Thrombosis Research 139 (2016) 44–49

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Prospective study of γ′ fibrinogen and incident venous thromboembolism: The Longitudinal Investigation of Thromboembolism Etiology (LITE)

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ARTICLE INFO

Article history:
Received 29 October 2015
Received in revised form 15 December 2015
Accepted 10 January 2016
Available online 12 January 2016

Keywords:
Venous thrombosis
Pulmonary embolus
Fibrinogen
Fibrinogen gamma
Prospective study

ABSTRACT

Introduction: Epidemiological studies generally have not found plasma total fibrinogen to be a risk factor for venous thromboembolism (VTE), but several have reported associations between variants in the fibrinogen gamma gene (*FGG*) and VTE. A case-control study in whites suggested plasma γ′ fibrinogen concentration may be associated inversely with VTE, but this was not replicated in African Americans.
Objective: To examine the prospective association between γ′ fibrinogen concentrations and occurrence of VTE.
Methods: We used the Longitudinal Investigation of Thromboembolism Etiology (LITE), involving two pooled population-based cohorts in the United States including 16,234 participants. The cohorts comprised white and African American men and women, aged 50 years and older at study onset in the early 1990s. We identified VTEs during follow-up and documented they met standardized diagnostic criteria.
Results: During two decades of follow-up, neither γ′ fibrinogen nor total fibrinogen nor their ratio was associated with VTE overall (n = 521 VTEs), in subgroups defined by race, or in other subgroups. In both race groups, the minor allele of *FGG* rs2066865 was associated with lower γ′ fibrinogen concentrations, but this allele was not associated with VTE.
Conclusions: A lower plasma concentration of γ′ fibrinogen in healthy adults does not appear to increase VTE risk.
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1. Introduction

Epidemiological studies have implicated a number of circulating procoagulant factors in the etiology of venous thromboembolism (VTE), that is, deep vein thrombosis (DVT) and pulmonary embolism (PE) [1]. However, existing studies generally have not found the concentration of fibrinogen associated with VTE [1]. This conclusion contrasts with consistent evidence that strongly associates higher fibrinogen concentrations with increased atherothrombotic events [1], although Mendelian randomization studies suggest the atherothrombotic association may not be causal [2,3].

Fibrinogen contains two copies each of Aα, Bβ, and γ chains. The γ chain, produced by the fibrinogen gamma chain gene (*FGG*), has two isoforms, γA and γ′. γ′ fibrinogen comprises approximately 10% of total plasma fibrinogen in the plasma, but the proportion varies among individuals and rises during an acute phase response. In the presence of factor XIII, clots made from γ′ fibrinogen are more resistant to lysis than normal clots; therefore the proportion of γ′ fibrinogen was hypothesized to be a risk factor for arterial thrombosis [4]. The Framingham Study reported that plasma γ′ fibrinogen was associated positively with prevalent arterial cardiovascular disease (odds ratio = 1.5 for the highest versus lowest γ′ fibrinogen tertiles), independent of total fibrinogen [5].

Despite a possible positive association of γ′ fibrinogen with atherothrombotic events in Framingham and several other epidemiological studies [4], studies of VTE have mostly reported the opposite association. For example, the Leiden Thrombophilia Study reported that having a *FGG*-H2 haplotype, which was strongly related to lower circulating γ′ fibrinogen, doubled the risk of VTE. Correspondingly,

Abbreviations: ARIC, Atherosclerosis Risk in Communities; BMI, body mass index; CHS, Cardiovascular Health Study; DVT, deep vein thrombosis; ELISA, enzyme-linked immunosorbent assay; *FGG*, fibrinogen gamma chain gene; GWAS, genome-wide association studies; HR, hazard ratio; LITE, Longitudinal Investigation of Thromboembolism Etiology; PE, pulmonary embolism; SD, standard deviation; SNP, single nucleotide polymorphism; VTE, venous thromboembolism.

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http://dx.doi.org/10.1016/j.thromres.2016.01.008
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plasma γ′ fibrinogen (and the ratio of γ′ fibrinogen to total fibrinogen) were associated negatively with VTE, while plasma total fibrinogen was associated positively with VTE [6]. Another study reported that the minor allele of the *FGG* single nucleotide polymorphism (SNP) rs1049636, which is associated with increased mean γ′ fibrinogen levels, is associated with decreased risk of VTE [7].

Supporting a potential etiological role for lower γ′ fibrinogen in increasing VTE risk, three [8–10] of five [8–12] genome-wide association studies (GWAS) and some candidate-gene studies [6,13–15] have linked SNPs in *FGG* to VTE risk in whites. Our GWAS consortium of VTE found the top *FGG* SNP to be rs6536024 [8], which is in modest linkage disequilibrium ($r^2 = 0.25–0.53$) with *FGG* variants linked to VTE in other studies, namely, three tightly linked SNPs (rs7659024, rs2066865, and rs2066854, $r^2 = 1.0$ among them) and rs1049636 [6,7,9,10,13–15]. The minor allele of rs2066865 seems key to the *FGG*-H2 haplotype [6,16].

We recently measured γ′ fibrinogen in the entire study population of the Longitudinal Investigation of Thromboembolism Etiology (LITE) in order to examine the associations of γ′ fibrinogen concentrations and total fibrinogen with VTE occurrence. Our hypothesis was that γ′ fibrinogen concentration would be associated with VTE incidence. In the Atherosclerosis Risk in Communities (ARIC) Study, we also examined the association of a top *FGG* genetic variant (rs2066865) with incidence of VTE. The novel aspects of our study are that it is the first prospective study of γ′ fibrinogen and VTE, as well as its large size, biracial sample, wide age range, and long follow-up.

2. Methods

2.1. Study population

The LITE study is a prospective study of VTE occurrence in 2 pooled, multi-center, longitudinal population-based cohort studies: the ARIC Study [17] and the Cardiovascular Health Study (CHS) [18]. We reported the LITE study design, methods, and VTE incidence rates in detail elsewhere [19,20]. In brief, 15,792 men and women aged 45 to 64 years enrolled in the ARIC study in 1987–1989, and had subsequent examinations in 1990–92, 1993–95, 1996–98, and 2011–13, along with annual telephone contact. In CHS, 5201 men and women aged ≥65 years enrolled in 1989–1990. In 1992–1993, CHS recruited 687 new African American participants. CHS contacted participants every six months for follow-up, alternating between a telephone interview and clinic visit for the first 10 years and by telephone interview only after that. The institutional review committees at each study center approved the methods and staff obtained informed participant consent.

2.2. Plasma total and γ′ fibrinogen measurements and FGG genotyping

ARIC and CHS had measured plasma total fibrinogen at participants′ baseline visits using the method of Clauss [21]. In addition, ARIC had remeasured total fibrinogen in a stratified sample of participants (n = 999) in 1993–95. Because total fibrinogen was not associated with VTE in an early LITE analysis [20], we did not remeasure total fibrinogen along with γ′ fibrinogen, and used the baseline value of total fibrinogen for this report.

By the time we undertook γ′ fibrinogen measurement in 2014, ARIC and CHS had exhausted most baseline citrate plasma samples. Therefore, we measured γ′ fibrinogen concentrations on fasting citrate plasma collected in ARIC in 1993–95 (6 years after baseline) and CHS in 1992–93 (3 years after baseline for the original cohort and at baseline for the African American supplemental cohort) and stored unthawed at −70 °C until analysis in 2014. The Laboratory for Clinical Biochemistry Research at the University of Vermont used the assay developed by Lovely et al. [22], made available by Gamma Therapeutics (Portland, OR). It is a standard sandwich enzyme-linked immunosorbent assay (ELISA) using anti-γ′ monoclonal antibody. The coefficient of variation for control samples averages 10.3%.

Because of the large number of samples, requiring 12 months of laboratory measurement for γ′ fibrinogen, and based on priorities, the laboratory first analyzed ARIC samples from the three study centers other than the Jackson, MS center, then the CHS samples, and finally the ARIC Jackson center. In addition to the laboratory's standard assay quality assurance procedures, we instituted two other quality checks on γ′ fibrinogen measurement. First, ARIC included blinded duplicate samples split at the time of blood draw to check on reliability. Second, the laboratory included a normal pool to check for long-term drift. The analysis of 75 split specimen pairs during the early ARIC phase yielded a coefficient of variation of 27% for the first specimen in each pair and an intra-class reliability coefficient of 0.59, and the measured mean on a normal pool showed a downward drift of 31% over the time the early assays were run. The laboratory attributed this drift to its learning curve with the γ′ fibrinogen assay and the pipette technique for it. For the later CHS and Jackson, MS center periods, the normal pool showed no significant drift and the reliability coefficient for Jackson samples was 0.79 (n = 99 pairs). We therefore used the normal pool results to adjust participants' γ′ fibrinogen concentrations from the earlier period to the later period, done by multiplying the observed lab values by the ratio of the mean γ′ fibrinogen levels in the normal pool during the later period to mean γ′ fibrinogen levels in the normal pool during the earlier period. Prior to adjustment, we excluded 61 values in ARIC and 10 in CHS that were above the limit of quantification (>400 mg/dL). Samples that were below the limit of detection (8 in ARIC and 2 in CHS) were set equal to the limit. The mean ± standard deviation (SD) γ′ fibrinogen level in ARIC was 35.8 ± 10.6 mg/dL before adjustment and 30.8 ± 9.0 mg/dL after.

The ARIC DNA Laboratory at the University of Texas-Houston genotyped rs2066865 with the iPLEX multiplex assay which utilizes the MassARRAY system (Sequenom, Inc., San Diego, CA).

2.3. Measurement of risk factors

We analyzed risk factors for VTE in LITE, measured at the ARIC or CHS visits in which γ′ fibrinogen was measured. We calculated body mass index as weight (kg)/height $(m)^2$. We defined diabetes as a fasting blood glucose of 126 mg/dl or higher, non-fasting blood glucose of 200 mg/dl or higher, a physician diagnosis of diabetes, or use of antidiabetic medication in the past 2 weeks. Participants reported smoking status, and women reported whether or not they were taking hormone replacement therapy.

2.4. VTE occurrence

Staff contacted ARIC and CHS participants annually or semi-annually by phone and asked about all hospitalizations in the previous year. They retrieved hospital records for possible VTE events through 2011 in ARIC and through 2001 in CHS. To validate VTE events, two physicians reviewed the records using standardized criteria [19], requiring positive imaging tests for diagnosis of DVT and PE. We restricted DVTs for this analysis to those in the lower extremity or vena cava, because upper extremity DVTs were relatively few and almost always the result of venous catheters. The reviewers sub-classified VTEs as unprovoked (no obvious cause) or provoked (associated with cancer, major trauma, surgery, marked immobility).

2.5. Statistical analysis

Of the 12,887 ARIC and 5265 CHS participants who attended the relevant exam, we excluded those not white or African American (n = 38 ARIC, 34 CHS), those with a VTE prior to γ′ fibrinogen assessment (n = 302 ARIC, 323 CHS), those taking anticoagulants (n = 124 ARIC, 107 CHS), those without γ′ fibrinogen measurement (n = 355 ARIC, 633

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