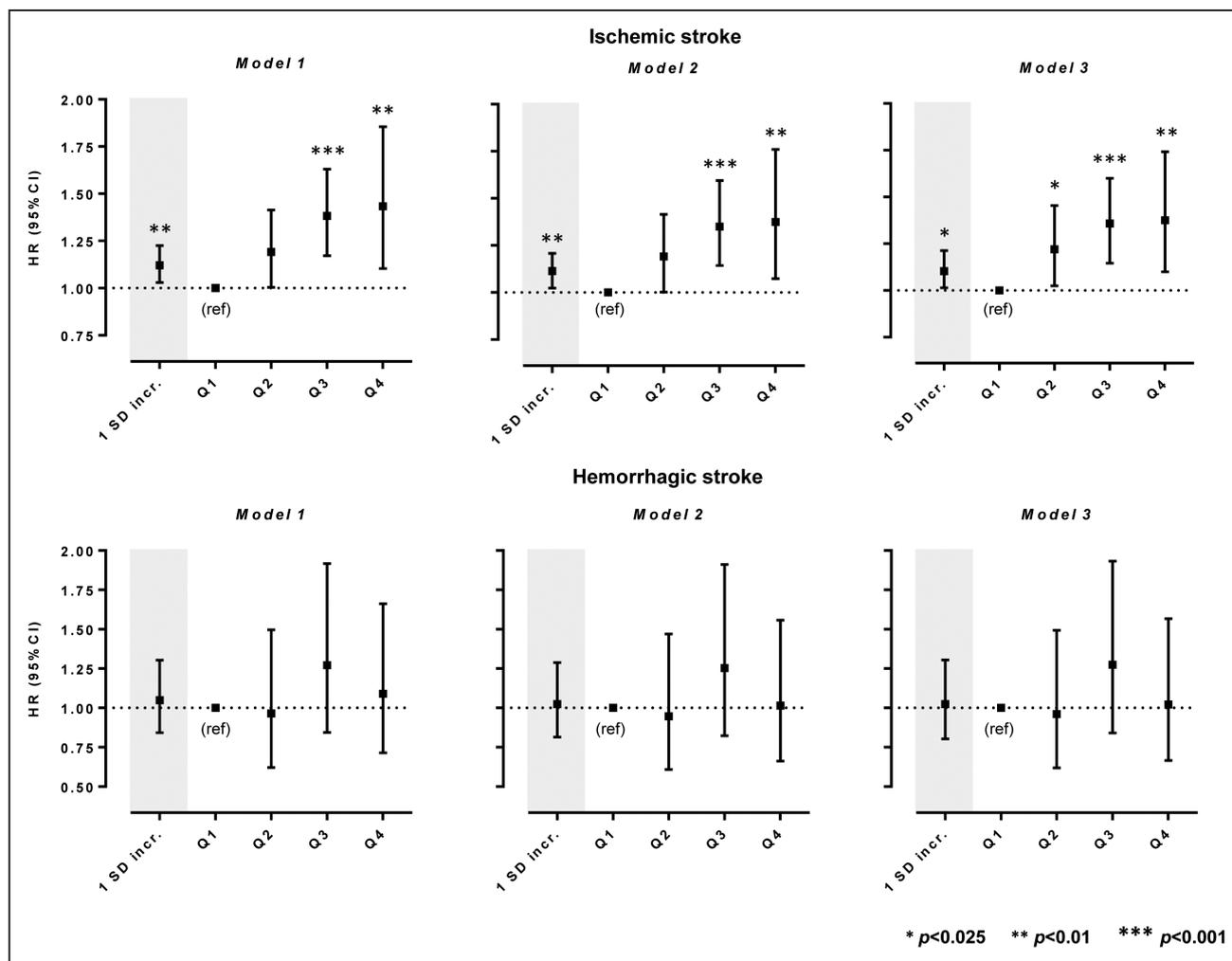
Figure 3. Associations between baseline circulating MCP-1 levels and risk of ischemic and hemorrhagic stroke.
Shown are the results from random-effects meta-analyses of the pooled sample consisting of 6 population-based studies for (**A**) ischemic and (**B**) hemorrhagic stroke. Model 1 is adjusted for age, sex, and race. Model 2 is adjusted for age, sex, race, and vascular risk factors including body mass index (1 kg/m^2 increment [incr.]), smoking (current vs noncurrent), estimated glomerular filtration rate (1 mL/min per 1.73 m^2 incr.), history of coronary artery disease, diabetes mellitus, hypercholesterolemia, hypertension, atrial fibrillation, and heart failure at baseline. Model 3 is additionally adjusted for circulating high-sensitivity CRP (C-reactive protein) levels. Analyses for 1-SD incr. correspond to ln-transformed MCP-1 levels. HR indicates hazard ratio. *Statistical significance threshold was set at $P < 0.05/2 = 0.025$ after correction for multiple comparisons (2 stroke subtypes).

for fatal stroke were wider probably because of lower statistical power (Online Figure V). The association estimates remained consistent in alternative models directly adjusting for the crude components of vascular risk factors (systolic blood pressure, fasting glucose levels, LDL cholesterol, and eGFR) and use of antihypertensive, glucose-lowering, or lipid-lowering medications (alternative model 2; Online Tables III through V). Furthermore, the results remained stable in sensitivity analyses omitting one study per time (leave-one-out analysis) showing that the results were not driven by any individual study (Online Figures VI through VIII). Meta-regression analyses showed that none of the examined study population characteristics nor the sample source (serum versus plasma) modified the associations of MCP-1 with the risk of any stroke, ischemic stroke, or hemorrhagic stroke (Online Table VI). Finally,

in subgroup analyses stratifying for sex, hypertension, diabetes mellitus, and body mass index (≥30 versus <30 kg/m^2), there was no indication for heterogeneity in the risk estimates for any stroke, ischemic stroke, and hemorrhagic stroke between subgroups (I^2=0%; Online Figure IX).

As a last step, we performed analyses with additional adjustments for IL-6 and hsCRP levels in 4 studies (12 516 individuals; 758 incident stroke events) with available data. Adjustment for IL-6 levels showed that the risk estimates between MCP-1 levels and risk of stroke and stroke subtypes remained stable, although with wider CIs than the main analysis, as would be expected given the smaller sample sizes (Online Table VII). Similarly, simultaneous adjustments for both IL-6 and hsCRP did not alter the risk estimates between MCP-1 and risk of stroke or stroke subtypes, even though both variables

were associated with the risk of any stroke and ischemic stroke (Online Table VII).

DISCUSSION

Pooling data from 6 population-based cohort studies involving 17 180 stroke-free individuals, we found higher circulating levels of MCP-1 at baseline to be associated with a higher long-term risk of stroke after accounting for age, sex, race, and vascular risk factors. In analyses for stroke subtypes, MCP-1 levels were specifically associated with the risk of ischemic stroke but not with hemorrhagic stroke. These associations followed a dose-response pattern, and risk estimates were stable after additional adjustments for serum levels of IL-6 or hsCRP.

Our results, which were obtained in studies with long-term follow-up, confirm and extend our recent Mendelian randomization finding of a higher stroke risk among individuals with genetic predisposition to higher lifetime MCP-1 levels.[9] The results were remarkably consistent between the 2 approaches: with Mendelian randomization, the odds ratio for stroke was 1.06 per SD increment in genetically determined MCP-1 levels, which is almost identical to the HR for incident stroke observed in the current meta-analysis of observational studies. In accord with the Mendelian randomization results, higher MCP-1 levels were further associated with a higher risk of incident ischemic stroke, but not hemorrhagic stroke, which is consistent with the established role of MCP-1 in experimental atherosclerosis. The magnitude of association of MCP-1 with incident ischemic stroke was modest suggesting that MCP-1 measurement is not likely to be of value as a risk marker for stroke although this would need to be formally examined. Of note, however, risk estimates compare well with those for lipoprotein (a),[32,33] which is established as a causal risk factor for atherosclerosis currently under investigation in clinical trials.[34,35] When viewed together with the genetic[9] and experimental data,[13–17] our findings provide triangulation of evidence regarding a role of MCP-1 as a causal risk factor for stroke.

Only limited human data exist supporting vascular benefits by reducing inflammation. Secondary analyses from the CANTOS trial showed that the reductions in vascular event rates after IL-1β inhibition were restricted to individuals with a substantial decrease in IL-6 or hsCRP levels.[31,36] Importantly, the risk estimates for stroke by MCP-1 levels in our study remained stable after additional adjustments for the baseline levels of IL-6, hsCRP, and both IL-6 and hsCRP. This observation provides indirect evidence suggesting that elevated levels of MCP-1 might influence risk of stroke independently of the IL-1β/IL-6/CRP axis. Thus, targeting the MCP-1/CCR2 pathway might serve as an alternative anti-inflammatory strategy with independent and complementary

effects in reducing vascular event rates on top of current approaches.

Deficiency of either MCP-1[15,17] or its receptor CCR2[16] decreases plaque burden and limits lipid deposition and macrophage infiltration in experimental models of atherosclerosis. Similar effects are observed with pharmacological treatment using MCP-1 competitors[13] or CCR2 antagonists.[14,37–39] In contrast, overexpression of MCP-1 promotes oxidized lipid accumulation, macrophage infiltration, and smooth muscle cell proliferation, thus accelerating atherosclerosis.[40] To our knowledge, there has been only one small phase II randomized controlled trial in the context of atherosclerosis in humans that targeted the MCP-1/CCR2 axis. Among 108 patients with cardiovascular risk factors and hsCRP levels >3 mg/L, those treated with a single intravenous infusion of MLN1202—a humanized monoclonal antibody against CCR2—exhibited significant reductions in hsCRP levels after 4 weeks and continuing through 12 weeks after dosing.[41] However, this study did not assess clinical outcomes, which would need to be examined in a larger trial.[41]

Our study has several strengths. The pooled analysis was based on a large sample size of >17 000 individuals from 6 previously unpublished population-based prospective studies with long follow-up intervals and a large number of incident events, thus providing sufficient statistical power to identify robust associations. The included studies fulfilled all of the criteria of quality assessment, which minimized the risk of several sources of bias. We further applied extensive adjustments for demographic and vascular risk factors thus accounting for confounding and enabling the identification of independent associations between MCP-1 levels and risk of stroke. Finally, in 4 of the cohorts, we had available data on IL-6 and hsCRP measurements, which allowed examining the associations between MCP-1 and stroke after adjusting for these biomarkers.

Our study also has limitations. First, the different assays used by individual studies to quantify circulating MCP-1 levels and the different sample sources (plasma versus serum) resulted in substantial variations in MCP-1 levels between studies. Although our analyses standardized MCP-1 levels across studies, it was not possible to explore associations between absolute MCP-1 values and risk of stroke. Second, studies differed in terms of demographic characteristics and prevalence of vascular risk factors. While we found no evidence of substantial heterogeneity between studies, there was moderate heterogeneity in the analyses for the highest quartiles of MCP-1, which could possibly be explained by the differences in baseline MCP-1 levels and in vascular risk profiles between studies. Third, we could not explore associations between MCP-1 levels and risk of ischemic stroke subtypes (large artery, cardioembolic, and small vessel stroke) because information on deeper phenotyping was not available for the majority of studies. Fourth, our analyses were based on predominantly

European ancestry individuals and do thus not necessarily apply to other ethnic groups. Fifth, we cannot exclude residual confounding. Finally, based on our a priori determined approach and power calculations, we corrected for multiple comparisons within each level of analysis but not across all analyses. Although this would not be expected to have any impact on the findings, future studies with even larger sample sizes would be useful in replicating our results

In conclusion, this meta-analysis demonstrates that higher circulating levels of MCP-1 among stroke-free individuals are associated with increased long-term risk of ischemic stroke. The results extend and corroborate experimental and genetic evidence suggesting a key role of MCP-1 in atherosclerosis and stroke. Additional work is needed to examine whether interventions aimed at interfering with MCP-1 signaling would lower stroke risk.

REFERENCES

1. DALYs GBD, HALE Collaborators. Global, regional, and national disability-adjusted life-years (dalys) for 315 diseases and injuries and healthy life expectancy (hale), 1990-2015: a systematic analysis for the global burden of disease study 2015. *Lancet*. 2016;388:1603–1658.

2. Mortality GBD, Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the global burden of disease study 2015. *Lancet*. 2016;388:1459–1544.

3. Esenwa CC, Elkind MS. Inflammatory risk factors, biomarkers and associated therapy in ischaemic stroke. *Nat Rev Neurol*. 2016;12:594–604. doi: 10.1038/nrneurol.2016.125

4. Libby P, Ridker PM, Hansson GK; Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. *J Am Coll Cardiol*. 2009;54:2129–2138. doi: 10.1016/j.jacc.2009.09.009

5. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, et al; CANTOS Trial Group. Antiinflammatory therapy with wanakinumab for atherosclerotic disease. *N Engl J Med*. 2017;377:1119–1131. doi: 10.1056/NEJMoa1707914

6. Ridker PM, Everett BM, Pradhan A, MacFadyen JG, Solomon DH, Zaharris E, Mam V, Hasan A, Rosenberg Y, Iturriaga E, et al; CIRT Investigators. Low-dose methotrexate for the prevention of atherosclerotic events. *N Engl J Med*. 2019;380:752–762. doi: 10.1056/NEJMoa1809798

7. Ridker PM. Anticytokine agents: targeting interleukin signaling pathways for the treatment of atherothrombosis. *Circ Res*. 2019;124:437–450. doi: 10.1161/CIRCRESAHA.118.313129

8. Aday AW, Ridker PM. Targeting residual inflammatory risk: a shifting paradigm for atherosclerotic disease. *Front Cardiovasc Med.* 2019;6:16. doi: 10.3389/fcvm.2019.00016

9. Georgakis MK, Gill D, Rannikmäe K, Traylor M, Anderson CD, Lee JM, Kamatani Y, Hopewell JC, Worrall BB, Bernhagen J, et al. Genetically determined levels of circulating cytokines and risk of stroke. *Circulation.* 2019;139:256–268. doi: 10.1161/CIRCULATIONAHA.118.035905

10. Lin J, Kakkar V, Lu X. Impact of MCP-1 in atherosclerosis. *Curr Pharm Des.* 2014;20:4580–4588. doi: 10.2174/1381612820666140522115801

11. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. *J Clin Invest.* 1991;88:1121–1127. doi: 10.1172/JCI115411

12. Lutgens E, Faber B, Schapira K, Evelo CT, van Haaften R, Heeneman S, Cleutjens KB, Bijnens AP, Beckers L, Porter JG, et al. Gene profiling in atherosclerosis reveals a key role for small inducible cytokines: validation using a novel monocyte chemoattractant protein monoclonal antibody. *Circulation.* 2005;111:3443–3452. doi: 10.1161/CIRCULATIONAHA.104.510073

13. Liehn EA, Piccinini AM, Koenen RR, Soehnlein O, Adage T, Fatu R, Curaj A, Popescu A, Zernecke A, Kungl AJ, et al. A new monocyte chemotactic protein-1/chemokine CC motif ligand-2 competitor limiting neointima formation and myocardial ischemia/reperfusion injury in mice. *J Am Coll Cardiol.* 2010;56:1847–1857. doi: 10.1016/j.jacc.2010.04.066

14. Bot I, Ortiz Zacarías NV, de Witte WE, de Vries H, van Santbrink PJ, van der Velden D, Kröner MJ, van der Berg DJ, Stamos D, de Lange EC, et al. A novel CCR2 antagonist inhibits atherogenesis in apoE deficient mice by achieving high receptor occupancy. *Sci Rep.* 2017;7:52. doi: 10.1038/s41598-017-00104-z

15. Gu L, Okada Y, Clinton SK, Gerard C, Sukhova GK, Libby P, Rollins BJ. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol Cell.* 1998;2:275–281.

16. Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. *Nature.* 1998;394:894–897. doi: 10.1038/29788

17. Combadière C, Potteaux S, Rodero M, Simon T, Pezard A, Esposito B, Merval R, Proudfoot A, Tedgui A, Mallat Z. Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6C(hi) and Ly6C(lo) monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice. *Circulation.* 2008;117:1649–1657. doi: 10.1161/CIRCULATIONAHA.107.745091

18. de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. *Circulation.* 2003;107:690–695. doi: 10.1161/01.cir.0000049742.68848.99

19. de Lemos JA, Morrow DA, Blazing MA, Jarolim P, Wiviott SD, Sabatine MS, Califf RM, Braunwald E. Serial measurement of monocyte chemoattractant protein-1 after acute coronary syndromes: results from the A to Z trial. *J Am Coll Cardiol.* 2007;50:2117–2124. doi: 10.1016/j.jacc.2007.06.057

20. Hoogeveen RC, Morrison A, Boerwinkle E, Miles JS, Rhodes CE, Sharrett AR, Ballantyne CM. Plasma MCP-1 level and risk for peripheral arterial disease and incident coronary heart disease: Atherosclerosis Risk in Communities study. *Atherosclerosis.* 2005;183:301–307. doi: 10.1016/j.atherosclerosis.2005.03.007

21. Deo R, Khera A, McGuire DK, Murphy SA, Meo Neto Jde P, Morrow DA, de Lemos JA. Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. *J Am Coll Cardiol.* 2004;44:1812–1818. doi: 10.1016/j.jacc.2004.07.047

22. van Wijk DF, van Leuven SI, Sandhu MS, Tanck MW, Hutten BA, Wareham NJ, Kastelein JJ, Stroes ES, Khaw KT, Boekholdt SM. Chemokine ligand 2 genetic variants, serum monocyte chemoattractant protein-1 levels, and the risk of coronary artery disease. *Arterioscler Thromb Vasc Biol.* 2010;30:1460–1466. doi: 10.1161/ATVBAHA.110.205526

23. Shoamanesh A, Preis SR, Beiser AS, Kase CS, Wolf PA, Vasan RS, Benjamin EJ, Seshadri S, Romero JR. Circulating biomarkers and incident ischemic stroke in the framingham offspring Study. *Neurology.* 2016;87:1206–1211. doi: 10.1212/WNL.0000000000003115

24. Herder C, Baumert J, Thorand B, Martin S, Löwel H, Kolb H, Koenig W. Chemokines and incident coronary heart disease: results from the MONICA/KORA augsburg case-cohort study, 1984-2002. *Arterioscler Thromb Vasc Biol.* 2006;26:2147–2152. doi: 10.1161/01.ATV.0000235691.84430.86

25. Schiopu A, Bengtsson E, Goncalves I, Nilsson J, Fredrikson GN, Bjorkbacka H. Associations between macrophage colony-stimulating factor and monocyte chemotactic protein 1 in plasma and first-time coronary events: a nested case-control study. *J Am Heart Assoc.* 2016;5:e002851.

26. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M. The newcastle-ottawa scale (NOS) for assessing the quality of nonrandomized studies in meta-analyses [Online]. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed September 13, 2019.

27. Williams B, Mancia G, Spiering W, Agabiti Rosei E, Azizi M, Burnier M, Clement DL, Coca A, de Simone G, Dominiczak A, et al; ESC Scientific Document Group. 2018 ESC/ESH guidelines for the management of arterial hypertension. *Eur Heart J.* 2018;39:3021–3104. doi: 10.1093/eurheartj/ehy339

28. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2019. *Diabetes Care.* 2019;42:S13–S28.

29. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, Grunberger G, Guerin CK, Bell DSH, Mechanick JI, et al. American Association of clinical endocrinologists And American College of endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocr Pract.* 2017;23:1–87. doi: 10.4158/EP171764.APPGL

30. Stevens PE, Levin A, Kidney disease: improving global outcomes chronic kidney disease guideline development work Group M. Evaluation and management of chronic kidney disease: synopsis of the kidney disease: improving global outcomes 2012 clinical practice guideline. *Ann Intern Med.* 2013;158:825–830

31. Ridker PM, Libby P, MacFadyen JG, Thuren T, Ballantyne C, Fonseca F, Koenig W, Shimokawa H, Everett BM, Glynn RJ. Modulation of the interleukin-6 signalling pathway and incidence rates of atherosclerotic events and all-cause mortality: analyses from the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study (CANTOS). *Eur Heart J.* 2018;39:3499–3507. doi: 10.1093/eurheartj/ehy310

32. Erqou S, Kaptoge S, Perry PL, Di Angelantonio E, Thompson A, White IR, Marcovina SM, Collins R, Thompson SG, Danesh J; Emerging Risk Factors Collaboration. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA.* 2009;302:412–423. doi: 10.1001/jama.2009.1063

33. Willeit P, Ridker PM, Nestel PJ, Simes J, Tonkin AM, Pedersen TR, Schwartz GG, Olsson AG, Colhoun HM, Kronenberg F, et al. Baseline and on-statin treatment lipoprotein(a) levels for prediction of cardiovascular events: individual patient-data meta-analysis of statin outcome trials. *Lancet.* 2018;392:1311–1320. doi: 10.1016/S0140-6736(18)31652-0

34. Nordestgaard BG, Langsted A. Lipoprotein (a) as a cause of cardiovascular disease: insights from epidemiology, genetics, and biology. *J Lipid Res.* 2016;57:1953–1975. doi: 10.1194/jlr.R071233

35. Khera AV, Kathiresan S. Genetics of coronary artery disease: discovery, biology and clinical translation. *Nat Rev Genet.* 2017;18:331–344. doi: 10.1038/nrg.2016.160

36. Ridker PM, MacFadyen JG, Everett BM, Libby P, Thuren T, Glynn RJ; CANTOS Trial Group. Relationship of C-reactive protein reduction to cardiovascular event reduction following treatment with canakinumab: a secondary analysis from the CANTOS randomised controlled trial. *Lancet.* 2018;391:319–328. doi: 10.1016/S0140-6736(17)32814-3

37. Winter C, Silvestre-Roig C, Ortega-Gomez A, Lemnitzer P, Poelman H, Schumski A, Winter J, Drechsler M, de Jong R, Immler R, et al. Chrono-pharmacological targeting of the CCL2-CCR2 axis ameliorates atherosclerosis. *Cell Metab.* 2018;28:175.e5–182.e5. doi: 10.1016/j.cmet.2018.05.002

38. Okamoto M, Fuchigami M, Suzuki T, Watanabe N. A novel C-C chemokine receptor 2 antagonist prevents progression of albuminuria and atherosclerosis in mouse models. *Biol Pharm Bull.* 2012;35:2069–2074. doi: 10.1248/bpb.b12-00528

39. Yamashita T, Kawashima S, Ozaki M, Namiki M, Inoue N, Hirata K, Yokoyama M. Propagermanium reduces atherosclerosis in apolipoprotein E knockout mice via inhibition of macrophage infiltration. *Arterioscler Thromb Vasc Biol.* 2002;22:969–974. doi: 10.1161/01.atv.0000019051.88366.9c

40. Aiello RJ, Bourassa PA, Lindsey S, Weng W, Natoli E, Rollins BJ, Milos PM. Monocyte chemoattractant protein-1 accelerates atherosclerosis in apolipoprotein E-deficient mice. *Arterioscler Thromb Vasc Biol.* 1999;19:1518–1525. doi: 10.1161/01.atv.19.6.1518

41. Gilbert J, Lekstrom-Himes J, Donaldson D, Lee Y, Hu M, Xu J, Wyant T, Davidson M; MLN1202 Study Group. Effect of CC chemokine receptor 2 CCR2 blockade on serum C-reactive protein in individuals at atherosclerotic risk and with a single nucleotide polymorphism of the monocyte chemoattractant protein-1 promoter region. *Am J Cardiol.* 2011;107:906–911. doi: 10.1016/j.amjcard.2010.11.005