[Frontiers in Bioscience E2, 559-565, January 1, 2010]

Haemostatic gene polymorphisms in young Sardinian with acute myocardial infarction

Lorena Musino[1], Renata Rossi[1], Adolfo Partenza[2], Giovanna Mureddu[3], Angelo Zinellu[1,6], Patrizia Pilo[3], Ignazio Maoddi[3], Liana Spazzafumo[4], Flavia Franconi[5, 6], Luca Deiana[1], Ciriaco Carru[1, 6]

[1]Department of Biomedical Sciences and Centre of Excellence for Biotechnology Development and Biodiversity Research, University of Sassari, Italy, [2]Laboratory Analysis Unit , C. Zonchello, Italy, [3]UTIC, ASL N.3 Nuoro, Italy, [4]INRCA Ancona, Italy, [5]Department of Pharmacology and Centre of Excellence for Biotechnology Development and Biodiversity Research, University of Sassari, Italy,[6]National Laboratory of the National Institute of Biostructures and Biosystems, Osilo, Italy

TABLE OF CONTENTS

1. ABSTRACT

Although the role of environmental factors in the development of acute myocardial infarction (AMI) has been clearly established, the role of genetic factors is still undefined. The aim of this study was to investigate the association between various gene polymorphisms in the haemostatic system and the risk of myocardial infarction in a very genetic restricted area population of Sardinian young adults with AMI. The study case-control involved 71 patients who had survived a first MI at a mean age of 47,2 years and 150 healthy subjects. No differences in the allele or genotype frequencies were seen between the study groups for the fibrinogen, prothrombin, factor V, factor VII, vWF, TM, PAI-1, TPO gene, and PLA and HPA-2 genes polymorphisms. Indeed differences statistically significant were detected for A5709G in the TPO gene (P= 0,041), and I/D dimorphism in the eNOS gene (P= 0,016). We therefore conclude that among all the investigated polymorphisms only the 5709G and eNOS4a alleles seem to confer protection against MI in the young age of Sardinian people.

2. INTRODUCTION

Myocardial infarction (AMI) is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual genetic background and various environmental factors (1). In general, the incidence of myocardial infarction increases additively as a function of the number of conventional risk factors, including hypertension, diabetes mellitus, and hypercholesterolemia (2). Although the role of environmental influences in the development of acute myocardial infarction has been thoroughly investigated, the role of genetic markers (particularly those related to thrombogenesis) is poorly defined. Acute myocardial infarction (AMI) is thought to be caused by thrombosis on the surface of a ruptured atherosclerotic plaque of a coronary artery (3). A number of reports have investigated the association between various genetic polymorphisms and the phenotypic expression of myocardial infarction. Endothelium-derived nitric oxide (NO) is synthesised by endothelial nitric oxide synthase (eNOS). Several studies suggest that the basal release of NO by the endothelium

contributes to basal vascular tone (4) and regulates blood flow and blood pressure. NO also inhibits the proliferation of smooth muscle cells (5), protects against platelet aggregation *in vitro* and *in vivo* (6-7), inhibits platelet adhesion to vascular endothelium (8) and leukocyte adhesion to endothelium (9). Because dysfunction of this important mechanism has been implicated in the development of coronary atherosclerosis and may increase the risk of thrombosis, leading to AMI, we hypothesised that polymorphisms of *NOS3* might be associated with increased susceptibility to this disorder. At the sites of vascular injury, when subendothelium is exposed, thrombus formation is initiated by binding of glycoprotein Ib-IX-V to von Willebrand factor (vWF). Stable adhesion, activation and aggregation of platelet are then mediated via GpIa/IIa binding to exposed collagen and GpIIb/IIIa binding to vWF and fibrinogen. The HPA-2a/b polymorphism in the GpIb gene, is adjacent to the vWF-binding site and might therefore influence the receptor-vWF interaction (10). A polymorphism of the gene encoding the IIIa subunit, with the two forms PlA1 and PLA2 has been identified and associated with an increased risk of MI. VWF is recognized as playing an essential role in the tethering and activation of platelets at sites of vascular injury under shear conditions typical of the arterial circulation. Elevated plasma vWF levels are associated with coronary artery disease, although the precise mechanism for this is unclear, and recent reports have shown a strong association between polymorphisms in the gene promoter and plasma vWF level. The primary regulator of platelet production is thrombopoietin (TPO), a haematopoietic factor which stimulates platelet production through its trophic effects on bone marrow megakaryocytes (11). Mutation in the TPO gene may influence its levels or activity and have profound effects on platelet production which in turn leads to disease. Polymorphisms in the factor VII (FVII) gene have been associated with either coagulant FVII, activated FVII, or mass factor VII levels. Recent data have reported that the R353Q and G73A polymorphisms were related with the risk of MI, the frequency of the rare allele Q and A respectively was higher in controls than in cases (12-13). Recently, a novel inherited risk factor for venous thrombosis was identified. A G→A transition at nucleotide 20210 in the 3' untranslated region of the prothrombin gene was associated with a higher prothrombin clotting activity and a 2.7-fold increased risk for venous thrombosis (14). The G1691A factor V point mutation renders factor V relatively insensitive to proteolytic degradation by activated protein C (15), thus creating a procoagulant state, which increases the risk of venous thrombosis approximately 2- to 4- fold compared with non-carriers. Fibrinogen is the last target of coagulation: its lysis by thrombin produces soluble fibrin fragments that are then stabilizes in a clot by FXIII. It is a mediator of platelet aggregation and a determinant of plasma viscosity. *In vitro* studies have suggested that β chain synthesis limits the rate of production of mature fibrinogen. As a consequence, polymorphisms affecting the production of this chain would more likely influence the levels of fibrinogen. Overall, the studies show a strong association between β chain fibrinogen genotypes and their plasma concentration: an *Hae*III -455G/A polymorphism (β chain) located in the promoter region of the gene is associated with increased fibrinogen levels. Thrombomodulin (TM) is a thrombin receptor expressed on the luminal surface of endothelial cells. The TM-thrombin complex can activate protein C, which then acts as a potent anticoagulant. Dysfunction of TM plays an important role in the pathogenesis of MI. Recent findings suggest that mutations in the promoter region of the TM gene may constitute a risk for arterial thrombosis. During fibrinolysis, plasminogen is converted to plasmin, which in turn lyses fibrin clots; this conversion is controlled to a large extent by the serine protease inhibitor plasminogen activator inhibitor-1 (PAI-1). A promoter polymorphism in the PAI-1 gene, at position -675 (4G/5G) influences PAI-1 levels. Acute myocardial infarction (AMI) at a young age (< 45 years) has shown significantly different risk profiles in young patients when compared with older patients: smoking and a family history of ischemic heart disease are more frequent. It is not clear whether AMI at a young age can be considered an independent model of infarction or a more premature and accelerated aspect of the same atherosclerotic process observed in older patients. It is characterized by low mortality rates, less extensive coronary artery disease (CAD), good residual left ventricular function, and a favourable prognosis (16). It is therefore biologically plausible that changes in haemostasis factors leading to prothrombotic phenotypes of hypercoagulability, heightened platelet function, hypofibrinolysis, and hyperomocystinemia (as well as their genetic determinants) play a relevant role in younger patients with myocardial infarction (17). Because coronary artery thrombosis over a ruptured atherosclerotic plaque is likely to be the prevailing pathogenetic mechanism of MI, especially of those occurring in young age, we chose to investigate the association between those genetic factors known to be related with an increased thrombosis tendency and the occurrence of myocardial infarction in a selected group of young survivors. The aim of this study was to investigate the association between various gene polymorphisms in the haemostatic system and the risk of young myocardial infarction in a selected population of Sardinian, characterized by a genetic background quite different from mainland Italy and from other European country. It is noteworthy that the epidemiological data available on CHD in Sardinia indicate the low mortality of this population, compared with mainland Italy.

3. MATERIALS AND METHODS

3.1. Subjects

The study population (61 Male/10 Female), belonged to the district of Nuoro, was recruited among patients ≤ 55 years of age (mean age 47,2; age range 31-55), consecutively admitted between June 2001 and April 2002 to the Coronary Care Unit of Hospital S. Francesco, ASL n3 Nuoro, who satisfied the World Health Organization criteria for the diagnosis of myocardial infarction (18). Healthy subjects (100 Male/ 50 Female) with the same age distribution (mean age 50, age range 35-61) randomly recruited among the general Sardinian population were examined as controls. The study was approved by the Hospital Ethics Committee,

ASL N°3 Nuoro and all the individuals were aware of the experimental nature of this study and gave their informed consent to use their samples for these specific DNA analyses.

3.2. DNA analysis

DNA was extracted from whole blood using the salting out method (19). The following polymorphisms of genes encoding proteins involved in blood coagulation, platelet function, and fibrinolysis were analyzed:

3.2.1. G-455A Polymorphism of the fibrinogen gene

The G to A transition located in the β fibrinogen gene promoter was detected by means of polymerase chain reaction (PCR) and digestion with *HaeIII* (New England BioLabs) restriction enzyme. The digestion products were visualized by electrophoretic separation on 2% agarose gel (20).

3.2.2. G20210A Polymorphism of the prothrombin (factor II) gene

The prothrombin 20210 G to A variation was detected by use of the 5' primer in exon 14 and a mutagenic primer in the 3' untranslated region to create a *HindIII* restriction site (14).

3.2.3. G1691A Polymorphism of the factor V gene

The mutation was determined by amplifying a region of exon 10 and the adjacent intron by PCR using forward primer as previously described (21) and reverse mismatch primer 5'GGTTACTTCAAGGACAAAATACCTGTATTCTT designed in our laboratory. The PCR products were digested with *Mbo*II, electrophoresed through 2% agarose gel and stained with ethide bromure.

3.2.4. G10976A and G73A Polymorphisms of the factor VII gene

The G10976A polymorphism in the codon for aminoacid 353 of exon 8 in the factor VII gene, responsible for the replacement of Arg by Gln, was determined as previously described (22). In this method, the fragment of DNA is amplified by PCR followed by digestion with *Msp*I restriction enzyme (New England BioLabs). The G to A substitution at position +73 was analysed using the method described by Peyandi (12). The digestion was performed with *MspI* enzyme and the fragments were separated on 2,5% agarose gel.

3.2.5. PLA1/PLA2 Polymorphism of the platelet glycoprotein IIIa gene

To detect the substitution of cytosine for thymidine responsible for the *PlA2* polymorphism at position 1565 in exon 2 of the glycoprotein IIIa gene, we used PCR to amplify the genomic DNA with primers flanking exon 2 (23).

3.2.6. Platelet glycoprotein HPA-2 polymorphism

The HPA-2 polymorphism, a substitution of Thr145Met in GpIα, was detected by PCR amplification of 587bp fragment using two oligonucleotide primers followed by *Bsa*HI digestion, as previously described (24).

3.2.7. 4G/5G Polymorphism of the Plasminogen Activator Inhibitor Type I Gene

The single-allele insertion/deletion located at nucleotide 675 in the promoter region was determined by PCR and digestion with *Bsl*I restriction enzyme (25)

3.2.8. G33A mutation in the Thrombomodulin gene

To identify the G33A mutation in the promoter region of the thrombomodulin gene, a 313bp fragment was amplified and digested using a PCR-RFLP method (26).

3.2.9. TPO A5713G AND C4830A Polymorphisms

The A to G transition in the 3' untranslated region and the C to A change at position 4830 in the TPO gene were detected by PCR. The amplified fragments were digested with *NlaIII* and *MvaI* restriction enzymes respectively (27).

3.2.10. The -1185 G/A and – 1051 A/G dimorphisms in the vWF gene

A 475bp fragment, spanning nucleotides -1380 to -906 of the vWF gene, was amplified from genomic DNA by PCR using the oligonucleotides primers described by Di Bitondo *et al.* (28). The -11185 G/A and -1051 A/G dimorphisms were detected by digestion of aliquots of the PCR products with *Bst*UI or *Nla*III respectively.

3.2.11. NOS

We used the PCR to identify the different alleles of a 27bp tandem repeat polymorphism in intron 4 of the NOS3 gene. The PCR products were directly visualized on 2,5% agarose gel (29).

3.3. Statistical Analysis

The characteristics of the sample were described by mean and standard deviation for continuous variables. Differences between non-fatal acute MI group and healthy controls were assessed by unpaired Student t test. The potential association between polymorphisms and myocardial infarction at young age was evaluated by calculating the odds ratios (OR) and 95% confidence intervals (CI). Throughout the study a two-side $p \leq 0.05$ was taken as level of statistical significance. All the analyses were performed using SPSS (Chicago, IL) version 11.5.

4. RESULTS

The fraction of female patients was low (14%) and in agreement with the prevalence of coronary disease in young women. The mean age was not different between patients and controls; nevertheless only the HDL concentration, as shown in Table 1, was significantly different. The distribution of the genotype and allele frequencies between the two study groups are shown in Table 2. The results indicate that there was no difference between the young survivors of myocardial infarction and controls in terms of genotype or allele frequencies for the following polymorphisms: G455A of the fibrinogen gene, G20210A of the prothrombin gene, G1691A of the factor V gene, G10976A and G73A of the factor VII gene, A1051G and G1185A of the von Willebrand factor gene, G33A of

Table 1. Lipid profile among young survivors of myocardial infarction and controls

	AMI patients N=71	*Controls N=150*	*P value*
Total cholesterol (mg/dL)	194,6 ± 43,6	202,3 ± 42,2	n.s.
HDL cholesterol (mg/dL)	46,6 ± 9,16	55, 9 ± 18,3	P <0.01
LDL cholesterol (mg/dL)	121,3 ± 32,17	124,9 ± 39,0	n.s.
Triglycerides (mg/dL)	131,5 ± 86,01	142,24 ± 72,16	n.s.

the thrombomodulin gene, 4G/5G of the PAI-1 gene, and C4826A of the thrombopoietin gene. The factor V 506Gln (G→A) allele occurred with very low frequency in MI patients (0,7%) and was not observed in the controls. Two polymorphisms showed statistically significant evidence of association with myocardial infarction: eNOS I/D and A5709G of the TPO gene. The A5709G transition was associated with a significant protective effect on myocardial infarction (OR 0,37, 95% CI 0,14 - 0,98) as that allele frequencies (OR 0,63, 95% CI 0,41- 0,96). Similar data were observed for eNOS I/D oxide nitric sinthase gene dimorphism: the allele D frequency was higher in the controls than cases (7% vs 16% respectively; OR 0,40, 95% CI 0,19 – 0,82).

5. DISCUSSION

Coronary heart disease has a notable genetic background, and genes have been thought to play an important role in the aetiology of CHD, especially in the youngest cases. The importance of haemostasis in the pathogenesis of acute myocardial infarction has been established by pathological and angiographic findings of coronary thrombosis and by knowledge that plasma levels of proteins involved in the haemostatic mechanism (such as fibrinogen, factor VII, tissue plasminogen activator antigen, and its principal inhibitor) are associated with susceptibility or protection against myocardial infarction, especially in younger patients. Isolated populations have been long a subject of interest for both population and medical genetics studies (30). They are subpopulations resulting from the founder effect of a small number of individuals as a consequence of some types of bottleneck (31). They lived in geographical and cultural isolation over many generations without genetic interchange with other subpopulations (32-33). The Sardinian population, such as those from the other Mediterranean islands, may well suit to case-control designs because they are perceived as homogeneous. It is an ancient genetic isolated population with a unique distribution of alleles at multiple loci and with one of the highest frequency of many common genetic disorders, such as type 1 diabetes mellitus and multiple sclerosis, in the world. In particular, several authors observed a very low degree of genetic differentiation between the population of the different island regions submitted to repeated invasions in the past and the population of the more isolated inland regions (34), others brought evidences of heterogeneity at microgeografic level, in which genetic features render the population of the centre of the island adapted for the genetic analysis for complex traits (35). With this background, our study was designed to assess the degree of association between genetic markers that previous studies linked to an increase thrombotic tendency and the occurrence of myocardial infarction in a selected group of young survivors of myocardial infarction, mainly men. We have shown that the frequency of the D allele in the eNOS gene is higher in controls than in the IMA patients, thus suggesting that this allele protects against premature myocardial infarction, but a large sample and other variant alleles need to be studied. Nitric oxide is synthesized from the aminoacid L-arginine by a family of enzymes known as NO synthases, of which eNOS is constitutively expressed in the endothelium. The biological effects of endothelial derived NO include vasodilatation, inhibition of vascular smooth muscle cell growth, anti-atherosclerotic properties, prevention of platelet aggregation and inhibition of adhesion of white cells to the blood vessel whole (5, 7). NO is also known to suppress the production of the potent vasoconstrictors endothelin and angiotensin II, which also induce vascular smooth muscle cell proliferation (36). In the present study the A5709G polymorphism in the 3^{I} UTR region of the thrombopoietin gene was associated with myocardial infarction. The high expressor genotype GG in the controls was linked to reduced risk of disease. This is in contrast to previous reports describing association of the G allele with increased risk of MI at a young age in the Italian sample (27). None of the remaining genetic markers evaluated in this study were associated with the occurrence of premature myocardial infarction. In particular, we did not succeed in observing the previously reported “protective” effect of the polymorphic alleles of the factor VII gene identified as Gln353 and 73A alleles. The results of studies correlating genetic variations with the risk of a disease are often too inconsistent to draw conclusions. This can be attributed to heterogeneity of the studies in terms of outcome definition, design, selection of cases and controls, study size, genetic makeup of the population study, and the interaction with acquired or environmental risk factors. A limitation of our study is that only patients who had survived a myocardial infarction were enrolled. It cannot be excluded that prothrombotic mutations may be associated with more severe myocardial infarctions and a large number of early deaths; thus, their effects on the myocardial infarction risk may be underestimated. However, our results should considered preliminary, based on a small number of patients and need confirmation by replication in a larger sample or by a prospective studies on a large series of young healthy individuals followed up until they reach the end points of myocardial infarction and cardiac death could overcome the limitation of our study. Nevertheless, our data suggest a possible association between the studied prothrombotic gene variants and AMI in a group that is characterized by a strong genetic homogeneity.

6. ACKNOWLEDGEMENTS

This study was supported by the “Fondazione Banco di Sardegna – Sassari – Italy”, and by the “Assessorato Sanità della Regione Autonoma Sardegna –

Table 2. Distribution of genetic polymorphisms in survivors of myocardial infarction at a young age and controls

Polymorphisms	Cases	Controls	Odds Ratio
	N= 71	N= 152	95% CI
PLA			
A1/A1	41 (59,4)	95 (65,5)	1 (Reference)
A1/A2	22 (31,9)	43 (29,7)	1,18 (0,63 – 2,23)
A2/A2	6 (8,7)	7 (4,8)	1,98 (0,63 – 6,27)
Allele Frequency			
A1	104 (75,4)	233 (80.3)	
A2	34 (24,6)	57 (19.7)	0,75 (0,46 – 1,21)
FI-455GA			
GG	40 (58,0)	78 (60,0)	1 (Reference)
GA	24 (34,8)	48 (36,6)	0,97 (0,52 – 1,81)
AA	5 (7,2)	5 (3,8)	1,95 (0,53 – 7,13)
Allele frequency			
G	104 (75,4)	204 (77,9)	
A	34 (24,6)	58 (22,1)	0,87 (0,54 – 1,41)
FVIIG10976A			
GG	48 (30,0)	106 (73,0)	1 (Reference)
GA	21 (29,6)	39 (26,7)	1,20 (0,63 – 2,23)
AA	52 (2,8)	1 (0,7)	4,42 (0,40 – 49,90)
Allele frequency			
G	117 (84,4)	251 (86,0)	
A	25 (17,6)	41 (14,0)	0,76 (0,44 – 1,32)
FVIIG73A			
GG	46 (64,8)	97 (64,0)	1 (Reference)
GA	23 (32,4)	51 (33,5)	0,95 (0,52 – 1,74)
AA	2 (2,8)	4 (2,6)	1,05 (0,19 – 5,97)
Allele frequency			
G	115 (81,0)	245 (80,6)	
A	27 (19,0)	59 (19,4)	1,03 (0,62 – 1,70)
WWF-1051AG			
AA	28 (39,4)	67 (45,0)	1 (Reference)
AG	36 (50,7)	69 (46,3)	1,25 (0,69 – 2,27)
GG	7 (9,9)	13 (8,7)	1,28 (0,46 – 3,57)
Allele frequency			
A	92 (64,8)	203 (68,1)	0,86 (0,56 – 1,31)
G	50 (35,2)	95 (31,8)	
WWF-1185GA			
GG	26 (36,6)	66 (45,0)	1 (Reference)
GA	37 (52,1)	69 (46,9)	1,69 (0,62 – 4,61)
AA	8 (11,3)	12 (8,1)	1,36 (0,74 – 2,49)
Allele frequency			
G	89 (62,7)	201 (68,4)	
A	53 (37,3)	93 (31,6)	0,77 (0,51 – 1,18)
Gplb			
AA	53 (74,6)	129 (86,0)	1 (Reference)
AB	16 (22,5)	20 (13,3)	1,65 (0,81 – 3,39)
BB	2 (2,8)	1 (0,7)	4,75 (0,42 – 53,57)
Allele frequency			
A	122 (85,9)	278 (92,7)	
B	20 (14,1)	22 (7,3)	0,48 (0,25 – 0,92)
PAI			
5G/5G	13 (18,3)	29 (19,2)	1 (Reference)
5G/4G	35 (49,3)	89 (58,9)	0,88 (0,41 – 1,88)
4G/4G	23 (32,4)	33 (21,8)	1,55 (0,67 – 3,61)
Allele frequency			
5G	61 (43,0)	147 (48,7)	0,79 (0,53 – 1,19)
4G	81 (57,0)	155 (51,3)	
NOS			
I/I	61 (85,9)	97 (72,0)	1 (Reference)
I/D	10 (14,1)	33 (24,4)	0,48 (0,22 – 1,05)
D/D	0 (0,0)	5 (3,6)	
Allele frequency			
I	132 (93,0)	227 (84,1)	0.40 (0.19 – 0.82)
D	10 (7,0)	43 (15,9)	
TPO A5709G			
AA	35 (49,3)	56 (37,0)	1 (Reference)
AG	30 (42,3)	70 (46,0)	0,69 (0,38 – 1,25)
GG	6 (8,4)	26 (17,1)	0,37 (0,14 – 0,98)
Allele frequency			0,63 (0,41 – 0,96)
A	100 (70,4)	182 (59,9)	
G	42 (29,6)	122 (40,1)	
TPO C4826A			
CC	19 (26,8)	24 (16,0)	1 (Reference)
CA	30 (42,3)	89 (59,3)	0,43 (0,21 – 0,88)
AA	22 (31,0)	37 (24,7)	0,75 (0,34 – 1,67)
Allele frequency			
C	68 (47,9)	137 (45,7)	1,09 (0,73 – 1,63)
A	74 (52,1)	163 (54,3)	

The values denote number of case patients or controls followed by percentage of the total for that group in parenthesis.

Progetto Ricerca Finalizzata" Italy. The manuscript language revision by Mrs Maria Antonietta Meloni is greatly appreciated.

7. REFERENCES

1. M. E. Maremberg, N. Risch, L. F. Berkman, B. Floderus, U. de Faire: Genetic susceptibility to death from coronary heart disease in a study of twins. *New England Journal of Medicine* 330, 1041-6 (1994)

2. J. J. Nora, R. H. Lortscher, R. D. Spangler, A. H. Nora, W. J. Kimberling: Genetic-epidemiologic study of early-onset ischemic heart disease. *Circulation* 61, 503-8 (1980)

3. A.G. Zaman, H.G. Helft, S. G. Worthley, J. J. Badimon: The role of plaque rupture and thrombosis in coronary artery disease. *Atherosclerosis* 149, 251-266 (2000)

4. S. Monkada, A. Higgs: The L-arginine-nitric oxide pathway. *New England Journal of Medicine* 329, 2002-2012 (1993)

5. U.C. Garg, A. Hassid: Nitric oxide-generating vasodilators 8-bromo-cyclic guanosine monophosphate inhibit mitogenesis and proliferation of cultured rat vascular smooth muscle cells. *Journal of Clinical Investigation* 83, 1774-1777 (1989)

6. D. I. Simon, J. S. Stamler, O. Jaraki, J. F. Keaney, J. F. Osborne, S. A. Francis, D. J. Singel, J. Loscalzo: Antiplatelet properties of protein S-nitrosothiols derived from nitric oxide and endothelium-derived relaxing factor. *Arterioscler Thromb.* 13, 791-799 (1993)

7. S. K. Yao, J. C. Ober, A. Krishnaswami, J. J. Ferguson, H. V. Anderson, P. Golino, L. M. Buja, J. T. Willerson: Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries. *Circulation* 86, 1302-1309 (1992)

8. M. W. Radomski, R. M. Palmer, S. Moncada: Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. *Lancet* 2, 1057-1058 (1987)

9. P. Kubes, M. Suzuki, D. N. Granger: Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proceedings of National Academy of Sciences of the USA* 88, 4651-4655 (1991)

10. F. Ishida, K. Furihata, K. Ishida, K. Kitano, K. Kiyosawa, S. Furuta: The largest variant of platelet glycoprotein Ib alpha has four tandem repeats of 13 amino acids in the macroglycopeptide region and a genetic linkage with methionine 145. *Blood* 86, 1357-60 (1995)

11. K. Kaushansky: Thrombopoietin: the primary regulator of platelet production. *Blood* 86, 419-431 (1995)

12. F. Peyvandi, P. M. Mannucci, P. Bucciarelli, S. Zeinali, S. Akhavan, E. Sacchi, P. A. Merlini, D. J. Perry: A novel polymorphism in intron 1a of the human factor VII gene (G73A): study of a healthy Italian population and of 190 young survivors of myocardial . infarction. *British Journal of Haematology* 108, 247-253 (2000)

13. C. J. Doggen, C. V. Manger, R. M. Bertina, P. H. Reitsma, J. P. Vandenbrouke, F. R. Rosendaal: A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. *Thrombosis and Haemostayis* 80, 281-285 (1998)

14. S. R. Poort, F. R. Rosendaal, P. H. Reitsma, R. M. Bertina: A common genetic variant in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increse in venous thrombosis. *Blood* 10, 3698-3703 (1996)

15. R. M. Bertina, B. P. Koeleman, T. Koster, F. R. Rosendaal, R. J. Dirven, H. de Ronde, P. A. van der Velden, P. H. Reitsma: Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 369, 64 (1994)

16. E. Brscic, S. Bergerone, A. Gagnor, E. Colajanni., G. Matullo, L. Scaglione, M. Cossander, G. Gaschino, M. Di Meo, A. Brusca, G. F. Pagano, A. Piazza, G. P. Trevi: Acute myocardial infarction in young adults: prognostic role of angiotensin-converting enzyme, angiotensin II type I receptor, apolipoprotein E, endothelial constitutive nitric oxidase synthase, and glycoprotein IIIa genetic polymorphisms at medium-term follow-up. *American Heart Journal*, 139, 979-84 (2000)

17. 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*. 107, 1117-1122 (2003)

18. WHO (Report of the Joint International Society and Federation of Cardiology/World Health Organization Task Force on standardization of clinical nomenclature) Nomenclature and criteria for diagnosis of ischemic heart disease. *Circulation* 59, 607-9 (1979)

19. S. A. Miller, D. D. Dykes, H. F. Polesky: A simple salting out procedure for extracting DNA from human nucleate cell. *Nucleic Acids Research* 16, 1215 (1998)

20. A. E. Thomas, F. R. Green, C. H. Kelleher, H. C. Wilkes, P. Brennan, T. W. Meade, & S. E. Humphries: Variations in the promoter region of the β -fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. *Thrombosis and Haemostasis*, 65, 487-490 (1991)

21. P. M. Ridker, C. H. Hennekens, K. Lindpainter, M. J. Stampfer, P. R. Eisenberg, J. P. Miletich: Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and deep venous thrombosis in apparently healthy men. *New England Journal of Medicine* 332, 912–7 (1995)

22. M. Hunault, A. A. Arbini, S. Lopaciuk, J. A. Carew, K. A. Bauer: The Arg353Gln polymorphism reduces the level of coagulation factor VII. *Artheriosclerosis, Thrombosis and Vascular Biology* 17, 2825-9 (1997)

23. E. J. Weiss, P. F. Bray, M. Tayback, S. P. Schulman, T. S. Kickler, L. C. Becker, J. L. Weiss, G. Gerstenblith, P. J. Goldschmidt,-Clermont: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. *New England Journal of Medicine* 334, 1090-1094 (1996)

24. R. Gonzalez-Conejero, M. L. Lozano, J. Rivera, J. Corral, J. A. Iniesta, J. M. Moraleda, V. Vicente: Polymorphisms of platelet membrane glycoprotein Ibα associated with arterial thrombotic disease. *Blood* 92, 2771-2776. (1998)

25. M. Margaglione, E. Grandone, G. Vecchione, G. Cappucci, N. Giuliani, D. Colaizzo, E. Cementano, S. Panico, G. Di Minno: Plasminogen activator inhibitor-1 (PAI-1) antigen plasma levels in subjects attendine a metabolic ward: relation to polymorphisms of PAI-1 and angiotensin convertine enzyme (ACE) genes. *Artheriosclerosis Thrombosis and Vascular Biology* 17, 2082-2087 (1997)

26. L. Le Flem, V. Picard, J. Emmerich, S. Gandrille, J. N. Fiessinger, M. Aiach, M. Alhenc-Gelas: Mutations in promoter region of thrombomodulin and venous thromboembolic disease. *Artheriosclerosis, Thrombosis and Vascular Biology* 19, 1098-1104 (1999)

27. K. E. Webb, J. F. Martin, A. Hamsten, P. Eriksson, L. Iacoviello, M. Gattone, M. B. Donati, A. Di Castelnuovo, J. Erusalimsky, S. E. Humphries: Polymorphism in the thrombopoietin gene are associated with risk of myocardial infarction at a young age. *Atherosclerosis* 154, 703-11 (2001)

28. R. Di Bitondo, C. L. Cameron, M. E. Daly, S. A. Croft, R. P. Steeds, K. S. Channer, N. J. Samani, D. Lillicrap, P. R. Winship: The -1185 A/G and -1051 dimorphisms in the von Willebrand factor gene promoter and risk of myocardial infarction. *British Journal of Haematology* 115, 701-706 (2001)

29. C. Tempfer, G. Unfried, R. Zeillinger, L. Hefler, F. Nagele, J. C. Huber: Endothelial nitric oxide synthase gene polymorphism in women with idiopathic recurrent miscarriage. *Human Reproduction* 16, 1644-47 (2001)

30. L. B. Jorde, W. S. Watkins, J. Kere, D. Nyman, A. W. Eriksson: Gene mapping in isolated populations: new roles for old friends?, *Human Heredity* 50, 57–65 (2000)

31. L. Peltonen, P. Pekkarinen, J. Aaltonen: Messages from an isolate lessons from the Finnish gene pool. *Biological Chemistry Hoppe-Seyler*, 376, 697–704 (1995)

32. G. Barbujani, G. Bertorelle: Genetics and the population history of Europe. *Proceedings of National Academy of Sciences of the USA* 98, 22–25 (2001)

33. A. M. Bowcock, J. R. Kidd, J. L. Mountain, J. M. Hebert, L. Carotenuto, K. K. Kidd, L. L. Cavalli-Sforza: Drift, admixture, and selection in human evolution: a study with DNA polymorphisms. *Proceedings of National Academy of Sciences of the USA* 88, 839–843 (1991)

34. R. Lampis, L. Morelli, M. Congia, M. D. Macis, A. Mulargia, M. Loddo, S. De Virgiliis, M. G. Marrosu, J. A. Todd, F. Cucca: The inter-regional distribution of HLA class II haplotypes indicates the suitability of the Sardinian population for case-control association studies in complex diseases. *Human Molecular Genetics* 9, 2959-2965 (2000)

35. N. Cappello, S. Rendine, R. Griffo, G. E. Mameli, V. Succa, G. Vona, A. Piazza: Genetic analysis of Sardinia: I. data on 12 polymorphisms in 21 linguistic domains. *Annals of Human Genetics* 60, 125-141 (1996)

36. M. Takemoto, K. Egashira, M. Usui, K. Numaguchi, H. Tomita, H. Tsutsui, H. Shimokawa, K. Sueishi, A. Takeshita: Important role of tissue angiotensin-converting enzyme activity in the pathogenesis of coronary vascular and myocardial structural changes induced by long-term blockade of nitric oxide synthesis in rats. *Journal of Clinical Investigation* 99, 278-287 (1997)

Key Words: Young, Myocardial Infarction, Genetic Polymorphism, Coagulation, Platelets, Fibrinolysis

Send correspondence to: Ciriaco Carru, Department of Biomedical Sciences, Chair of Clinical Biochemistry, University of Sassari, Viale San Pietro 43/B, I-07100 SASSARI, Italy, Tel: 39-079229775, Fax: 39-079228275, E-mail: carru@uniss.it

http://www.bioscience.org/current/vol2E.htm