No effect of polymorphisms in prothrombotic genes on the risk of myocardial infarction in young adults without cardiovascular risk factors

Martini, C.H.; Doggen, C.J.M.; Cavallini, C.; Rosendaal, F.R.; Mannucci, P.M.

9 Boresztajn K, Sobrier M-L, Fischer A-M, Chafa O, Amselem S, Tapon-Bretaudiere J. Factor VII gene intronic mutation in a lethal Factor VII deficiency: effects on splice-site selection. *Blood* 2003; **102**: 561–3.
10 Rosen E, Chan JCY, Idusogie E, Clotman F, Vlasuk G, Luther T, Jalbert LR, Albrecht S, Zhong L, Lissens A, Schoonjans L, Collen D, Costellino FJ, Carmeliet P. Mice lacking Factor VII develop normally but suffer fatal perinatal bleeding. *Nature* 1997; **390**: 290.
11 Peyvandi F, Mannucci PM, Jenkins PV, Lee A, Cappola R, Perry DJ. Homozygous 2 pb deletion in the human factor VII gene: a non lethal mutation that is associated with a complete absence of circulating factor VII. *Thromb Haemost* 2000; **84**: 635–7.
12 Marechetti G, Patracchini P, Gemmati D, De Rosa V, Pinotti M, Rodorigo G, Casonato A, Girolami A, Bernardi F. Detection of two missense mutations and characterization of a repeat polymorphism in the factor VII gene. *Hum Genet* 1992; **89**: 497–502.
13 Bernardi F, Liney DL, Patracchini P, Gemmati D, Legnani C, Arcieri P, Pinotti M, Redaelli R, Ballerini G, Pemberton S, Wacey AI, Mariani G, Tuddenham EGD, Marchetti G. Molecular defects in CRM + factor VII deficiencies: modelling of missenses mutations in the catalytic domain of factor VII. *Br J Haematol* 1994; **86**: 610–8.

No effect of polymorphisms in prothrombotic genes on the risk of myocardial infarction in young adults without cardiovascular risk factors

C. H. MARTINI,* C. J. M. DOGGEN,* C. CAVALLINI,† F. R. ROSENDAAL*‡ and P. M. MANNUCCI§
*Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands; †Cardiology Division, Hospital of Treviso, Treviso, Italy; ‡Hemostasis and Thrombosis Research Center, Leiden University Medical Center,Leiden, the Netherlands; and §Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Maggiore Hospital and University of Milan, Milan, Italy

To cite this article: Martini CH, Doggen CJM, Cavallini C, Rosendaal FR, Mannucci PM. No effect of polymorphisms in prothrombotic genes on the risk of myocardial infarction in young adults without cardiovascular risk factors. *J Thromb Haemost 2005*; **3**: 177–9.

Recently, the association between nine variants of prothrombotic genes, previously associated with myocardial infarction, were investigated in a large case–control study among young individuals [1]. The focus on the young stems from the assumption that atherosclerosis may play a minor role and that any risk related to a genetic predisposition for a prothrombotic state may be more easily detected. The study followed several reports on the effects of factor (F)V Leiden and prothrombin 20210A in young women [2,3]. The study included 1210 patients with myocardial infarction before age 50 and 1210 controls. Overall, there was no evidence supporting an association between these gene variants and the occurrence of myocardial infarction. However, even in these young individuals the presence of cardiovascular risk factors may have influenced the results. In young individuals who smoke or have hyperlipidemia, atheromatous plaque formation predisposes to myocardial infarction. The aim of the present study was to take the original reasoning even further by the most extreme inclusion criteria, and investigate whether in young individuals amongst whom atherosclerosis is least likely, i.e. in non-smoking, non-obese, non-hypertensive, non-hypercholesterolemic and non-diabetic persons, there would still be no association between prothrombotic gene variants and the development of myocardial infarction. Additionally, we evaluated the influence of a positive family history on developing myocardial infarction.

In the present study data from the same Atherosclerosis, Thrombosis and Vascular Biology Italian Study Group were used [1]. We selected individuals without major cardiovascular risk factors, including smoking, obesity [defined as a body mass index (BMI) > 30.0 kg m^{-2}], hypertension, hypercholesterolemia and diabetes, resulting in 54 cases and 55 controls. Information on cardiovascular risk factors and family history was collected by standardized questionnaires. To decrease statistical variation, we also contrasted the 54 cases with all 1210 controls, under the assumption that in non-diseased individuals the frequency of prothrombotic gene variants will not differ between those with and without cardiovascular risk factors. All participants provided a blood sample for DNA isolation. Subsequently, the following polymorphisms of genes encoding proteins involved in blood coagulation, platelet function, fibrinolysis and homocysteine metabolism were analyzed: the G455A polymorphism in the fibrinogen gene, the G1691A polymorphism in the FV gene (FV Leiden), the G20210A polymorphism in the

Correspondence: F. R. Rosendaal, Department of Clinical Epidemiology, C9-P, Leiden University Medical Center, PO Box 9600, NL-2300 RC Leiden, the Netherlands.
Tel.: +31 71 526 4037; fax: +31 71 526 6994; e-mail: f.r.rosendaal@lumc.nl

Received 7 September 2004, accepted 16 September 2004

prothrombin (factor II) gene, the G1097A polymorphism in the factor VII gene, the C807T polymorphism in the glycoprotein Ia gene, the C1565T polymorphism in the glycoprotein IIIa gene, the G185T polymorphism of the A subunit factor XIII gene, the 4G/5G polymorphism in the plasminogen activator inhibitor type 1 gene and the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene. The association of each of these genetic variants with risk was expressed as an odds ratio (OR) for developing myocardial infarction with a corresponding 95% confidence interval (CI). Adjustments were made for sex, age and geographic origin using unconditional logistic regression.

The 54 cases consisted of 38 men and 16 women, whereas the control group was composed of 38 men and 17 women. The mean age among cases was 35.6 years (range 20–47), and 38.1 (range 22–45) in the controls. Mean BMI among cases and control subjects was 24.5 kg m^{-2} and 23.6 kg m^{-2}, respectively. Despite our inclusion of patients without any cardiovascular risk factor, we failed to find evidence for an excess risk of myocardial infarction for any of the nine polymorphisms (Table 1). In contrast to the findings in other reports [4,5], there was no association of the selected polymorphisms with myocardial infarction, neither when compared with the control group without cardiovascular risk factors ($N = 55$), nor when compared with the overall control group ($N = 1210$). As the allele frequencies in both control groups were very similar, the choice of controls did not materially change the odds ratios. In the controls, allele frequencies of all polymorphisms were similar to those reported in literature among Caucasians [6], except for the MTHFR gene polymorphism, the frequency of which is higher in the Italian population [7].

Table 1 The effect of polymorphisms in prothrombotic genes in the young without cardiovascular risk factors and the influence of family history on the risk of developing myocardial infarction

Polymorphism	Cases ($N = 54$), *N* (%)	Controls ($N = 55$), *N* (%)	OR* (95% CI)	Controls ($N = 1210$), *N* (%)	OR (95% CI)
G455A fibrinogen gene					
GG	38 (70.4)	34 (61.8)		744 (61.5)	
GA	14 (25.9)	20 (36.4)	0.7 (0.3, 1.7)	398 (32.9)	0.7 (0.4, 1.3)
AA	2 (3.7)	1 (1.8)	0.9 (0.1, 11.9)	68 (5.6)	0.6 (0.1, 2.6)
G1691A factor V gene					
GG	51 (94.4)	53 (96.4)		1166 (96.4)	
GA	3 (5.6)	2 (3.6)	1.6 (0.2, 11.0)	44 (3.6)	1.6 (0.5, 5.2)
AA	0	0	–	0	–
G20210A prothrombin gene					
GG	52 (96.3)	52 (94.5)		1171 (96.8)	
GA	2 (3.7)	3 (5.5)	0.7 (0.1, 4.8)	38 (3.1)	1.2 (0.3, 5.1)
AA	0	0	–	1 (0.1)	–
G1097A factor VII gene					
GG	37 (68.5)	40 (72.7)		863 (71.3)	
GA	17 (31.5)	14 (25.5)	1.1 (0.5, 2.6)	325 (26.9)	1.2 (0.7, 2.2)
AA	0	1 (1.8)	–	22 (1.8)	–
C807T platelet glycoprotein Ia gene					
CC	25 (46.3)	24 (43.6)		483 (39.9)	
CT	21 (38.9)	26 (47.3)	0.8 (0.3, 1.7)	557 (46.0)	0.7 (0.4, 1.3)
TT	8 (14.8)	5 (9.1)	1.3 (0.4, 4.9)	171 (14.1)	0.9 (0.4, 2.1)
C1565T platelet glycoprotein IIIa gene					
CC	35 (64.8)	41 (74.5)		865 (71.5)	
CT	16 (29.6)	12 (21.8)	1.7 (0.7, 4.3)	307 (25.4)	1.3 (0.7, 2.4)
TT	3 (5.6)	2 (3.6)	1.7 (0.2, 11.5)	38 (3.1)	2.0 (0.6, 7.1)
G185T factor XIII gene					
GG	39 (72.2)	34 (61.8)		789 (65.2)	
GT	13 (24.1)	18 (32.7)	0.6 (0.3, 1.5)	363 (30.0)	0.7 (0.4, 1.4)
TT	2 (3.7)	3 (5.5)	0.7 (0.1, 4.3)	58 (4.8)	0.7 (0.2, 3.2)
4G/5G PAI-1 gene					
5G/5G	20 (37.0)	19 (34.5)		342 (28.3)	
4G/5G	19 (35.2)	29 (52.7)	0.7 (0.3, 1.7)	588 (48.6)	0.6 (0.3, 1.1)
4G/4G	15 (27.8)	7 (12.7)	2.1 (0.7, 6.5)	280 (23.1)	0.9 (0.5, 1.8)
C677T MTHFR gene					
CC	14 (25.9)	17 (30.9)		363 (30.0)	
CT	24 (44.4)	28 (50.9)	1.0 (0.4, 2.8)	620 (51.2)	1.0 (0.5, 2.0)
TT	16 (29.6)	10 (18.2)	2.0 (0.7, 5.9)	252 (20.8)	1.7 (0.9, 3.2)
Family history of myocardial infarction					
No	29 (53.7)	38 (69.1)			
Yes	25 (46.3)	17 (30.9)	1.9 (0.9, 4.2)		

*Odds ratio, adjusted for sex, age and geographical origin.

This study included only survivors of myocardial infarction. If prothrombotic mutations lead to severely altered plasma concentrations of hemostatic factors, this may have produced some early deaths and therefore an underestimation of the risk of developing myocardial infarction. Because of the extreme inclusion criteria we used, the study size has become too small to enable us to exclude reliably an effect of variants with low allele frequencies, such as FV Leiden and prothrombin 20210A mutation, that have previously been found to increase risk in young women [2,3]. Our results are therefore not inconsistent with a mild deleterious effect of these variants. Family history was a risk factor for myocardial infarction in young individuals without a cardiovascular risk factor, as a higher prevalence of familial myocardial infarction was found among cases (46.3%) compared with controls without any cardiovascular risk factor (30.9%), leading to an odds ratio of 1.9 (95% CI 0.9, 4.2). Whether this increased risk is the result of socio-economic circumstances and related lifestyle or an undiscovered genetic influence is unclear. In conclusion, in a subgroup of young survivors of myocardial infarction without any cardiovascular risk factor, we found no support for a causal role of nine prothrombotic genetic variants in the development of myocardial infarction.

References

1 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.

2 Rosendaal FR, Siscovick DS, Schwartz SM, Beverly RK, Psaty BM, Longstreth WT Jr, Raghunathan TE, Koepsell TD, Reitsma PH. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. *Blood* 1997; **89**: 2817–21.

3 Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood* 1997; **90**: 1747–50.

4 Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. *Blood* 2000; **95**: 1517–32.

5 Reiner AP, Siscovick DS, Rosendaal FR. Hemostatic risk factors and arterial thrombotic disease. *Thromb Haemost* 2001; **85**: 584–95.

6 Tang Z, Tracy RP. Candidate genes and confirmed genetic polymorphisms associated with cardiovascular disease. *J Thromb Thrombol* 2001; **11**: 49–81.

7 Margaglione M, D'Andrea G, d'Addedda M, Giuliani N, Cappucci G, Iannaccone L, Vecchione G, Grandone E, Brancaccio V, Di Minno G. The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin A^{20210} mutation. *Thromb Haemost* 1998; **79**: 907–11.

Hemostatic effects of diets containing olive or soy oil in hypertensive patients

A. TRIFILETTI, R. SCAMARDI, A. GAUDIO, A. LASCO and N. FRISINA

Department of Internal Medicine, University of Messina, Messina, Italy

To cite this article: Trifiletti A, Scamardi R, Gaudio A, Lasco A, Frisina N. Hemostatic effects of diets containing olive or soy oil in hypertensive patients. *J Thromb Haemost* 2005; **3**: 179–80.

Various studies have shown that the Mediterranean diet is associated with a low incidence of atherosclerotic disease [1–5]. A recent finding indicates that dietary interventions based on the use of extra-virgin olive oil and a significant reduction in total and saturated fatty acids intake favorably affect blood pressure (BP) control in pharmacologically treated hypertensive patients, significantly decreasing the required daily dosage of antihypertensives [6].

In the present study we evaluated the effect of extra-virgin olive oil (MUFA) and soy oil (PUFA) diet for 3 months on the hemostatic system, lipid profile and blood pressure in a group of hypertensive patients. This investigation was carried out in 44 consecutive patients [30 men and 14 women, mean age 49 ± 4 years, body mass index (BMI) 24.1 ± 3.0 kg m^{-2}] with essential hypertension with objective organic change according to international guidelines [7], admitted to the Division of Internal Medicine, University Polyclinic of Messina, Italy. Patients taking oral anticoagulants, with valvular heart disease, with severe heart failure or with any other serious pathology were excluded. Patients with symptoms of peripheral vascular disease or ischemic heart disease were also excluded. All patients were treated with antihypertensive drugs (ACE inhibitors, β-blockers, diuretics, Ca^{2+} channel blockers). Patients taking antiplatelets drugs (18/44) underwent a 4-week washout of these drugs. The study protocol was approved by the Ethics Committee of the University of Messina School of Medicine. Patients were randomly divided in two groups. These groups were prescribed two well-balanced diets with almost 6200 kJ daily, identical in daily content of proteins (17 ± 3%), carbohydrates (56 ± 5%), total lipids

Correspondence: A. Trifiletti, Via Monsignor D'Arrigo, n.23, 98121 Messina, Italy.
Tel.: +39 09 0221 2370; fax: +39 293 5162; e-mail: aldo.trifiletti@unime.it

Received 28 August 2004, accepted 27 September 2004