ARIC Manuscript Proposal #810

PC Reviewed: 07/03/01 **Status: __A__** **Priority: N/A**
SC Reviewed: _____ **Status: _____** **Priority: _____**

CHS Manuscript Proposal Form

1. Title of proposal: **Investigation of the protective effects of a Factor XIII Val34Leu polymorphism and a fibrinogen Hae III polymorphism in venous thromboembolism (VTE)**

2. Type of study: _____ Main _____ Substudy ___xx__ Ancillary (see below)

Ancillary Study title and name of PI: LITE Study (Dr. AR Folsom)

3. Type of data: __xx___ Events _____ Longitudinal _____ Cross-sectional (Baseline)

4. Genetic Information:

Genetic information is defined as any data from a participant's DNA. Please be advised that the Penultimate Draft of your paper must describe the IRB approval and informed consent process at each site. The number of cases removed from the data set due to a lack of specific consent for the analyses performed must also be stated in the Methods section.

a. Does your proposal contain the use of any genetic data? (please check one)
___ No (go to question 5) _xx__ Yes (see question 4b)

b. Is genetic information used to address a primary aim or secondary aim of the Cardiovascular Health Study? (please check one or both)

_x__ Primary aim (heart and vascular disease) __ _ Secondary aim (other health conditions) (Factor V Leiden will be a covariate.)

5. Location of analysis: _____ Central __x___ Local (Site)

6. Name, address, phone number, and email address of investigator:
Alexandra Cornell
Given Box 96
UVM College of Medicine
Burlington, VT 05405

7. Name, address, phone number, and email address of CHS sponsor, if applicable: M Cushman mcushman@salus.med.uvm.edu

8. Names, justification for inclusion as co-authors, addresses, phone numbers, and email addresses of co-authors, if this paper will not be centrally analyzed:

Dr. M. Cushman (mcushman@salus.med.uvm.edu)
Dr. A. Folsom (folsom@epi.umn.edu)
Dr. M Tsai (tsaix001@maroon.tc.umn.edu)
Dr. T. Zang (tangzh@msx.upmc.edu)

All of the above are justified for inclusion by virtue of being LITE study investigators who have contributed to this study.

9. Key words: venous thrombosis (VTE), factor XIII, fibrinogen

10.Introduction/background:

Venous thrombosis (VTE) occurs in approximately 1 of every 1000 people. Despite this common frequency, the etiology of VTE is still not well understood. Several recent studies have shown that a major risk factor for developing VTE, particularly deep vein thrombosis (DVT), is a mutation in the blood coagulation protein Factor V (FV Leiden or FVL), which causes an increased resistance to an anti-clotting factor, activated protein C (APC) (2, 3). Other risk factors include obesity, immobilization and pregnancy/labor, as well as deficiencies in a number of anti-clotting factors, including APC, protein S and antithrombin III (AT-III) (3).

Recent studies have also suggested that a common polymorphism in Factor XIII (FXIII Val34Leu) has a protective effect in the development of VTE, brain and myocardial infarctions (4-6, 12-14). Factor XIII (FXIII) is a tetrameric (A_2B_2), pro-transglutaminase that, once activated by thrombin, stabilizes fibrin clots by forming fibrin gamma-chain dimers (via an acyl-transfer reaction between Glu and Lys residues on adjacent fibrin monomers) and cross-linking α_2-plasmin inhibitor to the fibrin polymers (9, 11, 12). One hypothesis is that in individuals with the Val34Leu polymorphism, FXIII is activated prematurely by thrombin (i.e. before there is a substantial amount of fibrin present), thereby depleting the pool of available FXIII and resulting in fibrin clots that are more susceptible to fibrinolysis (12).

In addition, recent studies have disagreed on whether a fibrinogen polymorphism, Fgn Hae III or –455 G/A, is also protective against thrombosis (20-26). Fibrinogen is the plasma protein that, during the clotting cascade, is cleaved by thrombin to fibrin. Elevated levels of fibrinogen have been shown to increase the risk of both arterial and venous thrombosis (although this was not associated with venous thrombosis in the LITE study) (26). In the Leiden Thrombophilia Study (LETS), subjects with a fibrinogen level greater than 5 g/L had a 4-fold increase of thrombosis.

The fibrinogen –455 G/A polymorphism is located on the beta chain in the 5' promoter region and has been associated with higher levels of circulating plasma fibrinogen (20, 22, 23, 25). This would suggest that the fibrinogen variant is related to a hypercoagulable state and subsequent ischemic disease, but that association has not been conclusively demonstrated, and there is little information concerning venous thrombosis (21). The largest study of venous thrombosis was the LETS, which showed a protective effect with the A allele (26).

There are several problems in the existing literature, in addition to those mentioned above. First, the prevalence of the Val34Leu and Fgn Hae III polymorphisms and their ethnic heterogeneity has not been adequately examined, although one small study found that the prevalence of the Fgn Hae III polymorphism was lower among African Americans than Whites in the U.S. and Europe (21). The only studies that have investigated the prevalence of Val34Leu in ethnic minorities have

been in Brazil and in a UK Asian population (10, 18). Second, there is not yet agreement on whether the Val34Leu or Fgn Hae III polymorphisms are protective at all, and if so, by what mechanism (9, 11, 12, 20-26). There is also no consensus on whether the Factor XIII or fibrinogen variants are protective when other risk factors are present, such as FVL. Kohler and Grant suggest that increased levels of plasminogen activator inhibitor 1 (PAI-1), among other risk factors, negate the protective effects of the Val34Leu polymorphism (15). However, they also stated that further studies are needed to confirm this. Third, there have been no studies of the Val34Leu polymorphism and thrombosis in the U.S. population. Finally, all of the studies to date of these polymorphisms and venous thrombosis are retrospective case-control studies, with potential bias.

The Longitudinal Investigation of Thromboembolism (LITE) study addresses many of these concerns. LITE is a prospective study employing a nested case-control design. It combines two U.S. cohorts, the Cardiovascular Health Study (CHS) and the Atherosclerosis Risks in Communities (ARIC) study (1, 2). Both population-based studies examined the risk factors and subsequent clinical development of cardiovascular diseases in six communities. Details of both CHS and ARIC have been published previously (16, 17). Briefly, in 1987-89, 15,792 men and women between the ages of 45-64 were enrolled in ARIC, 27% African American. In 1989-90, 5,201 men and women over the age of 65 enrolled in CHS. However, only 3% of the participants were African American, so CHS enrolled another 687 African American participants in 1992-93. Baseline blood samples were taken and stored, including both DNA and plasma samples (2).

The proposed study will determine the prevalence of the FXIII Val34Leu and Fgn Hae III (-455 G/A) polymorphisms in LITE cases and controls. In addition, we will ascertain whether the Val34Leu and/or Fgn Hae III polymorphisms are protective, as well as whether they are protective when other risk factors are present, specifically FVL and obesity.

11. Hypotheses:

1. Val34Leu and Fgn Hae III are protective against VTE.
2. Val34Leu and Fgn Hae III are protective against VTE independent of other factors.
3. Val34Leu and Fgn Hae III are protective against VTE among subjects with and without FVL.
4. Val34Leu and Fgn Hae III are protective among obese and non-obese subjects.
5. Val34Leu and Fgn Hae III are protective to a similar degree among blacks and whites.

12. Analysis plan and methods:

Methods

LITE investigators validated cases of VTE through 31 December 1996 for the ARIC study, and through 30 June 1997 for the CHS (1). Incidence rates, predictive values for specific discharge codes, case characteristics, 28-day case fatality and recurrence rates were determined.

A total of 335 cases of VTE were identified in 304 individuals, using objective criteria in the evaluation of 938 hospitalization discharge codes (542 in ARIC, 396 in CHS) (1). DVT comprised 267 of the cases, while 58 were PE and 41 were concurrent PE and DVT. Approximately 50% of the cases were idiopathic, with cancer (n=91) and recent hospitalization (n=99) being the most common precipitants in secondary events. Controls were randomly chosen from the ARIC and CHS cohorts at a rate of 2.1 controls per case, yielding a total of 688 controls (2). The cases and

controls were frequency matched according to age, gender, race, follow-up time and study (ARIC/CHS). Several plasma and DNA measures have been performed in baseline samples from this case-control population, including FVL and APC resistance.

Data Analysis

Using the nested case control group from the LITE study, the following data analysis will be performed for each gene variant:

1. Describe and compare the characteristics of cases and controls.
2. Compare the prevalence of the polymorphism in both cases and controls, as well as the prevalence in ethnic minorities.
3. Estimate the relative risk of developing VTE in the presence of the polymorphism. (Logistic regression will be used to calculate the odds ratio (OR) and 95% confidence interval.)
4. Examine the subgroups as defined by case type (incident, recurrent; idiopathic, secondary) and parent study membership; FVL status, ethnic group and BMI >30 or <30.

The factor XIII gene polymorphism will be tested comparing Val/Val to Val/Leu and Leu/Leu separately. If appropriate based on the findings, participants with Val/Leu and Leu/Leu will be combined for secondary analyses, including stratifications (although separate analyses of Leu/Leu will be attempted – we may be limited by power). The fibrinogen variant will be analyzed in similar fashion comparing –455 GG to both GA and AA, and combing the heterozygote and homozygote wild type for secondary analyses, if appropriate.

13. Future Directions:

If the analysis confirms our hypotheses, further study of the roles of FXIII Val34Leu or Fgn Hae III status (with or without FVL) might be undertaken to determine the utility of testing in directing the course of treatment for an individual who has VTE.

14. References:

1. Cushman M, Tsai A, Heckbert S, White R, Rosamond WD, Enright P, Folsom AR. Deep vein thrombosis and pulmonary embolism in two cohorts: the Longitudinal Investigation of Thromboembolism Etiology (LITE). [In preparation]
2. Folsom AR, Cushman M, Tsai M, Aleksic N, Heckbert S, Boland L, Tsai A, Yanez ND, Rosamond WD. A prospective study of venous thromboembolism in relation to Factor V Leiden and related factors. [In preparation]
3. van der Meer FJ, Koster T, Vandenbroucke JP, Rosendaal FR. The Leiden Thrombophilia Study (LETS). *Thrombosis & Haemostasis* 1997;78(1):631-5.
4. Catto AJ, Kohler HP, Coore J, Mansfield MW, Stickland MH, Grant PJ. Association of a common polymorphism in the Factor XIII gene with venous thrombosis. *Blood* 1999;93(3):906-8.
5. Franco RF, Reitsma PH, Lourenco D, Maffei FH, Morelli V, Tavella MH, Araujo AG, Piccinato CE, Zago MA. Factor XIII Val34Leu is a genetic factor involved in the etiology of venous thrombosis. *Thrombosis &Haemostasis* 1999;81(5):676-9.

6. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the Factor XIII gene with myocardial infarction. *Thrombosis & Haemostasis* 1998;79(1):8-13.
7. Franco RF, Pazin-Filho A, Tavella MH, Simoes MV, Marin-Neto JA, Zago MA. Factor XIII Val34Leu and the risk of myocardial infarction. *Haematologica* 2000;85(1):67-71.
8. Corral J, Gonzalez-Conejero R, Iniesta JA, Rivera J, Martinez C, Vicente V. The Factor XIII Val34Leu polymorphism in venous and arterial thromboembolism. *Haematologica* 2000;85(3):293-7.
9. Balogh I, Szoke G, Karpati L, Wartiovaara U, Katona E, Komaromi I, Haramura G, Pfliegler G, Mikkola H, Muszbek L. Val34Leu polymorphism of plasma Factor XIII: biochemistry and epidemiology in familial thrombophilia. *Blood* 2000;96(7):2479-2486.
10. Attie-Castro FA, Zago MA, Lavinha J, Elion J, Rodriguez-Delfin L, Guerreiro JF, Franco RF. Ethnic heterogeneity of the Factor XIII Val34Leu polymorphism. *Thrombosis & Haemostasis* 2000;84:601-3.
11. Wartiovaara J, Mikkola H, Szoke G, Haramura G. Karpati L, Balogh I, Lassila R, Muszbek L, Palotie A. Effect of Val34Leu polymorphism on the activation of the coagulation Factor XIII-A. *Thrombosis & Haemostasis* 2000;84:595-600.
12. Trumbo TA, Maurer MC. Examining thrombin hydrolysis of the Factor XIII activation peptide segment leads to a proposal for explaining the cardioprotective effects observed with the Factor XIII V34L mutation. *The Journal of Biological Chemistry* 2000;275(27):20627-20631.
13. Wartiovaara U, Perola M, Mikkola H, Totterman K, Savolainen V, Penttila A, Grant PJ, Tikkanen MJ, Vartiainen E, Karhunen PJ, Peltonen L, Palotie A. Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. *Atherosclerosis* 1999;142:295-300.
14. Elbaz A, Poirier O, Canaple S, Chedru F, Cambien F, Amarenco P. The association between the Val34Leu polymorphism in the factor XIII gene and brain infarction. *Blood* 2000;95(2):586-91.
15. Kohler HP, Grant PJ. Clustering of haemostatic risk factors with FXIII Val34Leu in patients with myocardial infarction. *Thrombosis & Haemostasis* 1998;80:862.
16. The ARIC Investigators. The Atherosclerosis Risks in Communities (ARIC) Study: design and objectives. *American Journal of Epidemiology* 1989;129:687-702.
17. Fried LP, Bohrani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. *Annals of Epidemiology* 1991;1:263-276.
18. Warner D, Mansfield MW, Grant PJ. Coagulation Factor XIII and cardiovascular disease in UK Asian patients undergoing coronary angiography. *Thrombosis & Haemostasis* 2001;85:408-11.
19. Eekhoff EMW, Rosendaal FR, Vendenbroucke JP. Minor events and the risk of deep venous thrombosis. *Thrombosis & Haemostasis* 2000;83:408-11.
20. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000;95(5):1517-1532.
21. Austin H, Hooper WC, Lally C, et al. Venous thrombosis in relation to fibrinogen and factor VII genes among African-Americans. *Journal of Clinical Epidemiology* 2000;53(10):997-1001.

22. Behague I, Poirier O, Nicaud V, et al. β fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. *Circulation* 1996;93:440-449.
23. Tybjaerg-Hansen A, Agerholm-Larsen B, Humphries SE, et al. A common mutation (G_{-455}-A) in the β-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease: a study of 9,127 individuals based on the Copenhagen City Heart Study. *Journal of Clinical Investigation* 1997;99:3034-3049.
24. de Maat MPM, Kastelein JJP, Jukema JW, et al. –455 G/A polymorphism of the β-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men: proposed role for an acute phase reaction pattern of fibrinogen. *Arteriosclerosis Thrombosis Vascular Biology* 1998;18:265-271.
25. Carter AM, Mansfield MW, Stickland MH, Grant PJ. β-fibrinogen gene –455 G/A polymorphism and fibrinogen levels: risk factors for coronary artery disease in subjects with NIDDM. *Diabetes Care* 1996;19:1265-1268.
26. Koster T, Rosendale FR, Reitsma PH, et al. Factor VII and fibrinogen levels as risk factors for venous thrombosis: A case-control study of plasma levels and DNA polymorphisms – The Leiden Thrombophilia Study (LETS). *Thrombosis & Haemostasis* 1994;71:719-22.