7. Lao TT, Yin JA, Yuen PM. Coagulation and anticoagulation system in newborns. Gynecol Obstet Invest 1990;29:181-4.
8. Roman J, Velasco F, Fernadez F, Fernandez M, Villalba R, Rubio V, et al. Protein C, protein S and C4b binding protein in neonatal severe infection and septic shock. J Perin Med 1992;20:111-6.

Thrombosis

Five prothrombotic polymorphisms and the prevalence of premature myocardial infarction

We studied 5 functional hemostatic polymorphisms in 281 patients with premature myocardial infarction and in 530 control subjects. The role of these polymorphisms when analyzed independently is small, if any. However, the simultaneous combination of factor XIII and prothrombin polymorphisms exacerbated the risk. (OR=12.12; p=0.028). Moreover, combinations of factor V Leiden with prothrombin, and factor XII with prothrombin polymorphisms were only identified in patients. Our results support the relevance of gene-gene interactions in myocardial infarction.

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Unprecedented efforts are underway to identify polymorphisms and to establish their relationship to susceptibility for common thrombotic disorders, especially myocardial infarction (MI). The influence of polymorphisms in MI is contradictory, with positive and negative results, although no study has identified a relevant risk (OR>4) associated with any polymorphism.[1] Very recently two big case/control studies suggested a minor role in MI for many polymorphisms.[2,3] The role of common genetic changes affecting candidate genes is certainly weak in itself, and much weaker than that of traditional cardiovascular risk factors. Like any complex and multigenic disease, MI probably involves many different factors that might interact to result in an additive or a synergistic co-effect. Therefore, an accurate evaluation of MI would require analysis of multiple polymorphisms and environmental risk factors in order to identify specific combinations with a significant association with the disease. Functional polymorphisms affecting hemostatic proteins could unbalance this system, increasing the risk of developing thrombotic or hemorrhagic diseases.

We studied five polymorphisms affecting the level or function of key hemostatic proteins in 281 patients who survived an acute MI before 45 years old during the last 7 years, and in 530 controls. The patients were selected from our Cardiology outpatient clinic and the controls were recruited from blood donors and traumatology and ophthalmology patients. A personal interview was done, and the clinical history of these controls was evaluated to discard vascular disease or personal history of thromboembolic or hemorrhagic disease. All the patients and controls were Caucasian.

The study had been approved by the local ethics committee, and was carried out in accordance with declaration of Helsinki. Univariate statistical analysis was performed by the χ^2 test. Multiple analysis was performed using logistic regression adjusting for sex and cardiovascular risk factors (smoking habit, diabetes, hypertension, and hypercholesterolemia). Differences among groups for each individual test were considered significant when the uncorrected p<0.05. The strength of the association of major risk factors and polymorphisms with the occurrence of MI was estimated by calculation of the odds ratio (OR) and the Cornfield method for the calculation of 95% confidence intervals (CI). Gene interactions were determined by comparing the prevalence of combined carriership for 2 gene variants in patients and controls with respect to the whole population. There was no statistical deviation from Hardy-Weinberg equilibrium for any polymorphism in all groups of subjects. The frequencies of these polymorphisms in the control group did not differ from those reported in other Caucasian populations. Univariate analysis revealed that only the prothrombin polymorphism slightly increased the risk of premature MI (p=0.013; OR=2.73; 95%CI: 1.12-6.70),

Table 1. Prevalence of classic cardiovascular risk factors and genetic polymorphisms in patients and controls.

	MI patients	*Controls*	*Adjusted p; OR (95%CI)*
N	281	530	
Age	44±6	50±19	
(Age of first MI)	(40±5)		
Male sex (%)	90	55	<0.001
			3.62(2.25-5.82)
Smoking (%)	82	40	<0.01
			2.45 (1.81-3.34)
Hypertension (%)	29	27	0.420
			0.85(0.57-1.26)
Diabetes (%)	11	12	0.172
			0.67(0.39-1.18)
Hypercholesterolemia (%)	70	22	<0.01
			6.62(4.59-9.54)
FXIII Val34Leu			
Val/Val	180	354	0.085
Val/Leu & Leu/Leu	86+15	157+19	1.38(0.96-2.0.1)
FV Leiden			
-/-	270	511	0.547
-/+ & +/+	10+1	19+0	1.31(0.54-3.16)
PT G20210A			
-/-	267	520	0.241
-/+	14	10	1.78(0.68-4.68)
FXII C46T			
C/C	172	356	0.015
C/T & T/T	103+6	153+21	1.58(1.09-2.29)
FVII Del-323Ins			
A1/A1	207	397	0.312
A1/A2 & A2/A2	67+7	122+11	0.81(0.54-1.21)

Hypertension was defined as a systolic blood pressure > 140 mm Hg or a diastolic blood pressure > 90 mm. Hg on repeated observations over 3 months or, if no blood pressure values were available when the subject was under treatment with chronic antihypertensive therapy. Smoking habit was considered when the subject smokes more than 10 cigarettes per day. Hypercholesterolemia was defined as a total serum cholesterol level > 5.72 mmol/L (220 mg/dL). Diabetes was defined by a history of a fasting glucose of at least 7.8 mmol/L (140 mg/dL) or use of insulin or hypoglycemic medications.

Table 2. Joint effect of pairs of polymorphisms.

Combination of alleles	*MI 281*	*Controls 530*	*Adjusted p; OR (95% CI)*
FXIII Leu34 & PT 20210A	**7 (2.5%)**	**1 (0.2%)**	**0.028; 12.12 (1.33-112.46)**
FXIII Leu34 & FVL	1 (0.3%)	3 (0.6%)	0.903; 1.16 (0.11-11.82)
FXIII Leu34 & FXII 46T	37 (13.2%)	70 (13.2%)	0.377; 1.25 (0.76-2.06)
FXIII Leu34 & FVII -323Ins	24 (8.5%)	50 (9.4%)	0.420; 0.78 (0.43-1.43)
FXII 46T & PT 20210A	**4 (1.4%)**	**0**	
FXII 46T & FVL	2 (0.7%)	6 (1.1%)	0.966; 0.96 (0.16-5.81)
FXII 46T & FVII -323Ins	27 (9.6%)	42 (7.9%)	0.103; 0.59 (0.31-1.11)
FVII -323Ins & PT 20210A	3 (1.1%)	2 (0.4%)	0.103; 0.10 (0.01-1.60)
FVII -323Ins & FVL	4 (1.4%)	3 (0.6%)	0.175; 0.33 (0.06-1.64)
PT 20210A & FVL	**2 (0.7%)**	**0**	

but its effect was lost after multivariate analysis (Table 1). In contrast, FXII C46T was the only polymorphism with a significant association with premature MI after multivariate analysis (Table 1). The analysis of association between polymorphisms showed interesting results. One control (0.2%) simultaneously carried the *FXIII* and prothrombin polymorphisms but 7 patients did so (2.5%). Accordingly, co-inheritance significantly increased the risk of developing premature MI (OR=13.51; 95%CI: 1.67-293.76; p=0.001). After multivariate analysis, the effect of the combination of these two alleles remained significant (Table 2).Two patients simultaneously carried FV Leiden (FVL) (one case in homozygous state) and the prothrombin polymorphism, a combination that was not found among controls. Moreover, we found no control simultaneously carrying the prothrombin and FXII polymorphisms, a combination carried by 4 patients (Table 2). Synergism between polymorphisms has been identified in other diseases, including hemostatic disorders.[4-7] Our data support the belief that combinations of polymorphisms affecting the hemostatic system could strongly increase the risk of premature MI. All relevant data involve the procoagulant G20210A polymorphism of prothrombin, whose role in MI is still controversial.[8] We observed a significant synergism between the prothrombin and the FXIII polymorphisms, similar to the recently described by Butt and co-workers in consecutive case of MI from a different population.[5] These data support a relevant prothrombotic effect for this combination of one procoagulant polymorphism and one polymorphism affecting clot stability. Moreover, our data also suggest that co-inheritance of the procoagulant FVL and prothrombin polymorphisms could increase the risk of MI, synergism clearly demonstrated in venous thrombosis.[4,7] Finally, the combination of a procoagulant trait (prothrombin) with reduced fibrinolytic function (FXII) could also predispose to premature MI. Our study has important limitations. Association studies to evaluate polymorphisms with low frequency alleles involving hundreds of patients and controls are greatly underpowered, and the possibility of a chance result is real. Moreover, the cross-sectional design of the study only allows us to explore associations and no causality is implied. Finally, a survival bias cannot be avoided as the study was performed in patients who survived a MI. Accordingly, further studies including more patients from other populations are required to confirm the synergistic interactions observed in our study.

In conclusion, our study reinforces the possibility that specific combinations of genetic and environmental risk factors in each person will determine the susceptibility towards MI. Thus, certain polymorphism profiles, with a low prevalence in the general population, might behave as strong risk factors for MI.

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References

1. Lane DA, Grant PJ. Role of hemostatic gene polymorphism in venous and arterial thrombosis disease. Blood 2000;95:1517-32.
2. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. N Engl J Med 2002; 347:1916-23.
3. Atherosclerosis, Thrombosis and Vascular Biology Italian Study Group. No evidence of association between prothrombotic gene polymorphisms and the development of acute myocardial infarction at a young age. Circulation 2003; 107: 1117-22.
4. Lane DA, Mollica LR. Haemostatic gene polymorphisms in venous and arterial thrombosis. Pathophysiol Haemost Thromb 2002;32:213-5.
5. Butt C, Zheng H, Randell E, Robb D, Parfrey P, Xie YG. Combined carrier status of prothrombin 20210 A and factor XIII-A Leu34 alleles and a strong risk factor for myocardial infarction: evidence of a gene-gene interaction. Blood 2003; 101:3037-41.
6. Schwartz E, Demidova D, Sirotkina O, Kudinov S. The combination of glycoprotein IIIa PlA polymorphism with polymorphism of serotonin transporter as an independent strong risk factor for the occurrence of coronary thrombosis. Mol Genet Metab 2003;79:229-30.

7. De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Chiusolo P, Casorelli I, et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 1999;341:801-6.
8. Burzotta F, Paciaroni K, De Stefano V, Crea F, Maseri A, Leone G, et al. G20210A prothrombin gene polymorphism and coronary ischaemic syndromes: a phenotype-specific meta-analysis of 12034 subjects. Heart 2004;90:82-6.

Thrombosis

Importance of troponin T for the risk stratification of normotensive patients with pulmonary embolism. A prospective, cohort study with a three-month follow-up

To determine the prognostic importance of troponin T in normotensive patients with pulmonary embolism, we investigated the rate of adverse events in patients with normal and elevated troponin values, during the hospital period and at three months of follow-up. We also calculated the proportion of patients with abnormal troponin values and adverse outcomes who could have been treated with more aggressive therapy according to published criteria.

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Several studies have investigated the prognostic value of troponin T and I levels in patients with pulmonary embolism, showing an increased risk of in-hospital adverse outcomes in patients with elevated serum levels, as well as a high negative predictive value of normal levels for an unfavorable course.[1-7] However the importance of risk stratification by using troponin testing is not completely defined. In fact, it is still unclear whether the high negative predictive value for unfavorable outcomes is maintained after hospital discharge, and whether high troponin values in patients experiencing adverse events could translate into realistic therapeutic alternatives.

Moreover only one study[3] has focused on hemodynamically stable patients, for whom risk stratification is presumably more important.[8] In this prospective cohort study, which evaluated normotensive patients with pulmonary embolism, we aimed to investigate: (i) the rates of adverse outcomes in patients with normal and elevated troponin T values in the in-hospital period and at three months of follow-up; (ii) whether patients with high troponin T values, and experiencing adverse in-hospital events, could have been treated with more aggressive therapy. Adverse events were considered to be thrombolytic therapy, need for positive inotropic support, endotracheal intubation, cardiopulmonary resuscitation, and all-cause mortality, in the in-hospital period; thromboembolic recurrences and all-cause mortality at three months of follow-up.

The patients with unfavorable in-hospital outcomes were considered to have been suitable for more aggressive treatment if they had no contraindications to thrombolytic treatment according to the MAPPET 3 criteria.[9]

Troponin T was assessed with a highly sensitive test. Concentrations > 0.01 ng/mL were considered abnormal.

Fisher's exact test was used to compare the proportions of adverse events between the groups with normal and elevated values of troponin during the in-hospital period and at the end of follow-up. Quantitative variables were compared by Student's t-test. Sixty consecutive normotensive patients with objectively confirmed pulmonary embolism were enrolled. None received inotropic support on admission. The baseline clinical characteristics of the patients are displayed in Table 1.

Table 1. Base-line characteristics of the study patients.

Characteristics	*All patients*	*TT>0.01 ng/mL*	*TT≤0.01 ng/mL*	*p*
No. (%)	60	26 (43)	34 (57)	
Mean age, y (SD)	65 (18)	70 (16)	60 (19)	0.04
Female n.	39	15	24	
Underlying diseases n°(%)				
Cardiovascular	40 (67)	19 (73)	21 (62)	0.68
Pulmonary	22 (37)	11 (42)	11 (32)	0.10
Neurologic	15 (25)	7 (27)	8 (24)	0.97
Infectious diseases	11 (18)	4 (15)	7 (21)	0.79
Thromboembolic risk factors n°(%)*				
Previous VTE	2 (3)	1 (4)	1(4)	0.61
Recent surgery	14 (23)	8 (31)	6 (18)	0.38
Recent bed rest	19 (32)	13 (50)	6 (18)	0.02
Paralysis of the leg (s)	5 (8)	3 (11)	2 (6)	0.67
Malignancy	10 (17)	3 (11)	7 (20)	0.56
Obesity	4 (6)	3 (11)	1 (3)	0.47
Oral contraceptives	4 (6)	1 (4)	3 (9)	0.80
No risk factors	14 (23)	4 (15)	10 (29)	0.33
Systolic blood pressure, mmHg, mean (SD)	127 (23)	123 (24)	130 (22)	0.22
Heart rate, beats/min, mean (SD)	105 (20)	106 (19)	108 (19)	0.56
Partial pressure of arterial oxygen, mmHg, mean (SD)	61 (10)	57 (10)	64 (9)	0.01
Severe hypoxemia°	25 (42)	17 (65)	8 (23)	< 0.01
Echocardiography	41 (68)	15 (58)	26 (76)	0.23
Right ventricular dysfunction±	17 (41)	9 (60)	8 (30)	±0.12

*TT: Troponin T. *Some patients had more than one risk factor; °Partial pressure of arterial oxygen < 60 mmHg while breathing room-air; ± defined as the presence of either of the following criteria: (i) right ventricle/left ventricle >1 in the apical 4-chamber view; (ii) maximal tricuspid regurgitant velocity > 2.7 m/s.*

Variables between groups were comparable except for older age and more frequent bed rest and hypoxemia in the group with abnormal troponin levels. The mean age of the patients was 64.8 years (range: 23-90). Thirty-seven (62%) were outpatients and 39 (65%) were female. Abnormal troponin T values were detected in 26 (43%) patients of the entire cohort (range 0.02 to 1.5 ng/mL). All patients were treated with heparin(s) followed by oral anticoagulants. Eight patients (13%) had adverse events in the in-hospital period, and all of them died. All deaths were considered to have been due to pulmonary embolism, and all but one were in the abnormal troponin group (27% [95%CI, 10 to 44] versus 3% [95%CI, 0 to 9], p=0.02).

The main characteristics of the patients who died are