ARIC Manuscript Proposal #4224 (Amended)

PC Reviewed: 06/13/23	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Association of Clonal Hematopoiesis of Indeterminate Potential (CHIP) with Incident of Atrial Fibrillation and Potential Mechanisms: The Atherosclerosis Risk in Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): CHIP and Incidence of Atrial Fibrillation

2. Writing Group:
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3. Timeline:

The data needed for this analysis will be available in 6 months; we plan to submit for publication within 1.5 year.

4. Rationale:

Atrial fibrillation (AF) is the most common sustained cardiac dysrhythmia, and the global burden of AF is estimated to be about 60 million in 2019, double the number of estimated cases in 1990. (1) Age, male gender, HF, high blood pressure, diabetes, and structural heart disease are common risk factors of AF. (2, 3) Multiple longitudinal cohort studies have recently studied the associations between plasma proteomic profiling and the risk of AF. In all studies, N-terminal pro-B-type natriuretic peptide (NT-proBNP) was the strongest predictor of the incidence of AF, suggesting cardiac remodeling and cardiac tension as the primary mechanism leading to AF. (4-8)

Human aging creates a state of chronic inflammation defined by increased levels of circulating inflammatory markers like interleukin (IL)–6, IL-1β, and C-reactive protein. (9) Therefore, the concept of "inflammaging" was introduced, (10), in which clonal hematopoiesis of indeterminate potential (CHIP) might play a role. As we age, genetic mutations happen in our cells, causing changes that were not present in our germline DNA, a phenomenon named somatic mosaicism. Like other highly proliferating cells, hematopoietic stem cells (HSCs) are more likely to develop mosaicism. (11) A clone with survival benefits can expand and populate the peripheral blood (i.e., clonal hematopoiesis). (12) The subset of clonal hematopoiesis with a driver mutation in one of the genes implicated in hematologic malignancies, including *DNMAT3A, TET2,* and *ASXL1* among others, with variant allele frequency (VAF) of at least 2% in the absence

of known hematologic malignancy or other clonal disorders is called CHIP (13)

Recent studies have elaborated on the role of inflammation in the development of AF. (14-16) However, the association between CHIP and AF is not studied well. (17, 18)

Using UK-Biobank and Atherosclerosis Risk in Communities (ARIC) cohorts on visit 5 (V5), we will study the association of CHIP with AF and investigate if this risk varies based on the driver gene. Using the ARIC cohort, we will study if CHIP is associated with cardiac remodeling measured by cardiac markers, including high-sensitivity troponin I and T (hs-TnI and hs-TnI) and NT-proBNP, and if there is any structural difference between this two groups detected in echocardiography. We will compare inflammatory markers, including IL6 and IL18, as markers of NLRP3 inflammasome activity and high-sensitivity C-reactive protein (hsCRP) as a marker of general inflammation and investigate if these are part of the causal pathway. Previous proteomic studies in the ARIC V5 cohort identified 17 protein markers associated with the increased risk of AF. (8) As an exploratory analysis, we will compare these protein markers in patients with and without CHIP to identify the pathways in which CHIP and AF may overlap.

5. Main Hypothesis/Study Questions:

Aim 1: Study the association of CHIP with atrial fibrillation (AF)

Hypothesis 1: AF is more prevalent in individuals with CHIP

Hypothesis 2: ARIC participants with CHIP have a greater risk of developing AF

Hypothesis 3: The risk of AF associated with CHIP varies based on the driver gene

Hypothesis 4: The risk of AF associated with CHIP varies based on the allele frequency

Aim 2: Study the potential pathways in which CHIP might be associated with AF

Hypothesis 1: Inflammatory markers, including hsCRP, IL-6, and IL-18, are in the causal pathway of AF associated with CHIP

Hypothesis 3: Cardiac remodeling manifested by increased cardiac biomarkers, including hs-Troponin I, hs-Troponin T, and NT-BNP, and measured by echocardiography are in the causal pathway of AF associated with CHIP

Aim 3: Exploratory analysis of the 17 known proteomic markers reported to be associated with AF in patients with CHIP

Hypothesis 1: The concentration of proteomic markers of incident AF varies in patients with and without CHIP

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).

Study Design: Individuals with exome sequencing at visit 5 (n = 4233) and cross-validation of Aim 1 findings (association studies) in UK-Biobank

Exclusions:

- No exome sequencing available for CHIP calling
- Sex-mismatched sequencing samples
- Those with AF at baseline will be excluded from the incidence analysis

Exposure:

- Presence of CHIP

Outcomes:

1. Primary outcome: Incident AF after V5
2. Secondary outcome: Prevalent AF at V5
3. Exploratory outcomes: AF-related cardiovascular events after V5
4. Exploratory analysis: Cross-sectional study of cardiac remodeling and CHIP
5. Exploratory analysis: Cross-sectional study of inflammatory markers and CHIP
   Exploratory analysis: Cross-sectional study of proteomic markers that might predict the incidence of AF and CHIP

Covariates:

Demographic including age, sex, race, and center; clinical conditions including myocardial infarction, stroke, coronary revascularization, history of other atherosclerotic cardiovascular diseases, hematologic and solid organ malignancies, hypertension, diabetes mellitus, dyslipidemia; lifestyle factors including obesity, cigarette smoking, alcohol use, diet, physical activity, body mass index (BMI); lipid-lowering medication use, antihypertensive medication use, glucose-lowering medication use, antiplatelet and anticoagulant use; and laboratory markers including complete blood count (CBC, V5),

low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, estimated glomerular filtration rate (eGFR), high-sensitivity C-reactive protein (hsCRP), high-sensitivity cardiac troponin T (hs-cTnT), and N-terminal pro-brain natriuretic peptide (NT-proBNP), fibroblast growth factor 23, galectin 3, Interleukin 6, interleukin 18. Proteomic profile at V5. Echocardiography data at V5.

CHIP will be determined using exome sequencing (ES) of blood DNA using the GATK MuTect2 (19) somatic variant caller based on the 74 prespecified driver sequence variations in genes known to promote clonal expansion of hematopoietic stem cells. (20-22) A conventional variant allele frequency (VAF) of >2% will be used to identify CHIP. and those with VAF >10% will be considered large clones. CHIP calling will be conducted at BROAD institute.

Statistical Analyses:

For the current study, we will use the V5 visit as baseline. Baseline characteristics (V5) will be tabulated by CHIP status, with continuous variables expressed as mean±SD or median [25th percentile, 75th percentile], and categorical variables as percentages. P-value for trend will be calculated by rank-sum test for trend across ordered groups (23). In UKBB, AF will be defined using interview data (Filed ID 20002, disease code 1471) and ICD codes (ICD-9: 4273, ICD-10: I48). In ARIC, AF events are adjudicated using electrocardiograms, hospital discharge diagnoses, and death certificates. Those noted to have AF at baseline (V5) will be excluded from the prospective analyses of incident events. Unadjusted Kaplan-Meier curves will be used to depict time to event in the cohort. The event date will be considered the endpoint, and those without outcomes will be censored on 12/31/2017 in ARIC and 12/31/2021 in UKBB or death date. In both cohorts, Cox proportional hazard models adjusted for age, sex, race, diabetes, hypertension, smoking status, and body mass index (BMI), coronary artery disease, stroke and heart failure. We also will perform a gene-based analysis for the top three genes (i.e., DNMT3A, TET2, and ASXL1) to investigate further the different driver gene roles in the association of CHIP and AF. The mean follow-up of 6.21 years in ARIC and 11.96 years in UKBB. As all covariates are available in both cohorts, we then will conduct an inverse variance-weighted approach with a restricted maximum-likelihood estimation and a common effect model (with fixed treatment effect) meta-analysis to obtain the effect estimates for CHIP and AF in both ARIC and UKBB cohorts.
To investigate the potential mechanisms by which mutation in each gene may affect the

risk of AF, we will use echocardiographic measures, biomarkers of cardiac injury, including high-sensitivity troponin T, high-sensitivity troponin I, and N-terminal pro natriuretic peptide (hs-TnT, hs-TnI, and NT-proBNP), and inflammatory markers, including high-sensitivity C-reactive protein, Interleukin 6, and Interleukin 18 (hs-CRP, IL6, and IL18) available in ARIC (all measured at V5) and will compare these in those with no CHIP, small clones, and large clones in one of the top three genes.
To investigate how IL6 levels may affect the risk of AF associated with CHIP, we will divide individuals into four groups based on their CHIP status (none vs. large clones) and IL6 level (low vs. high) and compare the risk of incident AF using the adjusted Cox model described above. 17 protein markers known to be associated with incident AF will be tested against CHIP status to find those by which CHIP increases the risk of incident AF.

Sensitivity Analyses:

- The association finding of the ARIC (V5) cohort will be cross-validated in UKBB.

<u>Limitations</u>:

- Small sample size: gene-specific analyses may be underpowered.
- There is the potential for residual confounding.

7.a. Will the data be used for non-CVD analysis in this manuscript? 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? NA
(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

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"? Confirmed

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/search.php

   Yes

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

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)* (#2009.24, #2009.16, #2009.17, #2017.20)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

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.

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