1.a. Full Title: Genetic Polymorphisms of Tristetraprolin (TTP) in Cardiovascular Disease (CVD)

b. Abbreviated Title (Length 26 characters): TTP SNPs in CVD

2. Writing Group: Perry Blackshear, M.D, D.Phil  
Nishadi Rajapakse, PhD  
Kari North, PhD  
Eric Boewinckle, PhD  
Joe Coresh

Writing group members:

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

First author: Nishadi Rajapakse, PhD  
Address:  
NIEHS  
MD: F106  
P.O.BOX 12233  
RTP, NC 27709

Phone: (919)316-4531  
Fax:  
E-mail: rajapak1@niehs.nih.gov

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).  
Name: Kari North  
Address: Department of Epidemiology, University of North Carolina Chapel Hill, Bank of America Center, 137 E. Franklin Street, Suite 306, Chapel Hill, NC 27514.

Phone:  
Fax:  
E-mail: kari_north@unc.edu
3. **Timeline:** 3-6 months

4. **Rationale:** TNF-α is an important pro-inflammatory cytokine that contributes to the pathogenesis of inflammatory diseases, including rheumatoid arthritis (RA), asthma and other autoimmune diseases. This cytokine is released primarily from macrophages and mast cells via IgG-dependent mechanisms during the innate immune response to infection and plays a role in a wide variety of other cellular responses involving activation and recruitment of other inflammatory cytokines, differentiation and apoptosis.

Recent clinical studies have suggested the involvement of TNF-α in the development of coronary artery disease (CAD), since elevated levels of TNF-α have been observed in atherosclerotic lesions in patients with cardiovascular disease. This observation is especially notable in women, as there is reported to be a correlation between increased expression of TNF-α and an increased risk of CAD in females. In addition, TNF-α blockers that are efficacious in the treatment of RA have been shown to lower the risk of developing CAD in both male and female RA patients.

Tristetraprolin (TTP) is an inhibitor of TNF-α secretion, and plays this important role by stimulating the turnover of TNF-α mRNA. ZFP36 is the human gene that codes for TTP. TTP acts by binding to the 3'-untranslated region of TNF-α mRNA, inducing deadenylation and turnover of TNF-α mRNA. As seen in mice that over-express TNF-α, Zfp36 knockout mice develop severe inflammatory arthritis as a result of increased TNF-mRNA, and consequent chronic increased expression of TNF-α. Moreover, this phenotype is abolished in Zfp36 knockout mice treated with anti-TNF-α antibodies or interbred with mice lacking both types of TNF-α receptors.

Previous resequencing studies from our laboratory have identified sequence variants and predicted haplotypes in ZFP36 in patients with autoimmune disease. 18 novel ZFP36 polymorphisms were identified in 316 individuals with various autoimmune diseases, as well as in normal controls of varying ethnicities. Furthermore, frequencies of all known ZFP36 variants in 484 participants of a regional DNA registry (Environmental Polymorphisms Registry) were evaluated, and 13 of the 28 known ZFP36 polymorphisms were detected in this population. Three SNPs, ZFP36*2, *8 and *10, have been significantly associated as either risk or protective factors with asthma, rheumatoid arthritis and myositis. Nonetheless, the relationship between TTP and CAD has not been examined. Because TTP plays a significant role in TNF-α regulation, it is possible that it TTP may play an important role in CAD.

5. **Main Hypothesis/Study Questions:** Overall hypothesis is that one or more genetic variants in ZFP36 are associated with coronary artery disease.

Specific aim: Evaluate associations of known genetic variants in ZFP36 with development of cardiovascular disease in individuals enrolled in the Atherosclerosis Risk in Communities (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).

As outlined in Specific aim 1, we propose to test for genetic associations between ZFP36 and non-invasively measured atherosclerotic burden and/or risk of adverse cardiovascular events in individuals in the ARIC study (entire cohort, N=16571). Our previous studies in ~800 subjects indicate that there are 13 htSNPs of ZFP36 that are sufficient to distinguish 85% of the haplotypes. Therefore, we propose to genotype for these 13 variants of ZFP36 in individuals enrolled in the ARIC study.

We propose that all of the genotyping will be done in the ARIC facility at the University of Texas in order to minimize handling and loss of the valuable DNA samples. The specific details of these protocols will be determined in conversations with the scientists at the ARIC core laboratory.

The following variables and measurements from the ARIC main study database will be analyzed.

1. Incident coronary heart disease (non-fatal and fatal MI, acute coronary syndromes, and revascularization procedures)
2. Carotid atherosclerosis (increased carotid intima-media thickness assessed by B-mode ultrasound)
3. Peripheral arterial disease (ankle-arm blood pressure index assessed by DINAMAP)
4. Cerebrovascular disease (stroke and transient ischemic attacks).
5. Confounding variables: Age, sex and ethnicity.

The relationship between each polymorphism and incident CHD and stroke will be assessed with Cox proportional hazards regression, using Barlow’s method to account for ARIC’s stratified, case-cohort design. Prevalent CVD disease endpoints will be analyzed with logistic regression, using the weighted analytic technique described by Prentice to account for the stratified, case-cohort design. Final multivariable models will be adjusted for established risk factors for the development of atherosclerotic disease.

In one of our previous case-control studies in autoimmune disease, the odds ratio (OR) of 2.46 was detected for a common genetic variant (defined as a frequency greater than 10%). Based on these power calculations, 200 controls and 200 cases would be adequate in order to obtain a significance level of 0.05. Therefore, genotyping 16571 should be sufficient to detect significant associations while maintaining adequate power.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes ___ 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 ICTDER03 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 ___ 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”?  
___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/search.php

____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?  
___x_ Yes    ____ No

11.b. If yes, is the proposal
___x   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/

12. 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.