**ARIC Manuscript Proposal # 3290**

**PC Reviewed: 12/11/18** **Status: _____** **Priority: 2**
**SC Reviewed: _____** **Status: _____** **Priority: ____**

**1.a. Full Title**: Circulating MCP-1 levels and incident stroke: A meta-analysis of population-based cohort studies

**b. Abbreviated Title (Length 26 characters)**: MCP-1 and incident stroke

**2. Writing Group**:
Writing group members:
Myriam Fornage, PhD
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Other ARIC co-authors are welcome.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. __MF___ **[please confirm with your initials electronically or in writing]**

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**3. Timeline**: Analyses to be completed by end of December 2018 to be included in the meta-analysis

**4. Rationale**: Recently, using a Mendelian Randomization approach, we examined associations between the circulating levels of 41 cytokines and growth factors and the risk of stroke in the MEGASTROKE GWAS dataset (67,000 stroke cases and 450,000 controls) and we found MCP-1 as the cytokine showing the strongest association with stroke. Genetically upregulated MCP-1 levels were specifically associated with higher risk of large artery and cardioembolic stroke subtypes.
Despite the evidence arising from experimental and genetic studies supporting associations of MCP-1 with cardiovascular disease, there are only limited data from observational studies. MCP-1 levels have been found to be predictive of recurrent cardiovascular disease in patients with established myocardial infarction, whereas case-control studies consistently show that patients with coronary artery disease or stroke have higher MCP-1 levels, as compared to controls. However, there are no data from large population-based cohort studies examining the associations of MCP-1 levels with incident stroke events.

**5. Main Hypothesis/Study Questions**:
Leveraging data from prospective cohort studies with available data on baseline MCP-1 circulating levels and cardiovascular endpoints at follow-up we aim to examine whether baseline MCP-1 levels associate with the risk of incident stroke.

**6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).**

Studies included in this project:
1. MONICA/KORA study (Munich, Germany)
2. Atherosclerosis Risk in Community Study (Forsyth County, North Carolina; Jackson, Mississippi; Suburbs of Minneapolis, Minnesota; and Washington County, Maryland; USA)
3. Framingham Heart Study (Framingham, Massachusetts, USA)
4. EPIC-Norfolk study (Norfolk, UK)
5. Malmö Diet and Cancer Study – Cardiovascular Cohort (Malmö, Sweden)
6. PRIME study (Belfast, Northern Ireland and Lille, Strasbourg, and Toulouse, France)
7. Dallas Heart study (Dallas, Texas, USA)

MCP-1 circulating levels assessment
MCP-1 circulating levels across the studies are assessed either in the serum or in plasma, by either ELISA or multiplex assay methods. As MCP-1 levels do not follow a normal distribution, we propose that MCP-1 levels are log-transformed in every study.

Outcome
Incident stroke events (transient ischemic attacks not included), classified (if information available) to ischemic and hemorrhagic stroke events, and sub-classified (if information available) to ischemic stroke etiological subtypes (large artery, cardioembolic, and small vessel stroke). Time to event from baseline MCP-1 assessment (in months) will be used to run Cox proportional hazard models. Patients with prevalent stroke (history of stroke at baseline) will be excluded from the analysis.

Exclusion criteria

Patients with a history of stroke at baseline will be excluded from the analysis.

Covariates

1. Age (continuous in 1-year increment)
2. Sex (male vs. female)
3. Race/ethnicity (categorical, as appropriate for each study)
4. Study site/center (if applicable for the study)
5. Presence of hypertension at baseline (defined either as history of hypertension, SBP ≥140 mm Hg, DBP ≥90 mm Hg, or prescription of antihypertensive medications)

If information on hypertension is not available, SBP at baseline (continuous) could be used instead.

6. Presence of diabetes at baseline (defined either as a history of diabetes, administration of glucose lowering medication, HbA1c ≥6.5%, fasting glucose ≥126 mg/dl, or random glucose levels ≥200 mg/dl).

If data on presence of diabetes is not available fasting glucose or HbA1c levels at baseline could be used as a continuous variable instead.

7. Smoking (ever vs. never or current vs. not current smoking)
8. Hypercholesterolemia (defined as history of hypercholesterolemia, administration of lipid-lowering drugs, or LDL levels ≥130 mg/dl).

If hypercholesterolemia is not available, LDL levels (continuous) or HDL/total cholesterol levels could be used instead

9. Chronic kidney disease (yes/no, defined as eGFR<60 ml/min/1.73m2) or eGFR (continuous)
10. BMI (continuous) or waist-to-hip ratio (continuous)
11. Heart failure (yes/no)
12. History of coronary artery disease (stable/unstable angina or MI)
13. Atrial fibrillation (yes/no)
14. Physical activity (as assessed in the study)
15. Alcohol consumption (as assessed in the study)
16. CRP circulating levels (log-transformed, continuous)

Statistical analysis

Time to incident events of any stroke will be the primary outcome of the study and will be examined in Cox proportional hazard models. We suggest that each study provide us with the results of three models for the association between MCP-1 levels and incident stroke:

a. Model 1: adjusted for age, sex, race/ethnicity and (if applicable) study site
b. Model 2: same as model 1 plus hypertension or systolic blood pressure levels, diabetes or glucose or HbA1c levels, smoking, hypercholesterolemia or LDL levels, chronic kidney disease or eGFR, BMI or waist-to-hip ratio, heart failure, coronary artery disease, and atrial fibrillation
c. Model 3: same as model 2 plus CRP levels

If one or more of the variables in the models is not available or has a substantial proportion of missing values in the individual datasets, the analyses may be performed with the remaining variables.

The models should be repeated for 1 SD increment in log-transformed MCP-1 levels and for MCP-1 categorized in 4 quartiles with the lowest category as reference. In addition to any stroke, the analyses should be repeated for ischemic stroke, hemorrhagic stroke, and, if available across studies, for etiological ischemic stroke subtypes (large artery, cardioembolic, small vessel stroke). For the analyses for stroke subtypes, individuals should be censored in case of the occurrence of an alternative stroke event. Finally, we propose sub-analyses by sex, presence of hypertension, diabetes, and BMI (<30 vs. ≥30 kg/m2).
In the first sheet of the attached excel file (named 'baseline data'), we suggest that each study adds the summary baseline characteristics of the study. The results from the analyses (Hazard ratios, confidence intervals, p-values and number of individuals and events in every analysis) may be provided in the second and third sheets. Specifically, the second sheet (named '1 SD increment') should include the results of the models, in which MCP-1 is included as a continuous variable (1 SD increment), whereas the third sheet (named 'quartiles') should include the results of the models, in which MCP-1 is included as a categorical variable (in quartiles with 1st quartile as reference category).

Meta-analysis
The estimates derived from every study will be pooled in random-effects meta-analyses in order to deal with heterogeneity across studies. Heterogeneity will be evaluated with the I2 and the Cochran Q statistic ($p<0.05$ indicating statistically significant heterogeneity).

**7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X__ No**

**b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used? ____ Yes ____ No**
(This file ICTDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

**8.a. Will the DNA data be used in this manuscript? ____ Yes __X__ No**

**8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = "No use/storage DNA"? ____ Yes ____ No**

**9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.**
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html

___X___ Yes _____ No

**10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?**

**11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ____ Yes __X__ No**

**11.b. If yes, is the proposal**

**___ A. primarily the result of an ancillary study (list number* _____)**

**___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _____ _____ _____)**

*ancillary studies are listed by number at https://www2.cscc.unc.edu/aric/approved-ancillary-studies

**12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.**

**12b. The NIH instituted a Public Access Policy in April, 2008** which ensures that the public has access to the published results of NIH funded research. It is **your responsibility to upload manuscripts to PubMed Central** whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.cscc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to PubMed central.