ORIGINAL RESEARCH

Multilocus Genetic Risk Scores for Venous Thromboembolism Risk Assessment

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***Background*—**Genetics plays an important role in venous thromboembolism (VTE). Factor V Leiden (*FVL* or rs6025) and prothrombin gene G20210A (*PT* or rs1799963) are the genetic variants currently tested for VTE risk assessment. We hypothesized that primary VTE risk assessment can be improved by using genetic risk scores with more genetic markers than just *FVL*-rs6025 and prothrombin gene *PT*-rs1799963. To this end, we have designed a new genetic risk score called Thrombo inCode (TiC).

***Methods and Results*—**TiC was evaluated in terms of discrimination (Δ of the area under the receiver operating characteristic curve) and reclassification (integrated discrimination improvement and net reclassification improvement). This evaluation was performed using 2 age- and sex-matched case–control populations: SANTPAU (248 cases, 249 controls) and the Marseille Thrombosis Association study (MARTHA; 477 cases, 477 controls). TiC was compared with other literature-based genetic risk scores. TiC including *F5* rs6025/rs118203906/rs118203905, *F2* rs1799963, *F12* rs1801020, *F13* rs5985, *SERPINC1* rs121909548, and *SERPINA10* rs2232698 plus the A1 blood group (rs8176719, rs7853989, rs8176743, rs8176750) improved the area under the curve compared with a model based only on *F5*-rs6025 and *F2*-rs1799963 in SANTPAU (0.677 versus 0.575, $P<0.001$) and MARTHA (0.605 versus 0.576, $P=0.008$). TiC showed good integrated discrimination improvement of 5.49 ($P<0.001$) for SANTPAU and 0.96 ($P=0.045$) for MARTHA. Among the genetic risk scores evaluated, the proportion of VTE risk variance explained by TiC was the highest.

***Conclusions*—**We conclude that TiC greatly improves prediction of VTE risk compared with other genetic risk scores. TiC should improve prevention, diagnosis, and treatment of VTE. **(*J Am Heart Assoc*. 2014;3:e001060 doi: 10.1161/JAHA.114.001060)**

Key Words: genetics • risk factors • tests • thrombosis • veins

Thrombosis is the formation of a blood clot inside a blood vessel that obstructs the normal flow of blood. The clinical manifestations of thrombosis are myocardial infarction, stroke, and venous thromboembolism (VTE). The latter includes pulmonary embolism and deep vein thrombosis. VTE is a common cardiovascular illness associated with high mortality[1] that affects ≈0.2% of the US and European population annually.[1,2] Consequently, it is a considerable public health concern with a high economic burden.[3,4]

VTE is a multifactorial, complex disease that results from a combination of genetic and acquired risk factors. The heritability of VTE has been estimated at about 60%.[5] The genetic factors underlying the risk of thrombosis include some well-established mutations such as the *FVL* and *PT*

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Accompanying Tables S1 through S3 are available at http://jaha.ahajournals.org/content/3/5/e001060/suppl/DC1

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mutations, which give rise to deficient anticoagulant or gain-of-function proteins.[6] In addition, recent evidence shows several genetic variants that predispose someone to VTE by modifying components in the coagulation pathway.[7–15] Moreover, several common low-penetrance gene variants and unsuspected genes have been identified by genomewide association studies (GWASs) as contributing to a risk of thromboembolic disease.[16–18] These new variants still require proper clinical validation.

This new knowledge of the genetic profile of VTE could be used to increase the ability to more accurately predict the risk of a thrombotic event. In current clinical practice, only *FVL* and *PT* mutations are used as markers to assess a patient's risk of VTE. In a genetic risk score (GRS) described by de Haan et al,[19] 5 of 31 single nucleotide polymorphisms (SNPs) linked to VTE were found to improve the predictive capacity of clinical factors including family history assessment. Moreover, the similar discriminative capacity observed by these authors with the use of 5 of 31 SNPs associated with VTE clearly indicates a need to identify an appropriate panel of genetic variants and to determine the predictive capacity of such a panel as a risk assessment score for this complex disease.

Our study was designed to compare the predictive capacity of a new GRS, Thrombo inCode (TiC), with a risk score based on family history alone and another based on *FVL* and *PT* mutations.

Methods

Study Populations

The SANTPAU case–control study, conducted on a Spanish population, has been described extensively.[20] Briefly, 248 consecutive unrelated patients who had been referred to or had visited the Hospital de la Santa Creu i Sant Pau (Barcelona, Spain) for thrombophilia screening were recruited over the period from November 1997 to April 2002. The inclusion criterion was having suffered a first thrombotic event at an age younger than 68 years. Patients were excluded if they had cancer or a history of chronic or acute liver disease or nephrotic syndrome. A medical history was obtained for each patient, including the site of thrombosis and acquired predisposing factors. The diagnosis of deep vein thrombosis of the lower limbs was established objectively by ultrasonography or ascending venography. Pulmonary embolism was diagnosed by ventilation–perfusion lung scanning, pulmonary angiography, or spiral computed tomography. Intracranial venous thrombosis was diagnosed by magnetic resonance imaging. A patient's family history was scored as positive if at least 1 other first- or second-degree family member had a venous thrombosis. As controls, 249 unrelated, asymptomatic, and apparently healthy persons were recruited with no personal history of VTE or use of oral anticoagulants. The control group was matched to the patient group for age and sex. To avoid genetic stratification, both the case and control groups were recruited from the same geographical region; all participants were white, and all their family names were Spanish. The study protocol was approved by the hospital's institutional review board, and signed informed consent was obtained from each patient.

The Marseille Thrombosis Association study (MARTHA)[21] is a case–control study including 1150 patients and 801 controls. The patients were unrelated and white; were recruited consecutively from the Thrombophilia Center, Hôpital de la Timone (Marseille, France) over the period from January 1994 to October 2005; had VTE; and were without known risk factors including antithrombin, protein C or protein S deficiency, homozygosity for *FVL* or for *PT*, or the presence of lupus anticoagulant. Thrombotic events including deep vein thrombosis and pulmonary embolism were documented by venography, Doppler ultrasound, spiral computed tomographic scanning angiography, and/or ventilation–perfusion lung scan. The control group was comprised of 2 subgroups: one included 475 healthy French white subjects with no personal history of cardiovascular disease (VTE was also considered) who were from the Marseille area and another group made up of 326 healthy French white heterozygotes for the FV Leiden or FII 20210A variants. In the MARTHA case–control study, controls were older than patients (47.4 versus 38.0 years, respectively), and the proportion of men was also higher among controls than among patients (47.8% versus 30.1%, respectively). To avoid the paradoxical (and confounded, by design) protective association between age and VTE and between sex and control, we matched patients and controls 1:1 by age and sex. Patients and controls were randomly matched 1:1 for the same sex and similar age (±5 years). In the original MARTHA study, 1148 patients and 801 controls were included, but for our study, we considered 477 cases and 477 controls.

SNP Selection and Genotyping

We performed a systematic review and meta-analysis to select genetic variants that contribute to VTE risk (Table 1). Based on this information, we defined a panel, TiC (Table 1), with the variants rs6025 (*F5*, Factor V Leiden), rs118203906 (*F5*, Factor V Hong Kong), rs118203905 (*F5*, Factor V Cambridge), rs1799963 (*F2*, G20210A), rs5985 (*F13*, V34L), rs121909548 (*SERPINC1*, 384 Ala>Ser), rs2232698 (*SERPINA10*, 67 ARG>Stop), and rs1801020 (*F12*, 46 C>T) and the A1 carriers rs8176719, rs7853989, rs8176743, and rs8176750.[22] It is important to note that all of these

Table 1. Genetic Variants Included in the Different Genetic Risk Scores Assessed and Coefficients (Weights) Assigned to Each Risk Factor

SNP	Gene	Mutation	Risk Coefficient Assigned (ß)	GRS*			
				1	2	3	4
rs6025, FV Leiden	*F5*	R506Q	1.589	■	■	■	■
rs118203905, FV Hong Kong	*F5*	R306G	1.589		■		■
rs118203906, FV Cambridge	*F5*	R306T	1.589		■		■
rs1799963	*F2*	G20210A	0.293	■	■	■	■
ABO	*ABO*	A1 carriers	0.956		■		■
rs8176719	*ABO*		—			■	
rs1801020	*F12*	C46T	1.633		■		■
rs5985	*F13*	V34L	0.198		■		■
rs2232698	*SERPINE10*	R67X	1.358		■		■
rs121909548	*SERPINC1*	A384S	2.277		■		■
rs2036914	*F11*		0.293 0.519			■	■
rs2066865	*FGG*		0.344			■	■
rs710446	*KNG1*		0.182				■
rs2289252	*F11*		0.315 0.577				■

GRS indicates genetic risk score; SNP, single nucleotide polymorphism.
*GRS 1, *FVL+PT* based on *F5* and rs1799963 (*F2*, prothrombin); GRS 2: Thrombo inCode based on *F5*, rs1799963 (*F2*, prothrombin), ABO-A1 carriers (rs8176719, rs7853989, rs8176743, rs8176750), rs1801020, rs5985, rs2232698, and rs121909548; GRS 3, de Haan et al based on *F5*, rs1799963 (*F2*, prothrombin), ABO (rs8176719), rs2036914, and rs2066865; GRS 4, expanded based on *F5*, rs1799963 (*F2*, prothrombin), ABO-A1 carriers (rs8176719, rs7853989, rs8176743, rs8176750), rs1801020, rs5985, rs2232698, rs121909548, rs2036914, rs2066865, and rs2289252.

genetic variants have functional effects on the coagulation cascade.[7–15] All except the A1 carriers are gain- or loss-of-function variants.

In addition, 3 different panels of genetic variants were defined (Table 1):

1. *FVL+PT*: rs6025 (*F5*, Factor V Leiden) and rs1799963 (*F2*, 20210 G>A). This panel represents the genetic variants most commonly tested in current clinical practice.
2. de Haan et al panel: rs6025 (*F5*, Factor V Leiden), rs1799963 (*F2*, 20210 G>A), rs8176719 (*ABO*), rs2066865 (*FGG*, 10034 C>T), and rs2036914 (*F11*, 7872 C>T).
3. Extended panel: All the TiC variants plus rs2289252 (*F11*, 22771 T>C), rs2036914 (*F11*, 7872 C>T), rs710446 (*KNG1*, Ile581Thr), and rs2066865 (*FGG*, 10034 C>T). These SNPs were added because of their relationships to VTE that were detected recently by GWAS.

DNA samples from the 2 populations were genotyped. With the SANTPAU samples, the Thrombo inCode kit (Ferer inCode) was used to identify the variants included in this panel, and the remaining variants were detected by Taqman assays run in an ABI 7500 instrument. The MARTHA samples were genotyped by allele-specific polymerase chain reaction.

Genetic Risk Score

To take into account the association strengths between the selected SNPs and VTE, we created a weighted GRS for each of the panels described (Table 1). The weights assigned to each SNP were defined a priori and based on the results of published meta-analyses in the case of *FVL*[23] and *PT*[24] or data from individual reports for rs2232698,[13,25] rs1801020,[20,26,27] rs59852,[8,28,29] rs2289252,[30,31] and rs2036914[31,32] or meta-GWAS for the variants rs2066865,[19,33,34] *ABO*,[35] rs121909548,[14] and rs710446[36] (Tables S1 and S2). For *FV* Cambridge and Hong Kong, the same weights as FV Leiden were assigned. For the panel described by de Haan et al,[19] we used the weights cited by the authors.

The genetic variants were introduced considering the genetic risk, and all were weighted in the same direction. The only genetic variant with a minor allele associated with lower thrombosis risk and odds was rs5985; in this case, we considered the common homozygote group as the risk.

Family History

For SANTPAU populations, data were compiled on the family history of VTE.

Statistical Analysis

Continuous variables are designated as means and standard deviations, and categorical variables are designated as proportions. Odds ratios (ORs) for the different variables linked to VTE and their 95% confidence intervals were calculated by conditional logistic regression.

We constructed different predictive models based on the *FVL+PT* GRS, the TiC GRS, the de Haan et al GRS, the extended GRS, or family history and combinations of these.

As previously mentioned, the weights assigned to all variables included in the models were defined a priori based on prior evidence.

The different scores were assessed according to the scientific statement of the American Heart Association, which describes the steps to be taken to evaluate novel risk markers in the cardiovascular field.[37]

To assess whether the strengths of association between clinical or genetic factors and thrombosis were different for those observed in the literature (expected) and those we observed in our study (observed), we compared coefficients (ie, logarithm of the ORs) by the *z* test statistic; in the equation z=(b[E]−b[0])/SE, b[E] and b[0] are, respectively, the coefficients expected and observed, whereas SE is the standard error of the difference in the coefficient.

We used different measures of performance to test the quality of fit in the GRS models. *Discrimination* measures the ability of the model to discriminate between participants who will and will not have a VTE. We quantified this by calculating the area under the receiver operating characteristic curve.[38] This value represents an estimate of the probability that a model assigns a higher risk to those participants who will have a VTE than to those who will not have a VTE.

Reclassification measures how the inclusion of a new marker classifies as highest risk those participants with a VTE and as lowest risk those without a VTE. We used the methods described by Pencina et al.[39,40] Integrated discrimination improvement (IDI) considers changes in the estimated VTE prediction probabilities as a continuous variable. The IDI increases when a new marker is added and thus enhances the estimate of the risk in those with VTE and decreases in those without VTE. Similarly, net reclassification improvement (NRI) requires the classification of the participants in risk categories and considers changes in the predicted probabilities of estimated VTE that imply a change from one category to another. Risk categories required to estimate the net reclassification improvement were established according to risk tertiles.

The sensitivity and specificity of the different GRSs were calculated[41] using the cut points giving the highest sensitivity.

All tests were performed using R statistical software (version 3.0.1).[42]

Results

The sociodemographic, clinical, and genetic characteristics of the participants are listed in Table 2. In both populations, patients showed higher scores than controls in all of the GRSs examined.

Regression coefficients (ie, logarithm of the ORs) and their standard errors for associations between the different variables and VTE are shown in Table S3. In our case, not all SNPs were associated with VTE, although we included all SNPs in the GRS calculations because of consistent reports in the literature of their correlation with VTE. In Table S3, we also provide the expected regression coefficients based on a literature review and our own meta-analysis and *P* values for differences between observed and expected coefficients. We did not observe a significant difference between expected and observed coefficients except for mutations in the gene for *FVL* in the MARTHA population (expected coefficient 1.589 versus observed 0.805, $P=0.028$).

As shown in Table 3, in the SANTPAU population, the predictive model based on FVL and PT mutations showed an area under the curve of 0.575 (95% CI 0.547 to 0.604), which increased significantly when the model was based on the *TiC* GRS (0.575 versus 0.677, $P<0.001$). The de Haan et al GRS also was significantly better than the predicted model based on FVL and PT mutations (0.575 versus 0.645, $P=0.015$); however, this GRS did not improve the discriminative capacity of TiC (0.677 versus 0.645; $P=0.346$). Moreover, when we extended the TiC score (extended GRS) by adding 4 common VTE-associated SNPs (*F11* rs2289252 and rs2036914, *KNG1* rs710446, and *FGG* rs2066865), no improvement was observed over the TiC GRS area under the curve (0.677 versus 0.671, $P=0.848$).

The same approach was used to assess the validity and predictive improvement capacity of the different models when *FVL* and *PT* mutations (often used in clinical practice) were considered in addition to family history of VTE. The results in Table 4 indicate the capacity of each of the GRS models in addition to family history of VTE to improve the discrimination capacity when compared with family history of VTE alone. The discriminative capacity of TiC plus family history of VTE was not improved by the de Haan et al or extended GRSs plus family history of VTE.

Reclassification was improved by TiC, extended, or de Haan et al GRS when compared with the *FVL+PT* model, as measured by IDI (5.49, $P<0.001$; 2.56, $P=0.009$; and 2.43, $P=0.015$, respectively) (Table 3). The only GRS showing an improvement in reclassification (net reclassification improvement) over the simple *FVL+PT* model was the TiC GRS (19.17, $P=0.002$). Similar results were obtained when all GRSs plus family history of VTE were compared with family history of VTE alone (Table 4).

Table 2. Main Sociodemographic, Clinical, and Genetic Characteristics of the Study Participants

	Controls	Cases	*P* Value
	n=249	n=248	
SANTPAU			
Sex (male), n (%)	109 (44.0)	111 (44.6)	0.960
Age (y), mean (SD)	49.0 (14.9)	47.1 (14.0)	0.145
Smoker, n (%)	101 (40.7)	108 (43.7)	0.559
Diabetes, n (%)	9 (3.7)	14 (5.7)	0.404
Oral contraceptives, n (%)	74 (29.8)	83 (33.5)	0.440
Family history, n (%)	45 (23.2)	97 (40.9)	<0.001
*F5**[†], n (%)	5 (2.02)	32 (12.9)	<0.001
F2: rs1799963*, n (%)	7 (2.82)	19 (7.63)	0.027
ABO-A1 carriers/ABO*, n (%)	87 (35.7)	147 (59.0)	<0.001
F12: rs1801020*, n (%)	5 (2.02)	15 (6.02)	0.041
F13: rs5985*, n (%)	139 (56.5)	146 (58.6)	0.698
SERPINE10: rs2232698*, n (%)	4 (1.61)	10 (4.02)	0.178
SERPINC1: rs121909548*, n (%)	1 (0.40)	4 (1.61)	0.372
F11: rs2036914			
Hetero, n (%)	111 (46.2)	119 (48.0)	0.77
Homo, n (%)	54 (22.5)	43 (17.3)	0.189
FGG: rs2066865*, n (%)	92 (37.9)	98 (39.4)	0.804
KNG1: rs710446b[‡], n (%)	47 (19.5)	49 (19.8)	0.966
F11: rs2289252			
Hetero, n (%)	113 (47.1)	122 (49.4)	0.675
Homo, n (%)	39 (16.2)	46 (18.6)	0.568
GRS 1[§], mean (SD)	0.04 (0.23)	0.23 (0.54)	<0.001
GRS 2[§], mean (SD)	0.56 (0.61)	1.10 (0.89)	<0.001
GRS 3[§], mean (SD)	0.78 (0.56)	1.16 (0.76)	<0.001
GRS 4[§], mean (SD)	1.23 (0.66)	1.76 (0.95)	<0.001
MARTHA	n=477	N=477	
Sex (male), n (%)	198 (41.5)	198 (41.5)	1.000
Age (years), mean (SD)	44.2 (13.6)	43.9 (14.0)	0.681
Smoker, n (%)	143 (30.2)	124 (27.7)	0.447
BMI (kg/m^2), mean (SD)	23.8 (3.8)	25.0 (4.2)	<0.001
Oral contraceptives, n (%)	105 (22.1)	187 (39.4)	<0.001
*F5**[†], n (%)	103 (21.6)	168 (35.2)	<0.001
F2: rs1799963*, n (%)	92 (19.3)	86 (18.0)	0.678
ABO-A1 carries/ABO*, n (%)	28 (5.87)	47 (9.85)	0.030
F12: rs1801020*, n (%)	20 (4.19)	29 (6.08)	0.241
F13: rs5985*, n (%)	255 (53.5)	283 (59.3)	0.078
SERPINE10: rs2232698*, n (%)	8 (1.68)	15 (3.14)	0.205
SERPINC1: rs121909548*, n (%)	3 (0.63)	1 (0.21)	0.324
F11: rs2036914			
Hetero, n (%)	231 (49.7)	236 (50.1)	0.948
Homo, n (%)	118 (25.4)	100 (21.2)	0.155
FGG: rs2066865*, n (%)	178 (38.3)	220 (49.5)	0.001
KNG1: rs710446b[‡], n (%)	92 (19.6)	82 (18.2)	0.638
F11: rs2289252			
Hetero, n (%)	231 (48.4)	225 (47.2)	0.746
Homo, n (%)	72 (15.1)	126 (26.4)	<0.001
GRS 1[§], mean (SD)	0.40 (0.63)	0.61 (0.73)	<0.001
GRS 2[§], mean (SD)	0.67 (0.83)	0.97 (0.92)	<0.001
GRS 3[§], mean (SD)	0.85 (0.73)	1.12 (0.83)	<0.001
GRS 4[§], mean (SD)	1.34 (0.86)	1.70 (0.94)	<0.001

BMI indicates body mass index; GRS, genetic risk score; hetero, heterozygosis; homo, homozygosis.
*Carriers of the risk allele.
[†]Carrier of any risk allele (Leiden, Hong Kong, or Cambridge).
[‡]Homozygotes for the risk allele.
[§]GRS 1, *FVL+PT* based on *F5* and rs1799963 (*F2*, prothrombin); GRS 2, Thrombo inCode based on *F5*, rs1799963 (*F2*, prothrombin), ABO-A1 carriers, rs1801020, rs5985, rs2232698, and rs121909548; GRS 3, de Haan et al based on *F5*, rs1799963 (*F2*, prothrombin), *ABO*, rs2036914, rs2066865; GRS 4, expanded based on *F5*, rs1799963 (F2, prothrombin), *ABO*, rs1801020, rs5985, rs2232698, rs121909548, rs2036914, rs2066865, and rs2289252; ABO-A1 carriers: rs8176719, rs7853989, rs8176743, rs8176750; *ABO*: rs8176719.

In addition, using the GRS cut points to obtain maximal sensitivity, the sensitivity of TiC in the SANTPAU population was significantly higher than that of *FVL+PT* (0.85 versus 0.20%, respectively) (Table 5). The specificity of the *FVL+PT* GRS was higher than that of TiC (0.95 versus 0.25, respectively).

More important, the variance in VTE risk explained by the different GRSs in the SANTPAU population were 7.1%, 15.1%, 9.9%, and 13.0% for *FVL+PT*, TiC, de Haan et al, and extended GRSs, respectively. It is noteworthy that the TiC score explained the greatest amount of variance in thrombotic risk; in fact, it was >2-fold the variance explained by the conventional *FVL+PT* model.

Similar results were observed for the MARTHA population (Table 3). The AUC increased significantly with respect to the model based on *FVL* and *PT* alone when we used the TiC genetic variants (0.576 versus 0.605, *P*=0.008) and the extended genetic model (0.576 versus 0.629, *P*=0.037); however, the extended score did not improve the discriminative capacity of TiC (0.605 versus 0.629, *P*=0.361). Moreover, the de Haan et al GRS offered no improvement over the discriminative capacity of *FVL+PT* (0.576 versus 0.594,

Table 3. Predictive Capacities of the Different Models and Improvements Observed Including Different Genetic Variants Compared With the Simplest Model (*FVL+PT*)

	GRS 1 *FVL+PT* (95% CI)	GRS 2 TiC (95% CI)	GRS 3 de Haan et al (95% CI)	GRS 4 Extended (95% CI)
SANTPAU				
Discrimination				
AUC	0.575 (0.547; 0.604)	0.677 (0.631; 0.724)	0.645 (0.596; 0.694)	0.671 (0.623; 0.719)
P value ΔAUC	NA	<0.001	0.015	<0.001
Reclassification				
IDI	NA	5.49 (3.35; 7.63)	2.43 (0.47; 4.39)	2.57 (0.65; 4.49)
P value	NA	<0.001	0.015	0.009
NRI	NA	19.17 (7.01; 31.33)	−5.76 (−21.84; 10.32)	−8.66 (−25.61; 8.29)
P value	NA	0.002	0.483	0.317
MARTHA				
Discrimination				
AUC	0.576 (0.544; 0.609)	0.605 (0.570; 0.640)	0.594 (0.557; 0.631)	0.629 (0.592; 0.665)
P value ΔAUC	NA	0.008	0.478	0.037
Reclassification				
IDI	NA	0.96 (0.02; 1.90)	−0.06 (−0.86;0.75)	0.19 (−0.88; 1.25)
P value	NA	0.045	0.889	0.730
NRI	NA	4.94 (−1.46; 11.33)	−5.98 (−13.98; 2.02)	−7.11 (−16.35; 2.13)
P value	NA	0.130	0.143	0.131

AUC indicates area under the receiver operating characteristic curve; GRS, genetic risk score; IDI, integrated discrimination improvement; NA, not applicable; NRI, net reclassification improvement; TiC, Thrombo inCode.

P=0.47). In addition, when analyzing the reclassification, only the TiC score improved the reclassification capacity of *FVL+PT*, as assessed by the IDI (0.96, *P*=0.045).

The sensitivity and specificity of TiC scores were similar in the MARTHA population (0.850 and 0.264, respectively); however, the sensitivity and specificity of the *FVL+PT* GRS in the MARTHA population differed from that found in the SANTPAU population (sensitivity 0.532 and specificity 0.591 in MARTHA).

It should be emphasized that in the MARTHA study, the extended GRS explained a high proportion of the variance in VTE risk (5.3%), whereas the *FVL+PT*, TiC, and de Haan et al

Table 4. Predictive Capacity of the Different Models and Improvements Observed When Including Different Genetic Variants With Respect to Family History in the SANTPAU Population

	Family History (95% CI)	GRS 1 *FVL+PT* (95% CI)	GRS 2 TiC (95% CI)	GRS 3 de Haan (95% CI)	GRS 4 Extended (95% CI)
Discrimination					
AUC	0.589 (0.545; 0.632)	0.647 (0.602; 0.691)	0.701 (0.652; 0.749)	0.684 (0.633; 0.734)	0.700 (0.649; 0.750)
P value ΔAUC	NA	<0.001	<0.001	0.005	0.001
Reclassification					
IDI	NA	3.43 (2.10; 4.76)	6.63 (4.45; 8.82)	4.28 (2.18; 6.38)	2.57 (0.40; 4.74)
P value	NA	<0.001	<0.001	<0.001	0.020
NRI	NA	16.04 (9.50; 22.57)	29.42 (14.33; 44.53)	6.92 (−11.40; 25.24)	0.65 (−18.57; 19.88)
P value	NA	<0.001	<0.001	0.459	0.947

AUC indicates area under the receiver operating characteristic curve; GRS, genetic risk score; IDI, integrated discrimination improvement; NA, not applicable; NRI, net reclassification improvement; TiC, Thrombo inCode.

Table 5. Clinical Utility (Measured as the Sensitivity) and Specificity of TiC Compared With *FVL+PT* in the SANTPAU and MARTHA Populations

	Selected GRS Cut Points	SANTPAU		MARTHA	
		Sensitivity	Specificity	Sensitivity	Specificity
FV+PT	0.147	0.20	0.95	0.53	0.59
TiC	0.099	0.85	0.25	0.85	0.26

GRS indicates genetic risk scores; TiC, Thrombo inCode.

GRSs explained 3.1%, 3.9%, and 3.7%, respectively. This observation could be attributable to the fact that the extended panel included the genetic variants included in TiC plus 4 common SNPs (*F11* rs2289252 and rs2036914, *KNG1* rs710446, and *FGG* rs2066865) reported to be associated with VTE in the MARTHA study.

Discussion

As knowledge of disease improves, new biomarkers and new tests are changing the traditional concept of risk assessment. Given that thrombosis is the final outcome of many systemic disorders, it is not surprising that interest in this disease is increasing rapidly; however, accurately predicting a person's risk of developing a complex disease is very difficult. This difficulty results, in large measure, from the many risk factors that exist for a given disease. Most of these factors and their interactions are unknown. VTE is a case in point because it has a large number of risk factors related to genetic variability.[5] Despite this substantial genetic component of VTE and the new knowledge generated by GWASs, only 2 of these variants—*FVL* and *PT*—are used conventionally in clinical settings worldwide.

In our study, we assessed the predictive validity of 3 GRSs in 2 independent populations (SANTPAU and MARTHA) according to the American Heart Association's guidelines for the evaluation of novel cardiovascular risk markers.[37] The proof of concept derived from our study is that the *FVL+PT* model can be greatly improved by using new genomic information. More important, by comparing the use of 3 GRSs on the same populations, we were able to show conclusively that their predictive capacities are greatly augmented using the TiC GRS.

In an initial step, we performed a systematic review of the literature and meta-analysis to select genetic variants that contribute to VTE risk, and we assigned these variants a corresponding VTE risk coefficient (Tables 1, S1, and S2). The genetic variants selected have a direct functional effect on the blood-clotting proteins,[7–15] highlighting the role of coagulation in thrombosis risk. Based on these selected genetic variants, we defined 3 weighted GRSs and determined whether they could assess the risk of VTE better than a model based on family history or *FVL+PT* alone. The weights assigned to each genetic variant were defined a priori and based on the literature.

In the second step of our study, we selected 2 independent populations in which to compare the performance of these genetic scores: the Spanish population examined in the SANTPAU case–control study and the population of the French MARTHA study, with cases and controls that were rich in FV Leiden or FII 20210A mutations. Accordingly, the SANTPAU study participants better represented the general white population and formed the basis of our study, whereas the MARTHA study participants were considered a stricter sample in which to assess the role of new genetic markers because the effects of FV Leiden and FII mutations were overrepresented.

In this study, we were able to confirm the prior finding that *FVL+PT* improves the VTE predictive capacity of family history.[43] We were also able to demonstrate that TiC, a GRS including 12 low-frequency, high-impact genetic risk factors, was the only score of the 3 examined that significantly improved the VTE predictive capacity of family history of VTE (Table 4) and *FVL+PT* (Table 3). This improvement consisted of better discrimination and reclassification in both the SANTPAU and MARTHA study populations.

One of the GRSs that we examined was reported by de Haan et al.[19] Remarkably, these authors observed that a risk score including 31 SNPs independently associated with a VTE risk through GWAS showed a similar predictive capacity to a score including only 5 of these SNPs (*FVL*, *PT*, ABO blood group, *FGG* gene rs2066865, and *F11* gene rs2289252). Three of these 5 genetic variants (*FVL*, *PT*, *ABO* blood group) reported by de Haan et al were included in the TiC score. Importantly, these matching markers are low-frequency variants that show the highest individual ORs for VTE risk. This fact clearly indicates the limited value of risk scores composed of common SNPs that show low individual ORs, such as those identified by GWAS, when low-frequency variants with high ORs are also considered. In effect, the addition of 4 common SNPs (rs710446, rs2289252, rs2066865 and rs2036914) identified through GWAS to TiC (extended GRS) failed to improve the area under the curve.

This point is important regarding GWASs because the findings of both the study by de Haan et al and our study indicate the better predictive capacity of a GRS that considers low- frequency variants that returns high ORs. It should be underscored that the TiC includes rare variants featuring high individual ORs (eg, *SERPINC1* gene rs121909548 and *SERPIN10* gene rs2232698 polymorphisms). Based on these results and in agreement with a previous GWAS,[21] it is

unlikely that common risk alleles identified through GWAS (showing a Minon Allele Frequency [MAF] >0.05 and a modestly increased VTE risk with an OR in the range 1.10 to 1.35) alone account for a large proportion of the familial risk of VTE and its clinical variability, as observed for most other human diseases investigated through GWASs.[44]

These findings emphasize the need to pay special attention to low-frequency or rare variants rendering high ORs. In a recent study, the benefits of an in-depth sequencing strategy were emphasized because it permitted the identification of a rare mutation responsible for familial cases of early-onset VTE.[45,46] The important predisposing role played by low-frequency or rare variants in VTE was highlighted in a study based on multigenerational data by Zöller et al,[47] who observed a high familial risk of VTE in a small number of siblings, suggesting segregation of rare but strong genetic risk factors.

In our study, the clinical utility (measured as the sensitivity of the GRSs) of the TiC GRS compared with *FVL+PT* was also examined. Using the cut point obtaining the highest sensitivity for *FVL+PT*, the sensitivity of *FVL+PT* was 0.20 and 0.53 of patients with VTE in SANTPAU and MARTHA, respectively, whereas using TiC at the cut point for maximal sensitivity, the sensitivity of TiC was 0.85 and 0.85 in SANTPAU and MARTHA, respectively. High sensitivity is important when the test is used to identify a serious but preventable or treatable disease such as VTE.[41] This is especially true when low specificity may represent a genetic predisposition to VTE that will lead to VTE only when pro-VTE clinical conditions are also present. The identification of genetic thrombophilia has several clinical applications because most guidelines[48] advocate assigning a moderate risk of VTE in patients with thrombophilia.

Although our results point to a clear association between GRS and the risk of VTE, they should be interpreted with caution because of the limitations of our study. First, we did not include clinical data (apart from family history) in the scores because, as indicated by the guidelines,[46] several clinical scenarios exist for VTE but the genetic basis of the disease is common to all of them. Consequently, we focused our study on genetic factors and will be examining the use of the TiC GRS in combination with several clinical variables in future studies. This strategy of combining genetic and clinical data, as observed by de Haan et al,[19] is likely to further improve the performance of TiC, especially its sensitivity, specificity, and the area under the receiver operating characteristic curve.

Given the lack of universally acceptable risk categories for VTE, in our net reclassification improvement analysis, we established categories based on risk tertiles, which could be an overly demanding process. Nevertheless, this limitation was resolved using the IDI index, which measures risk as a continuum and thus should be considered a more powerful indicator for reclassification in our study.

Although we have described the differences in the design of the 2 populations, the differences in the predictive capacities of the TiC GRS in those 2 populations may raise concerns about the replicability of TiC in other populations. As mentioned, MARTHA has a special design to put the new markers under additional stress to evaluate whether they add value to *FVL+PT*. It is a case–control study enriched in carriers of *FVL* and *PT* mutations, for which >50% of participants are carriers of 1 of these genetic variants. It is expected that the area under the receiver operating characteristic curve and the IDI and net reclassification improvement results could be modest when comparing TiC with *FVL+PT* because these 2 mutations account for an important part of the risk, especially if half of the participants are carriers. Despite this big effect of these 2 mutations (due to the study design), it is important to emphasize that TiC also significantly improved the predictive capacity of *FVL+PT* in MARTHA population. Moreover, we must consider the sensitivity and specificity of TiC because those classification functions, contrary to other statistical functions, are more strongly associated with the capacity of the test than with the specific characteristic of the population in which it is tested.[41] The sensitivity and sensibility of TiC were the same in the SANTPAU and MARTHA populations (sensitivity 0.85 for both; specificity 0.25 versus 0.26 in SANTPAU and MARTHA, respectively). This supports the idea that TiC will have similar performance in other populations. Considering the number, weight, and pathological relevance of the genetic variants included in TiC, the significant improvement in area under the received operating character curve and IDI in MARTHA, and the similar sensitivity and specificity in both populations, it is reasonable to believe that TiC will be replicable.

Finally, replication of the present results in other populations, evaluation of the clinical utility (measured as reduction in VTE events by the use of TiC GRS), and analysis of the cost-effectiveness of TiC remain to be accomplished.

In summary, to the best of our knowledge, this study is the first to assess the efficiency of new genetic markers of VTE, such as TiC, according to the recommendations of experts in the field of cardiovascular disease.[37] By determining the discrimination and reclassification capacity of these markers and their clinical utility (measured as sensitivity) in comparison to conventional recognized risk models (*FVL+PT* or family history), we were able to conclude that by using new genetic markers, especially TiC, conventional VTE risk assessment algorithms are substantially improved. New risk scores such as the TiC GRS, proposed in this paper, should allow for more tailored thromboprophylaxis strategies and improve estimates of a patient's risk of VTE.

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Disclosures

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