Chronic Kidney Disease and the Risk of Venous Thromboembolism

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CHRONIC KIDNEY DISEASE AND THE RISK OF VENOUS THROMBOEMBOLISM

A Dissertation Presented

by

Katharine L Cheung

to

The Faculty of the Graduate College

of

The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
Specializing in Clinical and Translational Science

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ABSTRACT

Chronic kidney disease (CKD) affects more than 30 million adults in the U.S. and is strongly associated with cardiovascular events and mortality. Venous thromboembolism (VTE) is the third leading vascular disease, affects up to 900,000 Americans each year and contributes to as many as 100,000 deaths annually. The relationship of CKD and VTE has been described in patients receiving dialysis, kidney transplants recipients and in nephrotic syndrome, however, data supporting the association of VTE in mild to moderate CKD is conflicted. The overall goal of this research was to study the association of CKD and VTE and to understand the mechanisms of this association. To accomplish this goal we studied participants of the Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study, a nationally representative cohort of 30,239 blacks and whites in the U.S..

The first chapter provides a review of the state-of-the-science on CKD and VTE and potential mechanisms for this association. We focus on factor VIII as a potential mediator of VTE risk in CKD by reviewing the biochemistry and epidemiology linking factor VIII and CKD.

In Chapter 2, we use a cohort study design and a competing risk analysis to determine the risk of VTE with albuminuria (ACR) and with various equations for estimated glomerular filtration rate (eGFR). There was no association of ACR and VTE and the risk of VTE was similar among eGFR equations. Compared to a normal eGFR (>90 ml/min/1.73m²), eGFR < 45 ml/min/1.73m² was associated with a two-fold risk of VTE. The association of eGFR and unprovoked VTE was similar to the association with provoked VTE. The population attributable fraction of CKD (eGFR<60 ml/min/1.73m²) was modest at 5%.

In Chapter 3, we utilize a case-cohort study to determine if biomarkers of inflammation (C-reactive protein) and procoagulation (Factor VIII and D-dimer) attenuate the risk of VTE in CKD. These biomarkers were higher in lower kidney function and were also strongly associated with VTE. Adjustment for factor VIII fully attenuated the risk of VTE in CKD, thus factor VIII is a potential mediator of the association of CKD and VTE. We assessed whether lifestyle factors and medications mitigate the risk of VTE in those with and without CKD. Exercise frequency and use of statins were associated with reduced risk of VTE in the presence and absence of CKD, but normal BMI was associated with reduced VTE risk only in those without CKD.

We conclude that CKD is a risk factor for VTE, and findings shed light on the mechanisms of this association. Interventions that might lower VTE risk in CKD patients include exercise and statin therapy, but not weight loss. Factor VIII is a potential mediator of VTE in CKD and deserves further study. We suggest several avenues for future research to explore the relationship of Factor VIII and CKD.
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DEDICATION

I dedicate this to Christina.

Your belief in me is unquestionably the reason this dissertation is complete.
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First and foremost, I want to thank Dr. Mary Cushman, my research mentor and advisor, for sharing her passion for science, collaboration and advocacy; for being willing to teach a nephrologist about hematology, biochemistry and lab medicine; for warmly welcoming me into her research community, supporting my training and pushing me out of my shell; for her patience and understanding as I found my footing; and for introducing me to other exceptional scientists- Drs. Neil Zakai, Susan Gilchrist, Peter Callas and Nels Olson- who also supported me throughout my training. You have taught me what it means to be a mentor.

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To my friends and family, thank you for your love and understanding as I worked through countless holidays and weekends. I’ve missed you!
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CITATIONS</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>viii</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td><strong>CHAPTER 1: VENOUS THROMBOEMBOLISM, FACTOR VIII AND CHRONIC KIDNEY DISEASE</strong></td>
<td>1</td>
</tr>
<tr>
<td>1.1 Abstract</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Introduction</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Associations of Chronic Kidney Disease with Venous Thromboembolism</td>
<td>6</td>
</tr>
<tr>
<td>1.4 Mechanisms of Venous Thromboembolism in Chronic Kidney Disease</td>
<td>10</td>
</tr>
<tr>
<td>1.5 Biochemistry of Factor VIII</td>
<td>11</td>
</tr>
<tr>
<td>1.6 Association of Factor VIII and Chronic Kidney Disease</td>
<td>19</td>
</tr>
<tr>
<td>1.7 Research Gaps and Future Directions</td>
<td>27</td>
</tr>
<tr>
<td>1.8 Conclusions</td>
<td>28</td>
</tr>
<tr>
<td>1.9 Acknowledgements</td>
<td>28</td>
</tr>
<tr>
<td>References</td>
<td>36</td>
</tr>
<tr>
<td><strong>CHAPTER 2: MEASURES OF KIDNEY DISEASE AND THE ASSOCIATION OF VENOUS THROMBOEMBOLISM IN THE REGARDS STUDY</strong></td>
<td>47</td>
</tr>
<tr>
<td>2.1 Abstract</td>
<td>48</td>
</tr>
<tr>
<td>2.2 Introduction</td>
<td>49</td>
</tr>
<tr>
<td>2.3 Methods</td>
<td>50</td>
</tr>
</tbody>
</table>

vi
# LIST OF TABLES

## CHAPTER 1

Table 1.1: Case-control and cohort studies of Chronic Kidney Disease and Venous Thromboembolism......................................................................................................................... 29

Table 1.2: Genes and SNPs associated with Factor VIII, vWF and Kidney Disease. 30

Table 1.3: Cross-sectional and Cohort studies on Factor VIII and Chronic Kidney Disease................................................................................................................................................ 32

Table 1.4: Distribution of ABO blood group in hemodialysis patients and controls. 33

## CHAPTER 2

Table 2.1: Baseline characteristics by categories of eGFR\textsubscript{Cr-CysC} ................................. 62

Table 2.2: Baseline characteristics by categories of albumin-to-creatinine ratio (ACR) ................................................................................................................................. 63

Table 2.3: Adjusted Incidence Rate (IR) for VTE and mortality per 1000 Person-Years by kidney disease measures ........................................................................................................ 64

Table 2.4: Cause specific hazard ratios (95% CI) for total VTE, provoked and unprovoked VTE and mortality by kidney disease measures ................................................. 65

Table 2.5: Adjusted Cause-Specific Hazard Ratios and 95% Confidence Intervals for VTE and mortality by eGFR\textsubscript{Cr-CysC} stratified by race and region .................................................................. 66

Table 2.6: (Supplemental Table S2.1): Age, sex and race adjusted Incidence rate per 1000 Person-Years and Hazard Ratio (95% CI) of VTE for categories of estimated albumin excretion rate (eAER) ......................................................................................................................... 67

Table 2.7: (Supplemental Table S2.2): Adjusted Cause-Specific HRs (95% CI) for VTE and mortality by ACR stratified by race and region .................................................. 68

Table 2.8: (Supplemental Table S2.3): Incidence Rates Per 1000 PY and Hazard Ratio (95% CI) of VTE for eGFR equations .......................................................................................... 69
CHAPTER 3

Table 3.1: Baseline characteristics of REGARDS case-cohort participants. ........ 91

Table 3.2: Association of eGFR and biomarkers of inflammation and procoagulation in the cohort sample.......................................................... 92

Table 3.3: Association of biomarkers and risk of VTE. ................................. 93

Table 3.4: Association of eGFR and incident VTE with adjustment for biomarkers of procoagulation and inflammation. ........................................ 94

Table 3.5: The association of lifestyle factors and medications with the risk of VTE, stratified by CKD status. ................................................................. 95

Table 3.6: (Supplemental Table S3.1): Exclusion criteria ............................... 96

Table 3.7: (Supplemental Table S3.2): Methodological approaches to studying analytic drift in REGARDS case-cohort study of VTE risk ........................ 97

Table 3.8: (Supplemental Table S3.3): Association of eGFR and VTE at 1, 3, 5 years follow up ......................................................................................... 98
LIST OF FIGURES

CHAPTER 1

Figure 1.1: Potential mechanisms of Venous Thromboembolism and Chronic Kidney Disease……………………………………………………………………………… 34

Figure 1.2: Potential explanations for elevated factor VIII in Chronic Kidney Disease……………………………………………………………………………… 35

CHAPTER 2

Figure 2.1: Flow diagram of participants included in the study. Mutually exclusive counts in parentheses …………………………………………………………… 53

CHAPTER 3

Table 3.1: Potential mediators of the association of eGFR and Venous Thromboembolism: role of biomarkers of inflammation and procoagulation……… 93
CHAPTER 1: VENOUS THROMBOEMBOLISM, FACTOR VIII AND CHRONIC KIDNEY DISEASE

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1.1. Abstract

Chronic kidney disease (CKD) affects 30 million Americans and is associated with approximately a two-fold increased risk of venous thromboembolism (VTE). There is a graded increased risk of VTE across declining estimated glomerular filtration rate (eGFR) and increasing albuminuria, and the risk varies by cause of CKD. Compared to the general population, VTE in end-stage kidney disease (ESKD) is associated with greater hospitalization and higher mortality. Mortality associated with PE in ESKD also varies by cause of ESKD. Transplant patients with VTE are at a greater risk for death and graft loss than transplant patients without VTE. The reasons for VTE in CKD are not well understood but recent data suggest that Factor VIII (FVIII) is a mediator of VTE in CKD. FVIII is an essential cofactor in the coagulation cascade and a strong risk marker for VTE in general. It is inversely correlated with eGFR and prospective studies demonstrate that FVIII activity predicts CKD and rapid eGFR decline. This review summarizes the epidemiology of CKD with VTE, reviews the biochemistry and determinants of FVIII, including von Willebrand factor and ABO blood group, outlines the epidemiology of FVIII and CKD, and suggests future research directions.
1.2. Introduction

Chronic kidney disease (CKD) affects 15% of the adult U.S. population and the prevalence is increasing, in part due to the aging population. The two most common causes of CKD are diabetes and hypertension. CKD can lead to important health consequences including anemia, metabolic acidosis, bone disease, immune dysfunction and lower quality of life. There is also an increased risk of death and cardiovascular events including stroke, coronary disease, heart failure and peripheral arterial disease in CKD. The heightened risk of vascular diseases in CKD is not limited to the arterial circulation. Emerging evidence reveals an association between CKD and venous thromboembolism (VTE).

VTE consists of deep vein thrombosis (DVT) and pulmonary embolism (PE). It is the third leading vascular disease and costs the US healthcare system $7-10 billion each year. VTE affects up to 900,000 Americans annually and contributes to 60-100,000 deaths each year. Approximately 25% of people who have a PE experience sudden death and for VTE the 28-day case fatality is 11%. Among those who experience a DVT, up to one-half will go on to develop chronic venous insufficiency and/or post thrombotic syndrome. Also, VTE recurs in one-third of people within ten years. About half of VTE are provoked by surgery, trauma, cancer or immobilization and half are unprovoked. Given that there is no identifiable provoking factor in half of all VTE cases, research on novel risk factors and mechanisms of VTE is important. The risk of VTE increases with lower kidney function, and VTE carries a higher risk of death in CKD than the general population.
FVIII is an acute phase reactant\textsuperscript{16} that is elevated in stress\textsuperscript{17}, inflammation\textsuperscript{18} and exertion\textsuperscript{19}. Elevated FVIII also represents a procoagulant state and endothelial injury. It is elevated with increased age, BMI, total cholesterol and in diabetes.\textsuperscript{20} FVIII is a strong and independent risk factor for VTE; in the Leiden Thrombophilia Study (LETS) FVIII $\geq$ 150\% was associated with a 5-fold increased risk of VTE compared to FVIII <100\%, after controlling for BMI, diabetes, smoking, ABO blood group and vWF.\textsuperscript{21} FVIII remained a strong risk factor after controlling for fibrinogen, an acute phase reactant, and C-reactive protein, an inflammatory marker that is often elevated in the acute phase.\textsuperscript{22}

The reasons for VTE in CKD are not well understood. The classical pathophysiological explanation for VTE, as attributed to Virchow, implicated the triad of a hypercoagulable state, stasis of blood and vascular injury.\textsuperscript{23,24} Over the past few decades a greater understanding of the pathophysiology of VTE has been described including delineation of prothrombotic genetic factors (Factor V Leiden and prothrombin gene mutation 20210A),\textsuperscript{25} procoagulant and inflammatory pathways\textsuperscript{26} and the influence of obesity.\textsuperscript{27,28} Of these risk factors, procoagulant and inflammatory pathways are candidate mediators for VTE in CKD given the heightened procoagulant and inflammatory state that accompanies CKD. As the increased risk of VTE in CKD has been only recently reported, little research exists studying the mechanisms of this association. The Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study, a case-control study of patients with VTE and controls, identified that the relationship between CKD and VTE could be explained by von Willebrand factor and Factor VIII.\textsuperscript{29} In the Reasons for Geographic And Racial
Differences in Stroke (REGARDS) study, a case-cohort analysis showed that Factor VIII completely accounted for the association between CKD and VTE. In this review we provide an overview of the epidemiology of CKD and VTE. Given the importance of Factor VIII in this relationship, we summarize the literature on CKD and FVIII, identify potential pathogenic pathways involved, discuss limitations of current literature and explore research gaps for future investigations.

Definitions of Chronic Kidney Disease

CKD is defined as a reduced glomerular filtration rate (GFR) or elevated albuminuria that is present for a minimum of three months. Since glomerular filtration rate is not easily measured in practice, estimated GFR (eGFR) is calculated based on equations that include serum creatinine (and or cystatin C), age, sex and race. Less commonly studies cited in this review defined CKD based on a threshold of serum creatinine (or cystatin C). For the purposes of surveillance and in most epidemiological studies, CKD is defined based on one measurement of eGFR or albuminuria. Albuminuria is measured using the urinary albumin-to-creatinine ratio (ACR) and is categorized as <30 mg/g (normal to mildly increased), 30-<300 mg/g (moderately increased) and ≥ 300 mg/g (severely increased). The kidney has many functions beyond that of filtration including production of hormones such as erythropoietin and activated vitamin D, regulation of volume status, tonicity and pH and metabolism and excretion of drugs. A decline in eGFR is approximately paralleled by a decline in other kidney functions and is the most commonly used clinical measure of kidney function.
1.3. Association of Chronic Kidney Disease with Venous Thromboembolism

*Mild to moderate kidney disease and VTE*

There is growing evidence for an association of kidney disease and VTE, although some of the data are conflicting depending on how kidney disease was defined (Table 1.1). The Tromso study, which recruited community-dwelling adults age 25-84 years old from 1994-95, found that higher serum cystatin C was associated with a 2.5-fold increased risk of VTE over 12.5-year median follow-up. The Atherosclerosis Risk in Communities (ARIC) Study studied 10,700 self-identified white and black participants age 45-64 years and used cystatin C-based eGFR. As compared to eGFR ≥90 ml/min/1.73m², the adjusted hazard ratios (HRs) for eGFR 60-89 and 15-59 ml/min/1.73m² were 1.26 and 1.60. Conversely, creatinine-based eGFR was not associated with VTE occurrence in the ARIC study. In the Longitudinal Investigation of Thromboembolism (LITE) study, which combined participants from ARIC and the Cardiovascular Health Study (CHS), creatinine-based eGFR <60 ml/min/1.73m² was associated with an increased risk of VTE in middle age and older Americans. Given the prior conflicting reports, The Reasons for Geographic and Racial Differences in Stroke (REGARDS) study compared the risk of VTE among 30,239 adults age 45 and older across the US, using different eGFR equations, including the combined creatinine-cystatin C based eGFR. This study reported a two-fold increased risk of VTE in participants with creatinine-cystatin C based eGFR <45 compared to eGFR>90 ml/min/1.73m², and a similar risk for the other eGFR equations. In addition, a meta-
analysis of five cohort studies, including LITE and Tromso, confirmed an association of eGFR and VTE. Other types of studies have reported a relationship between eGFR and VTE. The MEGA study was a case-control study that examined risk factors for venous thrombosis. This study recruited consecutive patients from 1999-2004, aged 18-70 years, who experienced a first VTE, and a control group. This study found an increased risk of VTE with eGFR < 60 ml/min/1.73m², and this risk was augmented by additional genetic or acquired risk factors such as Factor V Leiden or immobilization. Interestingly, there was also a J-shaped relationship between eGFR and VTE, where very high eGFR was also associated with an increased risk of VTE. A large nationwide case-cohort study in Denmark of 128,096 patients with a hospital diagnosis of VTE demonstrated that kidney disease was associated with an increased risk of VTE. The risk also varied by etiology of kidney disease. The adjusted OR of VTE for hypertensive nephropathy was 1.41 (95% CI 1.22, 1.63) and for nephrotic syndrome was 2.89 (95% CI 2.26, 3.69). In summary, compared to normal eGFR, the risk of VTE for CKD is approximately 1.26-2.89-fold depending on the definition of eGFR and etiology of CKD.

Albuminuria has also been linked to a higher risk of VTE, even at mildly increased levels. In the Prevention of Renal and Vascular End-stage Disease (PREVEND) study, which was enriched for participants with albuminuria, as compared to minimal albuminuria (ACR < 30 mg/g) the adjusted HR (95% CI) of VTE for ACR >30 mg/g was 2.0 (1.34, 2.98). In contrast, there was no association of albuminuria and VTE in the REGARDS general population study. In the aforementioned meta-analysis, as compared to ACR 5 mg/g, the HR for VTE increased linearly across ACR: 1.34 for
ACR 30mg/g, 1.60 for ACR 300 mg/g and 1.92 for ACR > 1000mg/g. This is consistent with the marked increased risk of VTE in nephrotic syndrome,\textsuperscript{36,37} which presents with proteinuria >3.5 grams/day. The mechanisms promoting VTE in this disease state may be different.

\textit{End-stage kidney disease and VTE}

There is an increased risk of VTE in patients with end-stage kidney disease (ESKD) although it is not clear if the risk is similar or greater than in CKD. In the Nationwide Inpatient Sample from the U.S., the incidence of PE per 100,000 was 66 in those with normal kidney function, 204 in CKD and 527 in ESKD.\textsuperscript{15} Using data from the U.S. Renal Data System and National Hospital Discharge Survey, the age-adjusted PE-related hospitalization incidence rate ratio was 2.11 in dialysis patients compared to the general population.\textsuperscript{38} Similarly, in a study of National Health Insurance claims, the incidence rate for PE in Taiwanese dialysis patients versus age- and sex-matched controls was 0.92 versus 0.33 per 1000 person-years, and the adjusted HR (95% CI) for PE was 2.0 (1.63, 2.50),\textsuperscript{39} which is similar to the increased risk in CKD. It is unknown whether hemodialysis poses a greater risk for VTE than peritoneal dialysis.\textsuperscript{8,39} Importantly, PE in dialysis patients is associated with a high mortality rate. In a study comparing European dialysis patients to the general population, it was reported that the age and sex standardized mortality rate (SMR) for PE was 12 times higher in dialysis versus the general population. This exceeds the SMR for myocardial infarction or stroke, which was 11 and 8 times higher in dialysis patients versus the general population.\textsuperscript{40} The Nationwide
Inpatient Sample study reported in-hospital mortality associated with PE was 50% greater for CKD and 90% greater for ESKD compared to patients with normal kidney function. The cause of ESKD is also related to the risk of mortality associated with PE in dialysis patients, with diabetes posing a greater risk than polycystic kidney disease.

Kidney transplantation and VTE

There is an increased risk of VTE in kidney transplant patients as well and this is related to the time since transplantation. As compared to patients with eGFR>30 ml/min/1.73m², those with eGFR<30 have a 2-fold increased risk of VTE 1.5-3 years after transplantation. The immediate risk of symptomatic VTE within 90 days of transplant was 0.9% in a retrospective study of 441 consecutive kidney transplants from Argentina, similar to the risk associated with relatively low risk surgeries. A Korean study reported a 4% incidence of VTE within 90 days by screening for VTE with duplex ultrasound, however only one patient was symptomatic. An Italian study of 538 kidney transplant patients reported that 9% developed VTE with a median follow-up time of 17 months, and 40% were symptomatic. A larger, retrospective study of healthcare data from Ontario, Canada studied 4,343 kidney transplant patients compared to 17,372 matched controls from the general population. The incidence rate of VTE in kidney transplants was 7-fold greater than the general population but varied based on time since transplant. The incidence rate of VTE was greatest in the first three months post-transplant, and while it declined over time it remained higher than the matched cohort. Similar to patients with ESKD, VTE in kidney transplant patients confers significant
morbidity and mortality. Those who experience a post-transplant VTE had a four-fold higher risk of death and a two-fold increased risk of death-censored graft loss compared to matched recipients without VTE.\textsuperscript{45} Since kidney transplant patients with VTE were more likely to have coronary heart disease, diabetes, peripheral vascular disease and recent cancer than patients without VTE, and given that the median time to death and death-censored graft loss was 1.8 years and 1.4 years, respectively, it seems likely that death and graft loss were related to underlying disease rather than the VTE itself.

1.4. Mechanisms of Venous Thromboembolism in Chronic Kidney Disease

There is a myriad of risk factors for VTE, including older age, obesity, prior VTE, active cancer, immobilization, recent hospitalization, surgery, or trauma, and thrombophilia. Studies documenting associations of biomarkers with VTE risk, reveal the importance of inflammation and procoagulation in the development of VTE. For instance, higher D-dimer,\textsuperscript{46} FVIII\textsuperscript{47} and C-reactive protein\textsuperscript{26} are independently associated with VTE risk. Similar directional changes in these biomarkers are seen in CKD,\textsuperscript{48,49} and could be mediators for VTE in people with CKD. As previously noted, two recent studies implicated FVIII as a potential mediator in the relationship of CKD and VTE.\textsuperscript{29,30} In the MEGA and REGARDS studies, FVIII attenuated 100\% of the relationship of CKD and VTE. In comparison, reduced anticoagulant levels such as protein C, protein S and antithrombin did not explain VTE risk in CKD in the MEGA study, and inflammation had a more minor role than FVIII in the REGARDS study.\textsuperscript{29,30} Other potential mechanisms for VTE in CKD, such as greater platelet activation or decreased fibrinolytic
activity, have not been studied (Figure 1.1.). Given that FVIII appears to mediate the association of CKD and VTE, further study of the relationship between FVIII and CKD is warranted.

Conceptually, there are at least three potential ways in which FVIII and CKD may be related. FVIII may represent a manifestation of kidney disease itself, it may promote kidney disease progression and/or it may be elevated due to the consequences of CKD (Figure 1.2.). To explore this further, the remainder of this review will focus on the biochemistry of FVIII as this may be influenced by complications of CKD, the determinants of FVIII and what is known about shared determinants with CKD, and the epidemiology of FVIII and CKD.

1.5. Biochemistry of Factor VIII

Structure

The FVIII gene is located on the X chromosome and encodes a large precursor protein of 2,351 amino acids.\textsuperscript{50} FVIII is predominantly synthesized in the hepatic sinusoidal cells although the natural cell type that produces FVIII has not been identified and there are no known natural established cell lines that express FVIII.\textsuperscript{51} It is also believed to be synthesized by glomerular endothelial cells based on data locating FVIII mRNA in the kidney.\textsuperscript{52-54} The organizational structure of FVIII consists of six domains: A1-A2-B-A3-C1-C2.\textsuperscript{55} In the Golgi compartment, FVIII is cleaved at two sites in the B domain to produce the heavy (A1-A2-B) and light (A3-C1-C2) chains. It is then secreted as a heterodimer with a molecular weight of approximately 280 kDa. The three A
domains are involved in protein-protein interactions, the B domain is removed by thrombin activation and the two C domains are involved in phospholipid binding. Both chains of this heterodimer contribute to the interaction with lipoprotein receptor-related protein (LRP-1), a cell surface endocytic receptor expressed on a variety of human tissues including monocytes, fibroblasts and smooth muscle cells and is involved in FVIII clearance.\textsuperscript{56} The mature factor has 2,332 amino acids and circulates in plasma at a concentration of 0.7 nM.\textsuperscript{57} FVIII contains one calcium and two copper binding sites, and ion binding interactions may be important for both the structure and function of FVIII. FVIII is heavily glycosylated, which is also important for its structure and function.\textsuperscript{58}

\textit{Regulation by vWF}

After FVIII is secreted by exocytosis and once in the plasma, it is stabilized by non-covalent association with von Willebrand factor (vWF). This increases the half-life of vWF-bound FVIII to about 10 hours\textsuperscript{59} by preventing inactivation by activated protein C, FIXa and FXa.\textsuperscript{60,61} Deficiency of vWF-FVIII binding, as in von Willebrand disease type 2N, results in a secondary FVIII deficiency, due to more efficient clearance from the circulation.

\textit{FVIII activation, inactivation and clearance}

FVIII is activated by thrombin through proteolytic cleavage at three sites. This results in polypeptides A1 (50 kDa), A2 (43 kDa) and A3-C1-C2 (73 kDa). When A3 is cleaved, FVIII dissociates from vWF and a heterotrimeric FVIIIa forms. The A1 subunit
derived from the heavy chain exists in a stable association with the light chain-derived A3-C1-C2, which is weakly associated with the A2 subunit. In the presence of phospholipids and calcium, activated FVIII (FVIIIa) forms a membrane-bound complex with FIXa called the FXase complex. This complex then acts to convert FX to FXa, a process that is amplified 100,000-fold by the presence of FVIIIa. FXa and its cofactor FVa form the prothrombinase complex, which activates prothrombin to thrombin. Thrombin in turn catalyzes the conversion of soluble fibrinogen into fibrin.

FVIII is inactivated by two mechanisms. First, the A2 subunit spontaneously dissociates when FVIII is activated, with a half-life of 2 minutes. Second, FVIIIa undergoes proteolysis by activated protein C at two sites. The mechanisms for FVIII clearance are not yet well characterized but several potential mechanisms have been reported. Free FVIII is removed from circulation primarily in the liver by binding to LDL-receptors, heparan sulphate proteoglycans (HSPGs), asialoglycoprotein receptors and megalin. LRP-1 mediates cellular uptake and degradation of free FVIII through interaction with the C2 and A2 domains, and is generally inhibited when FVIII is bound to vWF with few exceptions. In mouse models, LRP-1-deficient mice have higher plasma FVIII levels, and blocking both LRP and HSPGs results in prolongation of the FVIII half-life. Stabilin-2, expressed on liver endothelial sinusoidal cells, is also involved in vWF dependent endocytosis of FVIII. Both FVIII and vWF are rapidly degraded when they are bound by macrophages. Given the molecular weight of FVIII, it could not be renally cleared by filtration in its intact form. In summary, FVIII is synthesized in endothelial cells primarily in the liver, activated upon endothelial injury,
inactivated spontaneously and by proteolysis and cleared by the reticuloendothelial system in the liver.

Studies to support direct relationships between the complications of CKD and the biochemistry of FVIII are lacking. Nevertheless, CKD may play a role in activation, inactivation or non-renal clearance indirectly by affecting enzymatic reactions, LRP-1 or immune function more generally. For example, metabolic acidosis is a common complication of CKD and the pH of the blood is important for regulating protein function. The dissociation of the A2 subunit of FVIIIa heavy chain is pH dependent in vitro, such that at lower pH there is less dissociation and greater FVIIIa activity. Immune function is impaired in patients with CKD and more specifically, the uremic toxin, P-cresyl sulfate, suppresses macrophage function. Since macrophage activity is responsible for clearance of FVIII, CKD might lead to reduced FVIII clearance through reduced activity of the reticuloendothelial system. The inflammatory cytokines prominent in CKD, including TNF-alpha and IL-6, are also considered the main mediators of inflammation-induced activation of the hemostatic system. Elevated TNF-alpha mediates downregulation of protein C, the anticoagulant that inactivates FVIII. Thus, higher levels of TNF-alpha in CKD may result in less inactivation of FVIII and greater FVIII activity. IL-6 promotes transcription activation of FVIII, and in this way CKD related IL-6 could lead to higher FVIII levels.
Determinants of FVIII blood levels

vWF is an important determinant of plasma FVIII levels. vWF is synthesized in endothelial cells and megakaryocytes as a pre-pro-polypeptide monomer of 2,813 amino acids. Each monomer contains N- and O- glycosylation sites, which are involved in vWF secretion and clearance.\(^8^3\) In endothelial cells, vWF is constitutively secreted mostly as dimers, and is stored as larger multimers in Weibel-Palade bodies. In platelets, vWF is stored in alpha-granules until platelets are activated.\(^8^4\) vWF multimers in the circulation are long filamentous strands that are loosely coiled until activation causes them to unwind and expose sites for platelet adhesion. There are two forms of vWF. High molecular weight vWF functions to promote hemostasis through facilitating platelet-platelet interactions and platelet-subendothelium adhesion, as well as platelet aggregation. Low molecular weight vWF functions to carry and protect FVIII from activated protein C mediated proteolytic degradation or early activation by thrombin. The vWF binding sites are located on the A3 domain of the light chain of FVIII. Interestingly, there is reciprocal regulation; Factor VIII serves as a cofactor to ADAMTS13, a metalloprotease that proteolytically cleaves large vWF multimers.\(^8^5\)

ABO blood group, a genetic trait, is also a determinant of plasma FVIII levels, primarily through ABO blood group effect on vWF. Twin studies indicate that approximately 66\% of the variation in plasma vWF is genetically determined, of which 30\% is explained by ABO group.\(^8^6\) The ABO antigens are carbohydrate molecules expressed on RBC membranes and other cells including epithelium, platelets and vascular endothelium. N-linked sugars on vWF also contain ABO blood group
determinants. Persons with blood group types A, B and AB (the non-O blood types) have higher vWF and FVIII levels. The level of vWF increases across blood types: O<A<B<AB. The level of FVIII also increases accordingly: O<A<AB<B. ABO type appears to affect clearance rather than synthesis or secretion of vWF. The receptor involved in ABO blood type facilitated clearance of vWF has not been identified. It has been suggested that ADAMTS13 may play a role in clearance of vWF since ADAMTS13-mediated proteolysis of vWF is much faster for O-blood group than non-O blood group. This is thought to be due to impaired ADAMTS13 cleavage since the A and B antigens are in close proximity to the ADAMTS13 cleavage site within the A2 domain. An experimental model of ADAMTS13-deficient mice however, failed to support the role of ADAMTS13 in vWF clearance, but mice do not express ABO antigens.

To understand whether FVIII and CKD might share common etiologic factors, we cross-referenced published reports of genetic variants associated with both FVIII and kidney disease. We did not identify any single nucleotide polymorphism (SNP) directly linked to both kidney disease and FVIII or vWF. Rather, we identified common genes important in kidney function or development and related to FVIII or vWF (Table 1.2.). Some of these findings were incongruous. For example, rs4981021 located at STAB-2, which encodes stabilin-2 protein, is associated with higher FVIII and lower vWF. Deficiency of stabilin 1 and 2 in mice causes glomerulofibrosis, albuminuria and early mortality. Other associations were congruous, such as a rs10102164 at SOX17, which is associated with FVIII and vWF. SOX17 is expressed throughout the urogenital tract and
mutations of which are associated with congenital anomalies of the kidney and urinary tract. Different SNPs at SYNGR1 are associated with lupus, lupus nephritis and higher vWF and FVIII. SYNGR1 encodes synaptogyrin1, which is an integral membrane protein involved in vesicle trafficking and exocytosis. Genetic risk variants for kidney disease may influence proinflammatory pathways that affect FVIII. For instance, APOL-1 genetic variants, which have a prevalence of 13% among African-Americans, are associated with a five-fold higher risk of progressive kidney disease.92,93 APOL1 encodes apolipoprotein 1 in macrophages, endothelial cells and epithelial cells and its expression is induced by inflammatory cytokines. In the ARIC study, the relationship of FVIII and ESKD varied by different APOL-1 risk variants. The HR (95% CI) of ESKD per IQR change in FVIII was 2.48 (1.39, 4.41) for African-Americans with two APOL-1 risk variants, 1.19 (0.92, 1.53) for African Americans with one risk variant or none, and 1.07 (0.83, 1.38) for European-Americans.94 The authors suggest that vascular injury might be a common determinant that leads to inflammatory cytokines, which promotes higher factor FVIII and APOL1 expression. rs4845625 at IL6R was associated with CKD in Japanese, and is also associated with biomarkers of inflammation including IL6, TNF-alpha and CRP, however FVIII and vWF were not tested.

**Measurement of FVIII**

As we describe the relationship of FVIII and CKD, a review of the means of measurement of FVIII is warranted. FVIII can be measured by quantifying the amount of antigen present or by determining its procoagulant activity. The most common way of
measuring FVIII is the one-stage activity assay based on the activated partial thromboplastin time (aPTT). In this assay, the length of time it takes for a patient’s plasma to correct the clotting time of plasma known to be deficient in FVIII is measured. This is compared to a reference plasma, with a value of 100% FVIII activity. The patient’s FVIII activity is expressed as a percent of normal activity. The normal range in healthy adults is 50-150%. This one-stage activity assay is used clinically to detect factor deficiencies so the ability to detect low levels of FVIII are critical. However, given that our interest in distinguishing higher levels of FVIII that might be associated with increased thrombotic risk, the choice of an assay should address the ability to detect modestly elevated values. Different methods were compared using samples with high FVIII. The one-stage activity assay, the chromogenic activity assays and immunoassays had similar intra-assay precision (CV 6-8%). Inter-assay precision was best with the chromogenic assay (CV 13%) compared to the one-stage activity assay (CV 21.9%) and the ELISA (CV 19.3%). The one-stage assay was affected by lepirudin, lupus inhibitors, low-molecular weight heparin and unfractionated heparin; while the chromogenic assay and immunoassay were unaffected by these factors. The majority of studies relating FVIII and CKD measure FVIII activity. Of note, Factor VIII measured by one-stage activity assay was highly correlated with FVIII measured by a two-stage ELISA, r= 0.87. Biological variation of FVIII has been examined in short and long-terms studies. Within individual variation was 4.8-11.4% (for repeated measures within a week) and 15.8% (for repeated measures monthly across a year). There was no
apparent difference in the mean values for FVIII activity throughout the day to suggest that there is a circadian pattern to FVIII.\textsuperscript{98}

1.6. Association of Factor VIII and Chronic Kidney Disease

*Epidemiological studies of FVIII, vWF and CKD*

Several cohort studies support an association of FVIII and kidney disease (Table 1.3). In CHS, renal insufficiency, which was defined as a serum creatinine \(\geq 1.3\) mg/dL in women and \(\geq 1.5\) mg/dL in men, was associated with higher mean (± SEM) FVIII (145 ± 2\%) compared to those without renal insufficiency (131 ± 2\%), among 5,888 older adults.\textsuperscript{49} Using a more contemporary measure of kidney function, the Multiethnic Study of Atherosclerosis (MESA) studied 6,751 self-identified Caucasian, Chinese, African-American and Hispanic participants who were free of cardiovascular disease at the time of study enrollment and had relatively preserved kidney function (mean eGFR 79ml/min/1.73m\(^2\)).\textsuperscript{100} Factor VIII was inversely associated with eGFR; for every 10 ml/min/1.73m\(^2\) decrement in eGFR, FVIII was 2\% (95\% CI 1, 3) higher and vWF was 3\% (95\% CI 1, 5) higher. The British Regional Heart Study included 4,029 men age 40-59 recruited from 1998-2000 with eGFR \(\geq 70\) ml/min/1.73m\(^2\).\textsuperscript{101} FVIII increased across eGFR categories; median (IQR) FVIII was 131 (130, 133)\% for eGFR \(\geq 70\), 132 (130, 133)\% for eGFR 60-69, and 135 (133, 138)\% for <60 ml/min/1.73m\(^2\), p-trend <0.001. Two prospective cohort studies reported an association of baseline FVIII and CKD risk. The ARIC study of 14,854 participants, demonstrated higher vWF and FVIII in participants with CKD compared to those without CKD.\textsuperscript{102} In this study, the HR (95\%
CI) for incident CKD over a mean follow-up time of 14.5 years in the highest versus the lowest quartile was 1.46 (1.26, 1.68) for vWF and 1.39 (1.20, 1.60) for FVIII. In MESA, FVIII was also associated with rapid kidney function decline (defined as eGFR decline >3 ml/min/year); the OR (95% CI) of rapid decline was 1.11 (1.03, 1.18) per standard deviation (SD) higher FVIII. In summary, across a wide spectrum of ages above 40 years, mild to moderate kidney disease was associated with higher levels of FVIII, and higher FVIII was also linked to progression of CKD.

Studies of FVIII, vWF and end-stage kidney disease

The association of FVIII and kidney disease has also been reported in ESKD and in patients receiving maintenance dialysis, albeit in small studies and without adjustment for covariates. Among 21 patients with kidney failure and 42 with kidney failure on established hemodialysis, FVIII and vWF were 2-3-fold higher than normal adult controls. Mean (± SD) FVIII was 233 ± 123% in kidney failure, 218 ± 10% in dialysis and 79 ± 27% in controls and vWF was 247 ± 154% in kidney failure, 265 ± 159% in dialysis and 94 ± 31% in controls. In an early study that compared FVIII in 38 patients with kidney failure, 19 hospitalized patients with normal kidney function and 17 normal subjects, FVIII was elevated in both patient groups, and mean FVIII was significantly higher in those receiving dialysis versus non-dialytic (conservative) treatment. Not all reports agreed with these findings; in a study of 57 patients with kidney failure, 49 patients with normal kidney function and 8 with impaired kidney function, the authors failed to find a correlation between vWF and FVIII and kidney function. Given the
individual variability in FVIII, a comparison of FVIII in the same patients before and after dialysis might clarify if commencing maintenance dialysis is associated with lower FVIII. In 16 patients with chronic kidney failure who later started dialysis, mean (± SEM) FVIII was *significantly lower* after being established on dialysis: FVIII was 279 ± 36% in chronic kidney failure versus 218 ± 28% in established dialysis, p <0.05. Mean vWF was not significantly different.\textsuperscript{104} A more recent study reported FVIII and vWF were elevated prior to starting dialysis and by six months mean FVIII and vWF were greatly reduced.\textsuperscript{107} The hemodialysis procedure may affect FVIII differently than vWF. In ten patients studied immediately before and after receiving dialysis, FVIII increased in some and decreased in others, while vWF levels were increased following dialysis.\textsuperscript{103} vWF also increases with duration of the dialysis procedure.\textsuperscript{104} As endothelial cells release both vWF and FVIII, whereas platelets release vWF but not FVIII, increased vWF in dialysis may be due to platelet activation as the blood comes in contact with the extracorporeal circuit in hemodialysis. There are fewer studies in peritoneal dialysis, but they support a similar pattern. Among 14 patients receiving continuous ambulatory peritoneal dialysis Factor VIII and vWF were significantly greater (301 ± 122% and 372 ± 112%, respectively) than in ten normal controls (101 ± 11%, and 99 ± 7%, respectively).\textsuperscript{108}

Small studies of kidney transplant recipients indicate that FVIII\textsuperscript{109} and vWF levels are elevated in the early post-transplant period.\textsuperscript{103} As in the studies of FVIII and vWF in dialysis patients, there was no adjustment for covariates. In a study of 17 consecutive deceased donor kidney transplant recipients on cyclosporine immunosuppression, pre-transplant FVIII activity was 224%, FVIII antigen was 248% and vWF was 206%. These
measures increased transiently to 360% for FVIII activity, 398% for FVIII antigen and 362% for vWF. By four months post-transplant they returned to pre-transplant levels.\textsuperscript{110} Similar findings were reported in eight kidney transplant patients before and 4 weeks post transplantation.\textsuperscript{109} Another small study of 11 patients with functioning kidney transplants, the reported mean FVIII (± SD) was 179 ±100 and vWF (±SD) was 136 ±64 six months to 6 years post-transplant. These values were similar to FVIII and vWF in hemodialysis patients.\textsuperscript{103} Despite presumed restoration of kidney function, FVIII levels are not returned to a normal range, perhaps due to immunosuppressive agents related prothrombotic effects or endothelial damage.

\textit{Epidemiology of FVIII, vWF and albuminuria or proteinuria}

Albuminuria, which is a marker of endothelial dysfunction\textsuperscript{111} as well as kidney disease, is associated with elevated levels of FVIII and vWF. In an early study of 57 patients with diverse forms of kidney disease, vWF and FVIII were positively correlated with magnitude of proteinuria.\textsuperscript{106} These findings were confirmed in two other studies,\textsuperscript{112,113} and this relationship between higher ACR and higher vWF levels, vWF activity and FVIII persisted after adjustment for risk factors.\textsuperscript{113} Among type 1 diabetics, vWF was elevated in those with albuminuria (ACR ≥ 30 mg/g) and/or reduced GFR (eGFR<60 ml/min/1.73m$^2$) as compared to those without albuminuria.\textsuperscript{114} For every 88.5 mg/g increase in UACR, the vWF and FVIII levels were 8.3% and 6.3% higher. In nephrotic syndrome where proteinuria exceeds 3.5 grams per day, vWF was much higher (287 ± 26%) than in controls (99 ± 5%), and this was independent of presence of chronic
renal failure. The relationship between FVIII and albuminuria may differ by race/ethnicity. In a study of 1,051 self-identified, African-Americans and 894 non-Hispanic whites with hypertension, albuminuria was associated with higher FVIII and vWF in African-Americans, but not in non-Hispanic whites.

**FVIII and vWF in different etiologies of CKD**

If there were distinct patterns of FVIII in different etiologies of kidney disease, independent of kidney function, this might indicate that FVIII elevation in kidney disease represents the underlying pathology of the kidney disease. There are only a few studies that address the relationship of kidney disease etiology and FVIII or vWF. The studies are limited by small sample size and lack of control for eGFR, albuminuria and other important covariates. In one study of 105 patients, vWF was higher in minimal change disease compared to membranous nephropathy or focal segmental glomerulonephritis among primary renal disorders, and vWF was higher in lupus and amyloid compared to Alport’s syndrome or mixed cryoglobulinemia among secondary causes of glomerulonephritis. A second study confirmed that vWF was higher in nephrotic syndrome, and noted it was also higher in lupus nephritis and hypertensive nephropathy compared to diabetic nephropathy and chronic glomerulonephritis, but a third study reported vWF was higher in lupus nephritis compared to nephrotic syndrome. Among non-insulin dependent diabetic patients, there is a heterogenous relationship of vWF to albuminuria, which is dependent on the kidney pathology. Patients with typical diabetic nephropathy changes had higher levels of vWF compared to those with more
atypical biopsy findings, such as advanced tubulointerstitial fibrosis. This study supports the concept that the underlying kidney pathology is related to vWF. The data exploring the etiology of kidney disease in relation to FVIII in dialysis patients is conflicting. In one study, Factor VIII levels did not differ between non-diabetic and diabetic hemodialysis patients, although FVIII activity was twice that of healthy controls. In a more recent study of 37 CAPD patients, mean (± SE) FVIII and vWF were significantly higher in diabetics (195 ± 4% and 169 ± 5%) compared to non-diabetics (159 ± 5% and 124 ± 9%). Larger studies are needed to determine whether kidney disease etiology influences FVIII.

**ADAMTS13 and CKD**

There is growing evidence to support a relationship between ADAMTS13 and CKD etiology as well as kidney function. Among type 1 diabetics, kidney function was positively correlated with ADAMTS13 antigen and activity; ADAMTS13 activity was 95 ± 16% in controls, 104 ± 20% in mild CKD, and 108 ± 19% in severe CKD. ADAMTS13 levels are also reduced in dialysis patients compared to controls; median (IQR) ADAMTS13 was 279 (238-329) ng/mL in HD patients and was 578 (486-690) ng/mL in controls. vWF levels are elevated and ADAMTS13 levels are reduced in a variety of etiologies of CKD including lupus nephritis, nephrotic syndrome, diabetic nephropathy and chronic glomerulonephritis compared to normal controls. In acquired or genetic deficiency of ADAMTS13, ultra-large multimers of vWF accumulate resulting in platelet aggregation and microthrombi. This clinical syndrome known as
thrombotic thrombocytopenia purpura (TTP) can be life-threatening and not uncommonly causes acute kidney failure. It is not known outside of TTP if lower levels of ADAMTS13 cause CKD, although one study reported that higher vWF:ADAMTS13 ratio is associated with a decline in kidney function.\textsuperscript{125}

\textit{Prospective studies of ABO, FVIII and CKD}

There is growing evidence linking ABO blood group to cardiovascular diseases. For instance, in a US study the risk of stroke was increased in blood type AB, independent of FVIII and typical stroke risk factors.\textsuperscript{126} In the same cohort, blood type AB was also associated with incident cognitive impairment, with only part of the association attenuated by FVIII.\textsuperscript{127} There is considerable data linking non-O blood groups and risk of coronary artery disease\textsuperscript{128} and VTE.\textsuperscript{129-132} Non-O blood type is associated not only with higher levels of vWF and FVIII but also several inflammatory cytokines that are related also to VTE risk, including sICAM-1,\textsuperscript{133} IL6, TNF-alpha, e-selectin\textsuperscript{134,135} and p-selectin.\textsuperscript{133,135} Thus ABO blood type may have an effect on VTE independent of vWF and FVIII.

The literature on ABO blood type as it relates to CKD is scarce. A few small studies compare the prevalence of ABO blood groups in CKD and ESKD using healthy donors as a comparison group (Table 1.4.). None of the published reports account for FVIII. In the US, veteran patients with CKD had blood group B more frequently and blood group O less frequently than healthy controls.\textsuperscript{136} In a German study of different etiologies of CKD, Blood group A was more prevalent among females with
pyelonephritis, than blood type O, but there were no differences among males. A cross-sectional study of 195 Brazilian hemodialysis patients and 80 healthy subjects, demonstrated no significant differences in ABO blood group distribution between patients and controls. In 231 Syrian hemodialysis patients, Blood group B was more common and A was less common in dialysis patients than blood donors. The sum of the literature suggests that B and AB are more common and O and A are less common in dialysis patients, but larger prospective studies are necessary to confirm the possible relationship between ABO blood group and kidney disease.

**Hemophilia and kidney function**

Several lines of evidence report that hemophilia might be protective against CKD. Hemophilia A is an X-linked disorder caused by mutations in the FVIII gene and results in low or absent FVIII. Given that higher FVIII is related to kidney disease, lower FVIII might be associated with less kidney disease. Two cross-sectional studies showed that people with severe hemophilia had higher kidney function than those with mild or moderate hemophilia. In a study of 532 hemophiliacs, the reported prevalence of CKD was 5.5%, which is less than the prevalence of CKD in the general US population. The study sample had a median age 52 (range 40-98), but there was no comparison group and CKD prevalence rates were not reported separately for Hemophilia A and B. A third study of 3,422 hospitalized hemophilia patients (79% Hemophilia A) found that CKD was a discharge diagnosis in just 31 patients. There was no association between CKD and severity of hemophilia; the OR of CKD with severe versus mild/moderate
hemophilia was 0.7 (95% CI 0.3-1.7), after multivariable adjustment. While these studies suggest that hemophilia may be associated with better kidney function, they are not conclusive.

1.7. Research gaps and future directions

While there is data to support a relationship of CKD and FVIII several questions remain. First, is FVIII elevated due to consequences of CKD or does FVIII cause CKD? Studies that describe this association are for the most part limited to a single measurement of FVIII and kidney function, which limits our understanding of how FVIII might influence kidney function and vice-versa. Second, are different etiologies of CKD associated with different levels of FVIII? Studies addressing this issue are small, present conflicting results and do not control for important covariates or kidney function. Identifying the etiology of CKD is not straightforward, however, as biopsies are not performed routinely and are not always conclusive. Accumulating sufficient biopsy-proven cases to compare FVIII levels across a range of CKD etiologies would be difficult. Third, do CKD and FVIII share similar genetic predisposition, including but not limited to the potential impact of ABO blood type? Finally, as FVIII appears to mediate the association of CKD and VTE, could efforts to lower FVIII reduce the risk of VTE? Future research should aim to characterize the mechanisms of elevated FVIII activity in CKD and assess the directionality of the relationship of FVIII and CKD, including evaluation of potential confounders and common predictors. For instance, ABO blood type might lead to CKD in addition to its impact on FVIII levels, but this has not
yet been studied prospectively. A focus on macrophage clearance of FVIII in CKD versus non-CKD might help elucidate if immune function is critical to this relationship. Investigations on the impact of specific uremic toxins, such as P-cresyl sulfate, on coagulation and inflammatory pathways, including effect on proteins critical for coagulation, are currently lacking.

1.8. Conclusions

CKD is an emerging risk factor for venous thromboembolism. The mechanisms of the association of kidney disease and VTE are understudied, but new evidence supports FVIII as a mediator. FVIII activity is inversely correlated with kidney function from mild to severe kidney disease, and remains high in dialysis and kidney transplantation. vWF and ABO blood group are recognized determinants of FVIII, and are also linked to CKD. A greater understanding of the pathophysiological mechanisms for higher FVIII in CKD is necessary, and could lead to discoveries that would improve FVIII levels and reduce VTE in CKD.

1.8. Acknowledgments

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<table>
<thead>
<tr>
<th>Country, Recruitment period</th>
<th>Study design, name</th>
<th>N participants</th>
<th>Patient characteristics</th>
<th>Measure of kidney disease</th>
<th>Findings HR or OR (95% CI)</th>
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<tr>
<td>Netherlands 1994-1995</td>
<td>Cohort, Tromso</td>
<td>3,251</td>
<td>Age 25-84</td>
<td>Cystatin C</td>
<td>HR 2.5 (1.27, 4.96) for top vs bottom quartile of cystatin C</td>
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<td>USA 1987-1989</td>
<td>Cohort, ARIC</td>
<td>10,700</td>
<td>Age 53-75; White (n=8,317) and African-American (n=2353)</td>
<td>Cystatin C based eGFR</td>
<td>HR 1.40 for eGFR 60-89, 1.94 for eGFR 15-59 compared to eGFR &gt;90 ml/min/1.73m². No association of creatinine based eGFR and VTE</td>
</tr>
<tr>
<td>USA</td>
<td>Cohort, LITE</td>
<td>19,073</td>
<td>Mean age 59 yrs; 75% white</td>
<td>Creatinine-based Cystatin C</td>
<td>HRs 1.29 (1.02, 1.62) for eGFR 60-89 and 1.71 (1.18, 2.49) for eGFR 15-59 compared to eGFR≥90 ml/min/1.73m². No association of cystatin C and VTE</td>
</tr>
<tr>
<td>USA 2003-2007</td>
<td>Cohort, REGARDS</td>
<td>30,239</td>
<td>Age 45 and older; 46% male; 60% white, 40% black</td>
<td>Creatinine-cystatin C based ACR</td>
<td>HRs 1.28 (0.94, 1.76) for eGFR 60-90, 1.30 (0.77, 2.18) for eGFR 45-60 and 2.13 (1.21, 3.76) for eGFR &lt;45, compared to eGFR ≥90 ml/min/1.73m². No association of ACR w VTE</td>
</tr>
<tr>
<td>Netherlands 1999-2004</td>
<td>Case-control, MEGA</td>
<td>2936 controls, 2473 cases</td>
<td>Age 18-70; cases 46% male</td>
<td>Creatinine-based eGFR</td>
<td>OR 1.2 (1.0, 1.4) for eGFR 60-90, 2.2 (1.5, 3.0) for eGFR &lt;90 ml/min/1.73m²</td>
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<tr>
<td>Denmark 1980-2010</td>
<td>Case-control</td>
<td>128,096 cases, 624,426 controls</td>
<td>Hospitalized patients; cases 47% male</td>
<td>Creatinine-based eGFR (MDRD)</td>
<td>OR 1.27 (1.10, 1.47) for hypertensive nephropathy and 2.17 (1.68, 2.80) for nephrotic syndrome compared to healthy controls</td>
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<tr>
<td>Europe &amp; USA</td>
<td>Meta-analysis, Tromso, ARIC, CHS, HUNT2, PREVEND</td>
<td>1,178 events, 95,154 participants</td>
<td>97% white, 47% male across 5 studies</td>
<td>Creatinine-based eGFR</td>
<td>HRs 1.29 (1.04, 1.59) for eGFR 75), 1.31 (1.00, 1.71) for eGFR 60, 1.82 (1.27, 2.60) for eGFR 45, 1.95 (1.26, 3.01) for eGFR 30, compared to eGFR 100 ml/min/1.73m². HRs were 1.34 (1.04, 1.72) for ACR 30, 1.60 (1.08, 2.36) for ACR 300 and 1.92 (1.19, 3.09) for ACR 1000 mg/g, compared to ACR 5.0 mg/g.</td>
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<tr>
<td>Netherlands</td>
<td>Cohort, PREVEND</td>
<td>40,856</td>
<td>99% white; 50% male</td>
<td>ACR</td>
<td>HR 2.0 for ACR 30-300mg/g compared to ACR &lt;30 mg/g</td>
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Table 1.2.: Genes and SNPs associated with Factor VIII and vWF and Kidney Disease

| Gene     | Protein function                                                                                                                                                                                                 | Association with FVIII or vWF                                                                                   | Association with Kidney disease                                                                                     |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| APOL1    | Apolipoprotein L1 is a minor apoprotein of HDL with a role in innate immunity (trypanolytic factor) and possibly the inflammatory response.                                                                                                                                  | FVIII is associated with ESKD and APOL1 risk alleles modify this association. If both APOL1 risk alleles are present, FVIII is associated with two-fold risk of ESKD, but is only one risk allele or none are present FVIII is not associated with ESKD.94 | APOL1 risk alleles (rs73885319 and rs60910145 are referred to as G1 and in perfect linkage disequilibrium; rs71785313 is referred to as G2) are associated with seven-fold increased risk ESKD in blacks G1 p=1.1 x 10^{-39} and G2 p=8.8 x 10^{-18} if both risk alleles are present compared to none.93 |
| SOX17    | SOX17 is a transcription factor essential for endothelial cell morphogenesis, necessary for function of stable endothelial cells, expressed throughout urogenital tract and regulates beta-catenin signaling. SOX17 is also a potent Wnt-signaling antagonist.143 | rs10102164 is associated with FVIII p=2.4 x 10^{-9} and vWF p=2.9 x 10^{-3}.144 It is unknown how SOX17 might regulate FVIII or vWF.                                                                                      | SOX17 mutations are associated with duplication of urinary tract, vesico-ureteric reflux, is associated with congenital anomalies of kidney and urinary tract (CAKUT).143 |
| TMEM171  | Transmembrane protein that is associated with urate levels.                                                                                                                                                                                                                 | rs548630 is associated with vWF p=1.2 x 10^{-10} and FVIII p=2.1 x 10^{-10}.145 TMEM171 in regulating vWF and FVIII is unknown, but it is associated with higher basal vWF and secretion of vWF under stimulus. | Rs17632159 is associated with serum urate levels p=3.5 x 10^{-11} and gout OR 0.91 p=6 x 10^{-3}.146 rs17632159 is associated with systolic and diastolic BP.147 Frequency A1 0.31. effect for serum urate - 0.039.                                      |
| STAB-2   | Stabilin 2 protein, large transmembrane receptor protein with potential functions in angiogenesis, receptor scavenging, cell adhesion, lymphocyte homing, endocytosis of AGE modified proteins.                                                                                                                   | rs4981021 is associated with FVIII, p=3 x 10^{-20} and vWF p=6 x 10^{-41}.145 rs4981021 serves as proxy for rs12229292 (r^2 = 0.879) and is associated with higher FVIII and lower vWF.144 | Deficiency of Stabilin 1 and 2 in mice causes glomerulofibrosis, albuminuria and early mortality.148 No GWAS linking specific SNPs to kidney disease.                      |
| SYNGRI1  | Synaptogyrin1 integral membrane protein associated with presynaptic vesicles in neuronal cells and may have a role in vesicle trafficking, synaptic machinery and exocytosis.                                                                                                               | rs5750823 is associated with FVIII p=1.5 x 10^{-14} and vWF p=6 x 10^{-14}.145 SYNGRI1 is associated with higher basal vWF.                                                                                           | rs61616683 is associated with lupus in East Asians P\textsubscript{meta} 5.73 x 10^{-10} and lupus nephritis in Chinese159,150                                                                                     |
| Interleukin 6 receptor (IL6R) | IL6 promotes transcription of FVIII and regulates inflammation                                                                                                                                                                                                                 | rs 4845625 (in full linkage disequilibrium with rs6689393) and other variants in IL6R are associated with biomarkers of inflammation including C-reactive protein, fibrinogen, soluble IL6R, IL6, IL8, TNF- | rs4845625 identified as candidate gene given association of CAD in GWAS in whites, was significantly related to CKD in cross-sectional study of 2247 Japanese (1,588 of whom had CKD defined as eGFR<60 ml/min/1.73m2).152 |
Rab GTPases regulate intracellular membrane traffic.\textsuperscript{153} Rab27A is recruited to weibel-palade bodies, where vWF is stored in endothelial cells. Rab27a and its effector slp4a regulate apical transport of Rab27 bearing vesicles in polarized renal epithelial cells and control cell size.\textsuperscript{154} Rab27a regulates vWF release from endothelial cells. STXBP5 promotes weibel-palade body exocytosis by interaction with Rab27a effector slp4a.\textsuperscript{155} Silencing STXBP5 impairs vWF secretion. rs9390459 (near STXBP5) is associated with higher vWF and FVIII.\textsuperscript{144} Rs1528472 (near RAB27A) is associated with proteinuria in adults, p=5 x 10^{-7}.\textsuperscript{156} Rs17830558, PLA2R1, OR = 2.2, P = 1.9 x 10^{-8}.\textsuperscript{158} The gene encoding PLA2R is expressed in podocytes and is the target of auto-antibodies responsible for \textasciitilde70\% idiopathic membranous nephropathy, a type of nephrotic syndrome.\textsuperscript{159}

<p>| <strong>RAB27A</strong> | Rab GTPases regulate intracellular membrane traffic.\textsuperscript{153} Rab27A is recruited to weibel-palade bodies, where vWF is stored in endothelial cells. Rab27a and its effector slp4a regulate apical transport of Rab27 bearing vesicles in polarized renal epithelial cells and control cell size.\textsuperscript{154} Rab27a regulates vWF release from endothelial cells. STXBP5 promotes weibel-palade body exocytosis by interaction with Rab27a effector slp4a.\textsuperscript{155} Silencing STXBP5 impairs vWF secretion. rs9390459 (near STXBP5) is associated with higher vWF and FVIII.\textsuperscript{144} Rs1528472 (near RAB27A) is associated with proteinuria in adults, p=5 x 10^{-7}.\textsuperscript{156} |
| <strong>STXBP5</strong> | |
| <strong>PLA2R1</strong> | PLA2R is a type 1 transmembrane receptor that binds and removes secreted PLA2 enzyme from circulation. PLA2 is proinflammatory. sPLA2-IIA correlates with FVIII and vWF\textsuperscript{157} but there are no identified PLA2R1 SNPs associated with FVIII and vWF. |
| | rs17830558, PLA2R1, OR = 2.2, P = 1.9 x 10^{-8}.\textsuperscript{158} The gene encoding PLA2R is expressed in podocytes and is the target of auto-antibodies responsible for \textasciitilde70% idiopathic membranous nephropathy, a type of nephrotic syndrome.\textsuperscript{159} |</p>
<table>
<thead>
<tr>
<th>Study year, country</th>
<th>Study design</th>
<th>N patients</th>
<th>Biomarker</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003, US</td>
<td>Cohort study, cross-sectional analysis</td>
<td>5,888 older adults</td>
<td>FVIII activity</td>
<td>FVIII was higher in CKD adjusting for age, sex, race, education, DM, HTN, SBP, DBP, triglycerides, smoking, alcohol, physical activity, BMI, CVD.</td>
</tr>
<tr>
<td>CHS&lt;sup&gt;29&lt;/sup&gt;</td>
<td></td>
<td>CKD defined as SCr &gt;-1.3 mg/dL in women, ≥1.5 mg/dL men</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006, UK</td>
<td>Cohort study, cross-sectional analysis</td>
<td>4,029 adults age 60-79</td>
<td>FVIII activity, vWF Antigen</td>
<td>FVIII and vWF were higher across categories of eGFR &gt;=70, 60-70, &lt;60, adjusting for age, DM, HTN, CVD, SBP, HDL, BMI, LVH, alcohol, smoking, physical activity.</td>
</tr>
<tr>
<td>Cohort, British Regional Heart Study&lt;sup&gt;101&lt;/sup&gt;</td>
<td></td>
<td>14,854 middle age adults from 4 different US communities</td>
<td></td>
<td>FVIII and vWF were associated with incident CKD, adjusting for age, sex, race, DM, SBP, antihypertensive use, CVD, smoking, alcohol use, TG, HDL, LDL, baseline eGFR. FVIII more strongly associated with CKD among those with DM vs without DM (p ixn &lt;0.01).</td>
</tr>
<tr>
<td>2009, US</td>
<td>Cohort study, prospective analysis</td>
<td>6,751 adults free of CVD at entry</td>
<td>FVIII activity, vWF Antigen</td>
<td>FVIII and vWF were higher in lower eGFR, adjusting for age, sex, race, BMI, DM, HTN, statin use, ACE-I, LDL, HDL, TG, fasting glucose and ln(albumin/creatinine).</td>
</tr>
<tr>
<td>Cohort, ARIC&lt;sup&gt;102&lt;/sup&gt;</td>
<td></td>
<td>Reference group: eGFR cystatin C &gt;90 ml/min/1.73m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2011, US</td>
<td>Cohort study, cross-sectional analysis</td>
<td>4,966 adults free of CVD at entry</td>
<td>FVIII activity</td>
<td>FVIII was associated with renal decline, and rapid decline, adjusting for age, sex, race, DM, HTN, HDL, LDL and albuminuria.</td>
</tr>
<tr>
<td>Cohort, MESA&lt;sup&gt;100&lt;/sup&gt;</td>
<td></td>
<td>Reference group: eGFR &gt;86 (50&lt;sup&gt;th&lt;/sup&gt; percentile)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2012, US</td>
<td>Cohort study, prospective analysis</td>
<td>2,936 controls. Reference group: eGFR &gt;86 (50&lt;sup&gt;th&lt;/sup&gt; percentile)</td>
<td>FVIII activity, vWF antigen</td>
<td>FVIII, vWF were higher in eGFR&lt;1&lt;sup&gt;st&lt;/sup&gt; percentile (eGFR &lt;53) vs ref. FVIII and vWF were higher with lower eGFR, adjusting for age and sex.</td>
</tr>
<tr>
<td>Cohort, MESA&lt;sup&gt;101&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2014, Netherlands</td>
<td>Case-control, cross-sectional analysis</td>
<td>949 cohort random sample</td>
<td>FVIII antigen</td>
<td>FVIII was higher in lower eGFR, adjusting for age, sex, race, BMI, DM, HTN, CVD, hyperlipidemia, and smoking.</td>
</tr>
<tr>
<td>MEGA study&lt;sup&gt;29&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2018, US</td>
<td>Case-Cohort study, cross-sectional</td>
<td>945 cohort random sample</td>
<td>FVIII antigen</td>
<td>FVIII was higher in lower eGFR, adjusting for age, sex, race, BMI, DM, HTN, CVD, hyperlipidemia, and smoking.</td>
</tr>
<tr>
<td>REGARDS&lt;sup&gt;30&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HTN hypertension; DM diabetes; CVD cardiovascular disease, SBP systolic blood pressure; BMI body mass index; LVH left ventricular hypertrophy; ACE-I angiotensin converting enzyme inhibitor; LDL low density lipoprotein; HDL high density lipoprotein, TG triglycerides;
Table 1.4.: Distribution of ABO blood groups in hemodialysis patients and controls

<table>
<thead>
<tr>
<th>Study country</th>
<th>N patients</th>
<th>A</th>
<th>B</th>
<th>AB</th>
<th>O</th>
<th>Reported differences in frequencies among patients and controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syria&lt;sup&gt;138&lt;/sup&gt;</td>
<td>231 hemodialysis patients</td>
<td>71 (30.7)</td>
<td>34 (14.7)</td>
<td>8 (3.5)</td>
<td>118 (51.1)</td>
<td>B more common in dialysis, A less common in dialysis compared to donors</td>
</tr>
<tr>
<td>11,320 blood donors*</td>
<td>4,528 (40)</td>
<td>906 (8)</td>
<td>566 (5)</td>
<td>5320 (47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>US&lt;sup&gt;136&lt;/sup&gt;</td>
<td>184 CKD patients including dialysis</td>
<td>75 (40.8)</td>
<td>27 (14.7)</td>
<td>6 (3.3)</td>
<td>76 (41.3)</td>
<td>B more common in renal patients, O less common compared to donors</td>
</tr>
<tr>
<td>37 dialysis patients</td>
<td>12 (32.4)</td>
<td>9 (24.3)</td>
<td>-</td>
<td>16 (43.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3,820 blood donors*</td>
<td>1540 (40.3)</td>
<td>325 (8.5)</td>
<td>135 (3.5)</td>
<td>1820 (47.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil&lt;sup&gt;123&lt;/sup&gt;</td>
<td>195 hemodialysis</td>
<td>60 (30.8)</td>
<td>28 (14.3)</td>
<td>9 (4.6)</td>
<td>98 (50.3)</td>
<td>No significant differences</td>
</tr>
<tr>
<td>80 healthy controls</td>
<td>28 (35)</td>
<td>8 (10)</td>
<td>7 (8.8)</td>
<td>37 (46.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jordan&lt;sup&gt;160&lt;/sup&gt;</td>
<td>197 kidney failure</td>
<td>90 (45.7)</td>
<td>34 (17.3)</td>
<td>13 (6.6)</td>
<td>60 (30.4)</td>
<td>No comparison group</td>
</tr>
<tr>
<td>No controls</td>
<td>90 (45.7)</td>
<td>34 (17.3)</td>
<td>13 (6.6)</td>
<td>60 (30.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iran&lt;sup&gt;161&lt;/sup&gt;</td>
<td>151 hemodialysis patients</td>
<td>55 (34.6)</td>
<td>26 (17.2)</td>
<td>62 (41)</td>
<td>8 (5.3)</td>
<td>No comparison group</td>
</tr>
<tr>
<td>No controls</td>
<td>55 (34.6)</td>
<td>26 (17.2)</td>
<td>62 (41)</td>
<td>8 (5.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iraq&lt;sup&gt;102&lt;/sup&gt;</td>
<td>60 hemodialysis patients</td>
<td>6 (10)</td>
<td>15 (25)</td>
<td>6 (10)</td>
<td>33 (55)</td>
<td>No comparison group</td>
</tr>
<tr>
<td>No controls</td>
<td>6 (10)</td>
<td>15 (25)</td>
<td>6 (10)</td>
<td>33 (55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>871 dialysis and kidney failure patients</td>
<td>294 (33.8)</td>
<td>146 (16.8)</td>
<td>98 (11.3)</td>
<td>333 (38.2)</td>
<td></td>
</tr>
</tbody>
</table>

*Number of blood donors per blood group inferred from total percentage reported.
Figure 1.1.: Potential mechanisms of Venous Thromboembolism in Chronic Kidney Disease
Figure 1.2.: Potential explanations for elevated Factor VIII in Chronic Kidney Disease
References


Lai JD, Georgescu MT, Hough C, Lillicrap D. To clear or to fear: An innate perspective on factor VIII immunity. *Cellular Immunology*. 2016;301:82-89.


CHAPTER 2: MEASURES OF KIDNEY FUNCTION AND THE ASSOCIATION OF VENOUS THROMBOEMBOLISM IN THE REGARDS STUDY

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Peter W. Callas, University of Vermont
Mary Cushman, Larner College of Medicine, University of Vermont
2.1. Abstract

**Background:** Kidney disease has been associated with venous thromboembolism (VTE) risk, but results conflict and there is little information in blacks.

**Study design:** Prospective cohort study

**Setting & participants:** 30,239 black and white adults age 45 and older enrolled in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study between 2003-7.

**Predictors:** Estimated GFR using the combined creatinine-cystatin C (eGFR<sub>Cr-CysC</sub>) equation and urinary albumin-creatinine ratio (ACR).

**Outcomes:** The primary outcome was adjudicated VTE and secondary outcomes were provoked and unprovoked VTE separately. Mortality was a competing risk event.

**Results:** Over 4.6 years of follow-up, 239 incident VTE events occurred over 124,624 person-years. Cause-specific hazard ratios (HRs) of VTE were calculated using proportional hazards regression adjusted for age, sex, race, region of residence and body mass index. The adjusted VTE HRs (95% CI) for eGFR<sub>Cr-CysC</sub> 60-<90, 45-<60 and <45 versus ≥90 ml/min/1.73m<sup>2</sup> were 1.28 (0.94, 1.76), 1.30 (0.77, 2.18) and 2.13 (1.21, 3.76). The adjusted VTE HRs (95% CI) for ACR 10-<30, 30-<300, >300 vs <10mg/g were 1.14 (0.84, 1.56), 1.15 (0.79, 1.69) and 0.64 (0.25, 1.62). Associations were similar for provoked and unprovoked VTE.

**Limitations:** Single measurement of eGFR and ACR may have led to misclassification. Smaller numbers of events may have limited power.
**Conclusions:** There was an independent association of low eGFR (<45 vs ≥90) with VTE risk, but no association of ACR and VTE.

### 2.2. Introduction

Chronic kidney disease (CKD) affects 13% of the general population\(^1\) and is a strong independent risk factor for death and cardiovascular disease including stroke, coronary disease, heart failure and peripheral arterial disease.\(^2\) There is an increased risk of venous thromboembolism (VTE) in nephrotic syndrome,\(^3\) in patients receiving maintenance dialysis,\(^4\) and in renal transplant recipients\(^5,6\) but the risk of VTE in patients with earlier stages of CKD is unclear.\(^7-9\) VTE consists of deep vein thrombosis (DVT) and pulmonary embolism (PE) and is the third leading vascular disease in the U.S., affecting approximately 500,000 Americans each year and contributing to >100,000 deaths annually.\(^10\) Prior studies linking measures of kidney function and VTE conflict, possibly due to varying composition and limited statistical power or the use of different measures of kidney disease in these studies, including various equations for estimated glomerular filtration rate (eGFR) and definitions of albuminuria.

To better elucidate the relationship between CKD and VTE, we assessed the associations of kidney disease measures with incident VTE in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. We had four primary aims. First, to determine the association of albuminuria and eGFR, in particular eGFR based on combining creatinine and cystatin C (eGFR\(_{Cr-CysC}\)), and VTE in the REGARDS study. Second, because results of prior studies disagreed as to whether CKD is more strongly associated with unprovoked or provoked VTE\(^9,11\) (which refers to VTE preceded
within 90 days by major trauma, surgery, or marked immobility or associated with active
cancer or chemotherapy), we evaluated associations of kidney disease measures with
each VTE type. We hypothesized that the association would be larger for provoked than
unprovoked VTE because persons with CKD might be more likely to be hospitalized and
require surgery. Third, since prior studies had limited numbers of black participants, and
CKD disproportionately affects blacks, we sought to evaluate whether race modified the
association of CKD and VTE. Finally, to contextualize the importance of CKD with
regards to VTE risk, we compared the population attributable fraction for a range of
kidney function to that of older age and obesity, which are established VTE risk factors.

2.3. Methods

Study population and design:
We studied participants in the REGARDS study, a prospective, nationally representative
cohort study of blacks and whites in the US, which has been previously described. REGARDS enrolled 30,239 black and white men and women age 45 and older between 2003-2007. Due to the goals of REGARDS to assess black-white disparities in stroke and
cognitive decline, only self-identified non-Hispanic white and black individuals were
included. Additional exclusion criteria included medical conditions preventing long-term
participation, active cancer, active treatment for cancer, residence in or awaiting
placement in a nursing home, or inability to communicate in English. For this analysis we
excluded participants who had baseline VTE, were missing measures of kidney disease,
or who reported being on dialysis.
Participants were identified from commercially available lists of residents and recruited through a mailing followed by telephone contact (response rate 33%). Of eligible participants contacted, the cooperation rate was 49%, similar to many other cohort studies. Participants first completed a computer-assisted telephone interview for self-reported demographic, risk factor and medications data. Then they underwent an in-home visit at which written informed consent was obtained, and anthropomorphic data, medication inventory, urine samples and fasting phlebotomy were performed. During the in-home examination, standardized protocols were followed to obtain 2 blood pressure measurements that were averaged for analysis, an electrocardiogram (ECG), height and weight. Blood and urine samples were collected after a 10 to 12 hour fast. Blood was centrifuged locally, and shipped with the urine samples on ice packs to the University of Vermont Laboratory for Clinical and Biochemistry Research for reprocessing and analysis or storage. The study was approved by all institutional review boards at participating universities, University of Alabama IRB 00000726, University of Vermont CHRMS 00-295.

Laboratory methods:

Serum creatinine was measured using an isotope-dilution mass spectrometry–traceable method using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Rochester, NY), with a coefficient of variance (CV) of 1.1%. Urinary albumin was measured with the BN ProSpec nephelometer (Dade Behring, Marburg,
Germany) with CV of 2.2% at 110 mg/L and 4.3% at 13 mg/L. Urinary creatinine was measured with a rate-blanked Jaffé procedure, using the Modular-P analyzer (Roche/Hitachi) with CV of 2.6% at 66 mg/dL and 8.6% at 156 mg/dL. Serum cystatin C was measured with high sensitivity particle-enhanced immunonephelometry (N Latex Cystatin C on the BNII, Dade Behring, Deerfield, IL), with an intra-assay CV of 2.0-2.8% and an inter-assay CV of 2.3-3.1%.

Definitions:

eGFR was calculated using the CKD-EPI equations based on creatinine (eGFR\text{Cr}), cystatin C (eGFR\text{CysC}) and combined creatinine-cystatin C (eGFR\text{Cr-CysC}).\textsuperscript{15} eGFR was categorized as <45, 45-<60, 60-<90, ≥90 ml/min/1.73m\textsuperscript{2} to correspond to clinical categories. Urinary albumin to creatinine ratio (ACR) was categorized as <10, 10-<30, 30-<300, ≥300 mg/g.

Covariates of interest were obtained at baseline and included age, sex, race (self-reported as black or white), region of residence (southeast or non-southeast), body mass index (BMI) in kg/m\textsuperscript{2}, hypertension, diabetes, hyperlipidemia, cardiovascular disease (CVD), smoking, self-report of current postmenopausal hormone therapy (HT), aspirin (ASA), statin or warfarin use. Hypertension is defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg and/or use of antihypertensive medication. Diabetes is defined as fasting glucose >126 mg/dL, non-fasting glucose >200 mg/dL, or use of diabetes medication. Hyperlipidemia was defined as cholesterol >240 mg/dL or low density lipoprotein >160 mg/dL or taking medications for high cholesterol.
CVD was defined as a self-report of pre-baseline myocardial infarction, aortic aneurysm, stent, coronary artery surgery or stroke.

*Identification and validation of outcomes:*

The primary outcome was incident VTE, ascertained as previously described.\(^\text{16}\) In brief, medical records were obtained from potential VTE events, each of which was reviewed by two physicians and major disagreements settled by a blinded third reviewer.\(^\text{16}\) Provoked VTE was defined as a VTE that was preceded within 90 days by major trauma, surgery, or marked immobility or associated with active cancer or chemotherapy. All other events were considered unprovoked. The competing event of death was identified through active surveillance including calls to participants, search of online sources e.g. Social Security Death Index and National Death Index. Further details of ascertainment were previously reported.\(^\text{17,18}\)

*Statistical Analysis:*

We compared baseline characteristics among participants across eGFR\(_{\text{Cr-CysC}}\) and ACR categories using ANOVA for continuous variables and \(\chi^2\) tests for categorical variables. Dichotomous variables were expressed as percentages and continuous variables as means ± standard deviation (SD).

We calculated age, sex and race adjusted incidence rates of VTE per 1000 person-years with 95% confidence intervals (CI) for each kidney disease measures using Poisson regression. We used Cox proportional hazards regression to estimate cause-
specific hazard ratios (HRs) with 95% CI for the competing risk of VTE and mortality. The observation time was defined as time from completing the baseline in-home visit to time of lost to follow-up, end of follow-up or event. Participants were censored for death in the VTE model, and censored for VTE for the mortality model. To control for confounding we created a multivariable model including demographics (age, sex, race, region of residence) and established VTE risk factors (BMI) to adjust associations of kidney disease measures and VTE. An interaction of race x region for VTE risk was previously reported in REGARDS so this interaction term was included in the model.

The same covariates were used to estimated adjusted HRs for provoked and unprovoked VTE. Mortality models were additionally adjusted for hypertension, diabetes, hyperlipidemia, history of cardiovascular disease and smoking. Violations of the Cox proportional hazards model assumptions were assessed by visual inspection of the log-minus-log survival plots and by testing global and individual Schoenfeld residuals. We tested for interactions among kidney disease measures and race (black/white), adjusting for age, sex and BMI, and stratified by region to account for known interaction between race and region. We also tested for an interaction between eGFR and ACR.

In a secondary analysis, we estimated the HR and 95% CI for VTE using eGFR$_{\text{Cr}}$, eGFR$_{\text{CysC}}$ using the CKD-EPI equation and eGFR$_{\text{Cr}}$ using the Modification of Diet in Renal Disease (MDRD) equation to understand whether a difference in estimating equations could account for conflicting reports in the association of kidney disease measures and VTE.
We calculated the population attributable fraction (PAF) of CKD (using a range of eGFR$_{Cr-CysC}$ cut points <45, <60 and <90 ml/min/1.73m$^2$), age $\geq$65 versus <65 years and obesity (BMI>30m/kg$^2$ vs <30 kg/m$^2$) for VTE using the punafcc command in Stata.

To reconcile our null findings with the literature showing an association of albuminuria and VTE we performed a post-hoc sensitivity analysis using estimated albumin excretion rate (AER), which is the product of ACR and estimated creatinine excretion rate (eCER), using Ix’s formula.$^{20}$

$$eAER = ACR \times (879.89 + 12.51 \times \text{weight(kg)} - 6.19 \times \text{Age} + (34.51 \text{ if black}) - (379.42 \text{ if female})$$

Statistical significance was considered as a two-tailed $P<0.05$, and for interaction analyses $p<0.10$. All statistical analyses were performed by the use of Stata software version 14 (StataCorp LP).

2.4. Results

The initial cohort included 30,239 participants. After exclusions for missing baseline home visit or follow-up data (n=684), self-report of dialysis (n=113) or baseline VTE (n=1,741), there were 27,703 participants. A total of 2,601 participants (8.6% of initial cohort) were missing any kidney disease measure with the following mutually exclusive counts: serum creatinine (n=1,871), serum cystatin C (n=2,518), urine albumin (2,002) and urine creatinine (n=1,929). The final analysis included 25,102 participants (Figure 2.1.).

55
The analytic cohort was 54% female, 40% black, and 48% age 65 or older. There were 9% with eGFR<sub>Cr-CysC</sub> < 60 ml/min/1.73 m<sup>2</sup>, 11% with eGFR<sub>Cr</sub> < 60 ml/min/1.73 m<sup>2</sup>, 15% with eGFR<sub>CysC</sub> < 60 ml/min/1.73 m<sup>2</sup> and 14% with ACR > 30 mg/g. There were 50 participants with ACR>3000 mg/g and only one VTE occurred in this category.

Compared to those with higher eGFR<sub>Cr-CysC</sub> at baseline, participants with lower eGFR<sub>Cr-CysC</sub> were older, more likely to have hypertension, diabetes, hyperlipidemia, be a former or never smoker, and use statins, aspirin or warfarin (Table 2.1.). For higher compared to lower ACR categories, diabetes, hypertension, CVD, current smoking were more common (Table 2.2.).

There were 239 VTE events (113 unprovoked, 126 provoked) during 124,624 person-years follow-up. The incidence rates of VTE with 95% CI are shown in Table 2.3. and increased monotonically across categories of declining eGFR<sub>Cr-CysC</sub>. The incidence rates of VTE increased across categories of ACR except for the highest category (≥ 300 mg/g), where the number of events was limited. The incidence rates of mortality increased across declining eGFR and higher ACR categories.

The risk of VTE increased with declining eGFR<sub>Cr-CysC</sub> (Table 2.4.); the unadjusted HRs (95% CI) for eGFR<sub>Cr-CysC</sub> 60-<90, 45-<60 and <45 compared to ≥90 ml/min/1.73 m<sup>2</sup> were 1.91 (1.45, 2.51), 2.24 (1.41, 3.57) and 3.38 (2.04, 5.60), p trend <0.001. The HRs were attenuated when adjusting for age, sex, race x region and BMI and remained statistically significant only for eGFR<sub>Cr-CysC</sub> <45 compared to ≥90 ml/min/1.73 m<sup>2</sup>, HR 2.13 (1.19, 3.76). Associations of eGFR<sub>Cr-CysC</sub> with provoked and
unprovoked VTE were similar with a two-fold increased risk for each type of VTE with eGFR_{Cr-CysC}<45 versus ≥90 ml/min/1.73 m² (Table 2.4.).

In the competing risk analysis, the adjusted cause-specific HRs for mortality increased with lower eGFR_{Cr-CysC} and were higher than the HRs for VTE in each category of eGFR (Table 2.4.).

ACR was not associated with VTE in univariate or multivariable analysis. As compared to ACR<10 mg/g, the unadjusted HRs (95% CI) for VTE for ACR 10-<30, 30-<300 and ≥300 mg/g were 1.30 (0.96, 1.76), 1.54 (1.06, 2.22), and 1.18 (0.52, 2.66). Multivariable HRs are shown in Table 2.4. The HRs were similar for provoked and unprovoked VTE (Table 2.4.), and for use of the estimated albumin excretion rate (Table 2.6., Supplemental Table S2.1.). The adjusted cause-specific HRs for mortality increased across higher levels of albuminuria (Table 2.4.).

Race-specific HRs for VTE by kidney disease measures were stratified by region given the known race x region interaction in REGARDS (Table 2.5.). For whites residing outside of the southeast, VTE risk increased across categories of eGFR_{Cr-CysC} with a HR (95% CI) 3.37 (1.34, 8.45) for eGFR_{Cr-CysC} <45 ml/min/1.73 m². For blacks residing outside of the southeast, eGFR_{Cr-CysC} was not associated with VTE, however the interaction p value for race*GFR was 0.70 outside the southeast. In the southeast, associations appeared larger in blacks than whites (p interaction = 0.1), with similar associations in blacks and whites for eGFR <45 (HR 1.68 and 1.95, respectively) but higher HRs among blacks than whites for eGFR 45-<90 ml/min/1.73 m². There were no significant differences in the association of ACR with VTE when stratified by race and
region (p interaction was 0.9 in southeast, p interaction was 0.3 in non-southeast) (Table 2.7., Supplemental Table S2.2.). There was also no significant interaction between ACR and eGFR (p interaction = 0.4).

The population attributable fractions of obesity (defined as BMI ≥30 kg/m²) and older age (defined as age ≥65 years), for VTE were 17% and 36%, respectively. The population attributable fractions of CKD for VTE by eGFR_{Cr-CysC} <90, <60 and eGFR_{Cr-CysC} <45 ml/min/1.73m² were 20%, 5% and 4%, respectively.

In a secondary analysis, the eGFR using alternate equations had similar associations with VTE (Table 2.8., Supplemental Table S2.3.). The adjusted HR (95% CI) for eGFR <45 compared to ≥90 ml/min/1.73m² was 1.93 (1.13, 3.32) for eGFR_{Cr}, and 1.58 (0.81, 3.08) for eGFR_{CysC}. The HR of VTE with eGFR_{Cr} <45 ml/min/1.73m² using the MDRD equation was 1.94 (1.07, 3.50).

2.5. Discussion

Estimated glomerular filtration rate was independently associated with an increased risk of future VTE in this large prospective cohort study. Compared to eGFR_{Cr-CysC} ≥90, an eGFR_{Cr-CysC} <45 ml/min/1.73m² was associated with a 2-fold increased risk of VTE, after adjusting for demographic and VTE risk factors. This relationship was similar for provoked VTE, but the association was not statistically significant in unprovoked VTE. The association of VTE and eGFR appeared to vary by race and region, but power limited the interpretation of these analyses. There was no association between ACR and incident VTE. The cause-specific hazard ratios for mortality were
higher for each eGFR or ACR category than the risk for VTE. The population attributable fraction of VTE for CKD was modest compared to that for accepted clinical cut points for BMI and older age.

Our results provide further evidence supporting the relationship between eGFR and VTE. A meta-analysis of five studies, the largest work thus far, demonstrated an association between eGFR and VTE risk and our findings extend this finding to a large, contemporary U.S. cohort. We hypothesized that different eGFR equations used in previous research, including individual studies from the meta-analysis, may have led to conflicting results on the association of kidney disease measures and VTE. In the ARIC and PREVEND studies, eGFR\textsubscript{Cr} by the MDRD equation was not associated with future VTE, however, in ARIC eGFR\textsubscript{CysC} was associated with VTE. We were able to clarify the relationship between kidney disease measures and VTE by simultaneously comparing the relationships of the CKD-EPI (eGFR\textsubscript{Cr}, eGFR\textsubscript{CysC}, eGFR\textsubscript{Cr-CysC}) and MDRD eGFR equations, and ACR, with VTE risk in one study. eGFR\textsubscript{Cr}, eGFR\textsubscript{Cr-CysC} and MDRD eGFR\textsubscript{Cr} were all significantly associated with VTE risk, in sum, supporting a link between kidney disease measures and VTE. With regards to type of VTE, the association of eGFR <45 ml/min/1.73m\textsuperscript{2} was somewhat stronger for provoked VTE than unprovoked. Uniformly, the risk of VTE begins at an eGFR below 45 ml/min/1.73m\textsuperscript{2}, suggesting the mechanism of the association between eGFR and VTE could relate to changes that occur at this level of clearance.

In contrast to eGFR, ACR was not associated with VTE in this study. This is consistent with the finding in ARIC, the only other epidemiologic study of the
relationship of ACR and VTE risk performed in the U.S.\textsuperscript{9} In the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study, a Dutch cohort that oversampled for albuminuria, urinary albumin excretion was associated with increased risk of future VTE. Differences in the cohort composition compared to the REGARDS and ARIC studies could account for the different results. In PREVEND there were more smokers (38\% vs 14\%), fewer diabetics (4\% vs 20\%) and lower BMI (mean BMI 26.1 vs 29.2 kg/m\textsuperscript{2}) compared to the REGARDS, and people with insulin treated diabetes were excluded.

Race and regional differences in the risk of VTE have been previously reported in the REGARDS study.\textsuperscript{16} Therefore, we studied whether race modified the association of eGFR and VTE within region (southeast/non-southeast). The association of eGFR and VTE appeared largest in whites residing outside of the southeast. While this may have been a chance finding and power to detect two-way interactions was limited, the reason for this finding may be due to the competing risk for death. For eGFR< 45 ml/min/1.73m\textsuperscript{2}, whites outside the southeast had the lowest cause-specific hazard ratio for death, compared to whites in the southeast or blacks in either region. In the aforementioned meta-analysis, there was no difference in the association of kidney disease and VTE by race. Conflicting results may be related to different definitions of kidney disease and limited number of black participants residing outside of the southeast included in the meta-analysis.

There are several strengths of the current study. The large, prospective, population based nature of the study enables our results to be generalized to black and white Americans living across a geographically diverse region of the U.S. We were able
to compare associations of kidney disease measures with VTE using several different equations in one study. The study was a contemporary cohort reflecting current trends in obesity and medication use (including widespread use of statins) that might alter relationships of CKD with VTE, and reflects current diagnosis patterns for VTE, which have changed over the past few decades.\textsuperscript{23} The presentation of competing risk of death is unique in the study of kidney disease measures and VTE, and informed our understanding of potential race and regional differences in the risk of VTE.

Limitations of this study require consideration. As with many other prospective cohort studies of general populations, GFR was estimated and not measured. The estimates of GFR and ACR were obtained once at baseline. This could have led to misclassification. The PREVEND study reported that ACR was less accurate and more biased than estimated albumin excretion rate,\textsuperscript{24} because urinary creatinine is influenced by muscle mass, introducing confounding based on non-albuminuric covariates.\textsuperscript{25} However, in a sensitivity analysis our results were consistent when utilizing estimated albumin excretion rate as a predictor. Our analysis may have been limited by low power to detect smaller differences in risk of VTE by ACR category. With 239 VTE events and a prevalence of 14\% ACR\textgreater{}30 mg/g we estimated 80\% power to detect a HR of 1.7 or higher using alpha\textless{}0.05. Despite a large cohort of blacks and whites, power was limited in studying both race and region stratified associations of kidney disease measures and VTE. The results may not be generalizable to the entire U.S. population as REGARDS was limited to self-identified black and white participants above age 45. Finally, while
we adjusted for factors known to be associated with VTE risk the results could be influenced by unmeasured factors and residual confounding could exist.

2.6. Conclusions

The present prospective study provides further clarification on a growing literature supporting a relationship between kidney function and VTE. In our study, eGFR < 45 ml/min/1.73m$^2$ was associated with a two-fold risk of VTE and a three-fold risk of mortality. Further study of the mechanisms of the association between low eGFR and VTE and potential modifiable risk factors is warranted.
Table 2.1: Baseline characteristics by categories of eGFR<sub>Cr-CysC</sub>

<table>
<thead>
<tr>
<th>Characteristic (mean, SD or frequency, %)</th>
<th>eGFR&lt;sub&gt;Cr-CysC&lt;/sub&gt; (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥90</td>
</tr>
<tr>
<td>Mean age, years</td>
<td>61.0±8.1</td>
</tr>
<tr>
<td>Sex, female</td>
<td>64.4</td>
</tr>
<tr>
<td>Race, black</td>
<td>35.5</td>
</tr>
<tr>
<td>Region of Residence, southeast</td>
<td>57.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td>48.9</td>
</tr>
<tr>
<td>Diabetes</td>
<td>16.1</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>42.1</td>
</tr>
<tr>
<td>CVD</td>
<td>11.1</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.9±6.1</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Current Smoking</td>
<td>14.9</td>
</tr>
<tr>
<td>Former Smoking</td>
<td>47.9</td>
</tr>
<tr>
<td>Never Smoking</td>
<td>37.1</td>
</tr>
<tr>
<td>Statin use</td>
<td>27.0</td>
</tr>
<tr>
<td>Aspirin use</td>
<td>37.8</td>
</tr>
<tr>
<td>Hormone Therapy use*</td>
<td>19.2</td>
</tr>
<tr>
<td>Warfarin use</td>
<td>1.4</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.7±0.1</td>
</tr>
<tr>
<td>Serum cystatin C (mg/L)</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;Cr&lt;/sub&gt; (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>96±13</td>
</tr>
<tr>
<td>eGFR&lt;sub&gt;cysC&lt;/sub&gt; (ml/min/1.73m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>102±14</td>
</tr>
<tr>
<td>Urine ACR (mg/g)</td>
<td>20±108</td>
</tr>
</tbody>
</table>

*Hormone therapy use for women only.
Table 2.2.: Baseline characteristics by categories of albumin-to-creatinine ratio (ACR)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ACR (mg/g)</th>
<th>&lt;10</th>
<th>10-&lt;30</th>
<th>30-&lt;300</th>
<th>≥300</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td>15,777</td>
<td>5,696</td>
<td>2,961</td>
<td>668</td>
</tr>
<tr>
<td>Mean age, years</td>
<td></td>
<td>63.4±9.0</td>
<td>66.5±9.5</td>
<td>67.3±9.9</td>
<td>66.6±9.3</td>
</tr>
<tr>
<td>Sex, Female</td>
<td></td>
<td>52.9</td>
<td>59.3</td>
<td>50.4</td>
<td>42.4</td>
</tr>
<tr>
<td>Race, Black</td>
<td></td>
<td>37.7</td>
<td>39.5</td>
<td>48.1</td>
<td>63.5</td>
</tr>
<tr>
<td>Region of Residence, Southeast</td>
<td></td>
<td>55.5</td>
<td>56.5</td>
<td>54.3</td>
<td>53.3</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td>51.44</td>
<td>65.5</td>
<td>75.2</td>
<td>87.4</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td>13.8</td>
<td>25.0</td>
<td>37.4</td>
<td>60.45</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td></td>
<td>42.6</td>
<td>46.4</td>
<td>48.7</td>
<td>59.0</td>
</tr>
<tr>
<td>CVD</td>
<td></td>
<td>14.1</td>
<td>20.9</td>
<td>26.3</td>
<td>37.7</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td>28.9±5.8</td>
<td>29.2±6.4</td>
<td>30.2±6.6</td>
<td>30.7±7.0</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td></td>
<td>13.2</td>
<td>14.7</td>
<td>17.6</td>
<td>20.7</td>
</tr>
<tr>
<td>Former</td>
<td></td>
<td>46.6</td>
<td>44.8</td>
<td>41.8</td>
<td>33.5</td>
</tr>
<tr>
<td>Never</td>
<td></td>
<td>40.2</td>
<td>40.5</td>
<td>40.6</td>
<td>44.7</td>
</tr>
<tr>
<td>Statin use</td>
<td></td>
<td>29.0</td>
<td>33.3</td>
<td>36.3</td>
<td>44.5</td>
</tr>
<tr>
<td>Aspirin use</td>
<td></td>
<td>41.3</td>
<td>45.4</td>
<td>46.8</td>
<td>52.9</td>
</tr>
<tr>
<td>HRT use*</td>
<td></td>
<td>19.4</td>
<td>12.2</td>
<td>11.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Warfarin use</td>
<td></td>
<td>2.0</td>
<td>3.4</td>
<td>4.7</td>
<td>4.6</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td></td>
<td>0.9±0.2</td>
<td>0.9±0.3</td>
<td>1.0±0.4</td>
<td>1.5±1.0</td>
</tr>
<tr>
<td>Serum cystatin C (mg/L)</td>
<td></td>
<td>0.9±0.2</td>
<td>1.0±0.3</td>
<td>1.1±0.4</td>
<td>1.6±0.9</td>
</tr>
<tr>
<td>eGFRcr (ml/min/1.73m²)</td>
<td></td>
<td>87±17</td>
<td>85±20</td>
<td>81±24</td>
<td>62±29</td>
</tr>
<tr>
<td>eGFRcysC (ml/min/1.73m²)</td>
<td></td>
<td>90±22</td>
<td>84±24</td>
<td>76±27</td>
<td>55±28</td>
</tr>
<tr>
<td>Urine ACR (mg/g)</td>
<td></td>
<td>5±2</td>
<td>16±5</td>
<td>84±60</td>
<td>1224±1422</td>
</tr>
</tbody>
</table>

Differences were significant (p <0.05) across categories of ACR for all variables except region of residence. *Hormone therapy use for women only.
Table 2.3: Adjusted Incidence Rate (IR) for VTE and mortality per 1000 Person-Years by kidney disease measures

<table>
<thead>
<tr>
<th>eGFR_{Cr-CysC} (ml/min/1.73m^2)</th>
<th>≥90</th>
<th>60-&lt;90</th>
<th>45-&lt;60</th>
<th>&lt;45</th>
<th>p trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. at risk</td>
<td>14,087</td>
<td>8,670</td>
<td>1,475</td>
<td>870</td>
<td></td>
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<tr>
<td>Person-time</td>
<td>66,171</td>
<td>41,580</td>
<td>6,788</td>
<td>3,720</td>
<td></td>
</tr>
<tr>
<td>No. VTE</td>
<td>90</td>
<td>110</td>
<td>21</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>VTE IR (95% CI)</td>
<td>1.50 (1.21, 1.85)</td>
<td>2.14 (1.72, 2.66)</td>
<td>2.18 (1.38, 3.46)</td>
<td>3.43 (2.09, 5.63)</td>
<td>0.02</td>
</tr>
<tr>
<td>No. deaths</td>
<td>666</td>
<td>1,132</td>
<td>428</td>
<td>378</td>
<td></td>
</tr>
<tr>
<td>Mortality IR (95% CI)</td>
<td>11.4 (10.6, 12.3)</td>
<td>18.5 (17.2, 19.8)</td>
<td>33.5 (30.0, 37.4)</td>
<td>53.4 (47.4, 60.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACR (mg/g)</td>
<td>&lt;10</td>
<td>10-&lt;30</td>
<td>30-&lt;300</td>
<td>≥300</td>
<td></td>
</tr>
<tr>
<td>No. at risk</td>
<td>15,777</td>
<td>5,696</td>
<td>2,961</td>
<td>668*</td>
<td></td>
</tr>
<tr>
<td>Person-time</td>
<td>75,273</td>
<td>26,732</td>
<td>13,347</td>
<td>2907</td>
<td></td>
</tr>
<tr>
<td>No. VTE</td>
<td>135</td>
<td>62</td>
<td>36</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>VTE IR (95% CI)</td>
<td>1.69 (1.42, 2.02)</td>
<td>1.97 (1.52, 2.56)</td>
<td>2.16 (1.54, 3.04)</td>
<td>1.70 (0.76, 3.81)</td>
<td>0.5</td>
</tr>
<tr>
<td>No. deaths</td>
<td>1,020</td>
<td>738</td>
<td>601</td>
<td>245</td>
<td></td>
</tr>
<tr>
<td>Mortality IR (95% CI)</td>
<td>11.4 (10.7, 12.2)</td>
<td>19.1 (17.6, 20.6)</td>
<td>28.0 (25.6, 30.7)</td>
<td>54.6 (47.8, 62.2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

eGFR is expressed in ml/min/1.73m^2 and ACR in mg/g. *Of the 50 participants with ACR>3000mg/g only one VTE in this category. Incidence rates are adjusted for age, sex and race.
Table 2.4.: Cause specific hazard ratios (95% CI) for total VTE, provoked and unprovoked VTE and mortality by kidney disease measures

<table>
<thead>
<tr>
<th>eGFR Cr-CysC</th>
<th>≥90</th>
<th>60-&lt;90</th>
<th>45-&lt;60</th>
<th>&lt;45</th>
<th>p trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude VTE HR</td>
<td>1.0 (ref)</td>
<td>1.91 (1.45, 2.51)</td>
<td>2.24 (1.41, 3.57)</td>
<td>3.38 (2.04, 5.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted VTE HR</td>
<td>1.0 (ref)</td>
<td>1.28 (0.94, 1.76)</td>
<td>1.30 (0.77, 2.18)</td>
<td>2.13 (1.21, 3.76)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total</td>
<td>1.0 (ref)</td>
<td>1.13 (0.73, 1.75)</td>
<td>1.39 (0.69, 2.79)</td>
<td>2.31 (1.07, 4.96)</td>
<td>0.03</td>
</tr>
<tr>
<td>Provoked</td>
<td>1.0 (ref)</td>
<td>1.47 (0.93, 2.32)</td>
<td>1.20 (0.55, 2.64)</td>
<td>1.98 (0.86, 4.61)</td>
<td>0.2</td>
</tr>
<tr>
<td>Unprovoked</td>
<td>1.0 (ref)</td>
<td>1.40 (1.26, 1.57)</td>
<td>2.28 (1.97, 2.64)</td>
<td>3.18 (2.72, 3.71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crude mortality HR</td>
<td>1.0 (ref)</td>
<td>2.53 (2.29, 2.78)</td>
<td>6.09 (5.39, 6.89)</td>
<td>10.04 (8.84, 11.41)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted mortality HR</td>
<td>1.0 (ref)</td>
<td>1.40 (1.26, 1.57)</td>
<td>2.28 (1.97, 2.64)</td>
<td>3.18 (2.72, 3.71)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ACR &lt;10</td>
<td>1.30 (0.96, 1.76)</td>
<td>1.54 (1.06, 2.22)</td>
<td>1.18 (0.52, 2.66)</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>10-&lt;30</td>
<td>1.14 (0.84, 1.55)</td>
<td>1.15 (0.79, 1.68)</td>
<td>0.65 (0.26, 1.64)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>30-&lt;300</td>
<td>1.38 (0.91, 2.09)</td>
<td>1.42 (0.85, 2.36)</td>
<td>0.53 (0.13, 2.25)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>≥300</td>
<td>1.92 (0.58, 1.45)</td>
<td>0.92 (0.52, 1.64)</td>
<td>0.78 (0.24, 2.59)</td>
<td>0.7</td>
<td></td>
</tr>
</tbody>
</table>

ACR is expressed in mg/g. VTE Cox models are adjusted for age, sex, race x region, BMI. Cox models for mortality are additionally adjusted for hypertension, diabetes, hyperlipidemia, cardiovascular disease and smoking. Adjusted ACR models are adjusted for eGFR and vice versa.
Table 2.5.: Adjusted Cause-Specific Hazard Ratios and 95% Confidence Intervals for VTE and mortality by eGFR<sub>Cr-CysC</sub> stratified by race and region

<table>
<thead>
<tr>
<th></th>
<th>eGFR</th>
<th>90</th>
<th>60-&lt;90</th>
<th>45-&lt;60</th>
<th>&lt;45</th>
<th>p trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VTE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.0</td>
<td>1.21 (0.72, 2.03)</td>
<td>0.30 (0.07, 1.31)</td>
<td>1.95 (0.76, 4.99)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.0</td>
<td>2.18 (1.06, 4.46)</td>
<td>3.45 (1.28, 9.31)</td>
<td>1.68 (0.36, 7.82)</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td><strong>Non-southeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.0</td>
<td>1.09 (0.61, 1.97)</td>
<td>1.64 (0.64, 4.11)</td>
<td>3.37 (1.34, 8.45)</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.0</td>
<td>0.89 (0.39, 2.02)</td>
<td>1.05 (0.33, 3.33)</td>
<td>1.04 (0.26, 4.10)</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.0</td>
<td>1.44 (1.20, 1.72)</td>
<td>2.25 (1.76, 2.88)</td>
<td>3.03 (2.31, 3.98)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.0</td>
<td>1.24 (0.95, 1.60)</td>
<td>1.99 (1.41, 2.81)</td>
<td>3.15 (2.20, 4.50)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Non-southeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>1.0</td>
<td>1.33 (1.06, 1.66)</td>
<td>2.12 (1.59, 2.84)</td>
<td>2.29 (1.64, 3.19)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>1.0</td>
<td>1.46 (1.14, 1.88)</td>
<td>2.37 (1.73, 3.25)</td>
<td>3.36 (2.40, 4.69)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

eGFR<sub>Cr-CysC</sub> in ml/min/1.73m<sup>2</sup>. VTE Hazard ratios are adjusted for age, sex and BMI. Mortality HR are additionally adjusted for hypertension, diabetes, hyperlipidemia, cardiovascular disease, smoking and albuminuria.
Table 2.6.: (Supplemental Table S2.1.): Age, sex and race adjusted Incidence rate per 1000 Person-Years and Hazard Ratio (95% CI) of VTE for categories of estimated albumin excretion rate (eAER)

<table>
<thead>
<tr>
<th>eAER</th>
<th>&lt; 15</th>
<th>15-&lt;30</th>
<th>30-&lt;300</th>
<th>≥300</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. at risk</td>
<td>848</td>
<td>2,548</td>
<td>12,875</td>
<td>8,831</td>
<td></td>
</tr>
<tr>
<td>Person-time</td>
<td>3,816</td>
<td>11,897</td>
<td>61,579</td>
<td>40,967</td>
<td></td>
</tr>
<tr>
<td>No. VTE</td>
<td>6</td>
<td></td>
<td>128</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>Incidence rate (95% CI)</td>
<td>1.54 (0.69, 3.44)</td>
<td>1.25 (0.76, 2.04)</td>
<td>1.88 (1.57, 2.26)</td>
<td>1.90 (1.52, 2.39)</td>
<td></td>
</tr>
<tr>
<td>Crude HR</td>
<td>1.0</td>
<td>0.84</td>
<td>1.27</td>
<td>1.35</td>
<td>0.3</td>
</tr>
<tr>
<td>Adjusted HR</td>
<td>1.0</td>
<td>0.84</td>
<td>1.35</td>
<td>1.22</td>
<td>0.4</td>
</tr>
</tbody>
</table>

eAER is estimated albumin excretion rate in mg/24hr. eGFR in ml/min/1.73m². Adjusted model is adjusted for age, sex, race, region and race*region and BMI and eGFRCr-CysC.
### Table 2.7 (Supplemental Table S2.2.): Adjusted Cause-Specific HRs (95% CI) for VTE and mortality by ACR stratified by race and region

<table>
<thead>
<tr>
<th></th>
<th>ACR</th>
<th>10-&lt;30</th>
<th>30-&lt;300</th>
<th>≥ 300</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VTE</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Southeast</td>
<td>White</td>
<td>1.0</td>
<td>1.04 (0.62, 1.75)</td>
<td>1.09 (0.56, 2.12)</td>
<td>0.54 (0.74, 3.93)</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.0</td>
<td>1.19 (0.60, 2.39)</td>
<td>1.35 (0.60, 3.01)</td>
<td>1.27 (0.30, 5.39)</td>
</tr>
<tr>
<td>Non-southeast</td>
<td>White</td>
<td>1.0</td>
<td>1.60 (0.94, 2.71)</td>
<td>1.15 (0.53, 2.49)</td>
<td>0.99 (0.14, 7.24)</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.0</td>
<td>0.64 (0.26, 1.61)</td>
<td>1.43 (0.65, 3.15)</td>
<td>0.55 (0.07, 4.11)</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td>Southeast</td>
<td>White</td>
<td>1.0</td>
<td>1.51 (1.28, 1.79)</td>
<td>2.02 (1.67, 2.44)</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.0</td>
<td>1.32 (1.03, 1.69)</td>
<td>1.82 (1.40, 2.37)</td>
<td>2.53 (1.76, 3.64)</td>
</tr>
<tr>
<td>Non-southeast</td>
<td>White</td>
<td>1.0</td>
<td>1.37 (1.11, 1.67)</td>
<td>1.84 (1.46, 2.33)</td>
<td>2.47 (1.66, 3.66)</td>
</tr>
<tr>
<td></td>
<td>Black</td>
<td>1.0</td>
<td>1.47 (1.16, 1.85)</td>
<td>1.99 (1.57, 2.54)</td>
<td>2.98 (2.20, 4.02)</td>
</tr>
</tbody>
</table>

ACR in mg/g. VTE Hazard ratios are adjusted for age, sex, BMI and eGFR\(\text{Cr-CysC}\). Mortality HR are additionally adjusted for hypertension, diabetes, history of cardiovascular disease, smoking.
Table 2.8.: (Supplemental Table S2.3.): Incidence Rates Per 1000 PY and Hazard Ratio (95% CI) of VTE for eGFR equations

<table>
<thead>
<tr>
<th>CKD-EPI eGFR&lt;sub&gt;eq&lt;/sub&gt;</th>
<th>≥90</th>
<th>60-&lt;90</th>
<th>45-&lt;60</th>
<th>&lt;45</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. events/N at risk</td>
<td>93/11,433</td>
<td>114/11,013</td>
<td>22/1,799</td>
<td>20/857</td>
<td></td>
</tr>
<tr>
<td>Person-time</td>
<td>53,464</td>
<td>52,855</td>
<td>8,244</td>
<td>3,696</td>
<td></td>
</tr>
<tr>
<td>Incidence Rate (95% CI)</td>
<td>1.91 (1.56, 2.36)</td>
<td>1.69 (1.37, 2.08)</td>
<td>1.77 (1.13, 2.76)</td>
<td>3.43 (2.15, 5.48)</td>
<td>0.02</td>
</tr>
<tr>
<td>Crude HR for VTE</td>
<td>1.0 (ref)</td>
<td>1.23 (0.93, 1.63)</td>
<td>1.62 (1.02, 2.59)</td>
<td>3.35 (2.06, 5.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adjusted HR for Total VTE</td>
<td>1.0 (ref)</td>
<td>0.86 (0.63, 1.17)</td>
<td>0.92 (0.56, 1.53)</td>
<td>1.93 (1.13, 3.32)</td>
<td>0.02</td>
</tr>
<tr>
<td>Provoked VTE</td>
<td>1.0 (ref)</td>
<td>0.76 (0.50, 1.17)</td>
<td>0.76 (0.36, 1.57)</td>
<td>1.96 (0.95, 4.06)</td>
<td>0.09</td>
</tr>
<tr>
<td>Unprovoked VTE</td>
<td>1.0 (ref)</td>
<td>1.00 (0.63, 1.57)</td>
<td>1.15 (0.57, 2.32)</td>
<td>1.94 (0.87, 4.31)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

| CKD-EPI eGFR<sub>Cryst</sub> | 84/11,971 | 103/9,401 | 35/2,316 | 27/1,414 |        |
| No. events/N at risk      | 56,159 | 45,322 | 10,740 | 6,039 |        |
| Incidence Rate (95% CI)   | 1.61 (1.30, 2.00) | 1.90 (1.54, 2.34) | 2.32 (1.61, 3.33) | 2.98 (1.97, 4.49) | 0.01 |
| Crude HR for VTE          | 1.0 (ref) | 1.45 (1.09, 1.95) | 2.07 (1.38, 3.10) | 3.01 (1.93, 4.68) | <0.001 |
| Adjusted HR for Total VTE | 1.0 (ref) | 1.02 (0.74, 1.39) | 1.10 (0.70, 1.74) | 1.58 (0.95, 2.62) | 0.08 |
| Provoked VTE              | 1.0 (ref) | 0.78 (0.50, 1.20) | 0.93 (0.50, 1.74) | 1.58 (0.81, 3.08) | 0.2 |
| Unprovoked VTE            | 1.0 (ref) | 1.36 (0.85, 2.18) | 1.36 (0.70, 2.63) | 1.62 (0.75, 3.47) | 0.2 |

| MDRD eGFR<sub>eq</sub> | 789,494 | 118/12,896 | 24/1,883 | 14/636 |        |
| No. events/N at risk      | 44,045 | 62,040 | 8,587 | 2,809 |        |
| Incidence Rate (95% CI)   | 1.79 (1.42, 2.24) | 1.69 (1.39, 2.05) | 2.06 (1.35, 3.14) | 3.54 (2.06, 6.09) | 0.42 |
| Crude HR for VTE          | 1.0 (ref) | 1.05 (0.79, 1.40) | 1.58 (1.00, 2.50) | 2.84 (1.61, 5.02) | <0.001 |
| Adjusted HR for Total VTE | 1.0 (ref) | 0.95 (0.70, 1.27) | 1.07 (0.66, 1.73) | 1.94 (1.07, 3.50) | 0.03 |
| Provoked VTE              | 1.0 (ref) | 0.88 (0.59, 1.32) | 0.94 (0.47, 1.88) | 1.81 (0.79, 4.16) | 0.2 |
| Unprovoked VTE            | 1.0 (ref) | 1.03 (0.66, 1.59) | 1.22 (0.62, 2.45) | 2.12 (0.92, 4.92) | 0.06 |

eGFR in ml/min/1.73m<sup>2</sup>. Incidence rates are adjusted for age, sex and race. Adjusted Cox models are adjusted for age, sex, race, region and race*region, BMI and ACR.
Figure 2.1: Flow diagram of participants included in the study. Mutually exclusive counts in parentheses.
References

13. Gillett SR, Boyle RH, Zakai NA, McClure LA, Jenny NS, Cushman M. Validating laboratory results in a national observational cohort study without field


CHAPTER 3: MECHANISMS AND MITIGATING FACTORS FOR VENOUS THROMBOEMBOLISM IN CHRONIC KIDNEY DISEASE: THE REGARDS STUDY

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Neil. A. Zakai, Larner College of Medicine at the University of Vermont

Peter. W. Callas, Larner College of Medicine at the University of Vermont

George Howard, University of Alabama Birmingham

B. Khan. Mahmoodi, University of Groningen

Carmen. A. Peralta, University of California San Francisco

Suzanne E. Judd, University of Alabama Birmingham

Manjula Kurella Tamura, Stanford University and VA Palo Alto Health Care System

Mary Cushman, Larner College of Medicine at the University of Vermont
3.1. Abstract

**Background:** Chronic kidney disease (CKD) is associated with venous thromboembolism (VTE) risk via unknown mechanisms. Whether strategies protective against VTE in the general population might work in CKD is unknown.

**Objectives:** To determine whether biomarkers of thrombosis risk attenuate VTE risk and if factors protective against VTE were similarly effective in people with CKD.

**Methods:** With 4.3 years of follow up, baseline biomarkers were measured in 294 incident VTE cases and 939 non-cases from the REGARDS cohort, a nationwide cohort of 30,239 persons age 45 and older. The hazard ratio (HR) of VTE per 10 ml/min/1.73m$^2$ lower eGFR, and the percent attenuation of this HR by each biomarker were calculated.

Associations of protective factors, physical activity, lower BMI, aspirin, warfarin and statin use, with VTE were estimated in those with and without CKD.

**Results:** The adjusted HR (95% CI) for VTE with lower eGFR was 1.13 (1.02, 1.25), and this was attenuated 23% (5, 100%) by D-dimer, 100% (50, 100%) by FVIII and 15% (2, 84%) by CRP. Normal BMI was associated with lower VTE risk in those without CKD (HR 0.47 (0.32, 0.70)) but not with CKD (HR 1.07 (0.51, 2.22) p interaction 0.07). Statin use, physical activity and warfarin use were associated with lower VTE risk in both groups.

**Conclusions:** Procoagulant and inflammatory biomarkers mediated the association of eGFR with VTE. Higher physical activity, statin and warfarin use mitigated risk of VTE in those with and without CKD, but normal BMI did not mitigate risk in CKD.
3.2. Introduction

Kidney disease has recently emerged as a risk factor for venous thromboembolism (VTE),[1-4] however little is known about mechanisms or clinical implications of this relationship. Low estimated glomerular filtration rate (eGFR) is associated with increased levels of biomarkers of inflammation and procoagulation, including higher levels of C-reactive protein (CRP), D-dimer and factor VIII (FVIII).[5-7]. Since these alterations are also associated with increased VTE risk,[8-19] they might account for the relationship of kidney disease with VTE. That is, CKD induced inflammation and procoagulation may explain part of the relationship between eGFR and VTE and thus could be considered as mediators. If this is the case, then interventions that have an impact on the biology represented by these biomarkers could lower VTE risk associated with CKD.

Epidemiology studies and clinical trials suggest a normal BMI, higher physical activity,[20-22] statins,[23-25] aspirin and anticoagulants[26-28] reduce the risk of VTE. However, it is not known if these factors that are protective in the general population are similarly protective in patients with CKD. Knowledge of whether a particular factor is equally, or more, effective in CKD could also shed light on potential mechanisms of VTE in CKD.

In this analysis, we evaluated whether higher levels of D-dimer, Factor VIII (FVIII) and C-reactive protein (CRP), would attenuate the association of eGFR and VTE. We also evaluated whether established risk factors and protective medication use exert similar associations with VTE risk in individuals with versus without CKD.
3.3. Methods

Study population and design:

We studied participants in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort, a prospective cohort study of self-reported blacks and whites in the United States, which has been previously described.[29] In brief, REGARDS enrolled 30,239 black and white women and men age 45 and older between 2003-2007. Exclusion criteria included medical conditions preventing long-term participation, active cancer or active treatment for cancer, residence in or awaiting placement in a nursing home, or inability to communicate in English. For this analysis, we excluded participants missing baseline measures of kidney function (serum creatinine and cystatin C), those with prior self-reported VTE or receiving dialysis at baseline.

Participants were identified from a commercially available list of U.S. residents and recruited through a mailing followed by telephone contact. The cooperation rate was 49% of eligible participants. After completing a computer assisted telephone interview, participants underwent an in-home visit, where informed consent was obtained, anthropomorphic data, medication inventory and urine samples were collected and fasting phlebotomy and an electrocardiogram were performed. Two blood pressure measurements were averaged for analysis, and blood samples were collected after a 10 to 12 hour fast. Blood was centrifuged locally and shipped on ice packs to the University of Vermont for reprocessing and analysis or storage.[30] The study was approved by all institutional review boards at participating universities.
Event ascertainment and definitions:

The primary outcome was incident VTE, consisting of deep vein thrombosis (DVT), pulmonary embolism (PE) or both occurring together. Events were ascertained and validated as previously described.[31] In brief, VTE events were identified by four methods: review of reasons for hospitalization from telephone calls every 6 months up to February 2010; a telephone interview administered from February 2010-February 2011 to identify any self-report of VTE from baseline to time of the call; review of all deaths using periodic searches of the National Death Index, exit interviews or records of last hospital stay; review of medical records for other events such as stroke. For potential cases, in-patient and outpatient medical records were retrieved and then reviewed and adjudicated by two physicians. Major disagreements were settled by a blinded third physician.

Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[32] based on creatinine and cystatin C.[33] Baseline covariates of interest included age, sex, race (self-reported black or white), region of residence (southeast or non-southeast), body mass index (BMI) in kg/m^2, diabetes, hyperlipidemia, cardiovascular disease (CVD), physical activity frequency (self-report of 0, 1-3 or 4 or more times per week), self-report of regular aspirin use, and statin or warfarin use determined by pill bottle review. Southeast was defined as residing in Alabama, Arkansas, Georgia, Louisiana, Mississippi, North Carolina, South Carolina or Tennessee. Diabetes was defined as fasting glucose $\geq$126 mg/dL, random glucose $\geq$200 mg/dL, or taking insulin or glucose lowering medications.
Hyperlipidemia was defined as cholesterol >240 mg/dL, low density lipoprotein >160 mg/dL or taking medications for high cholesterol. CVD was defined as self-report of pre-baseline myocardial infarction, coronary artery surgery, angioplasty, stenting, silent MI on electrocardiogram or stroke. CKD was defined as eGFR less than 60 ml/min/1.73m² by convention. In REGARDS, albuminuria was not associated with VTE[1] so this was not considered. Normal BMI was defined as 18.5-<25 kg/m².

Case-Cohort Study Design:

We used previously measured biomarker data for D-dimer and FVIII from a cohort random sample (CRS), selected as previously described.[34] The CRS was randomly selected within strata of race, sex and age to ensure sufficient representation of high-risk groups. To these data, we added measurement of these biomarkers in 294 VTE cases. For analyses conducted in this case-cohort sample we excluded participants missing lab data (Table 3.6., Supplemental Table S3.2.).

Laboratory:

In the full cohort, serum creatinine was measured using an isotope-dilution mass spectrometry-traceable method using the Vitros 950IRC instrument (Johnson & Johnson Clinical Diagnostics, Rochester, NY), with a coefficient of variation (CV) of 1.1%. Serum cystatin C was measured with high sensitivity particle-enhanced immunonephelometry (N Latex Cystatin C on the BNII, Dade Behring, Deerfield, IL), with an intra-assay CV of 2.0-2.8% and an inter-assay CV of 2.3-3.1%. CRP was
measured by particle enhanced immunonephelometry using the BNII nephelometer (N High Sensitivity CRP, Dade Behring, Deerfield, IL) with inter-assay CV of 2.1–5.7%. [35]

For this VTE case-cohort study, D-dimer and FVIII were measured in 2015 in VTE cases and harmonized with measurements made previously in the CRS in 2012. The CRS D-dimer was run in plasma using an immunoturbidometric assay on the STA-R analyzer (Diagnostica Stago, Asnières sur Seine, France; inter-assay CV 3.2–27.1%) and VTE cases were run on the STA-R Evolution analyzer (Diagnostica Stago, Asnières sur Seine, France; inter-assay CV 1.5–21.7%). FVIII was measured using an ELISA with a CV of 4-7% (Enzyme Research Laboratories, South Bend, IN), with units of % of normal.

Given that analytes in cases and the cohort sample were measured at two different times, and for D-dimer with two different analyzers, we evaluated for analytical drift in assay results using three approaches (Table 3.7., Supplemental Table S3.2.). We detected a laboratory shift in D-dimer values after the introduction of a new analyzer in 2014, which was verified in a set of experiments. There was no analytic drift observed for FVIII, however, the correlation between analytes in 2012 and 2015 was weaker, which we attributed to higher analytical CVs. To harmonize results, we adjusted the D-dimer values for VTE cases down by 0.05 µg/mL.
**Statistical analysis:**

In this report, we examine both the potential of mediation for selected biomarker and effect modification by CKD status.

For the mediation analysis, we compared baseline characteristics among participants with VTE and those in the cohort sample using chi-squared tests and t-tests as appropriate. D-dimer and CRP were highly skewed and thus natural log transformation was used for the analyses. We present back-transformed (geometric) means (95% CI) of log transformed variables in the results. We used linear regression to study the cross-sectional association of eGFR and biomarker levels (D-dimer, FVIII and CRP). We adjusted for demographics (age, sex, race and region of residence), and covariates that are known to be related to these biomarkers: BMI, hypertension, hyperlipidemia, diabetes, history of cardiovascular disease and smoking. Cox proportional hazards models were used to determine the hazard ratio (HR) for VTE per SD increment of each biomarker. Those without an event or who died of non-VTE related causes were censored at that time or at the time of last follow-up, whichever occurred first. We accounted for population weighting of the CRS using the Barlow method.[36] A base model adjusted for age, sex, race, region and BMI. We calculated the adjusted HR of VTE per 10-unit lower eGFR, then added each biomarker separately to the base model. To estimate the magnitude of the potential mediation of the association of CKD with VTE by each biomarker we calculated the percent attenuation of the HR: Percent attenuation = 100% x (HR without biomarker – HR with biomarker) /(HR without biomarker – 1). The 95% CI of the percent attenuation of the HR for eGFR and VTE was calculated using
bootstrapping with replacement with 1000 replicate samples. If the percent mediation was greater than 100% we reset that value to 100%. To verify that our results from the case-cohort study were representative of the full REGARDS study, we performed a sensitivity analysis by repeating the same analysis with CRP, which was measured in the entire cohort, and presented these results for qualitative comparison.

To study effect modification by CKD status, in the full cohort we obtained risk estimates for established VTE risk factors and protect medication use from stratified Cox models with CKD as the stratification variable. We formally tested for an interaction between each protective factor and CKD using interaction terms, interpreted as significant if p interaction <0.10. Models were adjusted for age, sex, race, region and BMI.

Violations of the proportional hazards assumption were tested visually and with Schoenfeld tests. For the primary predictor (eGFR), non-proportionality was further tested with an interaction term, eGFR x time, and HR were estimated at various time points. For FVIII, Schoenfeld testing demonstrated significant violation of the proportional hazards assumption but this was not confirmed with observed vs predicted plots or smoothed estimates of the log HR. All statistical analyses were performed using Stata software version 14 (StataCorp LP, College Station, TX) and SAS version 9.4 (SAS Institute Inc., Cary, NC).
3.4. Results

The case-cohort sample included 1,467 participants, with 386 being VTE cases. Among these, after excluding those with missing measures of kidney function (n=88), study biomarkers (n=9), receiving dialysis (n=9), or who reported pre-baseline VTE (n= 143), there were 1,233 participants in the case-cohort sample (294 VTE cases) (Table 3.6., Supplemental Table S3.1.).

The VTE cases were more likely to be male and white compared to non-cases. Comorbid conditions were similar between cases and non-cases other than BMI which was higher in cases. The eGFR was lower in VTE cases than the cohort sample, and markers of inflammation and procoagulation were higher (Table 3.1.).

Lower eGFR was associated with higher levels of biomarkers of inflammation and procoagulation. In the cohort sample, adjusting for demographics and cardiovascular risk factors, each 10 ml/min/1.73m² lower eGFR was associated with a higher level of natural log (ln) D-dimer 0.05 µg/mL (95% CI 0.01 0.08), Factor VIII 6.6% (95% CI 4.5, 8.4%) and ln-CRP 0.08 mg/L (95% CI 0.04, 0.15) (Table 3.2.). The adjusted HRs of VTE were 1.69 (95% CI 1.49, 2.02) per SD higher ln-D-dimer, 2.23 (95% CI 1.98, 2.62) per SD higher FVIII and 1.29 (1.09, 1.52) per SD higher ln-CRP (Table 3.3.).

Mean follow-up time was 4.3 years. The eGFR was inversely associated with VTE risk. For each 10 ml/min/1.73m² lower eGFR, the HR (95% CI) for VTE was 1.13 (1.02-1.25), after adjusting for demographics and VTE risk factors. After adjusting for ln-D-dimer, Factor VIII and ln-CRP individually, the HRs (95% CI) for VTE were attenuated 1.10 (0.99, 1.21), 0.98 (0.87, 1.11) and 1.11 (1.00, 1.22) respectively (Table

84
3.4). Including all three biomarkers the HR was 0.99 (0.88-1.11). The percent attenuation of the HRs (95% CI) were 23% (5,100%) for ln-D-dimer, >100% (50, 100%) for Factor VIII, 15% (2, 84%) for ln-CRP and >100% (44, 100%) for all three biomarkers (Figure 3.1.).

The proportional hazards assumption may have been violated for eGFR and FVIII. Stratification of FVIII at median FVIII values demonstrated proportional hazards for VTE. There was a statistically significant interaction between eGFR and time, thus we modeled the HR for VTE at 1, 3 and 5 years follow up. The association of eGFR and VTE decreased over time but attenuation by FVIII was similar across follow up time periods. (Table 3.8., Supplemental Table S3.3.).

In a sensitivity analysis utilizing the full cohort, for each 10 ml/min/1.73m$^2$ decrease in eGFR the age, sex, race and BMI-adjusted HR (95% CI) of VTE was 1.09 (1.02, 1.16). After the addition of ln-CRP the HR (95% CI) was 1.07 (1.00, 1.15), for a percent attenuation (95% CI) of 22% (5-69%).

The association of mitigating factors for risk of VTE were assessed in the full cohort, and stratified by CKD status, with results shown in Table 3.5.. Statin use was associated with a reduced risk of VTE; the HR was 0.68 (95% CI 0.50, 0.93) in the full cohort with a similar HR in the non-CKD group, and a weaker relationship in the CKD group, although this difference was not statistically significant (p interaction of statin use and CKD 0.61). In the full cohort and in those without CKD, normal BMI was associated with a ~50% lower risk of VTE, but there was no association in those with CKD (p interaction BMI and CKD = 0.07). We observed inverse associations of warfarin use and
physical activity with VTE which were similar in those with and without CKD, although
the 95% confidence intervals for these HRs included 1.0. Aspirin use was not associated
with risk of VTE in the full cohort, or in those with or without CKD.

3.5. Discussion

In this prospective study, the association of eGFR with VTE was attenuated
by D-dimer, CRP and in particular Factor VIII. Overall, normal BMI was strongly
protective against VTE, but this relationship was present only for those without CKD,
and was absent in those with CKD. Statin use was associated with reduced risk of VTE
overall, and this association was weaker in those with compared to without CKD,
although this difference was not statistically significant. Warfarin use and physical
activity were similarly associated with reduced risk of VTE in both those with and
without CKD. Baseline aspirin was not associated with lower risk of VTE in either those
with or without CKD.

Our findings suggest that CKD-induced activation of inflammation and
procoagulation are in the causal pathway between eGFR and VTE. We confirmed prior
literature that the studied biomarkers D-dimer[5, 7, 37] FVIII[4, 5, 7] and CRP[38, 39]
are more adverse with worsening kidney function (confirming our rationale to choose
them for this study). We also confirmed that D-dimer,[8] FVIII[10, 18] and CRP[14]
were strongly associated with VTE. We are aware of only one prior study of mediators of
the relationship between eGFR and VTE; The Multiple Environmental and Genetic
Association (MEGA) case-control study included 2,473 patients with recent VTE and
2,936 matched controls in the Netherlands between 1999-2004 and accounted for genetic mutations and confounders such as recent surgery or immobilization.[4] Similar to findings here, adjustment for FVIII or von Willebrand factor (vWF) measured after the VTE fully explained the association of eGFR and VTE, supporting the conclusion of both studies that FVIII and/or vWF may mediate the association of kidney function with VTE. In that study, there was no mediation by anticoagulant factors, including protein C, protein S or antithrombin. One limitation of the MEGA study is that blood samples were collected three months after the VTE, thus biomarkers might have been elevated due to the recent thrombosis, and eGFR might have changed after the VTE as well. VTE may also have caused greater increases in FVIII and vWF in those with versus without CKD, possibly explaining the observed mediation. In our study, kidney function and biomarkers were measured at baseline, prior to the VTE, and participants with previous VTE were excluded from the analyses. Our findings confirmed the MEGA findings, reducing the likelihood that the discussed factors were important limitations in that study, and strengthening a conclusion that FVIII and/or vWF are mediators of this association.

Unlike MEGA, we evaluated inflammation, as measured by CRP, and coagulation activation, measured by D-dimer, as potential mediators of the association of eGFR and VTE. The risk of VTE was attenuated by both biomarkers, but to a lesser extent than by FVIII. An alternative explanation for both studies findings is that these biomarkers are confounders but not mediators of the association of CKD with VTE. We are not aware of other studies addressing these two biomarkers.
Given our finding that FVIII played a larger role in the association of eGFR and VTE than CRP or D-dimer, further discussion is warranted, specifically on the potential role of FVIII as mediator in the association of eGFR with VTE. FVIII is a glycoprotein procofactor that is essential to coagulation. It is produced in liver sinusoidal cells and endothelial cells and circulates bound to vWF in an inactivated form. When blood vessel injury occurs, it separates from vWF and acts a cofactor in the conversion of Factor IX to Factor IXa. When not bound to vWF, FVIII is rapidly catabolized in circulation via LDL-receptor-related protein (LRP), a hepatic clearance receptor that is reduced in kidney disease.[40-42] Our model that included all three biomarkers had similar point estimates to that of the model with just FVIII. One possibility for this is that inflammation as measured by CRP and procoagulation as measured by D-dimer exert their effects upstream, by promoting FVIII activity or by impairing FVIII degradation. Despite the potential joint mechanisms by which inflammation and procoagulation may be increasing the risk of VTE in CKD, Factor VIII appears to play a larger role. FVIII has an established role in the etiology other cardiovascular diseases[43, 44] and in progression of CKD as well[45-47].

As CKD has only recently emerged as a risk factor for VTE, there is little data on primary prevention of VTE in CKD. Here, we studied the potential for lifestyle factors and medications to reduce the risk of VTE in those with and without CKD by determining if there was an interaction between CKD and a protective factor. If we found a factor was associated with reduced risk of VTE in CKD it might elucidate mechanisms of VTE in CKD and point to interventions that could be useful to lower the risk. Our
findings raise hypotheses that warfarin use, statin use and higher physical activity might mitigate VTE risk in those with CKD, although power limited our interpretations in some cases leading to confidence intervals that included 1.0. Considering warfarin use, we excluded those who had prior VTE, so participants presumably had a different indication for warfarin, e.g. atrial fibrillation or a mechanical heart valve. The wide confidence intervals (particularly in the CKD group) may be due to low numbers of events, imprecision of the predictor (i.e. baseline use not reflecting use at time of the VTE), under-dosing in CKD (due to concern for bleeding, alterations in non-renal clearance), or difficulties in achieving stable international normalized ratios in CKD. Whether warfarin would be practical in primary prevention of VTE in those with CKD would require a randomized controlled trial to determine, and must take into account the bleeding risk, which might offset any protective effect.[48, 49] Our finding related to statin use confirms prior work[24, 50] and suggests further study of statins (which do not cause bleeding) in VTE prevention in CKD patients. Our finding of no association of regular aspirin use with VTE risk agree with prior research.[51] An association of increased physical activity with lower VTE risk has been reported previously[20, 21] further study of its role in VTE prevention seems worthy. In contrast, our findings suggest that attaining a normal BMI might lower VTE risk in those without CKD, but not in those with CKD. One hypothesis to explain this is that the inflammatory or pro-coagulant pathways of CKD outweigh any impact that normal BMI might have in reducing VTE risk in CKD. Alternatively, lower BMI in CKD may be due to unmeasured confounding illness, which would mask a protective effect of lower BMI for VTE.
There are several strengths to this study. This was a prospective study where kidney function and biomarker levels were measured prior to onset of VTE, and this might better elucidate mechanistic pathways of CKD-related VTE risk. The study was a contemporary cohort that reflects current use of statins and trends in obesity and diabetes relevant to the U.S. population. We had excellent representation of white and black participants, unlike many studies of VTE epidemiology. In addition, we evaluated the role of lifestyle factors in risk of VTE stratified by CKD status, to attempt to develop hypotheses for how CKD patients could reduce their risk of VTE.

There are limitations of this study to consider. The biomarkers were measured at baseline which may have led to misclassification if biomarker level changed prior to the VTE. The biomarkers in cases were measured later than the cohort random sample, and in some cases with different analyzers, but we took several steps to harmonize the data. Also, we were unable to determine whether low eGFR preceded or was the result of inflammation and procoagulation. It is possible that inflammation and procoagulation cause both CKD and VTE through independent pathways. That is, misclassification of the mediator, unknown confounding between mediator and outcome and interaction between mediator and exposure are potential limitations. We demonstrated that the association of eGFR and VTE decreased with time, which may have led to underestimation of the effect size and the mediation by biomarkers. Due the blood sample processing methods in REGARDS we were unable to evaluate the role of platelet function, proteins released by platelets or vWF,[30] all of which may be other factors linking CKD with VTE. Warfarin is known to reduce the risk of VTE but the
association was weaker than might be expected in our study. Reasons for this could be insufficient power to detect this association given only 3% of the cohort reported warfarin use or failure of baseline use to reliably indicate continuous use up to the time of VTE. Direct oral anticoagulants could not be evaluated here due to the time when the study was performed. Finally, although we adjusted for established VTE risk factors, there may be other factors not considered that would lead to residual confounding.

### 3.6. Conclusion

In this study, markers of inflammation and procoagulation that are associated with both lower eGFR and VTE may mediate the association of eGFR and VTE. Normal BMI was protective against VTE but not in participants with CKD. Higher physical activity, statin and warfarin use mitigated risk of VTE in those with and without CKD, offering possible targets for intervention studies. Additional studies are necessary to confirm whether biomarkers like FVIII might serve as surrogate markers in trials to reduce the risk of VTE in CKD.
Table 3.1.: Baseline characteristics of REGARDS case-cohort participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No VTE N=939</th>
<th>VTE N=294</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68 ± 12</td>
<td>69 ± 8</td>
</tr>
<tr>
<td>Female</td>
<td>49</td>
<td>41</td>
</tr>
<tr>
<td>Black</td>
<td>49</td>
<td>38</td>
</tr>
<tr>
<td>Southeast</td>
<td>52</td>
<td>53</td>
</tr>
<tr>
<td>Hypertension</td>
<td>60</td>
<td>64</td>
</tr>
<tr>
<td>Diabetes</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>41</td>
<td>41</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>.0</td>
<td>28.7 ± 5.8</td>
<td>30.2 ± 5.7</td>
</tr>
<tr>
<td>Physical activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 0 x week</td>
<td>35</td>
<td>41</td>
</tr>
<tr>
<td>- 1-3 x week</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>- 4+ x week</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Current</td>
<td>15%</td>
<td>12%</td>
</tr>
<tr>
<td>- Never</td>
<td>48%</td>
<td>42%</td>
</tr>
<tr>
<td>- Past</td>
<td>37%</td>
<td>46%</td>
</tr>
<tr>
<td>eGFR ml/min/1.73m²</td>
<td>87 ± 23</td>
<td>80 ± 21</td>
</tr>
<tr>
<td>D-dimer (median, IQR) µg/ml</td>
<td>0.5 (0.3-0.9)</td>
<td>0.7 (0.4 - 1.2)</td>
</tr>
<tr>
<td>FVIII %</td>
<td>124 ± 45</td>
<td>165 ± 60</td>
</tr>
<tr>
<td>CRP (median, IQR) mg/L</td>
<td>2.1 (0.9- 4.9)</td>
<td>2.7 (3.1 - 6.1)</td>
</tr>
</tbody>
</table>

Abbreviations: BMI body mass index. eGFR estimated glomerular filtration rate. FVIII Factor VIII. CRP C-reactive protein. Continuous variables are presented as mean ± standard deviation (SD) unless noted. Frequency are presented as percentages unless noted.
Table 3.2.: Association of eGFR and biomarkers of inflammation and procoagulation in the cohort sample

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Difference in biomarker (95% CI) per 10 ml/min/1.73m² lower eGFR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>D-dimer (µg/ml)</td>
<td>0.12 (0.09, 0.15)</td>
</tr>
<tr>
<td>FVIII (%)</td>
<td>5.8 (4.5, 7.1)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.07 (0.03, 0.11)</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; FVIII, Factor VIII; CRP, C-reactive protein. Linear regression models were adjusted for age, sex, race, region, BMI, hypertension, diabetes, hyperlipidemia, cardiovascular disease, smoking. D-dimer and CRP were highly skewed and were log transformed.
### Table 3.3.: Association of biomarkers and risk of VTE

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Unadjusted HR (95% CI)</th>
<th>Adjusted HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer</td>
<td>1.77 (1.51, 2.07)</td>
<td>1.69 (1.41, 2.02)</td>
</tr>
<tr>
<td>FVIII</td>
<td>2.14 (1.80, 2.53)</td>
<td>2.23 (1.89, 2.62)</td>
</tr>
<tr>
<td>CRP</td>
<td>1.34 (1.16, 1.56)</td>
<td>1.29 (1.09, 1.52)</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval; SD, standard deviation; FVIII, Factor VIII; CRP, C-reactive protein. Cox models were adjusted for age, sex, race, region, race*region and BMI. D-dimer and CRP were log transformed.
Table 3.4.: Association of eGFR and incident VTE with adjustment for biomarkers of procoagulation and inflammation

<table>
<thead>
<tr>
<th>Model</th>
<th>HR (95% CI) of VTE per 10-unit lower eGFR</th>
<th>HR (95% CI) of VTE by quartile of eGFR (ml/min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Q1 (7≤71)</td>
</tr>
<tr>
<td><strong>Case-Cohort Study</strong></td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>Cases (n)</td>
<td></td>
<td>233</td>
</tr>
<tr>
<td>Cohort sample (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base Model*</td>
<td>1.13 (1.02, 1.25)</td>
<td>1.39 (0.77, 2.49)</td>
</tr>
<tr>
<td>Base Model + D-dimer</td>
<td>1.10 (0.99, 1.21)</td>
<td>1.12 (0.62, 2.05)</td>
</tr>
<tr>
<td>Base Model + FVIII</td>
<td>0.98 (0.87, 1.11)</td>
<td>0.69 (0.35, 1.37)</td>
</tr>
<tr>
<td>Base Model + CRP</td>
<td>1.11 (1.00, 1.22)</td>
<td>1.24 (0.68, 2.24)</td>
</tr>
<tr>
<td><strong>Full Cohort</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base Model</td>
<td>1.09 (1.02, 1.16)</td>
<td>1.46 (0.91, 2.36)</td>
</tr>
<tr>
<td>Base Model + CRP</td>
<td>1.07 (1.00, 1.15)</td>
<td>1.36 (0.84, 2.19)</td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval; Q, quartile; FVIII, Factor VIII; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate. eGFR as a predictor is presented as a continuous variable (HR per 10 ml/min/1.73m² decrement) and as quartiles with reference group as the highest quartile of eGFR. Cox models were adjusted for age, sex, race, region, race*region and BMI (Base Model*) and biomarkers individually. D-dimer and CRP were log transformed.
Table 3.5.: The association of lifestyle factors and medications with the risk of VTE, stratified by CKD status

<table>
<thead>
<tr>
<th></th>
<th>Overall (n = 25,936)</th>
<th>CKD (n = 2,473)</th>
<th>No CKD (n = 23,463)</th>
<th>P interaction of Protective Factor x CKD Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regular Aspirin Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>124</td>
<td>22</td>
<td>102</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>11,059</td>
<td>1,341</td>
<td>9,718</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.08 (0.81, 1.43)</td>
<td>0.96 (0.51, 1.79)</td>
<td>1.07 (0.80, 1.41)</td>
<td>0.64</td>
</tr>
<tr>
<td><strong>Statin Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>70</td>
<td>16</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>8,005</td>
<td>1,078</td>
<td>6,927</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.68 (0.50, 0.93)</td>
<td>0.86 (0.46, 1.64)</td>
<td>0.67 (0.49, 0.93)</td>
<td>0.61</td>
</tr>
<tr>
<td><strong>Warfarin Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>704</td>
<td>177</td>
<td>527</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.30 (0.07, 1.21)</td>
<td>0.70 (0.17, 2.94)</td>
<td>0.29 (0.07, 1.19)</td>
<td>0.41</td>
</tr>
<tr>
<td><strong>Physical activity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 x/week vs none</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>83</td>
<td>12</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>9,214</td>
<td>700</td>
<td>8,514</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.82 (0.59, 1.14)</td>
<td>0.88 (0.43, 1.81)</td>
<td>0.82 (0.59, 1.14)</td>
<td>0.98</td>
</tr>
<tr>
<td>4+/week vs none</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>66</td>
<td>8</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>7,622</td>
<td>539</td>
<td>7,083</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.76 (0.53, 1.07)</td>
<td>0.73 (0.32, 1.67)</td>
<td>0.76 (0.53, 1.08)</td>
<td></td>
</tr>
<tr>
<td><strong>BMI&lt;25 vs ≥25</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N cases</td>
<td>41</td>
<td>10</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>N non-cases</td>
<td>6,410</td>
<td>545</td>
<td>5,865</td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>0.48 (0.32, 0.70)</td>
<td>1.07 (0.51, 2.22)</td>
<td>0.47 (0.32, 0.70)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

CKD is defined as eGFR < 60 ml/min/1.73m². Models were adjusted for age, sex, race, region, race*region, BMI.
Table 3.6.: (Supplemental Table S3.1): Exclusion criteria

<table>
<thead>
<tr>
<th>Exclusion Criteria</th>
<th>Cohort Random Sample N=1,081</th>
<th>VTE cases N=386</th>
<th>Case-Cohort Sample N=1,467</th>
<th>Full Cohort N=30,239</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missing follow up</td>
<td>-684</td>
<td>-16</td>
<td>-88</td>
<td>-1767</td>
</tr>
<tr>
<td>Missing renal function</td>
<td>-72</td>
<td>-16</td>
<td>-88</td>
<td>-1767</td>
</tr>
<tr>
<td>Missing biomarker</td>
<td>-6</td>
<td>-3</td>
<td>-9</td>
<td>n/a</td>
</tr>
<tr>
<td>Receiving dialysis</td>
<td>-3</td>
<td>-5</td>
<td>-8</td>
<td>-113</td>
</tr>
<tr>
<td>Baseline VTE</td>
<td>-61</td>
<td>-68</td>
<td>-129</td>
<td>-1739</td>
</tr>
<tr>
<td>Final Cohort</td>
<td>939</td>
<td>294</td>
<td>1,233</td>
<td>25,936</td>
</tr>
</tbody>
</table>
Table 3.7.: (Supplemental Table S3.2): Methodological approaches to studying analytic drift in REGARDS case-cohort study of VTE risk

<table>
<thead>
<tr>
<th>Approach</th>
<th>Sample set</th>
<th>Duplicates</th>
<th>LITE study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description</td>
<td>Purchased set of donor samples are run along with each new set of analytes (FVIII and D-dimer in 2012 and 2015 with CRS and VTE cases, respectively)</td>
<td>FVIII and D-dimer were run in the CRS in 2012. All VTE cases were run in 2015. Some participants of the CRS developed VTE and had duplicate analytes in 2015.</td>
<td>D-dimer only. Two analyzers were used (Sta-R and Evolution). Repeat measures performed in 888 samples on both analyzers in 2014 over 5-day period.</td>
</tr>
<tr>
<td>Number of analytes</td>
<td>20</td>
<td>59</td>
<td>700</td>
</tr>
<tr>
<td>Results</td>
<td>-D-dimer was 0.10 µg/ml higher in 2015 vs 2012. Mean CV was 39.7% (range 5.4-73.9%). No difference comparing freeze thaw cycles x1 vs x5 in 2015 (r = 0.99; R² = 0.98).</td>
<td>-D-dimer was 0.06 µg/ml higher in 2015 (Evolution) vs 2012 (Sta-R). r = 1.00; R² = 0.97</td>
<td>-D-dimer was 0.05 µg/ml higher in Evolution vs Sta-R). r = 0.97; R² = 0.93.</td>
</tr>
<tr>
<td></td>
<td>-FVIII levels did not change over time. Mean CV 6.7% (5.11-21.30%).</td>
<td>-mean FVIII 26% higher in 2015. r = 0.84; R² = 0.71</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FVIII, Factor VIII; CRS, cohort random sample; vs, versus; r, correlation coefficient; R², coefficient of determination; LITE, Longitudinal Investigation of Thromboembolism Etiology study.
Table 3.8.: (Supplemental Table S3.3): Association of eGFR and VTE at 1, 3, 5 years follow up

<table>
<thead>
<tr>
<th>Case-Cohort</th>
<th>N cases, cumulative</th>
<th>Year 1</th>
<th>Year 3</th>
<th>Year 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base model</td>
<td>26</td>
<td>1.23 (1.11, 1.37)</td>
<td>1.13 (1.05, 1.22)</td>
<td>1.04 (0.95, 1.14)</td>
</tr>
<tr>
<td>Base + FVIII</td>
<td>1.10 (0.94, 1.29)</td>
<td>1.00 (0.89, 1.13)</td>
<td>0.91 (0.79, 1.06)</td>
<td></td>
</tr>
<tr>
<td>Full Cohort*</td>
<td>N cases, cumulative</td>
<td>25</td>
<td>108</td>
<td>250</td>
</tr>
<tr>
<td>Base model</td>
<td>1.18 (1.06, 1.31)</td>
<td>1.10 (1.03, 1.18)</td>
<td>1.03 (0.94, 1.12)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: HR, hazard ratio; CI, confidence interval; FVIII, Factor VIII; eGFR, estimated glomerular filtration rate. Base model includes age, sex, race, region, race*region and BMI. *FVIII was not measured in the full cohort.
Figure 3.1.: Potential mediators of the association of eGFR and Venous Thromboembolism: Role of biomarkers of inflammation and procoagulation

- FVIII 100% (50-100%)
- D-Dimer 23% (5-100%)
- CRP 22% (5-69%)

Inflammation and procoagulation

CKD

Unexplained factors

VTE
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