Vascular and haemostatic gene polymorphisms associated with non-fatal myocardial infarction: a critical review

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The importance of genetics to the pathogenesis of myocardial infarction is suggested by the frequent familial clustering of premature disease. Yet, studies associating myocardial infarction with gene polymorphisms of vascular proteins (angiotensinogen, angiotensin converting enzyme, angiotensin II type 1 receptor, endothelial nitric oxide synthase) and haemostatic factors (fibrinogen, coagulation factors II, V, VII and XIII, plasminogen activator inhibitor-1, tissue-type plasminogen activator, platelet glycoproteins IIb/IIIa, Ia/IIa and Ib-IX-V, or methylenetetrahydrofolate reductase) have revealed conflicting results. This is hardly surprising, given: 1) the multigenic nature of myocardial infarction, whereby single polymorphisms are bound to play at best only a limited role in the global risk of disease; 2) the multiple pathogenetic mechanisms of infarction (e.g., atheromatous obstruction, plaque rupture, thrombosis, vasospasm), each of which is likely influenced by a number of genes and by several environmental factors. The simultaneous investigation of a set of polymorphisms and of their interactions with environmental factors in extremely homogeneous sets of patients should offer a better understanding of the contribution of specific genes to the risk of myocardial infarction.

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Introduction

The multifactorial pathogenesis of myocardial infarction (MI) includes a combination of environmental and genetic factors. The role of inheritance is strongly supported by the predictive value of a family history for premature disease[1]. However, because many genetic traits are likely to influence the pathogenesis of MI, research in the field is admittedly complex.

It is estimated that 15 to 20% of the DNA sequence of the 50 000-100 000 human genes varies significantly among individuals, resulting in multiple alternative forms (alleles) for a given locus[2]. The term polymorphism refers to the presence of multiple alleles at a gene locus in a frequency 1% within a given population. Polymorphisms may occur in the coding (exons) or non-coding parts of genes (introns, 5 and 3 flanking regions) and may be determined by substitutions, deletions, insertions or unequal crossing-over of single bases or of longer DNA sequences[3]. When associated with disease, DNA polymorphisms can be used as markers. Many polymorphisms *per se* do not determine phenotypic changes, while others are known to influence the function or concentration of the encoded protein. Missense variations are DNA changes that cause a substitution in the encoded amino acid residue[3], which may or may not have a functional effect.

Candidate genes encode for proteins with a known function which, if altered, may explain at least in part the pathogenesis of, or the susceptibility towards, a given disease[3]. Using a population approach to investigate candidate genes, the frequency of the DNA marker (on the responsible gene itself or in close proximity) within the diseased population is compared to that of a control population. Examples of candidate genes are the angiotensin I converting enzyme or angiotensinogen genes for arterial hypertension[4], and the apolipoprotein genes for hyperlipidemia[5-8].

An increased risk of ischemic heart disease has been associated with polymorphisms of genes involved in vascular tone [angiotensinogen, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor, endothelial constitutive nitric oxide

synthase], homocysteine metabolism[9], coagulation (fibrinogen, factors II, V, VII and XIII), fibrinolysis [plasminogen activator inhibitor-1 (PAI-1) and tissue-type plasminogen activator (t-PA)], platelet membrane receptors (glycoproteins IIb/IIIa, Ia/IIa, Ib-IX-V), lipid metabolism (e.g., apolipoproteins, lipoproteinlipase and paraoxonase)[10,11] and the inflammatory system (interleukin family, tumor necrosis factor-alpha, HLA system)[12].

This article will focus on the association between MI and vascular and haemostatic gene polymorphisms and examine the complexities that characterize this area of research.

Vascular function

Angiotensinogen. Angiotensinogen is cleaved by renin to yield angiotensin I, which is then cleaved by the ACE to yield angiotensin II. Angiotensin II constricts vascular smooth muscle cells and stimulates smooth muscle cell migration and proliferation, macrophage-foam cell formation, platelet adhesion and aggregation, and the synthesis of PAI-1.

Several polymorphisms of the angiotensinogen gene on chromosome 1[13] have been identified. The most investigated is a missense variant, resulting in a methionine to threonine substitution (M235T); this variant has been associated with higher plasma angiotensinogen levels[14] which, in turn, are correlated positively with blood pressure levels[4]. Among 422 Caucasian patients with documented ischemic heart disease and 406 controls, TT homozygosity of this variant was reported to confer an odds ratio (OR), both for coronary artery disease and MI, of about 2[15]. Subsequent studies, however, have been inconsistent in relation to coronary artery disease (Ludwig et al.[16] and Gardemann et al.[17]: positive relation; Jeunemaitre et al.[18]: no significant relation) and to MI (Ludwig et al.[16] and Fomicheva et al.[19]: positive relation; Gardemann et al.[17]: no significant relation).

Angiotensin converting enzyme. ACE is a protease present in many tissues, in particular pulmonary vascular endothelium. It has an important role in cardiovascular homeostasis, because it converts angiotensin I to angiotensin II and degrades bradykinin.

The ACE gene, located on chromosome 17, shows a polymorphism caused by the insertion/deletion (I/D) of a 287 base pair (bp) segment in intron 16. Cambien et al.[20] in the ECTIM study (Etude Cas-TØmoin sur l Infarctus du Myocarde) found that the DD genotype was associated with higher ACE plasma levels and was more frequent in 610 MI survivors (especially in those considered at low risk, defined as lean and with low plasma apolipoprotein B levels) compared with 733 control subjects ($p = 0.007$). Some[21] but not other[22] reports have supported these findings. Anderson et al.[23] found a weak but significant association [OR 1.5, 95% confidence interval (CI) 1.0-2.3, $p < 0.04$] between the DD genotype and previous MI in 141 women compared with 338 controls. Gardemann et al.[24], among 2267 Caucasian men undergoing coronary arteriography, found an association between the D allele (ID + DD) and non-fatal MI in 56 older patients (75 years, OR 4.4, CI 1.7-11, $p < 0.01$) especially with low body mass index but not in younger patients (< 62 years), compared with controls; conversely, a relation with coronary artery disease was seen in younger but not in older subjects[24]. The relation between this polymorphism and MI has recently been tested in 5000 infarct survivors enrolled in the ISIS (International Studies of Infarct Survival)-2, compared with healthy spouses or siblings; the OR for homozygous carriers of the D allele was 1.1 (CI 1.0-1.2), and did not change substantially in the lean and low apolipoprotein B subgroups[25].

Angiotensin II type 1 receptor. The angiotensin II type 1 receptor plays a key role in mediating the vasoconstrictive and growth-promoting effects of angiotensin II. A common polymorphism in the 3 untranslated region of the angiotensin II type 1 receptor gene on chromosome 3[26] consists in the substitution of adenine by cytosine at nucleotide 1166 (A1166C). It is unclear whether this polymorphism is functional[27]. A synergistic effect between the C allele of the angiotensin II type 1 receptor and the I/D polymorphism of the ACE gene was observed in the ECTIM population of 613 MI survivors and 723 controls[28]: among subjects with the ACE DD genotype, the OR was 1.1 in the absence of the C allele, 1.5 for AC heterozygotes, and 3.9 for CC homozygotes ($p < 0.02$), increasing further in the subgroups with low body mass and low apolipoprotein B levels[28]. Berge et al.[29], among 235 MI survivors and 384 controls, found CC homozygosity associated with MI in the male subgroup ($p = 0.001$), especially in the presence of low body mass index and low apolipoprotein B levels ($p < 0.001$). Rice et al.[30], among 311 white Caucasian patients with chest pain and 287 controls, found a weak synergistic relation of the ACE DD and angiotensin II type 1 receptor CC genotypes with coronary artery stenosis but not with a history of MI. Gardemann et al.[31], on the other hand, among 2244 male Caucasians with and without significant coronary artery disease or previous MI, found no significant association between the A1166C polymorphism and either coronary artery disease or MI.

Endothelial nitric oxide synthase. Endothelial nitric oxide synthase is present in vascular endothelium, platelets, and several other cell types that continuously produce modest amounts of nitric oxide. Endothelium-derived nitric oxide is a major physiological regulator of systemic resistance vessels (inhibition of nitric oxide production decreases local blood flow) and also has vasoprotective effects by sweeping superoxide radicals and by inhibiting platelet aggregation, leukocyte adhesion and smooth muscle cell proliferation. Impaired nitric oxide func-

tion may thus contribute to the development of coronary atherosclerosis and thrombosis.

The endothelial nitric oxide synthase gene is on chromosome 7[32]. Ichihara et al.[33] identified the presence of a variant allele in intron 4 (characterized by a 27 bp repeat) as an independent risk factor for MI in the Japanese population (455 patients vs 550 controls, respective prevalence 0.14 vs 0.10, OR 1.5, CI 1.1-2.1, p = 0.007), especially in those without traditional cardiovascular risk factors (OR 2.5, CI 1.4-4.7, p = 0.0035). Shimasaki et al.[34], in 285 Japanese infarct survivors and 607 controls, found a significant association (OR 1.7) between MI and a missense variant in exon 7 (guanine at nucleotide 894 replaced by thymine, resulting in Glu298Asp). Another Japanese study[35] of 226 infarct survivors and 357 controls confirmed the association between the homozygous TT variant and MI (p = 0.009); the polymorphism, however, was not related to the severity of coronary artery disease. In the English CHAOS (Cambridge Heart Antioxidant Study) reports (the first one, of 298 patients with positive coronary angiograms vs 138 healthy controls, and CHAOS II, of 249 patients with recent MI vs 183 healthy controls), homozygosity for TT (Asp298) compared with GG (Glu298) was associated with an OR of 4.2 (CI 2.3-7.9) for angiographic coronary artery disease and 2.5 (CI 1.3-4.2) for MI[36]. In the ECTIM study, on the other hand the homozygous GG variant was associated with MI in the French (OR 1.5, CI 1.0-2.0, p < 0.009) but not in the Northern Irish patients[37].

Hyperhomocysteinemia

Homocysteine, a metabolite of methionine, is considered an independent risk factor for atherothrombotic diseases through its potential cytotoxic effects[9]. Hyperhomocysteinemia is associated with methionine-rich diets, low intake of vitamin B and folate, impaired renal function, and gene defects of key enzymes involved in homocysteine metabolism. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a methyl donor for remethylation of homocysteine back to methionine[9].

A common missense variant in the MTHFR gene on chromosome 14 [cytosine replacement by thymine at nucleotide 677 (C677T) causing the substitution of an alanine by valine] results in a thermolabile, †50% less active, enzyme[38]. Homozygous individuals for this variant have reduced MTHFR activity and increased plasma homocysteine concentrations[38]. Kang et al.[39] first reported a 17% prevalence of the TT genotype in 212 patients with proven coronary artery disease compared with a 5% prevalence in 202 control subjects (p < 0.01); this led the authors to propose it as an inherited coronary risk factor. Several subsequent studies, however, have not confirmed this finding (van Bockxmeer et al.[40] among 358 patients < 50 years with angiographically documented coronary artery disease: TT genotype in 10.6 vs 10.5% in 143 controls; Ma et al.[41] among 293 men from the Physicians Health Study who subsequently developed MI vs 290 controls: CT vs CC: OR 1.1; TT vs CC: OR 0.8; Schmitz et al.[42] among 190 MI survivors and 188 controls: TT vs CC + CT: OR 1.1). Therefore, despite a clear effect of this gene polymorphism on homocysteine levels, its association with the risk of MI is less clear.

Coagulation

Fibrinogen (factor I). Fibrinogen, a dimeric glycoprotein synthesized by the liver, is a major constituent of platelet aggregates and is converted by thrombin to form the fibrin clot. Each dimer consists of three chains (A-α, B-β, and γ) encoded by three separate genes located on chromosome 4. Many studies have consistently shown an independent association between plasma fibrinogen concentrations and the risk of ischemic heart disease[43-46]. Fibrinogen may contribute to atherosclerosis and thrombosis by increasing blood coagulability, plasma viscosity, platelet aggregability, and by promoting fibrin deposition in the vessel wall[46]. Plasma fibrinogen concentrations are influenced by many environmental factors, including smoking, obesity, lack of exercise, use of oral contraceptives, trauma, and the acute phase reaction. The basal levels and the response to environmental factors are genetically determined, partly through cytokine-mediated mechanisms[47].

Most studies have focused on polymorphisms of the β-fibrinogen chain: two in the 5 promotor region upstream of the start of transcription: -148C/T (*Hind*III) and -455G/A (*Hae*III), and two downstream: G1689T (*Bcl*I) and Arg448Lys. In particular, the -148C/T polymorphism is close to an interleukin-6 responsive element and appears to affect the binding of hepatic nuclear factors[47]. Several independent studies[48-50] have reported an association between the -455G/A polymorphism and fibrinogen plasma levels (with higher levels associated with the -455A allele), but not with the risk of MI (Scarabin et al.[48] among 533 patients vs 648 controls from ECTIM: -455A frequency 19 vs 21%; Green et al.[49] among 123 young MI survivors vs 86 controls: -455A frequency 25 vs 25%; Gardemann et al.[50] among 923 individuals investigated by coronary angiography: no relation to coronary artery disease or previous MI).

In a sample of 102 Italian MI survivors selected from the GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell Infarto Miocardico)-2 study for having one or more first degree relatives with a history of MI or stroke before the age of 65 a significantly higher prevalence of the B2 allele of the *Bcl*I polymorphism was found compared with 173 controls[51]: 28 vs 17%, OR 2.4, CI 1.2-4.6, p = 0.002. This allele was also associated with significantly higher fibrinogen levels.

Prothrombin (factor II). Prothrombin, the precursor of thrombin, is the final zymogen of the coagulation cascade; thrombin activates platelets, converts fibrinogen into fibrin, amplifies the coagulation process by activating coagulation factors V and VIII and concurrently promotes anticoagulation by binding to thrombomodulin and activating protein C.

Prothrombin is encoded by a 21-kb gene (on chromosome 11) composed of 14 exons and 13 introns. Poort et al.[52] described a G to A substitution at nucleotide 20210 in the 3 untranslated region; this region is probably involved in gene expression, as carriers of the variant have higher plasma prothrombin concentrations and are at increased risk of venous thrombosis. Rosendaal et al.[53] found a higher prevalence of the 20210A allele among 79 young women with MI vs 381 control women (5.1 vs 1.6%, OR 4, CI 1.1-15); in this case, the risk was much higher in the presence of smoking (OR 43, CI 6.7-281) or a metabolic risk factor (OR 34, CI 5.5-209). Other studies have failed to confirm the relation between this variant and MI (Ardissino et al.[54] in 200 infarct survivors < 45 years vs 200 controls: 20210A allele in 5.5 vs 4%, OR 1.4, CI 0.5-3.9; Ridker et al.[55] in 404 healthy male physicians who subsequently developed MI vs 1774 controls: $p = 0.5$).

In contrast, one recent study, investigating 247 patients presenting with a first acute coronary syndrome before the age of 65 and 247 matched controls[56], found a significantly increased prevalence of the 20210A allele among those with a family history of MI (OR 3.3, CI 1.2-9.1) or without other major coronary risk factors (OR 5.1, CI 1.2-21).

Factor V. Resistance to activated protein C. Protein C, a serine protease with powerful anticoagulant properties, is synthesized by endothelial cells. During normal haemostasis, protein C is activated on the endothelial surface by the thrombin-thrombomodulin complex[57]. Activated protein C limits thrombin formation by degrading coagulation factors Va and VIIIa[57]. Resistance of factor V to the degradation by activated protein C has been found in 20-50% of patients with venous thrombosis (compared to 5% of a general white population) and is associated with an 8-fold increase in the risk of venous thrombosis[58-61]. Activated protein C resistance is easily detected in the laboratory as a poor anticoagulant response of the sample (inadequate prolongation of the activated partial thromboplastin time) after addition of activated protein C[57]. It is largely determined by a missense variation of the factor V gene on chromosome 1[62,63] (guanine at nucleotide 1691 replaced by adenine) causing the substitution of arginine 506 by glutamine (factor V Leiden). Heterozygous carriership of factor V Leiden has not emerged as a clear predisposing condition for arterial thrombosis[64,65].

Factor VII. Factor VII, a vitamin K-dependent serine protease synthesized by the liver, plays a pivotal role in tissue factor-induced coagulation. The levels of factor VII vary widely in the general population and are influenced by several environmental factors, including use of oral contraceptives, high fat diet, and body weight[66]. Factor VII coagulant activity is associated with both triglyceride and cholesterol levels, and increases immediately after fat assumption, in close association with post-prandial hypertriglyceridemia[67,68]. The increase with age occurs in both sexes, being greater in women (who have lower levels compared to men before menopause). The Northwick Park Heart Study of 1511 middle-aged men found increased factor VII coagulant activity in plasma to be a strong predictor of major coronary events[69].

Genetic factors strongly influence factor VII plasma levels and regulate the response to environmental stimuli[66,70-73]. Three different polymorphisms within the factor VII gene (on chromosome 13) explain up to one third of factor VII plasma variability[71]. One of these, identified by the restriction enzyme *Msp*I[72], causes a guanine to adenine substitution, with arginine replacement by glutamine at position 353 (Arg353Gln or R353Q). An English study, performed in 123 white Europeans, 123 black Afro-caribbeans, and 142 Gujarati Indians, found this polymorphism to be strongly associated with factor VII coagulant levels in all groups, with Gln353 carriers showing levels 20-25% below the group mean[73]. The Gujarati Indians had the highest triglyceride levels which, however, were positively correlated with factor VII levels only in Arg353 carriers, an example of gene-environment interaction[73].

Studies on the association between the Arg353Gln polymorphism and the risk of MI have yielded varying results: in ECTIM, only a non-significant trend was found between Arg353 homozygosity and risk of MI[70]. Iacoviello et al.[74], among 165 Italian infarct survivors with a family history of MI or stroke (selected from GISSI-2) and 225 controls, found a significant relation between MI and several factor VII gene polymorphisms (the Arg353Gln or R353Q, and those involving the hypervariable region 4 with different length alleles termed H5, H6 and H7), probably mediated by modifications of factor VII plasma levels. In particular, the Gln353 homozygotes (QQ) and the H7H7 genotypes had a significantly lower risk (OR 0.08, CI 0.01-0.9, and OR 0.22, CI 0.08-0.65, respectively) while the Arg353 homozygotes (RR) and the H7H5 and H6H5 genotypes showed the highest risk ($p < 0.001$). Recently, an intronic polymorphism (G73A), in linkage disequilibrium with the Q353 allele, was investigated in 190 MI survivors who experienced the event < 45 years and in 179 controls; the A73 allele was associated with lower factor VII levels and tended to confer protection against MI (OR 0.5, CI 0.3-1)[75].

Factor XIII. Factor XIII, a tetrameric protein composed of two A and two B subunits, is activated by thrombin to form cross-linked fibrin which is more resistant to fibrinolysis. The gene on chromosome 6 is or-

ganized in 15 exons and 14 introns. A common single-point missense variation (G→T) in exon 2 of the A subunit encodes for the amino acid change Val34Leu near the thrombin activation site[76]. Recently Kohler et al.[77] demonstrated an inverse association between this polymorphism and MI: among 398 Caucasian patients who underwent coronary angiography for suspected coronary artery disease (with or without previous MI), matched with 196 healthy controls, the prevalence of the variant was 32% in patients with MI, compared to 50% in those without MI ($p = 0.0009$) and to 48% in controls ($p = 0.005$). Carriers of the T allele with a history of MI had higher plasma levels of PAI-1 ($p = 0.004$) and a higher prevalence of the PAI-1 4G/4G genotype (see below), a possible example of gene-gene interaction. Other studies from Finland[78] and Brazil[79] also reported a protective effect against MI associated with the T allele.

Platelets

Glycoprotein IIb/IIIa. The glycoprotein IIb/IIIa is a heterodimeric platelet membrane surface complex that mediates adhesion and aggregation. Its ligands include fibrinogen, von Willebrand factor, vitronectin, and fibronectin. A common missense variant in the glycoprotein IIIa gene on chromosome 17 results in the replacement of Leu by Pro at position 33 (Leu = PlA1, Pro = PlA2). Weiss et al.[80], in a case-control study of 71 patients with MI or unstable angina, vs 68 controls, first showed a significant association between the PlA2 polymorphism and acute coronary events. In particular, the prevalence of the PlA2 polymorphism was 2.1 times higher among patients than controls (OR 2.8, CI 1.2-6.4, $p = 0.01$), and 3.4 times higher in a subgroup of patients who had coronary events before the age of 60 years (OR 6.2, CI 1.8-22, $p = 0.002$). Subsequently, Walter et al.[81] among 318 patients who underwent coronary stenting (PlA1/PlA1 80.2%, PlA1/PlA2 19.8%) found a significant association between PlA2 carriership and risk of thrombotic occlusion (9.5 vs 1.9%, OR 5.3, CI 1.6-18). With the exception of one study by Ardissino et al.[54] (of 200 young survivors of MI vs 200 healthy controls) where the PlA2 resulted significantly related to MI (OR 1.8, CI 1.1-3.0), these findings, however, have not been confirmed by others (the Physicians Health Study[82] of 374 patients with subsequent MI vs 704 controls, $p = 0.4$; the ECTIM study[83] of 620 MI survivors and 700 controls; Joven et al.[84] in 250 MI patients and 250 controls, $p = 0.9$; Durante-Mangoni et al.[85] in 114 patients with a history of angina-like chest pain admitted for coronary arteriography, of which 43 with a previous MI and 71 without, $p = 0.6$). In particular, a study of 2250 individuals undergoing coronary angiography found no association between the PlA2 allele and MI in the overall population, although, in a subgroup of patients at low risk, the PlA2 allele was significantly associated with severity of disease[86].

Glycoprotein Ib-IX-V complex. The glycoprotein Ibα is one of four transmembranous platelet surface proteins (together with the glycoproteins Ibβ, IX and V) forming a complex that binds to von Willebrand factor and to thrombin. Two polymorphisms of the glycoprotein Ibα gene have been described: a C to T substitution at codon 145 (Thr145Met)[87] and a variable number of tandem repeats of 39 bp in the glycosylated region[88] (1, 2, 3 or 4 repeats, designated D, C, B and A alleles, respectively). Murata et al.[89] determined the prevalence of the Thr145Met variant in 91 patients with MI or angiographically documented angina pectoris and in 105 controls; although no significant difference was found in the total patient sample vs controls, there was a higher Met145 frequency in the subgroup < 60 years (31.8 vs 16% in controls < 60 years, OR 2.5, $p < 0.05$). Further studies have yielded controversial data: Gonzales-Conejero et al.[90], in a case-control study of 101 patients with acute coronary syndromes vs 101 controls, found a significant association of the Met145 allele ($p = 0.038$) and the C/B genotype of the variable number of tandem repeats with disease, whereas Ardissino et al.[54], among 200 young infarct survivors vs 200 healthy controls, did not (for Met145: OR 1.1, CI 0.4-3.3).

Glycoprotein Ia/IIa. The glycoprotein Ia/IIa (also known as α2β1 integrin, VLA-2, or CD49b/CD29) is a heterodimeric platelet membrane protein complex that mediates adhesion to collagen. Kunicki et al.[91] described two polymorphisms of the glycoprotein Ia gene, on chromosome 5, associated with a variable expression of the platelet surface receptor: the 807T allele (linked with 873G) was associated with a higher receptor density compared with the 807C allele (linked with 873A), although neither change altered the protein amino acid sequence. Several studies have investigated whether the 807T allele carries an increased risk of cardiovascular disease. Moshfeg et al.[92] in a 2:1 case/control study (177 MI survivors vs 89 healthy controls) found a significant association between the TT genotype and MI compared with the CC + CT genotypes (OR 3.3, CI 1.2-8.8, $p = 0.02$). This was subsequently confirmed by Santoso et al.[93] who found, among 2237 male patients undergoing coronary angiography, an increased risk of MI in carriers of the 807T allele in the subgroups under 62 years (OR 1.6, CI 1.1-2.1, $p = 0.04$) or under 49 years (OR 2.6, CI 1.3-5.4, $p = 0.009$), but no relation with the extent of coronary artery disease. In contrast, Croft et al.[94], investigating 546 infarct survivors and 507 healthy controls, did not find any significant association with MI, neither of the TT genotype ($p = 0.22$) nor of the 807T allele ($p = 0.24$).

Fibrinolysis

Plasminogen activator inhibitor-1. Reduced fibrinolytic capacity of plasma, mainly related to increased PAI-1 activity, is common in patients with ischemic

heart disease[95,96]. PAI-1 is a rapid inhibitor of both tissue-type and urinary-type plasminogen activators. Increased PAI-1 expression has been demonstrated in atherosclerotic plaques[97]. In the prospective epidemiological Northwick Park Heart Study of men aged 40-64 years, low fibrinolytic activity predicted the risk of future cardiovascular events[43].

The human PAI-1 gene, on chromosome 7, includes 9 exons and 8 introns. Eriksson et al.[98] found the 4G allele of a 4G/5G polymorphism in the promoter region associated with higher plasma PAI-1 activity: although both alleles bind a transcriptional factor, only the 5G binds a repressor protein, such that basal PAI-1 transcription is increased in the presence of the 4G allele. The prevalence of 4G was significantly higher among 94 young male survivors of a first MI (< 45 years) compared with 131 controls (63 vs 53%)[98]. Subsequent studies have yielded conflicting results: Ossei-Gerning et al.[99] found the 4G/4G genotype associated with MI among 453 subjects (with and without previous MI) (OR 2.0, CI 1.1-3.7, $p < 0.03$), and Iwai et al.[100] found a lower prevalence of the 5G/5G genotype among 204 survivors of MI vs 296 controls ($p = 0.003$). In contrast, data from the ECTIM[101], the GISSI-2[102] or the US Physicians Health[103] studies failed to show a correlation between MI and 4G. A meta-analysis[104], including more than 1500 cases and 2100 controls, showed that carriers of the 4G allele had a modestly increased risk of MI (OR 1.2, CI 1.0-1.5), which became 2-fold in the subgroup of patients considered at higher risk.

Tissue-type plasminogen activator. t-PA is the main endogenous profibrinolytic enzyme[96]. Increased plasma antigen concentrations have been associated with a higher risk of cardiovascular events, presumably in relation to higher PAI-1 levels and to reduced net t-PA activity[105]. The t-PA gene, on chromosome 8, shows an I/D polymorphism due to an Alu sequence in intron h[106]. There are few and conflicting data about the possible association of this polymorphism and the risk of MI: van der Bom et al.[107], in a case-control study of 121 survivors of MI and 250 controls from the Rotterdam Study, showed a significantly increased prevalence of the insertion sequence in cases (OR 2.2, CI 1.1-4.5); in contrast, Ridker et al.[108] did not find any relation between the Alu sequence and the risk of MI among 369 patients vs 369 controls, considering both a dominant (OR 1.0, CI 0.7-1.1, $p = 0.8$) and a recessive transmission (OR 1.1, CI 0.8-1.0); Steeds et al.[109], among 529 English Caucasian patients with MI and 525 controls, also found no relation with the risk of disease (OR 1.0, CI 0.6-1.4, $p = 0.56$).

Perspectives and implications

This review highlights the diversity of results generated by studies of vascular and haemostatic gene polymorphisms in patients with MI. In our view, the diversity stems from the complexity of molecular genetics applied to a multifactorial disorder such as MI. Some aspects of this complexity can be summarized as follows.

One phenotype - many mechanisms. The multifactorial nature of MI implies that a single clinical phenotype can be determined by a multitude of pathogenetic mechanisms acting individually if of sufficient intensity or, more commonly, concertedly, in varying degree. For MI, some of these mechanisms include thrombosis, atheromatous obstruction, vasomotion, and vasculitis.

One mechanism - many genes. Each pathogenetic mechanism for instance, thrombosis will in turn be influenced by many genes, regulating for example the dynamics of platelet function and of fibrin formation and dissolution. Even a single protein may be the result of different genes (e.g., fibrinogen).

One gene - mild effect. In the pathogenesis of a multifactorial disease, any one functional genetic variation will, on its own, result at most in a mild phenotypic defect[110]. It is hardly surprising, therefore, that for most studies, the OR for disease conferred by any given polymorphism (especially when investigated in unselected patient groups) has ranged from 1 to 2.

One gene - several polymorphisms. Several polymorphisms within a given locus may affect the levels of the protein product. As recently stated by Lane and Grant[111], although clinical studies consistently relate homocysteine, fibrinogen, factor VII or PAI-1 levels to acute coronary events, and genetic studies relate MTHFR, fibrinogen, factor VII and PAI-1 polymorphisms to plasma levels, the relation of genotypes with disease is less consistent. This is not surprising, considering that the contribution of a single polymorphism to the protein levels is only a fraction of the entire heritable variance[111].

Low specificity of DNA markers. In classic monogenic disorders a single gene defect causes full-blown disease. The relation between diseased phenotype and mutated genotype is exclusive and fully reciprocal. All carriers of the mutation will have the disease and, conversely, no unaffected individual will be found to have the mutation[3]. In contrast, in multifactorial disorders, a single gene defect, by definition, will not be entirely causal but will contribute only in part to the pathogenesis of, or susceptibility towards, the disease. Not surprisingly, therefore, the same gene defect may exist within an apparently healthy phenotype, if other cocausal genes or environmental factors are absent or if protective elements are present. The specificity of a DNA marker (i.e., its absence in the healthy phenotype) will be greatest when cases and controls come from the same genetic and environmental background and conversely will be lowest when the genetic and environ-

mental heterogeneity among cases and controls is high. Hence, the importance of ensuring a homogeneous racial and geographical background for cases and controls.

Survival bias. Because MI can be rapidly lethal after its onset, cross-sectional studies almost inevitably face a survival bias, whereby deadly genes of patients who dye at the very onset of disease escape analysis[112].

Interaction with traditional risk factors. An unresolved issue is whether the effects of vascular and haemostatic gene polymorphisms may be enhanced or obscured by the co-existence of traditional coronary risk factors. Among patients with a family history of MI, the risk conferred by genetic components, not surprisingly, appears enhanced[51,56,74]. For the environmental risk factors (obesity, dyslipidemia, smoking, diabetes, hypertension), analyses of subgroups with and without these factors have yielded conflicting results[20,33,53,54,56]. In the case of haemostatic gene polymorphisms, when the risk of disease conferred by the polymorphic variant is *enhanced in the absence* of environmental risk factors[33,56] but is *reduced in their presence*, the following possibilities are suggested: 1) MI occurring in the absence of environmental risk factors recognizes a strong, genetically-determined, prothrombotic component; 2) traditional environmental risk factors are so dominant that haemostatic gene polymorphisms provide a negligeable contribution to the overall risk[111].

Implications. The diversity of results from current investigations in this field is likely a consequence not so much of different sample sizes but more importantly of different selection criteria adopted for patients and controls in various studies, with special reference to clinical presentation, extent of coronary artery disease, age, race, geographical area, and concomitant risk factors. The simultaneous analysis of multiple gene polymorphisms and of environmental factors within very homogeneous groups (standardized for the above variables) should be useful to better understand the true contribution of these factors to the risk of disease.

Conclusions. The high incidence and potentially devastating consequences of MI justify all efforts to unravel the multiple and complex pathogenetic mechanisms of this disease. MI runs in families and the genetic component is substantial. Indeed, a positive family history is among the strongest traditional major risk factors[1]. Not uncommonly, an obvious cause of disease, in the form of hypercholesterolemia, hypertension, smoking and diabetes, is lacking. Conversely, not all hypercholesterolemic, hypertensive, smoking or diabetic subjects develop MI. This suggests that other factors, partly genetic, play a role.

Despite the many complexities, it can be anticipated that, in due course, molecular genetics will become a distinct branch of cardiology and a major tool to prevent, diagnose and treat cardiovascular diseases, including MI.

References

1. Roncaglioni MC, Santoro L, D Avanzo B, et al, for the GISSI-EFRIM Investigators. Role of family history in patients with myocardial infarction. An Italian case-control study. Circulation 1992; 85: 2065-72.
2. Willard HF. Human genetics. In: Leder P, Clayton DA, Rubenstein E, eds. Introduction to molecular medicine. New York, NY: Scientific American, 1994: 1-26.
3. Brock DJH. Molecular genetics for the clinician. Cambridge, UK: Cambridge University Press, 1993.
4. Caulfield M, Lavender P, Farrall M, et al. Linkage of the angiotensinogen gene to essential hypertension. N Engl J Med 1994; 330: 1629-33.
5. Hansen PS, Klausenic, Lemming L, Gerdes N, Faergeman O. Apolipoprotein B gene polymorphism in ischemic heart disease and hypercholesterolemia: effects of age and sex. Clin Genet 1994; 45: 78-83.
6. Hansen PS, Gerdes LU, Klansen IC, Gregersen N, Faegerman O. Polymorphisms in apolipoprotein B-100 gene contributes to normal variation in plasma lipids in 464 Danish men born in 1948. Hum Genet 1993; 91: 45-50.
7. Katzel LI, Fleg JL, Paidi M, Ragoobarsingh N, Goldberg AP. Apo E4 polymorphism increases the risk for exercise-induced silent myocardial ischemia in older men. Arterioscler Thromb 1993; 13: 1495-500.
8. Nakata Y, Katsuya F, Rakugi H, et al. Polymorphism of the apolipoprotein E and angiotensin converting enzyme genes in Japanese subjects with silent myocardial ischemia. Hypertension 1996; 27: 1205-9.
9. Andreotti F, Burzotta F, Manzoli A, Robinson K. Homocysteine and risk of cardiovascular disease. J Thromb Thrombolysis 2000; 9: 13-21.
10. Gerdes C, Gerdes LU, Hansen PS, Faergman O. Polymorphisms in the lipoprotein lipase gene and their associations with plasma lipid concentrations in 40-year-old Danish men. Circulation 1995; 92: 1765-9.
11. Ombres D, Pannitteri G, Montali A, et al. The Gln-Arg192 polymorphism of human paraoxonase gene is not associated with coronary artery disease in Italian patients. Arterioscler Thromb Vasc Biol 1998; 18: 1611-6.
12. Dahlen GH, Boman J, Birgander LS, Lindblom B. Lp(a) lipoprotein, IgG, IgA and IgM antibodies to Chlamydia pneumoniae and HLA class II genotype in early coronary disease. Atherosclerosis 1995; 114: 165-74.
13. Gaillard-Sanchez I, Mattei MG, Clauser E, Corvol P. Assignment by in situ hybridization of the angiotensinogen gene to chromosome band 1q4, the same region as the human renin gene. Hum Genet 1990; 84: 341-3.
14. Winkelmann BR, Russ AP, Nauck M, et al. Angiotensinogen M235T polymorphism is associated with plasma angiotensinogen and cardiovascular disease. Am Heart J 1999; 137: 698-705.
15. Katsuya T, Koike G, Yee TW, et al. Association of angiotensinogen gene T235 variant with increased risk of coronary heart disease. Lancet 1995; 345: 1600-3.
16. Ludwig EH, Borecki IB, Ellison RC, et al. Associations between candidate loci angiotensin-converting enzyme and angiotensinogen with coronary heart disease and myocardial infarction: the NHLBI Family Heart Study. Ann Epidemiol 1997; 7: 3-12.
17. Gardemann A, Stricker J, Humme J, et al. Angiotensinogen T147M and M235T gene polymorphisms are associated

with the extent of coronary atherosclerosis. Atherosclerosis 1999; 145: 309-14.
18. Jeunemaitre X, Ledru F, Battaglia S, et al. Genetic polymorphisms of the renin-angiotensin system and angiographic extent and severity of coronary artery disease: the CORGENE study. Hum Genet 1997; 99: 66-73.
19. Fomicheva EV, Gukova SP, Larionova-Vasina VI, Kovalev YR, Schwartz EI. Gene-gene interaction in the RAS system in the predisposition to myocardial infarction in elder population of St Petersburg. Mol Genet Metab 2000; 69: 76-80.
20. Cambien F, Poirier O, Lecerf L, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992; 359: 641-4.
21. Singer DRJ, Missouris CG, Jeffery S. Angiotensin-converting enzyme gene polymorphism: what to do about all the confusion. Circulation 1996; 94: 236-9.
22. Lindpaintner K, Pfeffer MA, Kreutz R, et al. A prospective evaluation of an angiotensin-converting-enzyme gene polymorphism and the risk of ischemic heart disease. N Engl J Med 1995; 332: 706-11.
23. Anderson JL, Carlquist JF, Gretchen JK, et al. Angiotensin-converting enzyme genotypes and risk for myocardial infarction in women. J Am Coll Cardiol 1998; 31: 790-6.
24. Gardemann A, Fink M, Stricker J, et al. ACE I/D gene polymorphism: presence of the ACE D allele increases the risk of coronary artery disease in younger individuals. Atherosclerosis 1998; 139: 153-9.
25. Keavney B, McKenzie C, Parish S, et al, for the International Studies of Infarct Survival (ISIS) Collaborators. Large-scale test of hypothesised associations between the angiotensin-converting enzyme insertion/deletion polymorphism and myocardial infarction in about 5000 cases and 6000 controls. Lancet 2000; 355: 434-42.
26. Szpirer C, Riviere M, Szpirer J, et al. Chromosomal assignment of human and rat hypertension candidate genes: type 1 angiotensin II receptor genes and the SA gene. J Hypertens 1993; 11: 919-25.
27. Poirier O, Georges JL, Ricard S, et al. New polymorphisms of the angiotensin II receptor gene and their associations with myocardial infarction and blood pressure: the ECTIM study. Etude Cas-TØmoin de l Infarctus du Myocarde. J Hypertens 1998; 16: 1443-7.
28. Tiret L, Bonnardeaux A, Poirier O, et al. Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. Lancet 1994; 344: 910-3.
29. Berge KE, Bakken A, Bohn M, Erikssen H, Berg K. A DNA polymorphism at the angiotensin II type 1 receptor (AT1R) locus and myocardial infarction. Clin Genet 1997; 52: 71-6.
30. Rice GI, Foy CA, Grant PJ. Angiotensin converting enzyme and angiotensin II type 1-receptor gene polymorphisms and risk of ischaemic heart disease. Cardiovasc Res 1999; 41: 746-53.
31. Gardemann A, Nguyen QD, Humme J, et al. Angiotensin II type 1 receptor A1166C gene polymorphism. Absence of an association with the risk of coronary artery disease and myocardial infarction and of a synergistic effect with angiotensin-converting enzyme gene polymorphism on the risk of these diseases. Eur Heart J 1998; 19: 1657-65.
32. Robinson LJ, Weremowicz S, Morton CC, Michel T. Isolation and chromosomal localization of the human endothelial nitric oxide synthase (NOS3) gene. Genomics 1994; 19: 350-7.
33. Ichihara S, Yamada Y, Fusimura T, Nakashima N, Yokota M. Association of a polymorphism of the endothelial constitutive nitric oxide synthase gene with myocardial infarction in the Japanese population. Am J Cardiol 1998; 81: 83-6.
34. Shimasaki Y, Yasue H, Yoshimura M, et al. Association of the missense Glu298Asp variant of the endothelial nitric oxide synthase gene with myocardial infarction. J Am Coll Cardiol 1998; 31: 1506-10.
35. Hibi K, Ishigami T, Mizushima S, et al. Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. Hypertension 1998; 32: 521-6.
36. Hingorani AD, Liang CF, Fatibene J, et al. A common variant of the endothelial nitric oxide synthase (Glu298→Asp) is a major risk factor for coronary artery disease in the UK. Circulation 1999; 100: 1515-20.
37. Poirier O, Mao C, Mallet C, et al. Polymorphisms of the endothelial nitric oxide synthase gene: no consistent association with myocardial infarction in the ECTIM study. Eur J Clin Invest 1999; 29: 284-90.
38. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for cardiovascular disease: a common missense mutation in methylenetetrahydrofolate reductase. Nat Genet 1995; 10: 111-3.
39. Kang SS, Wong PW, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary disease. Am J Hum Genet 1991; 48: 536-45.
40. van Bockxmeer FM, Mamotte DC, Vasikaran SD, Taylor RR. Methylenetetrahydrofolate reductase gene and coronary artery disease. Circulation 1997; 95: 21-3.
41. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996; 94: 2410-6.
42. Schmitz C, Lindpaintner K, Verhoef P, Gaziano JM, Buring J. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. Circulation 1996; 94: 1812-4.
43. Meade TW, Ruddock V, Stirling Y, Chakrabarti R, Miller GJ. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. Lancet 1993; 342: 1076-9.
44. Humphries SE, Panahloo A, Montgomery HE, Green F, Yudkin J. Gene-environment interaction in the determination of levels of haemostatic variables involved in thrombosis and fibrinolysis. Thromb Haemost 1997; 78: 457-61.
45. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J Med 1995; 332: 635-41.
46. Andreotti F, Burzotta F, Maseri A. Fibrinogen and inflammation: a clinical view. Blood Coagul Fibrinolysis 1999; 10 (Suppl 1): S3-S4.
47. Humphries SE. Genetic regulation of fibrinogen. Eur Heart J 1995; 16 (Suppl A): 16-20.
48. Scarabin PY, Bara L, Ricard S, et al. Genetic variation at the fibrinogen locus in relation to plasma fibrinogen concentration and risk of myocardial infarction. The ECTIM Study. Arterioscler Thromb 1993; 13: 886-91.
49. Green F, Hamsten A, Blomback M, Humphries S. The role of beta-fibrinogen genotype in determining plasma fibrinogen levels in young survivors of myocardial infarction and healthy controls from Sweden. Thromb Haemost 1993; 70: 915-20.
50. Gardemann A, Schwartz O, Haberbossch W, et al. Positive association of the beta fibrinogen variation to basal fibrinogen levels and to the increase in fibrinogen concentration during acute phase reaction but not to coronary artery disease and myocardial infarction. Thromb Haemost 1997; 77: 1120-6.
51. Zito F, Di Castelnuovo A, Amore C, D Orazio A, Donati MB, Iacoviello L. BclI polymorphism in the fibrinogen beta-chain gene is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels. A case-con-

trol study in a sample of GISSI-2 patients. Arterioscler Thromb Vasc Biol 1997; 17: 3489-94.
52. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3 -untranslated region of the prothrombin gene is associated with elevated levels and an increase in venous thrombosis. Blood 1996; 88: 3698-703.
53. 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.
54. Ardissino D, Mannucci PM, Merlini PA, et al. Prothrombotic genetic risk factors in young survivors of myocardial infarction. Blood 1999; 94: 46-51.
55. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation 1999; 99: 999-1004.
56. Burzotta F, Paciaroni K, Chiusolo P, et al. The prothrombin G20210A mutated allele in patients with acute coronary syndromes as first presentation of disease. (abstr) Circulation 1999; 100 (Suppl I): I-518.
57. Griffin JH, Heeb MJ, Kojima Y, et al. Activated protein C resistance: molecular mechanisms. Thromb Haemost 1995; 74: 444-8.
58. Koster T, Rosendaal FR, de Ronde H, et al. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. Lancet 1993; 342: 1503-6.
59. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994; 330: 517-22.
60. Griffin JH, Evatt B, Wideman C, et al. Anticoagulant protein C pathway defective in majority of thrombophilic patients. Blood 1993; 82: 1989-93.
61. De Stefano V, Martinelli I, Mannucci PM, 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.
62. Bertina RM, Koeleman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369: 64-7.
63. Voelkerding KV. Resistance to activated protein C and novel factor V gene mutation. Clin Lab Med 1996; 16: 169-86.
64. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and risk of myocardial infarction, stroke and venous thrombosis in apparently healthy men. N Engl J Med 1995; 332: 112-7.
65. Verheggen P, Slagboom E, de Maat M, Haverkate F, Cats VM. Normal distribution of a single point-mutation in the factor V gene in patients with severe unstable angina pectoris. (abstr) Circulation 1995; 92 (Suppl): I-624.
66. Iacoviello L, Zito F, Di Castelnuovo A, de Maat M, Kluft C, Donati MB. Contribution of factor VII, fibrinogen and fibrinolytic components to the risk of ischaemic cardiovascular disease: their genetic determinants. Fibrinolysis and Proteolysis 1998; 12: 259-76.
67. Simpson HC, Mann JI, Meade TW, Chakrabarti R, Stirling Y, Woolf L. Hypertriglyceridaemia and hypercoagulability. Lancet 1983; 1: 786-90.
68. Miller GJ, Walter SG, Stirling Y, Thompson SG, Esnouf MP, Meade TW. Assay of factor VII activity by two techniques: evidence for increased conversion of factor VII in hyperlipidaemia, with possible implications for ischaemic heart disease. Br J Haematol 1985; 59: 249-58.
69. Meade TW, Mellows S, Brozovic M, et al. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986; 2: 533-7.
70. Lane A, Green F, Scarabin PY, et al. Factor VII Arg/Gln353 polymorphism determines factor VII activity in patients with myocardial infarction (MI) and control subjects in Belfast and in France but is not a strong indicator of MI risk in the ECTIM study. Atherosclerosis 1996; 119: 119-27.
71. Bernardi F, Marchetti G, Pinotti M, et al. Factor VII gene polymorphisms contribute about one third of the factor VII level variation in plasma. Arterioscler Thromb Vasc Biol 1996; 16: 72-6.
72. Green F, Kelleher C, Wilkes H, Temple A, Meade T, Humphries S. A common genetic polymorphism associated with lower coagulation factor VII levels in healthy individuals. Arterioscler Thromb 1991; 11: 540-6.
73. Lane A, Cruickshank JK, Mitchell J, Henderson A, Humphries S, Green F. Genetic and environmental determinants of factor VII coagulant activity in ethnic groups at differing risk of coronary artery disease. Atherosclerosis 1992; 94: 43-50.
74. Iacoviello L, Di Castelnuovo A, de Knijff P, et al. Polymorphism in the coagulation factor VII gene and the risk of myocardial infarction. N Engl J Med 1998; 338: 79-85.
75. Peyvandi F, Mannucci PM, Bucciarelli P, et al. 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. Br J Haematol 2000; 108: 247-53.
76. Suzuki K, Henke J, Iwata M, et al. Novel polymorphisms and haplotypes in the human coagulation factor XIII A-subunit gene. Hum Genet 1996; 98: 393-5.
77. Kohler HP, Stickland MH, Ossei-Gerning N, Carter A, Mikkola H, Grant PJ. Association of a common polymorphism in the factor XIII gene with myocardial infarction. Thromb Haemost 1998; 79: 8-13.
78. Wartiovaara U, Perola M, Mikkola H, et al. Association of FXIII Val34Leu with decreased risk of myocardial infarction in Finnish males. Atherosclerosis 1999; 142: 295-300.
79. Franco RF, Pazin-Filho A, Tavella MH, Simoes MV, Marin-Neto JA, Zago MA. Factor XIII Val34Leu and the risk of myocardial infarction. Haematologica 2000; 85: 67-71.
80. Weiss EJ, Bray PF, Tayback M, et al. A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996; 334: 1090-4.
81. Walter DH, Schachinger V, Elsner M, Dimmeler S, Zeiher AM. Platelet glycoprotein IIIa polymorphisms and risk of coronary stent thrombosis. Lancet 1997; 350: 1217-9.
82. Ridker PM, Hennekens CH, Schimtz C, Stampfer MJ, Lindpaintner K. PlA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. Lancet 1997; 349: 385-8.
83. Herrmann SM, Poirier O, Marques-Vidal P, et al. The Leu33/Pro polymorphism (PlA1/PlA2) of the glycoprotein IIIa (GPIIIa) receptor is not related to myocardial infarction in the ECTIM Study. Thromb Haemost 1997; 77: 1179-81.
84. Joven J, Sim JM, Vilella E, et al. Lipoprotein(a) and the significance of the association between platelet glycoprotein IIIa polymorphisms and the risk of premature myocardial infarction. Atherosclerosis 1998; 140: 155-9.
85. Durante-Mangoni E, Davies GJ, Ahmed N, Ruggiero G, Tuddenham EG. Coronary thrombosis and the platelet glycoprotein IIIA gene PLA2 polymorphism. Thromb Haemost 1998; 80: 218-9.
86. Gardemann A, Humme J, Stricker J, et al. Association of the platelet glycoprotein IIIa PlA1/A2 gene polymorphism to coronary artery disease but not to nonfatal myocardial infarction in low risk patients. Thromb Haemost 1998; 80: 214-7.
87. Kuijpers RW, Faber NM, Cuypers HT, Ouwehand WH, von dem Borne AE. NH2-terminal globular domain of human platelet glycoprotein Ib alpha has a methionine 145/threonine 145 amino acid polymorphism, which is associated with

the HPA-2 (Ko) alloantigens. J Clin Invest 1992; 89: 381-4.
88. Moroi M, Jung S, Yoshida N. Genetic polymorphism of platelet glycoprotein Ib. Blood 1984; 64: 622-9.
89. Murata M, Matsubara Y, Kawano K, et al. Coronary artery disease and polymorphisms in a receptor mediating shear stress-dependent platelet activation. Circulation 1997; 96: 3281-6.
90. Gonzales-Conejero R, Lozano ML, Rivera J, et al. Polymorphisms of platelet membrane GP Ib associated with arterial thrombotic disease. Blood 1998; 92: 2771-6.
91. Kunicki TJ, Kritzik M, Annis DS, Nugent D. Hereditary variation in platelet integrin $\alpha 2\beta 1$ density is associated with two silent polymorphisms in the alpha 2 gene coding sequence. Blood 1997; 89: 1939-43.
92. Moshfeg K, Wuillemin WA, Redondo M, et al. Association of two silent polymorphisms of platelet glycoprotein Ia/IIa receptor with risk of myocardial infarction: a case-control study. Lancet 1999; 353: 351-4.
93. Santoso S, Kunicki TJ, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807T gene polymorphism with non fatal myocardial infarction in younger patients. Blood 1999; 93: 2449-53.
94. Croft SA, Hampton KK, Sorrell JA, et al. The GPIa C807T dimorphism associated with platelet collagen receptor density is not a risk factor for myocardial infarction. Br J Haematol 1999; 106: 771-6.
95. Wiman B. Plasminogen activator inhibitor 1 (PAI-1) in plasma: its role in thrombotic disease. Thromb Haemost 1995; 74: 71-6.
96. Andreotti F, Manzoli A. Circadian fluctuation of fibrinolytic factors in blood. Investigation, importance and clinical consequences. In: Muller J, Willich S, eds. Triggering of acute coronary syndromes: implications for prevention. Dordrecht: Kluwer Academic Publishers, 1995: 185-99.
97. van Leeuwen RT, Kol A, Andreotti F, Kluft C, Maseri A, Sperti G. Angiotensin II increases plasminogen activator inhibitor type 1 and tissue-type plasminogen activator messenger RNA in cultured rat aortic smooth muscle cells. Circulation 1994; 90: 362-8.
98. Eriksson P, Kallin B, van t Hooft FM, Bavenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proc Natl Acad Sci USA 1995; 92: 1851-5.
99. Ossei-Gerning N, Mansfield MW, Stickland MH, Wilson IJ, Grant PJ. Plasminogen activator inhibitor-1 promoter 4G/5G genotype and plasma levels in relation to a history of myocardial infarction in patients characterized by coronary angiography. Arterioscler Thromb Vasc Biol 1997; 17: 33-7.
100. Iwai N, Shimoike H, Nakamura Y, Tamaki S, Kinoshita M. The 4G/5G polymorphism of the plasminogen activator inhibitor gene is associated with the time course of progression to acute coronary syndromes. Atherosclerosis 1998; 136: 109-14.
101. Ye S, Green FR, Scarabin PY, et al. The 4G/5G genetic polymorphism in the promoter of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with differences in plasma PAI-1 activity but not with risk of myocardial infarction in the ECTIM study. Thromb Haemost 1995; 74: 837-41.
102. Burzotta F, Di Castelnuovo A, Amore C, D Orazio A, Donati MB, Iacoviello L. 4G/5G polymorphism in the promoter region of the PAI-1 gene is not a risk factor for familial myocardial infarction: a case-control study in a sample of GISSI-2 patients. Thromb Haemost 1997; 78: 1294-5.
103. Ridker PM, Hennekens CH, Lindpaintner K, Stamfer MJ, Miletich JP. Arterial and venous thrombosis is not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of US men. Circulation 1997; 95: 59-62.
104. Iacoviello L, Burzotta F, Di Castelnuovo A, Zito F, Marchioli R, Donati MB. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of myocardial infarction: a meta-analysis. Thromb Haemost 1998; 80: 1029-30.
105. Kluft C. Constitutive synthesis of tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1): conditions and therapeutic targets. Fibrinolysis 1994; 8 (Suppl 2): 1-7.
106. Ludwig M, Wohn KD, Schleuning WD, Olek K. Allelic dimorphism in the human tissue-type plasminogen activator (t-PA) gene as a result of an Alu insertion/deletion event. Hum Genet 1992; 88: 388-92.
107. van der Bom JG, de Knijff P, Haverkate F, et al. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. Circulation 1997; 95: 2623-7.
108. Ridker PM, Baker MT, Hennekens CH, Stampfer MJ, Vaughan DE. Alu-repeat polymorphism in the gene coding for tissue-type plasminogen activator (t-PA) and risk of myocardial infarction among middle-aged men. Arterioscler Thromb Vasc Biol 1997; 17: 1687-90.
109. Steeds R, Adams M, Smith P, Channer K, Samani NJ. Distribution of tissue plasminogen activator insertion/deletion polymorphism in myocardial infarction and control subjects. Thromb Haemost 1998; 79: 980-4.
110. Kluft C. Genetic research stimulates development of individual treatment. Cardiologia 1999; 44: 119-24.
111. Lane DA, Grant PJ. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood 2000; 95: 1517-32.
112. Carluccio M, Vannuzzo D, Pilotto L, et al. La componente genetica del rischio coronarico - Parte I. Cardiologia 1999; 44: 39-50.