Manuscript Proposal #2771

PC Reviewed: 6/7/16	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1. a. Full Title: Galectin-3 and Cardiovascular Outcomes

b. Abbreviated Title (Length 26 characters): Galectin-3 and CV outcomes

2. Writing Group:
David Aguilar
Christie M. Ballantyne
Vijay Nambi
Scott D. Solomon
Amil Shah
Eric Boerwinkle,
John W. McEvoy
Elizabeth Selvin
Kunihiro Matsushita
Wensheng Sun
(others welcome)

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

First author:
David Aguilar, MD
Baylor College of Medicine, Houston, TX
Email: daguilar@bcm.edu

ARIC author and Corresponding author
Christie M. Ballantyne
Professor
Baylor College of Medicine
Houston, TX, US
Phone: 713-798-5800
Fax: 713-798-7885

3. Timeline:
Analysis to start immediately; Manuscript to be written and sent for publication within one year of approval

4. Rationale:
Galectin-3 is a beta-galactoside binding lecitin that is produced and secreted primarily by activated macrophages, mast cells, and eosinophils and is involved in important regulatory roles

in adhesion, inflammation, immunity, and fibrosis.[1-3] Galectin-3 functions as a paracrine signal that is secreted in the blood stream and directly in the extracellular matrix and leads to macrophage proliferation, fibroblast proliferation, and the development of fibrosis. Galectin-3 has been implicated in numerous disease pathways including cancer progression, renal fibrosis and may play a significant role in the development of cardiovascular disease, including heart failure. Indeed, administration of galectin-3 in animal models results in myocardial fibrosis and heart failure, [4] while pharmacologic or genetic disruption of galectin-3 attenuates animal models of myocardial fibrosis, LV dysfunction, and heart failure development.[5] Galectin-3 also appears to be involved with aldosterone-mediated fibrosis.[6]

Several epidemiologic studies have demonstrated that plasma galectin-3 levels are associated with adverse cardiovascular outcomes in individuals with established heart failure, [7-10], and more recent studies have extended the association of galectin-3 and adverse cardiovascular outcomes to the general population.[11-15] Nonetheless, studies performed to date in the general population have several limitations. The majority of studies have been performed in mostly Caucasian populations,[11-14] which limits the generalizability to other populations. In addition, most previous studies have predominantly evaluated the relationship between galectin-3 and incident HF and/or mortality[12-15] and have not assessed the association of galectin-3 and incident CHD and stroke, in large part due to modest sample sizes of the cohorts. The large, biracial composition of the Atherosclerosis Risk in Communities (ARIC) Study will allow us to address these limitations and evaluate the relationship between plasma galectin-3 and incident coronary heart disease, heart failure hospitalization, stroke, and mortality in patients free of cardiovascular disease at Visit 4.

In addition, a previous genome-wide association study in a Dutch cohort has identified genetic loci associated with plasma galectin-3 levels [one including the galectin-3 encoding gene (LGALS3)].[16] We have confirmed the findings of an association of plasma galectin-3 levels and LGALS3 in a preliminary analysis of a Mexican-American population in Starr-County, Texas (pilot data). The extensive genotyping already completed in ARIC will allow us to determine genetic variation contributing to plasma galectin-3 levels in a large, biracial population. In addition, the association between genetic variants contributing to plasma galectin-3 levels and cardiovascular outcomes can be assessed. Identification of genetic variation contributing to both galectin-3 levels and adverse cardiovascular outcomes provides greater evidence for a direct functional role in the development of cardiovascular disease**.**[17-20]

5. Main Hypothesis/Study Questions:

Main hypothesis: Plasma levels of galectin-3 will be associated with incident cardiovascular disease outcomes (incident coronary heart disease, incident heart failure, incident stroke, and mortality) in patients free of overt cardiovascular disease at Visit 4.

Study Aims:

a. To determine the distribution of galectin-3 in a community-based population
b. To determine the association of galectin-3 and baseline clinical, laboratory, and biomarker data
c. To assess the association between galectin-3 and incident cardiovascular disease, including incident coronary heart disease, incident heart failure, incident ischemic stroke, and total mortality.

d. To assess the prognostic utility of galectin-3 as a biomarker for the cardiovascular outcomes described.
e. To determine whether the association of galectin-3 with outcomes is modified by gender or race.
f. To explore the genetic determinants of galectin-3 levels.
g. To investigate if the genetic determinants of galectin-3 are also associated with adverse cardiovascular outcomes (Mendelian randomization).

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 methodological limitations or challenges if present).

Study Design, Inclusion/exclusion:

Study design: The proposed study will be an observational study design using data acquired from all ARIC study centers on visit 4 and subsequent incident cardiovascular disease.

Inclusions: Participants in ARIC visit 4 who have plasma galectin-3 levels measures available who do not have prevalent HF, CHD, or ischemic stroke at Visit 4.

Exclusions: Standard ARIC exclusions (race exclusions for different communities) will apply. Individuals with prevalent HF, prevalent CHD, and prevalent stoke at Visit 4 will be excluded. Subjects will be excluded if there is missing information on galectin-3.

Summary of data analysis:
The distribution of galectin-3 will be determined. Continuous variables will be presented as means with standard deviations, unless otherwise specified. Baseline characteristics for the study population will be tabulated according to quartiles of galectin-3. P-values for linear trend will calculated by using trend test across ordered groups. Cox-proportional hazard regression models will be used to estimate the hazard ratios (HRs) and their 95% CI for outcomes. The basic model (model 1) will be adjusted by age, gender, and race-center. Model 2 will include all variables in model 1 plus total cholesterol, HDL, systolic blood pressure, anti-hypertensive medication, current smoking, and diabetes status. Model 3 will include all variables in model 2 plus eGFR. Model 4 will include all variables of model 3 plus NT-proBNP. Finally, model 5 will include all the variables in Model 4 plus hs-Troponin T. Potential interactions between sex and race will be ascertained adding a cross-product variable to the final model. Galectin-3 will be modeled as a categorical (quartile) variable. We will also model galectin-3 using linear splines in fully-adjusted models. C-statistics will be calculated to test the incremental model discrimination and to determine the incremental benefit of Gal-3 in the prediction of cardiovascular events. Integrated discrimination improvement (IDI) and net reclassification improvement metrics will also be measured.

A race-stratified genome wide association for plasma galectin-3 levels will be performed. Galectin-3 will be log transformed if needed. The GWAS will be performed both unadjusted and adjusted for age and sex under dominant models (ie, 0, 1, or 2 risk-raising alleles). To test the

association of genetic variants with clinical outcomes (CHD, heart failure, ischemic stroke and total mortality), Cox-proportional hazards modeling will be performed. Covariates included in this model will include age and sex. Further testing of the association of galectin-associated SNP and clinical outcomes can be performed utilizing the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium database.

Limitations:
Despite adjustment for baseline differences, residual confounding may persist.

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

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 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 found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.

_____ Yes ___x___ 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 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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