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Plasminogen Activator Inhibitor-1-675 4G/5G and Methylenetetrahydrofolate Reductase Gene Variants in Young Acute Myocardial Infarction and Juvenile Ischemic Stroke

Giulia Bivona, Chiara Bellia, Salvatore Cammarieri, Bruna Lo Sasso, Rosachiara Carollo, Rosalinda Raineri, Gaia Chiarello, Antonietta Caruso and Marcello Ciaccio
Department of Clinical Biochemistry, Faculty of Medicine, University of Palermo, Palermo, Italy

Abstract: Cardiovascular diseases often recognize a hereditary-familial genetic risk patterns and a substantial proportion of variability in clinical features of atherosclerosis could probably be explained by genetic factors. Aim of this study is to compare prevalence of factor V Leiden, factor II 20210A, PAI-1-675 4G/5G and MTHFR C677T gene variants in 2 cardiovascular disease clinical features: young acute myocardial infarction (<50 years) and ischemic stroke. Plasminogen Activator Inhibitor-1-675 4G/5G didn't show significant difference between patients and controls while, between patient groups, the polymorphism was most present in myocardial infarction group than in juvenile stroke. Polymorphisms in Homocysteine metabolism: MTHFR C677T variants was significantly higher in patients with myocardial infarction compared with those affected by ischemic Stroke.

Key words: Thrombophilia, stroke, myocardial infarction, gene variants, plasminogen

INTRODUCTION

Cardiovascular diseases often recognize a hereditary-familial genetic risk patterns and a substantial proportion of variability in clinical features of atherosclerosis could probably be explained by genetic factors (Lane and Grant, 2000; Voetsch and Loscalzo, 2004). It still remains controversial (Juul *et al*., 2002; Rasche, 2004) the role of 6 haemostatic gene polymorphisms that alters the function or plasma levels of the proteins involved in coagulation and fibrinolysis pathways; the following gene variants are the most interesting to investigate (Ye *et al*., 2006): factor V G1691A, Prothrombin G20210A, factor XIII Val34Leu, Plasminogen Activator Inhibitor-1 (PAI-1)-675 4G/5G, β-fibrinogen-455G<A and Methylene-tetrahydrofolate Reductase (MTHFR) C677T which is related to Homocysteine metabolism. Among these, factor V Leiden, factor II 20210A, PAI-1 and MTHFR C677T variants seem to be the most strongly associated with coronary disease (Kim and Becker, 2003; Lalouschek *et al*., 2005), regarding on the other polymorphisms much weaker association has been indicated (Endler and Mannhalter, 2003). Aim of this study is to compare prevalence of factor V Leiden, factor II 20210A, PAI-1-675 4G/5G and MTHFR C677T gene variants in 2 cardiovascular disease clinical features: young acute myocardial infarction (<50 years) and ischemic stroke.

MATERIALS AND METHODS

Ninty eight subjects (54 females, 44 males; age 39±4) were selected among all patients consecutively referred to our unit from March 2005 to April 2008 for genotype analysis and Homocysteine assessment. In all patients indications for genetic analysis were: documented atherosclerotic cardiovascular disease, young acute myocardial infarction (<50 years), ischemic stroke, familial history for early Coronary Artery Disease (CAD), deep venous thrombosis and Venous Thromboembolism (VTE).

Including criteria for the study were: documented previous young myocardial infarction or juvenile ischaemic stroke. Fifty two subjects with history of myocardial infarction and 46 ischemic stroke were selected for the study; all of them underwent complete anamnesis and objective examination and traditional risk factors, including smoking, hypertension, diabetes, dyslipidaemia were assessed; biochemical analysis for Homocysteine plasma levels assessment and genotype analysis for: Factor V G1691A, Prothrombin G20210A, PAI-1-675 4G/5G, MTHFR C677T. We also enrolled 100 sex and age-matched healthy subjects (40 males and 60 females) as control group. Plasma Homocysteine was determined with Axsym instrument (Abbott, IL, USA). The Imx Hcy assay is based on reduction of the plasma samples with dithiothreitol and subsequent conversion of free Hcy to S-adenosyl homocysteine by hydrolase in the presence of

Corresponding Author: Prof. Marcello Ciaccio, M.D., Chair of Clinical Biochemistry, Faculty of Medicine, University of Palermo, Policlinico Universitario-Via del Vespro, 129-90127 Palermo, Italy

added adenosine. The sample and the tracer compete in binding to the monoclonal antibody. This reaction is followed by detection of S-adenosyl homocysteine by a fluorescence polarization immunoassay. The concentration of tHcy in plasma is inversely related to the intensity of the polarized light. The intra-run CV was 2.1%. Genomic DNA was extracted from 300 μL of peripheral blood collected in EDTA-tubes with the Puregene Blood kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All blood samples were kept at -20°C until DNA isolation and analysis. Genotyping for Factor V G1691A, Prothrombin G20210A, MTHFR C677T and PAI-1 4G/5G was conducted by real time-PCR with Light Cycler instrument (Roche, Mannheim, Germany) according to the method described and provided with the kits by the manufacturer. Light Cycler is based on use of fluorescence resonance energy transfer (FRET) probes and melting curve analysis. To compare the difference between median ages McNemar test was performed. To compare the difference between distribution of each gene variant in patient subgroups Wilcoxon U test was performed using SPSS software (Chicago, IL, USA).

RESULTS

Baseline parameters of patients and controls are given in Table 1. Most conventional risk factors were more prevalent in the patient group. Significant difference in serum Homocysteine levels was observed in patients compared with controls, while between patient groups Homocysteine levels were similarly increased. Polymorphisms in coagulation and fibrinolysis genes: factor V G1691A and factor II G20210A prevalence was higher in patients than in controls; Plasminogen Activator Inhibitor-1-675 4G/5G didn't show significant difference between patients and controls while, between patient groups, the polymorphism was most present in myocardial infarction group than in juvenile stroke (Table 2 and 3). Polymorphisms in Homocysteine metabolism: MTHFR C677T variants was significantly higher in patients with myocardial infarction compared with those affected by ischemic Stroke.

Table 1: Classical risk factors for cardiovascular disease in patients enrolled

	Cases (n 98)	Controls (n 100)	p-value
Age median (IQR)	39±4	40 (29-47)	ns**
Female (%)	54 (55)	56 (56)	ns *
Hypertension	58 (59)	30 (30)	<0.05*
Diabetes	20 (21)	12 (12)	<0.05*
Hyperlipaemia	45 (46)	25(25)	<0.05*
BMI > 30	49 (50)	22 (22)	<0.05*
Current cigarette smoking	47 (48)	29(29)	<0.05*
Hyperomocysteinemia	20 (20.4)	12(12)	<0.05*

**Wilcoxon Test; ** McNemar Test

Table 2: Prevalence of gene polymorphisms in study patients and controls

Gene variants	Cases (98)	Controls (100)	p-value
Factor V G1691A			
GG	86 (88)	99 (99)	ns*
GA	12 (12.2)	1 (1)	ns*
AA	/	/	
PAI-1 -675			
5G/5G	37 (37.7)	52 (52)	ns*
4G/5G	36(36.7)	29 (29)	p<0.05*
4G/4G	25(25.5)	19 (19)	ns*
Factor II G20210A			
GG	89 (90.8)	100 (100)	ns*
GA	9(9.2)	/	ns*
AA	/	/	
MTHFR C677T			
CC	66(67.3)	78(78)	ns*
CT	28(28.6)	22(22)	p<0.05*
TT	4(4)	/	

Table 3: Prevalence of gene polymorphisms in patients groups

Gene variants	Stroke (46)	Myocardial infarction (52)	p-value
Factor V G1691A			
GG	43(95)	43(82.7)	ns*
GA	3 (6.5)	9 (17.3)	ns*
AA	/	/	
PAI-1 -675			
5G/5G	30(65.2)	7(13.5)	p<0.05*
4G/5G	11(23.9)	25(48.07)	p<0.05*
4G/4G	5(10.9)	20(38.5)	
Factor II G20210A			
GG	43(93.5)	46(88.5)	ns*
GA	3(6.5)	6(11.5)	ns*
AA	/	/	
MTHFR C677T			
CC	42(91.3)	24(46)	ns*
CT	4(8.7)	24(46)	p<0.05*
TT	/	4(7.7)	ns*

DISCUSSION

Such a haemostatic gene variants were considered involved in arterial disease pathogenesis, but the role of these polymorphisms was not clearly defined (Lane and Grant, 2000). Acute myocardial infarction (<50 years) and ischemic stroke in young people represent clinical contests underlying endotelial dysfunction. Our findings of higher prevalence of coagulation and fibrinolysis gene polymorphisms in patients with acute myocardial infarction and iuvenile stroke are consistent with recent literature data (Voetsch *et al*., 2000); particularly regarding on 1691A factor V and G20210A factor II variants, both produce increased circulating thrombin generation and it can explain a highly significant association between coronary and cerebrovascular risk and 1691A factor V and G20210A factor II variants; as reported by previous studies (Madonna *et al*., 2002) and confirmed in our study prothrombin and factor V polymorphisms are associated with a higher rate of cardiovascular and cererbrovascular risk in patients presenting classical risk factors as cigarette smoking (Lopaciuk *et al*., 2001). However, this

association remains controversial, as reported in previous studies performed by others Authors (Hankey *et al*., 2001); furthermore, our study is probably affected by the small samples of patient subgroups (coronary and cerebrovascular events) that result in a lack of significance of differences between prevalence of gene variants. Regarding on PAI-1 gene variants previous studies (Ye *et al*., 2006) reported controversial association with myocardial infarction; in this study, we observed a higher prevalence in 4G/5G and 4G/4G polymorphisms in myocardial infarction subgroup than in stroke one, finding a significant difference between distribution of PAI gene variants in spite of small sample size; in the same patient subgroup which represents the myocardial infarction population, we found a higher prevalence of MTHFR C677T compared with juvenile stroke subpopulation. The thermolabile variant of MTHFR C677T is a common cause of Hyperhomocysteinemia, which is considered a laboratory feature of cardiovascular disease spectrum (Lane *et al*., 2005); although several clinical and experimental *in vitro* studies (Al-Obaidi *et al*., 2000) suggested that the role of the molecule in arterial thrombosis remains unclear and controversial. Our finding is consistent with some Authors (Cleophas *et al*., 2000), who reported an association of statistical significance between coronary risk and MTHFR C677T variant-related Hyperhomocysteinemia; however, literature data provided from other studies (Christen *et al*., 2000) showed weak association between cerebrovascular risk and high Homocysteine levels. Finally, our purpose for future is to investigate whether exist an association between myocardial infarction and PAI 1 and MTHFR gene variants which may sinergically induce cogulation imbalance and endothelial dysfunction.

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