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Genetic variation in *F3* (tissue factor) and the risk of incident venous thrombosis: meta-analysis of 8 studies

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Keywords

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The coagulation factor III gene (*F3*) produces tissue factor (TF), a cell-surface glycoprotein that is expressed throughout the body including inside the vascular wall; it also circulates in microparticles.¹ Tissue factor and its activated cofactor, coagulation factor VII (FVIIa), initiate the human extrinsic coagulation cascade.² The TF-FVIIa complex is also involved in other biologic processes such as inflammation, angiogenesis, and tumor growth.^{3, 4} The *F3* locus was 1 of 3 associated with plasma levels of fibrin fragment D-dimer in a recent genome-wide association study.⁵ The *F3* rs12029080 was associated with the smallest p-value (6.4×10^{-22}) at this locus and is located 46.0 kb upstream from the start of

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transcription. Each copy of the G allele was associated with a 10.4% (genome-wide confidence interval [CI]: 6.6-14.3%) increase in median D-dimer level. The *F3* association was novel and suggests that TF has a role in coagulation propagation and possibly thrombotic risk beyond its well characterized role in coagulation initiation. We hypothesize that the G allele of the *F3* rs12029080 variant, which was associated with elevated plasma D-dimer levels, would also be associated with an increased risk of incident VT.

A meta-analysis of 9 datasets from 8 epidemiologic studies of incident VT was performed. The 8 studies included the Heart and Vascular Health Study,⁶ the Longitudinal Investigation of Thromboembolism Etiology (LITE),⁷ the Marseille Thrombosis Association (MARTHA) Study,⁸ a second French case-control study on early-onset VT (EOVT),⁹ the Mayo VTE Study,¹⁰ the Rotterdam Study,¹¹ and the Women's Genome Health Study.¹² The LITE study included data from 2 large cohort studies, the Cardiovascular Health Study and the Atherosclerosis Risk in Communities Study.^{13, 14} Each study used regression methods to characterize the association between rs12029080 and incident VT in European-ancestry participants using an additive genetic model that adjusted for age, sex, and study-design variables. The resultant coefficients and standard errors from the 9 association-studies were meta-analyzed using fixed-effect inverse-variance weights. A 1-sided level of 0.05 determined statistical significance. Given the positive association between the rs12029080 G-allele and D-dimer levels, a negative beta-coefficient between the G-allele and VT risk (representing a decreased risk of VT) would not be considered a significant finding.

Characteristics of the studies and populations are provided in the Table. There were 5117 incident VT events across the studies, 1809 (35%) were for a PE, with or without DVT, and 2731 (53%) were diagnosed in the absence of a recent hospitalization (90 days before) or cancer (within 5 years). The average age of the subject at the time of VT onset was 54.1 years and 34% of the events were among men. (The HVH and WGHS were exclusively women.) Results are also provided in the Table. The average minor allele frequency of the rs12029080 G allele was 0.299 (range 0.286-0.317). The inverse-variance weighted beta-coefficient was 0.026 (range -0.108 to 0.199) with a standard error of 0.029 (range 0.060 to 0.353). Expressed as an odds ratio with 1-sided 95% confidence intervals, the coefficient for the G-allele was not associated with a significantly increased risk of incident VT: 1.03 (95% CI: 0.98-1.08); p-value = 0.190. The Q-statistic for the heterogeneity test across studies was 10.6 and yielded a p-value of 0.22. In 2 of the larger studies (HVH and MARTHA), we estimated the risk in idiopathic cases and found no meaningful difference in risk estimates or consistency of the risk estimates for idiopathic cases.

Our inability to find strong evidence for an association between the G allele of *F3* rs12029080 and an increased risk of incident VT stands apart from our findings when we investigated other genetic loci that predicted plasma D-dimer levels, factor V (*F5*) and fibrinogen alpha chain (*FGA*).⁵ The *F5* Leiden R506Q (rs6025) variant accounted for the signal at the *F5* locus and was associated with a 24% (genome-wide CI: 1.3-52%) difference in median D-dimer level in a subset of the cohorts where this variant was measured. The *FGA* T331A (rs6050) variant, also known as T312A, accounted for the signal at the *FGA* locus and was associated with a 6.3% (genome-wide CI: 2.3-10.4%) difference. The *F5* and *FGA* D-dimer associations had been previously reported and both the rs6025 and rs6050 variants are associated with incident venous thrombosis (VT).¹⁵⁻²⁰

The D-dimer measure quantifies by-products of fibrin clot lysis and is considered a global measure of coagulation. The *F5* Leiden rs6025 variant increases D-dimer levels by inducing resistance to activated protein C thus inhibiting anti-coagulation, promoting fibrin production, and increasing the risk of VT. The *FGA* rs6050 variant is not associated with increased fibrinogen production rather it enhances factor XIIIa cross-linking in fibrin

clots.^{21, 22} The enhanced crosslinking increases the risk of VT but its relation to D-dimer production is not known.

Our hypothesis that the increased D-dimer levels associated with the *F3* rs1209080 variant would also be associated with increased risk of VT was not well supported by our data. There are several possible explanations for this, of which the most likely is that the pathway leading to increased D-dimer production is independent of any pathway leading to an increase in the risk of thrombosis. The *F3*-D-dimer pathway may be more related to fibrinolysis or to constitutional properties of the fibrin clot than to coagulation propensity. It is also possible that the sample-size of this meta-analysis was too limited to detect a small association between *F3* rs1209080 and risk of incident VT. Nonetheless, the upper confidence limit of the odds ratio was 1.08 which suggests that any association would likely be modest and less than an 8% increase in the relative risk. The lack of statistical significance for the heterogeneity test across studies suggests that random variation is a more likely explanation of the range of odds ratios than any true underlying difference in relative risk.

In summary, we did not find that the *F3* rs1209080 variant, which has previously been shown to be associated with D-dimer levels, was also associated with risk of incident VT in participants of European ancestry.

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Table 1

Associations between *F3* rs12029080 G-allele and incident venous thrombosis

	Design	Events, n	Male, %	Age, * yrs	PE, %	Idiopathic, %	MAF	Beta coefficient (SE)	Odds ratio (95% CI)
HVH	CC	656	0	64.7	48	59	0.304	-0.073 (0.092)	0.93 (0.8, 1.08)
EOVT	CC	419	55	36.0	0	100	0.327	0.091 (0.089)	1.10 (0.95, 1.27)
LITE (ARIC)	Cohort	248	47	56.0	42	36	0.299	0.040 (0.097)	1.04 (0.89, 1.22)
LITE (CHS)	Cohort	119	43	73.0	30	39	0.317	0.199 (0.138)	1.22 (0.97, 1.53)
MARTHA	CC	1542	34	47.0	22	55	0.316	0.023 (0.060)	1.02 (0.93, 1.13)
Mayo VTE	CC	1503	50	54.9	50	43	0.299	-0.068 (0.063)	0.93 (0.84, 1.04)
RS-1	Cohort	177	43	69.5	44	53	0.291	-0.108 (0.120)	0.90 (0.74, 1.09)
RS-2	Cohort	19	63	66.3	42	53	0.286	0.161 (0.353)	1.17 (0.66, 2.10)
WGHS	Cohort	434	0	65.4	41	44	0.299	0.173 (0.076)	1.19 (1.05, 1.35)
Combined		5117	34	54.1	35	53	0.299 ^{**}	0.026 (0.029) ^{***}	1.03 (0.89-1.08)

CI = confidence interval; MAF = minor allele frequency; SE = standard error. The Rotterdam Study analyzed 2 subcohorts separately: RS-1 and RS-2.

* Age at the time of the event among case subjects.

** Averaged.

*** Weighted by inverse of variance.