1.a. **Full Title**: ADAMTS-13 antigen as a measure of ULVWF proteolysis in patient with peripheral artery disease

b. **Abbreviated Title (Length 26 characters)**: ADAMTS-13 and PAD

2. **Writing Group**:
   Writing group members: Jing-fei Dong, Christie Ballantyne, Ron Hoogeveen, LE Chambless, Gerardo Heiss, Hanyu Ni, Eric Boerwinkle, Josef Coresh, Carol Sun, Angelia Bergeron, Paul Bray

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

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3. **Timeline**: Glycocalicin measurements on Visit 3 and 4 EDTA plasma samples of PAD patients and controls were completed in May of 2007 and data submitted to the ARIC CC. Manuscript preparation will start upon approval by the ARIC publication committee. We anticipate that the manuscript will be completed in 2007.

4. **Rationale**:
   In vascular endothelial cells, von Willebrand factor (VWF) is initially synthesized as monomers, which form dimers and then multimers through covalent means (1:2). The newly synthesized VWF multimers are either constitutively secreted or packaged into the storage granule Weibel-Palade bodies, where the multimerization process continues. VWF multimers stored in the Weibel-Palade bodies are, therefore, rich in the ultra-large (UL) forms as compared
to VWF multimers found in plasma (3;4). Furthermore, ULVWF multimers are also prothrombotic because they spontaneously binding and agglutinating platelets, leading to thromboembolism. The stored ULVWF is released when endothelial cells are activated by various agonists, including inflammation. Upon release, the prothrombotic ULVWF is subjected to rapid proteolysis that converts these hyperactive multimers to the forms that are smaller and less adhesive, but remain hemostatically active. Should the proteolysis become defective, the uncleaved or only partially cleaved ULVWF will accumulate on the surface of vascular endothelial cells and in plasma, resulting in systemic thrombotic microangiopathy such as thrombotic thrombocytopenic purpura (TTP) (5). This critical proteolysis occurs at a single peptide bond between Tyr842 and Met843 in the A2 domain of mature VWF and is the function of the plasma metalloprotease ADAMTS-13 (6-8).

Severe ADAMTS-13 deficiency, due to genetic mutations or autoantibodies against the metalloprotease, is closely associated with thrombotic thrombocytopenic purpura (TTP) (8;9). However, mild-to-moderate deficiency has also been reported in conditions other than TTP, including post-surgery, pregnancy (pre-eclampsia and HELLP), bacterial and viral infections (10-16). Although diverse in their causes, these conditions share a common characteristic of systemic inflammation that has increasingly been recognized as playing a critical role in atherothrombosis, including PAD. Deficient ULVWF proteolysis in systemic inflammation attributes to lower ADAMTS-13 activity due to consumption, proteolytical inactivation by thrombin and plasmin (17), and direct inhibition by proinflammation cyto- and chemokines such as IL-6 (18), thrombospondin (19), soluble P-selectin (20), and VWF (21). Human plasma contains about 8-10 µg/ml of VWF during resting state (22), but the VWF level increases 2-3 fold during acute systemic inflammation (23;24). The increase results in about 10–20 µg/ml of newly released VWF that is highly enriched in the hyperactive ULVWF. Facing this amount of ULVWF is approximately 1 µg/ml of circulating ADAMTS-13 (25), a molar ratio of approximately 8 to 1 to ADAMTS-13 deficit. This intrinsic mismatch suggests that the plasma level of ADAMTS-13 can serve as a critical marker for the metalloprotease activity and VWF adhesion activity. Plasma ADAMTS-13 varies significantly among individuals (26) and expected to be lower in patient with PAD. As a result, VWF multimers found in patients with PAD are likely to be much more adhesive and prothrombotic than normal subjects. The presence of such overly adhesive VWF contributes to the initiation and propagation of atherothrombosis (27;28).

5. Main Hypothesis/Study Questions:
Because ADAMTS-13 is a newly characterized molecule, the study serves to answer one question and test one hypothesis.
We will first answer the question regarding the plasma levels of ADAMT-13 in healthy adults and whether there are different between genders and among ethnic groups. Based on a study of a small cohort, we expect the difference between patients and controls to be 20-30%.
The working hypothesis is that ADAMTS-13 level is lower in patients with PAD. The ADAMTS-13 deficiency is more severe in female patients.

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).
We will test the hypothesis by measuring plasma ADAMTS-13 antigen in PAD patients and controls. This is a case–control study of design. For the study, PAD was defined by ankle–
brachial index (ABI), which, together with ultrasonography, was measured on individuals at the third or fourth exams.

The inclusion criterion for the case–control study was individuals who had valid ABI data at exam 3 or 4. Exclusion criteria included individuals in the exam 3 and 4, who were diagnosed as either coronary heart disease or stroke before exam 3. Patients with congenital or acquired thrombotic thrombocytopenic purpura, von Willebrand disease, or severe liver disease (cancer, cirrhosis) were exclusions applied to the selected cases and controls prior to analysis. The participants were divided into two groups. The Case group included individuals, whose ABI measurements were below the sex-specific cutpoints at exam 3 and 4. For each exam group, an equal number of noncase individuals were chosen as controls, stratified by field center.

We proposed to analyze data from individuals consisted of a 1:1 case:control ratio. We had 148 PAD cases at exam 3 and 234 new PAD cases at exam 4. The sample size selected is 296 at visit 3 and 448 at visit 4 (total 744).

ADAMTS-13 antigen was measured by an ELISA method using EDTA plasma obtained at the time of ABI measurement (exam 3 or exam 4).

Multivariable logistic regression models will be used to evaluate the relation between PAD status and plasma ADAMTS-13 antigen levels, modeled both as continuous variables and in tertiles. Consideration will be given to the effect of measurement error on the results of the analyses. Consideration will also be given to using SUDAAN software to adjust for sampling strategy in the logistic regression models, and SUDAAN will definitely be used for descriptive statistics. The established cardiovascular risk factors evaluated as potential confounders in the logistic regression models will include age, gender, race, center, BMI, years of cigarette smoking, incident CHD, incident diabetes, lipids (triglycerides, total cholesterol, LDL-C, HDL-C). We will also plan to analyze the ADAMTS-13 antigen together with ADAMTS-13 activity to distinguish consumptive or secretion defects to those caused by direct inhibition by inflammatory mediators. The analysis will be conducted after we obtain ADAMTS-13 activity data (expected to be completed in FY 08 using citrate plasma).

There have been increasing numbers of studies on correlation between acquired ADAMTS-13 deficiency and conditions other than TTP (cancer, transplantation, eclampsia/HELLP syndrome, and sepsis). Because of the small sample size, the normal distribution of ADAMTS-13 antigen in control population varied significantly among the studies. We therefore propose to use ARIC control population to determine basic ADAMTS-13 antigen and its correlation with age, gender, and ethnicity (and genotypes of selected SNPs).

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 ICTDER02 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 ICTDER02 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 ICTDER02 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/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)?
We did not find any manuscript proposal in ARIC that relates to the study topic (ADAMTS-13). However, several manuscripts are related for the ADAMTS-13 substrate VWF being the focus of the studies. These include, but not limited to #010c: VWF & Factor 8 Correlates and #777: Activity of coagulation and fibrinolytic factors and inhibitors in coronary heart disease. VWF is one of most widely used marker for endothelial cell damage so it appears in multiple manuscript proposals, but we propose to study its proteolytic enzyme.

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* _2004.09_) ___ 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.
Reference List


