# ARIC Manuscript Proposal #3605

**PC Reviewed:** 4/14/20   **Status:** _____   **Priority: 2**
**SC Reviewed:** _____   **Status:** _____   **Priority:** ____


**1.a.  Full Title**: Replication of CHIP (Clonal Hematopoiesis of Indeterminate Potential) associated EWAS (epigenome-wide association study) loci in the Atherosclerosis Risk in Communities (ARIC) Study

  **b. Abbreviated Title (Length 26 characters)**: Replication of CHIP associated EWAS loci in the ARIC study

**2.   Writing Group**:
    Writing group members: Md Mesbah Uddin, Akhil Pampana, Alexander Bick, Jan Bressler, Myriam Fornage, Varuna Chander, Richard Gibbs, Jennifer Brody, Eric Boerwinkle, Bing Yu, Joanne Murabito, Bruce Psaty, Christie Ballantyne, Karen Conneely, Pradeep Natarajan


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


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**3.   Timeline**: We propose to perform targeted replication per below within 2 months of P&P approval, and submit a manuscript within 4 months of P&P approval.


**4.   Rationale**:

Aging is the dominant risk factor for many chronic diseases in humans including hematologic cancers (Genovese et al. 2014; Jaiswal et al. 2014) and cardiovascular diseases (Jaiswal et al. 2017; Jaiswal et al. 2014), but its underlying molecular mechanisms are largely unknown. Clonal hematopoiesis of indeterminate potential (CHIP) is an age-related phenomenon where blood cells are predominantly derived from a single clone in individuals without hematologic malignancy (Jaiswal et al. 2017). Interestingly, the 3 most frequently mutated CHIP genes (*DNMT3A*, *TET2*, and *ASXL1*) are involved in epigenetic regulations. Furthermore, epigenetic changes such as differential DNA methylation (DNAm) at CpG dinucleotide sites (CpGs) also strongly associate with human aging (Hannum et al. 2013; Horvath 2013).

However, very little is known how these somatic and epigenetic changes contribute to aging and age-related diseases. Identification of differentially methylated CpGs in CHIP carriers vs. noncarriers will provide novel insight into the mechanisms of aging by facilitating the identification of DNAm that accelerates (or decelerates) accumulation of CHIP mutations and related burdens.

The aim of this replication study is to identify true associations from the discovery EWAS between DNA methylation and CHIP, conducted using Cardiovascular Health Study (CHS) cohort of TOPMed, and to rule out false positive associations using ARIC study population as an independent cohort.

**5. Main Hypothesis/Study Questions**:

We hypothesized that
1) DNA methylation (DNAm) profiles will differ in cells harboring CHIP mutations
2) These differences will be detectable in epigenome-wide association analysis
3) CHIP-associated DNAm differences may help explain the widespread differential DNAm observed in aging

Aim: To replicate CHIP-associated EWAS loci in the ARIC Study

**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.**
<u>Data</u>:
- CHIP calls on CHARGE-S Freeze 5 whole-exome sequence data (WES) from Bick et al. 2019 will be used.
- DNA methylation:
  o For the 1,092 European American participants (EA) with methylation data, 800 also have CHARGE-S WES data (methylation: visit 2: 605; visit 3: 195).
  o For the 2,822 African American participants (AA) with methylation data, 2,375 also have CHARGE-S WES data (methylation: visit 2: 2,064; visit 3: 311)
- ~10K Bonferroni significant CHIP and age associated CpG sites will be considered for replication

Inclusion/exclusion: Samples without hematologic malignancy; age and sex matched

Outcome: differential DNA methylation profile (DNAm)

Predictor: CHIP mutation (called from WES) at time of blood sampling

Covariates: Age, sex, cell type proportions, repeated measures/batch effect, first five (genomic) principal components of ancestry

Summary of data analysis: We performed the discovery EWAS between CHIP (or VAF: CHIP with large clone where variant allele fraction is greater than 10%) and DNAm in the CHS cohort of TOPMed (n=589; aged 64-90; 61% female). The mixed effects model in CpGassoc R package was used for EWAS:

$$DNAm \sim CHIP + age + sex + cell\ type\ proportion + individual\ random\ effect$$

We observed significant associations between DNAm and CHIP, even after accounting for potential confounders in the model, such as gender, cell types, repeated measures/batch effect, and chronological age—which is a strong predictor of epigenetic changes. A total of 275 CHIP, 280 VAF and 7144 age associated CpGs were identified in CHS cohort at epigenome-wide significance level (after Bonferroni correction). These CpG sites will be replicated in this study. We will analyze AA and EA data separately, with relevant covariates (listed) included in the model.

**7.a.  Will the data be used for non-ARIC analysis or by a for-profit organization in this manuscript? ____ Yes    __x__ No**

  **b. If Yes, is the author aware that the current derived consent file ICTDER05 must be used to exclude persons with a value RES_OTH and/or RES_DNA = "ARIC only" and/or "Not for Profit" ? ____ Yes    ____ No**
     (The 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? ___x_ Yes    ____ No**

**8.b.  If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the current derived consent file ICTDER05 must be used to exclude those with value RES_DNA = "No use/storage DNA"? __x__ 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     ____ 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 https://sites.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.

# References

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