Predicting the risk of thromboembolic disease by analyzing multiple genetic variants: incorporating risk profiles into clinical practice

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INTRODUCTION

Venous thromboembolism (VTE), i.e. Deep Vein Thrombosis (DVT) or pulmonary embolism (PE), is an important cause of mortality and morbidity in the developed world and the main preventable cause of mortality in hospitals[1]. Each year around 300.000 people in the United States die from acute PE, many cases of them being only diagnosed at autopsy[1]. Similarly, an European study estimates in 370.012 VTE-related deaths in 2004 in a group of 6 European countries[2]. DVT affects approximately two million Americans annually, while PE is the most common cause of preventable hospital death accounting for 60.000 deaths in the United States every year[3].

VTE has an important genetic component. In addition to the classic variants FV Leiden (FVL) and prothrombin G20210A (PT), currently determined in routine, there are new genetic variants associated with thrombosis but not yet added to the assessment of the genetic risk of thrombophilia[4,5,6]. Moreover, there are no interpretation protocols combining all thrombophilia genetic factors with the classic prothrombotic factors and there is not any commercial service suggesting therapeutic measures taking into account all these factors.

Given the high incidence and recurrence rates of VTE, a better identification of patients at risk of thrombosis might improve the preventive strategy and decrease the incidence of DVT and associated complications.

OBJECTIVES

The objective of this study was to determine whether the genetic variants included in THROMBOINCODE (genetic profile) improve the capacity of FVL and PT to predict the development of thrombosis.

METHODS

Two case-control studies of VTE were included:

- MARTHA[7]: 1,150 cases (347 males, 803 females; 38.0±13.9 years old) / 801 controls (383 males, 418 females; 47.4±14.0 years old) designed to assess the association of FV Leiden and Prothrombin with other risk factors.

- A study with Spanish population (SP): 249 cases (111 males, 138 females; 47.1±14.0 years old) / 248 controls (109 males, 139 females; 49.0±14.9 years old).

Genetic profile analyzed

- Genetic variants analyzed in this study are located in the following genes: FXII, ABO Group (A1 carriers), Serpin A10, Serpin C1, Factor V and PT.

Statistical Analysis

The association between variants and thrombosis was calculated using the OR adjusted for age and sex: [OR (95%)].

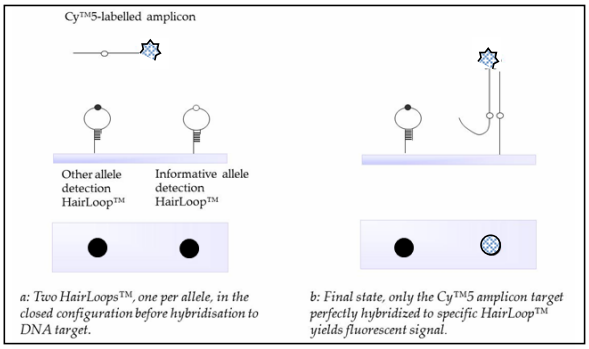
The predictive capacity was calculated using the c-statistic (AUC-ROC) observed when using the FVL, PT or the genetic profile.

The reclassification capacity was calculated using NRI (*net reclassification improvement*) and IDI (*integrated discrimination improvement*) observed when using the FVL, PT or the genetic profile.

Thrombo inCode

Thrombo inCode test kit is and IVD-CE marked kit for the simultaneous allele determination of 12 variants in 7 genes (PT, FV, FXII, FXIII, ABO, Serpin A10, and Serpin C1) associated with thrombosis in genomic DNA, extracted from either saliva or blood samples.

The calculated sensibility and specificity of the kif is ≥98% per SNP. The allele detection is performed by using HairLoop™ Technology.



a: Two HairLoops™, one per allele, in the closed configuration before hybridisation to DNA target.

b: Final state, only the Cy™5 amplicon target perfectly hybridized to specific HairLoop™ yields fluorescent signal.

RESULTS

Table 1: Association between variants and thrombosis [OR (95%)] and the proportion of FVL and PT carriers compared with carriers of the genetic profile assessed by THROMBOINCODE.

		FV Leiden	Prothrombin	ABO (A1)	FXII	Serpin C1	Serpin A10
MARTHA	OR	2.3	0.9	1.8	0.9	0.9	2.3
Cases	Carriers	50.4 %					
		87.5 % ← THROMBOINCODE					
SP	OR	7.2	2.8	2.62	3.1	4.1	2.5
Cases	Carriers	19.7 %					
		71.5 % ← THROMBOINCODE					

Table 2: c-statistic and reclassification [NRI (net reclassification improvement) and IDI (integrated discrimination improvement)] comparing the use of the genetic profile assessed by THROMBOINCODE to FVL and PT.

	c-statistic		NRI		IDI	
	MARTHA	SP	MARTHA	SP	MARTHA	SP
FV Leiden + Prothrombin	0.54	0.58	ref	ref	ref	ref
THROMBOINCODE	0.58	0.69	5.3	23.4	1	5.9
P-value	<0.001	<0.001	>0.05	<0.001	<0.05	<0.001

CONCLUSIONS

- The selected genetic profile included in THROMBOINCODE improves significantly the discrimination of VTE risk by identifying a genetic thrombophilia in **37.1-51.8%** of the subjects who developed VTE, but who had not showed genetic thrombophilia when analysing only FV Leiden and PT (Table 1).

- The predictive capacity of the FV Leiden and PT was significantly improved by the inclusion of the genetic panel (assessed using the c-statistic) both in MARTHA and the Spanish Population studies. Moreover, in the Spanish Population study, the use of the genetic profile improves in a very significant way the risk assessment done by the use of only FV Leiden and PT as evidenced by the very high values of both NRI and IDI. In the case of the MARTHA study, only IDI achieves statistical significance.

- THROMBOINCODE is the first IVD-CE commercial tool that integrates and automates in a single kit the detection of the most relevant genetic risk variants that significantly enhances our predictive capacity and will improve the prevention strategy for patients (and their relatives) who are at risk of developing thromboembolic events and also the diagnosis and the treatment of the thromboembolic disease.

References

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