UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

Estudo dos Fatores Genéticos de Risco para o Infarto Agudo do Miocárdio em Idade Precoce

Crislaine Aparecida Paludo

Tese apresentada ao Programa de
Pós-graduação em Genética e Biologia Molecular da
Universidade Federal do Rio Grande do Sul
para a obtenção do grau de Doutor em Ciências.

Orientador: Dr. Israel Roisenberg

Colaboradora: Dra. Vera Lúcia Portal

Porto Alegre, 2003.

Este trabalho foi realizado no Laboratório de Hemostasia
do Departamento de Genética
da Universidade Federal do Rio Grande do Sul,
com o apoio financeiro do CNPq, PRONEX e FINEP.

Agradecimentos

Ao Dr. Israel Roisenberg pela orientação, conhecimentos transmitidos, e por sua amizade. Por saber ouvir os eventuais lamentos durante os últimos anos, pelos cafés e por suas piadas.

À Dra. Vera Lúcia Portal do IC-FUC, pela co-orientação e ajuda inestimável, e principalmente por ter oferecido as condições necessárias para essa tese sair do papel.

Ao Dr. Iseu Gus chefe do serviço de Epidemiologia do IC-FUC, por ter aberto as portas do IC-FUC.

À Josi Vidart, acadêmica de medicina, pela ajuda com os pacientes, entrevistas, banco de dados, análises, e pelas coisas que esqueço de mencionar.

Ao Ricardo Petraco, acadêmico de medicina, pela ajuda na entrevista com os pacientes.

À Vânia, do setor de pesquisa do IC-FUC, pela tremenda ajuda na parte estatística dessa tese.

À Sandra Souza, secretária do Serviço de Epidemiologia do IC-FUC, pelo inestimável auxílo em diversos (ou muitos?) momentos.

À Amália e Elaine, do Ambulatório de Convênios do IC-FUC, pela paciência na coleta de sangue dos pacientes em muitas segundas-feiras de manhã.

Ao Ricardo e sua equipe do Laboratório Central do IC-FUC, pelas realização das dosagens bioquímicas dos pacientes.

Aos 283 pacientes infartados, que felizmente concordaram em fazer parte desse estudo.

Aos 96 indivíduos controle que se dispuseram a ir ao IC-FUC e participar desse estudo.

Ao Elmo e Helen, da Secretaria da Pós-graduação do departamento de Genética, pela ajuda em diversos momentos.

Aos colegas do laboratório de hemostasia pelo convívio diário, que deixaram os últimos anos bem mais divertidos.

Agradecimentos especiais

À minha família, pelo apoio, carinho e infra-estrutura necessária para a realização de mais essa etapa, tão importante da minha vida. Pelas últimas semanas (que foram muitas) em que a minha presença em casa foi meramente ilustrativa.

Ao Leandro, por todo amor, carinho, paciência, dedicação, e por todos os outros motivos que (por pura falta de espaço) não cabem aqui. Pelos fins de semana de trabalho e por entender o significado dos termos dedicação exclusiva e tempo integral. Mas, principalmente por ser essa pessoa tão maravilhosa.

A todos aqueles que de alguma forma contribuíram para a realização desse trabalho.

ÍNDICE

LISTA DE ABREVIATURAS

AMI: acute myocardial infarction

APC: proteína C ativada

AVC: acidente vascular cerebral

CAD: coronary artery disease

DAC: doença aterosclerótica coronariana

DCV: doença cardiovascular

eNOS: endothelial nitric oxide synthase, óxido nítrico sintetase endotelial

FvW: fator von Willebrand

FV: fator V

FV leiden: mutação fator V Leiden (Arg506Gln)

FVII: fator VII

HT: 5-hidroxitriptamina ou serotonina

IAM: infarto agudo do miocárdio

I/D: polimorfismo de inserção/deleção

IM: infarto do miocárdio

MMP: metaloproteinase de matriz

NO: nitric oxide

NOS: nitric oxide synthase

ON: óxido nítrico

PAI-1: inibidor tipo 1 do ativador do plasminogênio

TIMP: inibidor tissular específico de metaloproteinase de matriz

t-PA: ativador tipo tissular do plasminogênio

CAPÍTULO 1

Introdução

As doenças cardiovasculares (DCV), principalmente o infarto do miocárdio, permanecem como uma das principais causas de morbidade e mortalidade nos Estados Unidos, Europa e Brasil. No Brasil, as doenças cardiovasculares são responsáveis por cerca de 30% dos óbitos, e por 35% dos óbitos no Rio Grande do Sul. No Rio Grande do Sul, o número anual de óbitos por causa isquêmica é de aproximadamente 8.500, por causa cerebrovascular, de 7.350 e decorrentes de infarto agudo do miocárdio (IAM) somam 5.750 (dados do Ministério da Saúde referentes a 2001, http://datasus.gov.br).

Uma forma de dimensionar a importância da identificação e prevenção dos fatores de risco no Rio Grande do Sul, é a comparação dos dados referentes à mortalidade (GIANINI, 1998). Os coeficientes de mortalidade por coronariopatia em homens (número de óbitos/100.000 habitantes) observados em Porto Alegre (402,2 óbitos/100.000 habitantes), Rio de Janeiro (402,2/100.000) e Curitiba (389,3/100.000) são muito similares aos observados em países como a Finlândia (481,5/100.000), Hungria (445,8/100.000) e Inglaterra (429/100.000). Em relação às mulheres, Porto Alegre está em quinto lugar quanto ao número de óbitos decorrentes de coronariopatia (123.8/100.000), precedido por Rio de Janeiro (143,2/100.000), Curitiba (135,4/100.000), Campo Grande (SP) (133,9/100.000), Hungria (131,4/100.000) e Inglaterra (114,2/100.000).

Os esforços de prevenção e tratamento das DCV são baseados no reconhecimento e controle dos fatores de risco. Além dos fatores de risco clássicos (hipertensão, dislipidemia, tabagismo, sedentarismo e história familiar), outros têm sido propostos, a fim de explicar a incidência das doenças cardiovasculares e mais especificamente o infarto do miocárdio, principalmente em idade precoce.

O IAM resulta da necrose miocárdica, causada pela interrupção do suprimento sangüíneo por um período prolongado de tempo. O diagnóstico é feito quando encontramos pelo menos 2 dos seguintes critérios: características clínicas (dor torácica típica), eletrocardiográficas e bioquímicas (elevação das enzimas creatinofosfoquinase fração MB e troponina, principalmente).

A nova classificação para as síndromes coronarianas agudas proposta recentemente

compreende uma ampla variedade de formas clínicas de apresentação da doença coronariana, incluindo angina instável, infarto do miocárdio sem supradesnivelamento do segmento ST (IAM sem supra), infarto agudo do miocárdio tipo Q (IAM) e morte súbita (The Joint European Society of Cardiology/American College of Cardiology Committee, 2000).

Embora os mecanismos patogênicos do infarto possam ser múltiplos, cerca de 50% dos infartos do miocárdio resultam da formação de um trombo sobre o sítio de ruptura de uma placa aterosclerótica (FUSTER *et al.*, 1990; 1992; DAVIES, 1996). Em 1958, o processo aterosclerótico era definido como uma combinação de alterações degenerativas da íntima das artérias, acúmulo de moléculas lipídicas, tecido fibroso, cálcio, sangue e alterações na camada média das artérias (FUSTER, 1994). Desde 1973, a hipótese da resposta à lesão é aceita e revista nos seus detalhes. De acordo com essa hipótese a aterosclerose é vista como um processo inflamatório progressivo que inicia geralmente na infância e se manifesta na idade adulta, na forma de eventos clínicos como a angina instável, o infarto agudo do miocárdio e a morte súbita (ROSS & GLOMSET, 1973; ROSS, 1993; 1999).

A ruptura da placa aterosclerótica e posterior trombose são complicações de lesões ateroscleróticas em estágio avançado, cuja ruptura ativa a cascata de coagulação sangüínea, iniciando-se a formação do trombo e a subseqüente oclusão da artéria. Geralmente, a ruptura ocorre nos sítios de menor espessura da placa e os sítios mais propensos à ruptura ou erosão resultam da ativação de macrófagos que liberam enzimas proteolíticas que irão degradar a matriz extracelular.

As lesões ateroscleróticas iniciam como um processo inflamatório, através do acúmulo de macrófagos (derivados de monócitos) e linfócitos T no subendotélio. Após, essas lesões iniciais se transformam em lesões proliferativas através da infiltração de fibroblastos e acúmulo de matriz extracelular (FIGURA 1).

Modelos animais e observações em humanos demonstram que o fator que desencadeia o processo aterosclerótico é a disfunção ou lesão endotelial. As causas mais prováveis de disfunção endotelial são modificações oxidativas nas partículas lipídicas, radicais livres, infecção por microrganismos (como Chlamidia e herpesvírus) e diferentes combinações desses fatores. A disfunção endotelial resultante altera as propriedades hemostáticas do endotélio, que incluem o aumento da adesividade e permeabilidade a leucócitos e plaquetas. O endotélio assume um estado procoagulante através da formação de moléculas vasoativas, citocinas e fatores de crescimento. A resposta inflamatória estimula a migração e proliferação de células musculares lisas, espessamento e dilatação graduais da parede arterial, o que caracteriza o processo de remodelamento.

O acúmulo contínuo de células mononucleadas, a proliferação e migração de células musculares lisas e a formação de tecido fibroso reestruturam a lesão aterosclerótica, recobrindo-a com uma capa que protege o chamado núcleo necrótico (formado por leucócitos, lipídios e restos celulares). Na maioria dos casos, a ruptura de uma placa aterosclerótica está associada à hemorragia intraplaca e à formação de trombos no núcleo necrótico exposto (revisto em ROSS, 1999).

O processo de adesão plaquetária é crucial para a progressão destas lesões ateroscleróticas. As plaquetas têm a capacidade de aderir ao subendotélio, colágeno e macrófagos e uma vez ativadas, liberam o conteúdo de seus grânulos (citocinas e fatores de crescimento). A ativação das plaquetas também libera substâncias vasoconstritoras e outras substâncias que podem amplificar a resposta inflamatória. Plaquetas ativadas acumulam-se na parede arterial e recrutam plaquetas circulantes. Um dos principais componentes desse processo é o complexo IIb/IIIa, situado na superfície das plaquetas.

Assim, alterações relacionadas à função endotelial, atividade plaquetária, coagulação e fibrinólise poderiam ser consideradas potenciais fatores de risco para o desenvolvimento de um quadro de infarto do miocárdio. O presente estudo pretende avaliar o papel de diversos fatores de risco genéticos, possivelmente associados ao IAM, numa população caucasóide com idade inferior a 60 anos.

1. O Fator von Willebrand

O fator von Willebrand (fvW) é uma glicoproteína multimérica composta por diversas subunidades (com cerca de 250 kDa cada), ligadas por pontes dissulfídicas; podendo alcançar mais de 20.000 kDa. O gene do fvW localiza-se no cromossomo 12, apresenta aproximadamente 178 kb e contém 52 éxons. O fvW é produzido por células endoteliais e megacariócitos, podendo ser encontrado no plasma, grânulos plaquetários e endotélio. O fvW possui duas funções essenciais na hemostasia: mediar a adesão plaquetária ao subendotélio e ligar-se ao fator VIII, protegendo-o da degradação (ZIMMERMAN & MEYER, 1982; GIDDINGS, 1988; NICHOLS *et al.*, 1991; TUDDENHAM & COOPER, 1994; SADLER, 1998). No caso de lesão endotelial, o fvW liga-se a constituintes do tecido conectivo e plaquetas, retendo-as no sítio da lesão; assim o fvW tem sido referido como um marcador de lesão endotelial (SADLER, 1998).

Boneu, em 1975, foi o primeiro autor a propor a dosagem do fvW como um índice de lesão endotelial em indivíduos com isquemia ou septicemia (MANNUCCI, 1998), desde então,

diversos trabalhos analisaram a relação desse fator em situações clínicas envolvendo o endotélio vascular.

JANSSON *et al.* (1991) verificaram que altas concentrações de fvW foram independentemente associadas tanto com IAM recurrente, como com mortalidade pós-IAM. CORTELLARO *et al.* (1992) estudaram o efeito dos níveis de fvW em uma amostra de indivíduos com doença vascular preexistente e verificaram uma associação positiva entre os níveis apresentados e a ocorrência de eventos subseqüentes. Entretanto, SCHMITZ-HUEBNER *et al.* (1988) não observaram uma associação significativa dos níveis de fvW e a extensão da aterosclerose, em um estudo com pacientes anginosos.

THOMPSON *et al.* (1995) estudaram pacientes com angina, provenientes de 18 centros da Europa, em relação à incidência de eventos vasculares (IAM ou morte súbita) e observaram um aumento na incidência de tais eventos associado a níveis elevados de fvW. OSSEI-GERNING *et al.* (1998) sugeriram que os níveis de fvW poderiam ser utilizados como um marcador de risco, por sua associação com a presença de lesões ateroscleróticas. Da mesma forma, níveis elevados de fvW foram significativamente associados à doença aterosclerótica coronariana em caucasóides do Rio Grande do Sul (PALUDO, 1999).

LOPES *et al.* (1998) observaram que em pacientes com disfunção endotelial pulmonar (causada por hipertensão pulmonar), alterações nos níveis de fvW circulante apresentaram valor prognóstico, uma vez que os pacientes que apresentaram acentuadas elevações desse fator foram aqueles com menores possibilidades de sobrevida no período de um ano.

Em nosso grupo, observamos aumentos significativos nos níveis plasmáticos de fvW em mulheres portadoras de câncer de mama (RÖHSIG *et al*., 2001) e em pacientes com carcinoma coloretal (DAMIN *et al*., 2002). Eleveções nos níveis circulantes de fvW também foram associadas ao estadiamento e progressão do tumor durante o processo angiogênico por SCHWARTZMANN *et al*. (2001).

Recentemente, um polimorfismo de DNA localizado na região promotora do gene do fvW denominado G–1185A foi associado a elevações nos níveis plasmáticos desse fator. Aparentemente, esta associação é mediada pela ligação diferencial de proteínas nucleares envolvidas na regulação da expressão do gene (KEIGHTLEY *et al.*, 1999). No entanto, SIMON *et al*. (2002) mostraram que essa variante não foi associada aos níveis circulantes de fvW, apesar de apresentar diferentes freqüências gênicas em dois grupos étnicos brasileiros. Di BITONDO *et al.* (2001) e SIMON *et al.* (2003) não encontraram associação entre a variante G-1185A com os níveis circulantes de fvW e com o risco de doença coronariana.

2. A Serotonina

Em caso de lesão endotelial, imediatamente ocorre vasoconstrição, formação do tampão plaquetário e reparo vascular (WU & THIAGARAJAN, 1996). Na superfície plaquetária são expressos diferentes receptores glicoprotéicos de membrana, que irão interagir com fatores de coagulação e substâncias de adesão e agregação plaquetárias. Geralmente, eventos coronarianos como angina instável ou IAM resultam da formação de um agregado plaquetário e da ativação da cascata de coagulação no local onde o endotélio foi danificado (FUSTER *et al.*, 1992).

Durante a agregação plaquetária, são liberadas diversas substâncias como a serotonina (5-hidroxitriptamina, 5-HT), tromboxane e nucleotídeos de adenina (ADP e ATP), o que desencadeia o recrutamento e ativação das plaquetas circulantes e culmina na formação do trombo (ZUCKER *et al.*, 1985). A serotonina apresenta diversos efeitos sobre a parede vascular, favorecendo a trombogênese, a mitogênese, além de ter efeito vasoconstritor (YOKOYAMA *et al.*, 1983; McFADDEN *et al.*, 1991; VANHOUTTE, 1991; KAUMANN *et al.*, 1994).

Em indivíduos com hipercolesterolemia familar, onde a doença aterosclerótica ocorre precocemente, concentações de serotonina plaquetária e circulante mostraram-se alteradas (SMITH & BETTERIDGE, 1997). Concentrações elevadas de serotonina foram associadas à doença coronariana e eventos cardíacos, principalmente em pacientes com idade precoce, no trabalho de VIKENES *et al.* (1999). KOROVESIS *et al.* (2000) observaram um aumento nos níveis de serotonina durante o procedimento de angioplastia coronariana, indicando a ativação do sistema plaquetário, comum durante esse tipo de procedimento invasivo.

O efeito vasoconstritor da serotonina é principalmente regulado através da ação dos receptores 5-HT_2 situados na superfície de plaquetas e células musculares lisas. Até o momento, foram descritos 3 subtipos de receptores: 5-HT_2A, 5-HT_2B e 5-HT_2C. O gene do receptor 5-HT_2A é expresso nas plaquetas e artérias coronárias (VANHOUTTE *et al.*, 1991; HUMPHREY *et al.*, 1993; ULLMER *et al.*, 1995). Os receptores 5-HT também interagem sinergisticamente com molélulas lipídicas, estimulando a mitogênese e ativação de plaquetas (KOBA *et al.* 1999; MACCARRONE *et al.*, 2003). O gene 5-HT_2A localiza-se no cromossomo 13, apresenta um tamanho de 20kb e está organizado em 3 éxons e 2 introns (SPARKES *et al.*, 1991; CHEN *et al.*, 1992). Um polimorfismo de DNA denominado T102C situado no éxon 1 da região codificante do gene do receptor 5-HT_2A foi descrito (WARREN *et al.*, 1993) e parece afetar a função receptora.

Anormalidades no sistema serotoninérgico têm sido associadas a diversas doenças,

tais como depressão, epilepsia, transtorno obsessivo-compulsivo e transtornos afetivos. INIAYAMA *et al.* (1996) e WILLIAMS *et al.* (1996; 1997) encontraram associação significativa entre o polimorfismo T102C e esquizofrenia. Uma meta-análise da associação da serotonina em doenças comuns pode ser encontrada em LOHMUELLER *et al.* (2003).

YAMADA *et al.* (2000) verificaram que o polimorfismo T102C foi associado, de forma independente, com a ocorrência de IAM em homens, sendo sugerido como um novo marcador de risco.

3. A Estromelisina-1

As metaloproteinases de matriz (MMPs) são enzimas responsáveis pela manutenção da integridade estrutural dos tecidos, remodelamento fisiológico e coordenação entre síntese/degradação dos limites teciduais. Para tanto, as MMPs degradam as proteínas estruturais e adesivas da matriz extracelular (SELLERS & MURPHY, 1981; VISSE & NAGASE, 2003). A matriz extracelular é composta por colágeno, proteoglicanos e glicoproteínas, que além de manter o suporte estrutural dos tecidos, também interagem com as células circundantes. A matriz extracelular deve ser degradada fisiologicamente nos casos de reabsorção, reparo celular, crescimento e em tecidos que mudam de forma ou função (McCAWLEY & MATRISLAN, 2000). As MMPs são expressas constitutivamente e em níveis bastante reduzidos, porém sua secreção é rapidamente induzida no caso de remodelamento ativo, e inibidas por inibidores tissulares específicos (TIMPs). A atividade e expressão das MMPs devem ser rigorosamente controladas, a fim de evitar a destruição ou acúmulo de tecido conectivo (SHAH *et al*., 1995).

Deficiências na regulação da MMPs (ao nível de transcrição, ativação dos zimogênios ou inibição dos TIMPs) são associadas com diversas patologias proliferativas como artrite, doença arterial coronariana, úlcera, enfisema e câncer (revisto em McCAWLEY & MATRISLAN, 2000). Aparentemente as interações celulares que ocorrem em doenças proliferativas são as mesmas do processo aterogênico, e a resposta parece ser dependente do órgão atingido, ou seja, a aterosclerose irá se desenvolver em artérias e a artrite reumatóide em articulações. As MMPs também são associadas à progressão ou metástase de tumores invasivos (STERNLICHT *et al*., 1999; McCAWLEY & MATRISLAN, 2000; HINODA *et al*., 2002; KUYVENHOUVEN *et al.*, 2003). Como a ruptura de uma placa aterosclerótica é um fator crítico na patogênese do IAM (FUSTER *et al*., 1990; 1992), tem sido sugerido que as MMPs participariam dos processos de enfraquecimento e posterior ruptura da placa de ateroma. Macrófagos e linfócitos participam de processos inflamatórios e ateroscleróticos, e

estes induzem a ação das MMPs. SHAH *et al*. (1995) e MACH *et al*. (1997) demostraram que a ativação de fagócitos e macrófagos induzem a atividade das MMPs em placas de ateroma.

A estromelisina do tipo 1 é uma MMP do tipo 3 (MMP-3), é secretada pelas células endoteliais e fagócitos na forma de zimogênio e apresenta um peso molecular de 52 kDa. O gene da estromelisina-1 está localizado no cromossomo 11 e apresenta um tamanho de 135 kb (SPURR *et al.*, 1988; FORMSTONE *et al.*, 1993). A estromelisina-1 apresenta um amplo espectro de substratos sobre os quais pode atuar, podendo degradar proteoglicanos, laminina, fibronectina, elastina, gelatina, caseína, colágenos dos tipos III, IV, IX e X, além de ativar outras MMPs (LU *et al*., 1999). A degradação desses componentes se faz necessária em processos (pato)fisiológicos, como a formação da neoíntima após lesão tecidual, aterosclerose, doença do enxerto, infecção ou inflamação.

A regulação da expressão da estromelisina-1 ocorre principalmente ao nível de transcrição, como confirmada por QUINONES *et al.* (1989; 1994) e YE *et al.* (1999). HUMPHRIES *et al*. (2002a; b) relatam que o remodelamento vascular é uma condição essencial no desenvolvimento de alterações ateroscleróticas na parede vascular e que as MMP provavelmente estariam envolvidas nesse processo. O gene da estromelisina-1 também foi associado à regulação do desenvolvimento do tecido adiposo por MAQUOI *et al.* (2003). Um polimorfismo de DNA localizado na região promotora (-1171) do gene da estromelisina-1, denominado 5A/6A, foi descrito por YE *et al.* (1995) e parece estar relacionado aos níveis circulantes e à atividade da estromelisina-1. Estudos *in vitro* têm demonstrado que o alelo composto por 5 adeninas (5A) apresenta uma atividade promotora mais eficiente do que o alelo com 6 adeninas (6A) (YE *et al.* 1996). Aparentemente, portadores do alelo 6A apresentariam níveis reduzidos de estromelisina-1 na parede arterial, o que poderia favorecer a deposição de matriz extracelular em lesões ateroscleróticas, resultando em uma progressão mais rápida da doença, como confirmado por YE *et al.* (1995), HUMPHRIES *et al.* (1998) e de MAAT *et al.* (1999).

O polimorfismo 5A/6A da estromelisina-1 foi associado à angina instável por KIM *et al.* (2002) e à ocorrência de aneurisma coronariano por YOON *et al.* (1999) e LAMBLIM *et al.* (2002). Esse polimorfismo também foi associado a uma resposta diferencial à administração de estatinas (de MAAT *et al*., 1999). Assim, o polimorfismo 5A/6A seria um indicador, não somente da progressão da doença coronariana, mas também do risco de reestenose e resposta à medicação. Como a estromelisina-1 pode agir no enfraquecimento da placa aterosclerótica, favorecendo sua ruptura e posterior trombose, essa enzima também pode ser associada ao risco de IAM, como demonstrou TERASHIMA *et al.* (1999), analisando pacientes com história de infarto e YAMADA *et al.* (2002), em mulheres japonesas.

Provavelmente, a homozigosidade para o alelo 5A estaria associada a níveis elevados de estromelisina-1 na parede vascular, o que poderia predispor à instabilidade da placa aterosclerótica e assim associar-se ao infarto.

4. O Óxido Nítrico

O óxido nítrico (ON) é gerado a partir da L-arginina pela enzima óxido nítrico sintetase (NOS), seus efeitos resultam de reações de nitrosilação ou de espécies reativas de oxigênio, por oxidação (MacALLISTER, 1998). Até o momento foram descritas três isoformas de NOS: NOS induzível (relacionada a processos inflamatórios), NOS neuronal constitutiva e NOS endotelial (eNOS). A expressão das NOS e seus cofatores deve ser apropriadamente regulada a fim de garantir uma produção eficiente de ON. A eNOS é encontrada no endotélio e plaquetas, o gene localiza-se no cromossomo 7, apresenta 21 kb e 26 éxons (MARSDEN *et al.*, 1993).

O ON aumenta a permeabilidade do endotélio a lipoproteínas e outros constituintes do plasma, atua no controle do tônus vascular, inibe a adesão e agregação plaquetárias, a adesão de leucócitos ao endotélio e a proliferação de células musculares lisas, sendo por isso, relacionado ao processo aterosclerótico e trombótico (ROSS, 1999; CHIANG *et al.*, 2000; 2001).

Desde que foi sugerido como um mediador endógeno de diversas respostas celulares, o ON tem sido associado aos processos patogênicos envolvidos em doenças do sistema cardiovascular, sendo um candidato a fator de risco coronariano.

A atividade do ON pode ser afetada por alguns fatores exógenos como lipopolissacarídeos de membrana, fosfolipídeos e compostos derivados do cigarro. Reduções na liberação de ON podem predispor à hipertensão, trombose, vasoespasmo e aterosclerose, e a restauração da atividade do ON parece contribuir para o processo de regressão de lesões ateroscleróticas preexistentes (WANG & WANG, 2000).

Devido a seu efeito sobre o endotélio, diversos estudos têm avaliado o papel de variantes genéticas que poderiam causar deficiência na função da eNOS. Alterações na produção de ON poderiam contribuir para a patogênese dos processos ateroscleróticos e trombóticos.

Uma variante genética localizada na região promotora do gene da eNOS, denominada T-786C, parece afetar a atividade promotora do gene. NAKAIAMA *et al.* (1999) e YOSHIMURA *et al.* (2000) observaram que essa mutação foi um fator de risco para espasmo

coronariano. NAKAYAMA *et al.* (2000) verificaram que a variante T–786C foi associada à incidência de infarto em japoneses, especialmente em pacientes sem estenose arterial. Entretanto, quando investigada em relação ao risco de trombose venosa, a variante T–786C não foi preditiva (GONZÁLEZ-ORDÓÑES *et al.*, 2000).

Uma outra mutação, denominada Glu298Asp localizada no éxon 7 do gene da eNOS, foi descrita recentemente (YOSHIMURA *et al.*, 1998) e associada independentemente à ocorrência de IAM por SHIMASAKI *et al.* (1998). Aparentemente, esse polimorfismo resulta em alterações na produção de ON. O polimorfismo Glu298Asp também foi considerado como um fator de risco independente para doença coronariana e IAM por HINGORANI *et al.* (1999). MIYAMAMOTO *et al.* (1998) e SHOJI *et al.* (2000) observaram associação entre a variante Glu298Asp e hipertensão em japoneses. Esse polimorfismo também foi associado à incidência de reestenose intra-*stent* por SUZUKI *et al.* (2002).

5. O Sistema de Coagulação

O processo de coagulação sangüínea é essencial para evitar a perda excessiva de sangue e manter o fluxo sangüíneo nos vasos. Uma seqüência de reações enzimáticas, aceleradas por cofatores, ativam zimogênios ou precursores inativos e culminam na formação do coágulo de fibrina. A cascata de coagulação pode seguir duas rotas: a *intrínseca* ou de contato, que inicia com a ativação do fator XII, e a *extrínseca*, dependente de fator tissular, liberado em casos de inflamação ou lesão endotelial (FIGURA 2). Além do envolvimento na formação do trombo, os fatores de coagulação também participam do processo aterosclerótico, pois resíduos de fibrina são encontrados no interior de lesões ateroscleróticas.

O aumento nos níveis circulantes de determinados fatores da coagulação tem sido associado a um estado hipercoagulável e portanto relacionado ao risco cardiovascular.

5.1 O Fator VII

O fator VII (fVII) é uma glicoproteína de cadeia simples com um peso molecular de 45 kDa. O gene que codifica o fVII tem cerca de 13 kb, apresenta 9 éxons e está localizado no cromossomo 13. O fVII é sintetizado principalmente no fígado, e sua concentração plasmática é de aproximadamente 0,5 mg/ml.

O fVII é o primeiro fator plasmático da via extrínseca da coagulação, sendo ativado

na presença do fator tissular, o qual é liberado no caso de lesão endotelial (TUDDENHAM & COOPER, 1994). Níveis elevados de fVII foram considerados como um fator de risco cardiovascular, pela primeira vez, no *Nortwick Park Heart Study* (MEADE *et al.*, 1986). Desde então, diversos estudos investigam a possível associação entre níveis elevados de fVII e o conseqüente risco cardiovascular (MEADE *et al.*, 1993; JUNKER *et al.*, 1997).

Um polimorfismo de DNA denominado R353Q, localizado no éxon 8 do gene do fVII resulta de uma troca de base (G/A) no códon 353, substituindo uma arginina (R) por uma glutamina (Q) na proteína. Diversos estudos têm associado o polimorfismo R353Q com os níveis de fVII, e o mecanismo sugerido é que a substituição do aminoácido resultaria em uma alteração conformacional na proteína, o que causaria uma redução na concentração da proteína ativa (ARBINI & BAUER, 1994). De acordo com HEYWOOD *et al.* (1996a; b) e HUNAULT *et al.* (1997), os níveis de fVII parecem ser aproximadamente 25% menores nos portadores do alelo 353Q. HEYWOOD *et al.* (1996b) e WANG *et al.* (1997) estudaram o polimorfismo R353Q em pacientes com isquemia coronariana, e verificaram uma associação significativa com os níveis de fVII, porém sem associação com a severidade da doença. Uma associação positiva com os níveis de fVII também foi detectada por LANE *et al.* (1996), entretanto sem relação com a história de infarto. BERNARDI *et al.* (1997) estudaram o efeito do polimorfismo R353Q e sua influência nos níveis do fVII em europeus. Os autores observaram que os genótipos associados a níveis mais reduzidos foram mais raros no norte da Europa, onde o risco cardiovascular é maior. Um polimorfismo do tipo inserção/deleção (I/D), causado pela inserção de um decanucleotídeo na posição 323 da região promotora do gene do fVII (na seqüência CCTATATCCT) parece estar em desequilíbrio de ligação com o polimorfismo R353Q. Aparentemente o polimorfismo I/D altera a taxa de transcrição do gene e assim regularia os níveis plasmáticos de fVII (HUMPHRIES *et al.*, 1996, POLLACK *et al.*, 1996).

Diversos trabalhos têm relacionado o genótipo do polimorfismo I/D aos níveis plasmáticos de fVII, nos quais indivíduos portadores do alelo de inserção apresentariam níveis de fVII reduzidos (HEYWOOD *et al.*, 1996a; b; HUMPHRIES *et al.*, 1996; HEYWOOD *et al.*, 1997; di CASTELNUOVO *et al.*, 1998). Apesar da associação com os níveis de fVII, a relação da variante I/D com a doença cardiovascular ainda não foi estabelecida. HEYWOOD *et al.* (1996b; 1997) não observaram associação entre o polimorfismos I/D e a doença isquêmica coronariana, assim como LIEVERS *et al.* (2000) em relação à progressão da doença aterosclerótica coronariana. Di CASTELNUOVO *et al.* (2000) sugeriram que o polimorfismo I/D poderia influenciar o risco de IAM. Esse polimorfismo também não foi associado à doença coronariana em pacientes submetidos à angiografia em um estudo

realizado no Rio Grande do Sul, embora não tenham sido analisados os níveis de fVII (PALUDO, 1999).

5.2 O Fibrinogênio

O fibrinogênio é a proteína que se apresenta mais concentrada no plasma sangüíneo, sendo sintetizada pelas células parenquimais do fígado. Aproximadamente 80% do fibrinogênio circulante está contido no plasma, o restante pode ser encontrado nas plaquetas, líquido intersticial e linfa. A concentração de fibrinogênio em condições normais varia de 200 a 400 mg/dl (MARDER *et al.*, 1982; ERNST, 1993; ERNST & RESCH, 1993).

A molécula do fibrinogênio é constituída por três pares de cadeias polipeptídicas, denominadas Aα, Bβ e γ, e o heterohexâmero formado apresenta um peso molecular de 340 kDa. As cadeias polipeptídicas que compõem a molécula do fibrinogênio são codificadas por genes diferentes, situados num "cluster" de aproximadamente 50 kb localizado no cromossomo 4 (FIGURA 3) (MARDER *et al.*, 1982; TUDDENHAM & COOPER, 1994).

A conversão do fibrinogênio em fibrina é a última etapa da cascata de coagulação sangüínea e compreende três processos: a liberação dos fibrinopeptídeos A e B das cadeias Aα e Bβ respectivamente, a união não-covalente de monômeros de fibrina formando protofibrilas; e, por fim, a estabilização do coágulo pelo fator XIII (MARDER *et al.*, 1982; TUDDENHAM & COOPER, 1994).

Além de ser um fator de coagulação, o fibrinogênio é uma proteína de fase-aguda, e sua concentração pode aumentar em resposta a infecções, neoplasias, hormônios e ação da trombina (MARDER *et al.*, 1982; GREEN & HUMPHRIES, 1989). A literatura tem referido uma série de fatores que afetariam os níveis de fibrinogênio, entre os quais: raça, sexo, idade, obesidade, dislipidemia, hipertensão, diabetes, tabagismo e estresse (MEADE *et al.*, 1986; FOLSOM *et al.*, 1991; YARNELL *et al.*, 1991; ERNST, 1993; ERNST & RESCH, 1993; HEINRICH *et al.*, 1995; MANNUCCI, 1995; JUHAN-VAGUE, 1996; de MAAT *et al.*, 1996b; POTRON *et al.*, 1996; ROSENGREN & WILHELMSEN, 1996; THOMAS *et al.*, 1996). MEADE *et al.* (1986), HUMPHRIES *et al.* (1987) e HUMPHRIES (1995) consideram que o risco coronariano atribuído ao hábito tabagista seria decorrente de seu efeito nos níveis de fibrinogênio. Desde 1980, diversos estudos epidemiológicos têm demonstrado que níveis elevados de fibrinogênio estão associados ao risco de doença coronariana (MEADE *et al.*, 1980; 1986; KANNEL *et al.*, 1987; YARNELL *et al.*, 1991; EICHNER *et al.*, 1996; ROSENGREN & WHILHELMSEN, 1996; FOLSOM *et al.*, 1997; WOODWARD *et al.*, 1998), doença cerebrovascular (WHILHELMSEN *et al.*, 1984; CARTER *et al.*, 1997), infarto agudo do

miocárdio (WHILHELMSEN *et al.*, 1984; CORTELLARO *et al.*, 1992; SCARABIN *et al.*, 1993; THOMPSON *et al.*, 1995; ZITO *et al.*, 1997) e mortalidade de causa cardiovascular (THOMPSON *et al.*, 1995; BENDERLY *et al. al.*, 1996; ROSENGREN & WILHELMSEN, 1996; WOODWARD *et al.*, 1998).

Altas concentrações plasmáticas de fibrinogênio ocasionam mudanças reológicas no sangue, aumento da viscosidade e indução da agregação plaquetária, além da formação da rede de fibrina (MANNUCCI, 1995; BEHAGUE *et al.*, 1996; JUHAN-VAGUE, 1996). Apesar das associações observadas, o papel do fibrinogênio ainda não foi totalmente estabelecido em relação à patogênese das doenças cardiovasculares. Níveis elevados de fibrinogênio poderiam ser decorrentes da doença aterosclerótica preexistente (VASSE *et al.*, 1996; de MAAT *et al.*, 1996) ou ser diretamente responsáveis pelo risco de eventos cardiovasculares (MEADE *et al.*, 1986; HUMPHRIES *et al.*, 1987).

Existem diversos polimorfismos de DNA localizados no *cluster* dos genes do fibrinogênio, tanto na região codificadora dos genes α e β como nas regiões flanqueadoras e intrônicas dos genes. Somente um estudo investigou o efeito do polimorfismo *Kpn*I/*Sac*I presente no gene γ (CONNOR *et al.*, 1992). Os polimorfismos mais investigados são os do gene β, pois a síntese da cadeia Bβ parece ser limitante para a formação da proteína. O controle da expressão do gene β conta com a ação de diversos fatores de transcrição, e as seqüências às quais se ligam ficam próximas da região promotora (HUMPHRIES, 1995). De

acordo com HEINRICH *et al.* (1995), os alelos menos freqüentes dos polimorfismos estariam relacionados a níveis mais elevados de fibrinogênio plasmático.

O polimorfismo G–455A está localizado na região promotora do gene β-fibrinogênio, sendo o polimorfismo mais estudado em relação aos níveis plasmáticos de fibrinogênio. O alelo -455A foi associado a níveis mais elevados de fibrinogênio por THOMAS *et al.* (1991), SCARABIN *et al.* (1993), HEINRICH *et al.* (1995), HUMPHRIES *et al.* (1995), BEHAGUE *et al.* (1996), GARDEMANN *et al.* (1997), TYBJÆRG-HANSEN *et al.* (1997), de MAAT *et al.* (1998), MARGAGLIONE *et al.* (1998b) e BRULL *et al*. (2002). Entretanto, CONNOR *et al.* (1992), SOSEF *et al.* (1994) e CARTER *et al.* (1996) não detectaram associações significativas entre os genótipos do polimorfismo G–455A e os níveis plasmáticos de fibrinogênio. Apesar da aparente influência nos níveis de fibrinogênio, quando o polimorfismo G–455A foi investigado quanto à sua possível associação com a doença cardiovascular, diversos autores não verificaram efeito dessa variante no risco de infarto ou isquemia coronariana (SCARABIN *et al.*, 1993; HUMPHRIES *et al.*, 1995; BEHAGUE *et al.*, 1996; GARDEMANN *et al.*, 1997; TYBJÆRG-HANSEN *et al.*, 1997; LEANDER *et al.*, 2000).

O polimorfismo *Taq*I está localizado na região 3' do gene α-fibrinogênio, e também parece estar associado aos níveis de fibrinogênio (HEINRICH *et al.*, 1995). Contrariamente, os trabalhos de HUMPHRIES *et al.* (1987), THOMAS *et al.* (1995), VAISÄNEN *et al.* (1997) e de MAAT *et al.* (1998) não demonstraram associação entre esse polimorfismo e os níveis de fibrinogênio. Os polimorfismos *G–455A* e *Taq*I não foram associados aos níveis plasmáticos de fibrinogênio, nem à obstrução coronariana no Rio Grande do Sul (PALUDO, 1999).

5.3 O Fator V

O fator V (fV) é uma glicoproteína de 330 kDa, sintetizada nos hepatócitos e megacariócitos, podendo ser encontrado no plasma e grânulos plaquetários. O gene do fV localiza-se no cromossomo 1, apresenta 80 kb de comprimento e está organizado em 25 éxons. O fV é ativado pela trombina, e atua como cofator na ativação da protrombina pelo fator X (TUDDENHAM & COOPER, 1994).

A proteína C ativada (APC) é uma glicoproteína que inibe fisiologicamente o fV através da clivagem específica dos resíduos Arg 306, Arg 506 e Arg 679, além disso, a APC age sobre o sistema fibrinolítico, inativando os inibidores desse sistema. Uma mutação no gene do fV (conhecida como fator V Leiden), uma troca de base G/A no nucleotídeo 1691 (G1691A), causa a substituição de uma arginina por uma glutamina na posição 506 da proteína. Essa mutação faz com que o fV seja resistente à ação da proteína C ativada, não

sendo apropriadamente inativado (BERTINA *et al.*, 1994). Até 1993, somente 5 a 20% dos pacientes com trombose idiopática apresentavam trombofilias hereditárias. A partir dessa data, com a descoberta do controle genético da resistência do fator V à APC, essa situação mudou consideravelmente.

O fator V Leiden é a causa genética mais comum para trombose venosa entre caucasóides (BURICK *et al.*, 1997). A prevalência da mutação varia de 2 a 15% na população em geral, diferindo de acordo com o componente étnico da população (JEFFERY *et al.*, 1996; LEE *et al.*, 1996; GREGG *et al.,* 1997; TAMIM *et al.*, 2002). ARRUDA *et al.* (1995) verificaram que a mutação fV Leiden em brasileiros caucasóides apresentou uma distribuição similar a de outros trabalhos, verificada em cerca de 20% de pacientes com trombose e em cerca de 3-5% de indivíduos da população em geral.

Diversos trabalhos demonstraram uma associação significativa entre a mutação fV Leiden e a incidência de trombose venosa e tromboembolismo (BEAUCHAMP *et al.*, 1994; SVENSSON *et al.*, 1997; SIMIONI *et al.*, 1997; BONDUEL *et al.*, 2002), inclusive durante a gestação (HELLGREN *et al.*, 1995; HALLAK *et al.*, 1997; MURPHY *et al.*, 2000). MANDEL *et al.* (1996) observaram que pacientes com homocistinúria (freqüentemente associada à trombose em idade precoce) e portadores da mutação fV Leiden apresentavam um risco elevado de trombose, e ALHENC-GELAS *et al.* (1999) associaram a mutação fV Leiden com eventos trombóticos recurrentes. WÅHLANDER *et al.* (2002) não observaram uma associação significativa entre a incidência de tromboembolismo venoso no período pós-operatório em relação à mutação fV Leiden. OGER *et al.* (2002) relataram que a resistência à proteína C ativada, relacionada ao fV Leiden, não foi um fator de risco importante para tromboembolismo em pacientes com mais de 70 anos. YANQING *et al.* (2003) também não encontraram associação de polimorfismos genéticos no fator V (incluindo o fV Leiden), associados à resistência à proteína C ativada, com trombose em chineses. Enquanto INIESTA *et al.* (1997) não observaram diferenças quanto à prevalência do fV Leiden em pacientes com doença cerebrovascular em comparação a indivíduos controle, DESCHIENS *et al.* (1996), MARTINELLI *et al.* (1996) e MARGAGLIONE *et al.* (1999) verificaram uma associação positiva entre o fV Leiden e acidente vascular cerebral em idade precoce e AKAR *et al.* (1999b) associaram o fV Leiden com infarto cerebral em crianças na Turquia.

A associação entre o fV Leiden e trombose venosa é clara, entretanto o papel dessa mutação na trombose arterial permanece controverso. RIDKER *et al.* (1995) e JUNKER *et al.* (1998) não observaram associação entre o fV Leiden e a incidência de IAM precoce em homens. REDONDO *et al.* (1999) não observaram efeito no risco de IAM associado à mutação, entretanto os níveis de fV foram um fator de risco independente para infarto

nesses pacientes. Uma alta prevalência do fV Leiden em homens com IAM precoce foi observada por HOLM *et al.* (1996). ROSENDAAL *et al.* (1997a) demonstraram que essa mutação aumentou em cerca de três vezes o risco de IAM em mulheres jovens, principalmente quando outros fatores metabólicos de risco também estavam presentes.

WANG *et al.* (1997) não observaram associação significativa entre o fV Leiden e a severidade da doença coronariana em australianos. HOLM *et al.* (1999) avaliaram o efeito do fV Leiden no prognóstico de pacientes admitidos em hospitais por infarto ou episódio de angina instável. Os autores observaram que pacientes tabagistas portadores da mutação apresentaram uma maior incidência de re-IAM ou morte pós-IAM em 30 dias ou 2 anos após a entrada no estudo.

DUNN *et al*. (1998) não associaram o fV Leiden ao risco de doença aterosclerótica coronariana (DAC) em pacientes submetidos à angiografia e PETROVICK *et al*. (2001) não observaram associação significativa quanto ao risco de DAC antes dos 55 anos e a mutação fV Leiden, entretanto quando o fV Leiden foi analisado juntamente com o polimorfismo R353Q do fator VII, o efeito foi significativo.

MANSOURATTI *et al*. (2000) avaliaram pacientes com infarto prematuro com e sem evidência de doença aterosclerótica em comparação a um grupo controle composto por indivíduos saudáveis, e observaram que o fV Leiden foi mais prevalente nos indivíduos infartados e sem doença aterosclerótica. JUUL *et al*. (2002) avaliaram a associação entre o fV Leiden e o risco de IAM, doença isquêmica e acidente vascular cerebral em dinamarqueses participantes de estudos prospectivos, perfazendo um total de 10.000 indivíduos. Os autores não observaram associação significativa, portanto a mutação não poderia ser considerada um fator de risco nessa população.

5.4 A Protrombina

A protrombina (fator II) é uma glicoproteína de 72 kDa sintetizada pelos hepatócitos, que circula no sangue em sua forma inativa. O gene da protrombina localiza-se no cromossomo 11, apresenta 21 kb e está organizado em 14 éxons. A protrombina é o precursor da trombina, a enzima responsável pela etapa final da cascata da coagulação. A protrombina é ativada pelo fator X, na presença do fator V. A ativação da protrombina se dá através da clivagem dos resíduos Arg 271 e Arg 320 (TUDDENHAM & COOPER, 1994).

A trombina é uma enzima extremamente importante nos processos de hemostasia e trombose, exibindo atividades procoagulantes, anticoagulantes e antifibrinolíticas. A trombina

também ativa diversas respostas celulares, que são essenciais no processo atero-trombótico (MARAGANORE, 1993).

O polimorfismo G20210A localizado na região 3' não-traduzida do gene da protrombina, foi descrito e associado à trombose venosa (POORT *et al.*, 1996). Indivíduos portadores do alelo 20210A apresentariam níveis de protrombina cerca de 20% mais elevados. Diversos estudos têm confirmado a associação do alelo 20210A com trombose venosa (ARRUDA *et al.*, 1997; BROWN *et al.*, 1997; CORRAL *et al.*, 1997; CUMMING *et al.*, 1997; FERRARESI *et al.*, 1997; HILLARP *et al.*, 1997; HOWARD *et al.*, 1997; KAPUR *et al.*, 1997; BLOEM *et al.*, 1998; BUCCIARELLI *et al.*, 1998; SIMIONI *et al.*, 1998), entretanto em relação à trombose arterial os resultados permanecem controversos.

ROSENDAAL *et al.* (1997b) e FRANCO *et al.* (1998) associaram o alelo 20210A à ocorrência de IAM precoce, e de STEFANO *et al.* (1998) à incidência de AVC. De acordo com os autores essas associações estariam relacionadas ao efeito nos níveis circulantes de protrombina. No Brasil, FRANCO *et al.* (1999) estudando pacientes com doença aterosclerótica precoce, também encontraram associação entre a variante G20210A e níveis elevados de protrombina, assim como com a incidência de infarto.

RIDKER *et al.* (1999) não observaram associação entre o alelo 20210A em relação à trombose arterial, infarto ou acidente vascular cerebral, entretanto observaram uma associação positiva em relação aos casos de trombose venosa. CROFT *et al.* (1999) observaram que o polimorfismo G20210A não conferiu um risco aumentado de IM, em pacientes com menos de 75 anos. A mutação G20210A também não foi associada com a ocorrência de DAC em pacientes submetidos à angiografia (PROHASKA *et al.*, 1999). DURANTE-MANGONI *et al.* (2002) também não observaram associação entre essa variante e doença isquêmica (infarto ou angina).

No entanto, van der WATER *et al.* (2000) observaram que a mutação da protrombina, assim como o fV Leiden, foi associada ao risco de IAM em pacientes sem estenoses significantes, ou seja, sem DAC. von AHSEN *et al.* (2000) observaram que a atividade da protrombina em indivíduos saudáveis com o alelo 20210A foi significativamente maior quando comparados aos não-portadores. Entretanto, SMILES *et al.* (2002) em um estudo caso-controle em pacientes com síndromes isquêmicas agudas não observaram relação entre o genótipo do polimorfismo G20210A e os níveis de protrombina e AKHAVAN *et al.* (2002) não observaram associação entre mutações na região promotora do gene da protrombina e os respectivos níveis plasmáticos da proteína. MIKKELSSON & KARHUNEN (2002) avaliaram as mutações da protrombina, fV Leiden e R353Q do fator VII em indivíduos

cuja causa do óbito foi IAM, e não observaram associação com nenhuma dessas variantes.

A freqüência da mutação da protrombina em caucasóides é de aproximadamente 2%, elevações na prevalência dessa variante são relatadas no sul da Europa em comparação ao norte europeu (ROSENDAAL *et al.*, 1998). Aparentemente, a variante G20210A é muito rara em populações não-caucasóides (DILLEY *et al.*, 1998; ROSENDAAL *et al.*, 1998; MATHONNET *et al.*, 2002), não podendo portanto ser considerada com um fator de risco cardiovascular preditivo nessas populações. FRANCO *et al.*, (1998) verificaram que a freqüência do alelo 20210A é extremamente baixa em populações brasileiras não-caucasóides.

6. O Sistema Fibrinolítico

Enquanto o sistema de coagulação é responsável pela formação da rede de fibrina, o sistema fibrinolítico restaura o fluxo sangüíneo através da degradação proteolítica da matriz de fibrina (FIGURA 2). A ativação restrita ao local do trombo e a participação de inibidores específicos limitam a fibrinólise e impedem a proteólise das proteínas circulantes.

A formação do coágulo de fibrina desencadeia a ativação do sistema fibrinolítico e a geração da plasmina ativa, que irá degradar a fibrina em fragmentos solúveis, desintegrando assim o coágulo sangüíneo. O sistema fibrinolítico é composto pela proenzima plasminogênio, seus ativadores e inibidores, cujas interações específicas determinarão a taxa de dissolução do coágulo de fibrina. A fibrina não deve ser considerada simplesmente como um substrato à ação da plasmina, pois participa dos processos de ativação e inibição do sistema fibrinolítico através de domínios específicos na molécula, impedindo a degradação do fibrinogênio e outras proteínas da coagulação na circulação sangüínea (revisto em MEDVED & NIEVWENHUIZEN, 2003).

Distúrbios na atividade fibrinolítica poderiam aumentar o risco de trombose vascular, uma vez que uma atividade reduzida poderia resultar na manutenção da rede de fibrina. Um estado hipofibrinolítico poderia ser decorrente de uma síntese e/ou liberação deficiente de ativadores, de uma deficiência na molécula do plasminogênio ou de níveis elevados de inibidores (THOMAS & ROBERTS, 1997).

Como na trombose, o evento crítico é o bloqueio do fluxo sangüíneo pelo coágulo de fibrina, alterações na atividade fibrinolítica poderiam afetar a taxa de dissolução do coágulo formado.

6.1 O Ativador Tipo Tissular do Plasminogênio

O ativador tipo tissular do plasminogênio (t-PA) é uma serino-protease com 68 kDa, secretada por células endoteliais. A concentração plasmática de t-PA é de aproximadamente 0,005 mg/l. O gene do t-PA está localizado no cromossomo 8, apresenta 33 kb e está organizado em 14 éxons (TUDDENHAM & COOPER, 1994; COLLEN, 1999).

O t-PA é a principal enzima fibrinolítica, sua função é transformar o plasminogênio em plasmina, que degradará o coágulo de fibrina. Como o t-PA é sintetizado por células endoteliais, sua concentração aumenta na presença de lesões e a expressão desse fator parece ser regulada pela ação de interleucinas (JENKIS *et al.*, 1997). Níveis elevados de t-PA foram associados ao risco de IAM nos trabalhos de RIDKER *et al.* (1993) e THOMPSON *et al.* (1995), e ao risco de acidente vascular cerebral por LINDGREN *et al.* (1996). Níveis elevados de t-PA poderiam ser indicativos de uma capacidade fibrinolítica aumentada, entretanto, no plasma, a concentração de t-PA é menor do que a de seu principal inibidor (inibidor tipo 1 do ativador do plasminogênio, PAI-1), portanto níveis elevados de t-PA estariam complexados ao PAI-1, reduzindo a atividade fibrinolítica. GRAM *et al.* (1995) estudaram pacientes com história prévia de trombose venosa profunda e verificaram que os níveis de t-PA foram mais elevados e que a atividade fibrinolítica global foi significativamente menor nestes pacientes.

Um polimorfismo de DNA do tipo inserção/deleção (I/D) de uma seqüência *Alu*, localizado no intron h do gene do t-PA, tem sido associado ao risco de IAM. van der BOM *et al.* (1997) estudando pacientes infartados, observaram uma associação significativa entre o alelo de inserção e a ocorrência de IAM, entretanto essa associação não pareceu ser dependente das concentrações plasmáticas de t-PA.

RIDKER *et al.* (1997) em um estudo prospectivo, não observaram associação entre o genótipo do polimorfismo *Alu* I/D e o risco de IAM subseqüente. Também STEEDS *et al.* (1998) não encontraram associação significativa entre esse polimorfismo e infarto, em um estudo tipo caso-controle.

6.2 O Inibidor Tipo 1 do Ativador do Plasminogênio

O inibidor tipo 1 do ativador do plasminogênio (PAI-1) é uma glicoproteína de 50 kDa, sintetizada no fígado e células endoteliais, que circula no sangue na sua forma inativa. O gene que codifica o PAI-1 localiza-se no cromossomo 7 e apresenta 9 éxons. O PAI-1 é uma proteína de fase aguda e o principal inibidor do t-PA e u-PA (ativador do plasminogênio tipo uroquinase) do sistema fibrinolítico. Os níveis plasmáticos de PAI-1 são de

aproximadamente 0,05 mg/l (TUDDENHAM & COOPER, 1994; COLLEN, 1999).

Os níveis de PAI-1 são regulados por citocinas, lipopolissacarídeos, hormônios, VLDL-colesterol e insulina (GREEN & HUMPHRIES, 1994). Idade, tabagismo, ingestão de álcool e dieta também podem aumentar os níves de PAI-1 (de MAAT *et al.*, 1996a). Como o PAI-1 é o principal inibidor do sistema fibrinolítico, altos níveis plasmáticos poderiam ter um efeito protrombótico. HAMSTEN *et al.* (1985; 1987) observaram uma associação positiva entre altos níveis de PAI-1 e o risco de infarto, inclusive recurrente. No entanto, FOLSOM *et al.* (1998) não verificaram diferenças significativas nas concentrações de PAI-1 em um estudo de famílias com alta incidência de doença aterosclerótica coronariana, quando comparadas a famílias em geral.

Já foram descritos diversos polimorfismos de DNA no gene do PAI-1, que parecem estar associados às concentrações plasmáticas desse inibidor, o polimorfismo de inserção/deleção (4G/5G) localizado na posição –675 da região promotora do gene, é o mais investigado. O alelo de deleção (4G) estaria associado a níveis mais elevados de PAI-1 (MARGAGLIONE *et al.*, 1997; 1998a).

MANSFIELD *et al.* (1995) estudando indivíduos diabéticos observaram uma freqüência significativamente maior de homozigotos 4G/4G em pacientes que desenvolveram doença arterial coronariana. ERIKSSON *et al.* (1995) demonstraram uma associação significativa entre o alelo 4G do polimorfismo do PAI-1 e IAM em idade precoce. De acordo com os autores, os alelos 4G e 5G ligam-se a ativadores de transcrição, entretanto o alelo 5G também pode ligar-se a uma proteína repressora. Assim, na ausência do repressor, o nível de transcrição é aumentado. No trabalho de OSSEI-GERNING *et al.* (1997), o genótipo do polimorfismo 4G/5G foi considerado um fator de risco independente para infarto, particularmente naqueles indivíduos com doença aterosclerótica preexistente. Indivíduos homozigotos 4G/4G apresentaram os níveis mais elevados de PAI-1 e uma maior incidência de infarto. IWAI *et al.* (1998) num estudo em japoneses, relataram uma associação positiva entre o alelo 4G e os níveis de PAI-1, e também quanto ao tempo de progressão de síndromes coronarianas agudas. Contrariamente, RIDKER *et al.* (1997) em um grande estudo prospectivo, verificaram que o polimorfismo 4G/5G não foi um fator de risco para trombose venosa ou arterial. Da mesma forma, JUNKER *et al.* (1998), DOGGEN *et al.* (1999) e GARDEMANN *et al.* (1999) não encontraram associação entre o polimorfismo 4G/5G e a incidência de infarto. CATTO *et al.* (1997) também não verificaram diferenças significativas quanto ao genótipo do polimorfismo 4G/5G e o risco de AVC, entretanto os níveis de PAI-1 foram associados à mortalidade decorrente de AVC.

MANNUCCI *et al.* (1997), verificaram que italianos com mais de cem anos de idade (e sem sintomas cardiovasculares) apresentaram uma freqüência significativamente mais elevada do alelo 4G, em comparação a um grupo controle mais jovem. Assim, um marcador de fibrinólise reduzida parece ser compatível com a idade avançada.

Justificativa e Objetivos

Devido às altas taxas de morbidade e mortalidade decorrentes do IAM, as estratégias de prevenção primária e secundária incluem o reconhecimento e controle dos fatores de risco associados. Os fatores de risco tradicionais (como hipertensão, tabagismo e sedentarismo) justificam somente 30% da incidência dos IAMs (HELLER *et al.*, 1984). A história familiar é considerada um fator de risco independente, e a identificação do componente genético associado ao risco de IAM tem sido cada vez mais investigada. A análise de características genéticas e sua inter-relação com fatores ambientais pode identificar precocemente indivíduos com alto risco de desenvolver um IAM, principalmente em idade precoce, além de poder determinar o grau de resposta à terapêutica proposta.

Algumas anormalidades pró-trombóticas herdáveis como o fator V Leiden e a mutação G20210A da protrombina já estão estabelecidas em relação ao risco de trombose venosa, porém seus efeitos na trombose arterial permanecem à espera de confirmação. Polimorfismos genéticos presentes nos genes de outros fatores de coagulação têm sido constantemente investigados em relação ao risco de IAM e à sua interação com os fatores de risco metabólicos, e os resultados têm sido controversos.

O endotélio, por estar localizado entre o sangue circulante e as células musculares lisas, responde a sinais mecânicos como mudanças no fluxo sangüíneo e à ação de diversas substâncias como neurotransmissores, hormônios, plaquetas e fatores de coagulação. Dessa forma o endotélio regula a estrutura e a função vascular. Distúrbios que caracterizem a chamada disfunção endotelial são potencialmente aterogênicos e/ou trombogênicos.

A proposta do presente trabalho foi avaliar a associação entre diversos polimorfismos genéticos em genes candidatos a conferir suscetibilidade aumentada ao IAM antes dos 60 anos de idade. A escolha das variantes foi baseada no significado biológico/fisiológico dos produtos gênicos e sua provável influência nos processos patogênicos do IAM. Os objetivos específicos desse trabalho foram:

1. Investigar a associação entre os polimorfismos genéticos T-786C e Glu298Asp da óxido nítrico sintetase, T102C do receptor da serotonina, 5A/6A da estromelisina e A-1185G do fator von Willebrand, todos associados à disfunção endotelial, ao risco de infarto agudo do miocárdio.

2. Avaliar o efeito do polimorfismo A-1185G na região promotora do gene do fator von

Willebrand sobre os níveis plasmáticos desse fator.

3. Avaliar o efeito de polimorfismos genéticos presentes em fatores da coagulação: G-455A no gene β-fibrinogênio e *Taq*I no gene α-fibrinogênio, R353Q e inserção/deleção no gene do fator VII e as mutações fator V Leiden e G20210A da protrombina em relação ao risco de infarto agudo do miocárdio.

4. Avaliar o efeito dos polimorfismos G-455A no gene β-fibrinogênio e *Taq*I no gene α-fibrinogênio sobre os níveis plasmáticos de fibrinogênio.

5. Investigar se os polimorfismos inserção/deleção no gene do ativador tipo tissular do plasminogênio (t-PA) e 4G/5G no gene do inibidor tipo 1 do ativador do plasminogênio (PAI-1), que são fatores fibrinolíticos, estão associados ao risco de desenvolver um infarto agudo do miocárdio.

capítulo 2

Polymorphisms of Nitric Oxide Synthase, Serotonin (5-HT), Stromelysin-1 and von Willebrand Factor in Endothelial Dysfunction: Risk factors for Acute Myocardial Infarction

Capítulo 2

Polymorphisms of Nitric Oxide Synthase, Serotonin (5-HT), Stromelysin-1 and von Willebrand Factor in Endothelial Dysfunction: Risk factors for Acute Myocardial Infarction

Crislaine A. Paludo[1], Josi Vidart[2], Vera L. Portal[2] and Israel Roisenberg[1]

[1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, [2]Serviço de Epidemiologia, Instituto de Cardiologia do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Corresponding author:

Israel Roisenberg
Departamento de Genética
Instituto de Biociências
Universidade Federal do Rio Grande do Sul
Caixa Postal 15053
91.501-970 Porto Alegre, RS
Brazil
FAX n° + 55 51 3316 7311
Phone n° + 55 51 3316 6728
e-mail: israberg@ufrgs.br

Abstract

Acute myocardial infarction (AMI) is caused by thrombus formation at site of atheromatous plaque and the endothelial dysfunction may play a role in this process. Endothelial-derived nitric oxide (NO) plays an important role in hemostasis. The T-786C and Glu298Asp variants of endothelial nitric oxide synthase (eNOS) gene have been associated with impaired NO production. Serotonin released from activated platelets induces platelet aggregation through receptor activation at sites of coronary atherosclerosis. A variant T102C in the 5-HT_2A serotonin receptor might be important in the receptor function. Stromelysin-1 (a matrix metalloproteinase) can play a role in the rupture of atherosclerotic plaque, because it can cleave extracellular matrix components. The variant 5A/6A might modulate the activity of this enzyme. von Willebrand factor (vWf) is related to platelet thrombus formation, and levels of this factor have been suggested as a marker for AMI risk. The variant A-1185G has been associated with the vWf levels. We conducted a case-control study of 283 AMI cases and 96 control subjects, all aged $\leq$ 60 years, to associate the contribution of eNOS T-786C and Glu298Asp, serotonin T102C, stromelysin-1 5A/6A and vWf A-1185G polymorphisms to the risk of AMI. In the present study the distribution of eNOS T-786C, serotonin T102C and fvWf A-1185G variants did not differ significantly between patients and control subjects. We found that the Glu298Asp variant of eNOS was associated to the AMI risk, but not independently of other risk factors and the genotype of this variant contributes to AMI risk only in patients with major coronary risk factors. In conclusion, the present study shows that the 5A/6A variant of stromelysin-1 was an independent risk factor for AMI in Brazilian Caucasians.

Introduction

Acute myocardial infarction (AMI) is caused by thrombotic occlusion of an epicardial coronary artery, and thrombus formation is initiated by platelet adhesion and aggregation at sites of ruptured atheromatous plaque (1). The endothelial dysfunction is characterized by an imbalance between contracting and relaxing substances, anticoagulants and procoagulantes factors and anti-inflammatory and pro-inflammatory mediators, which may play a significant role in the pathogenesis of atherosclerosis and consequently acute myocardial infarction.

The nitric oxide (NO) is generated from the aminoacid L-arginine by nitric oxide synthase enzyme (NOS). NO plays a key role in the regulation of vascular tone and vascular hemostasis it has a vasoprotective effect by scavenging superoxide radicals, prevents platelet adhesion and aggregation to the endothelium, leucocyte adhesion and smooth muscle cells proliferation (2,3). Three isoforms of NO have been identified so far: inducible NOS, constitutive neuronal NOS, and endothelial eNOS. Properly regulated expression of NOS enzymes and cofactors are essential for appropriate biological NO production. If a variant genetic causes deficient NOS, either on levels or in activity, it can lead to reduced or excessive production of NO, which can contribute to atherosclerotic processes. The eNOS is found in the endothelium and platelets. It has been implicated in the pathophysiology of several diseases including hypercholesterolemia, hypertension, diabetes mellitus, renal disease, stroke, atherosclerosis and coronary spasm as reviewed by Wang & Wang (4). A polymorphism in promoter region of eNOS gene was described as T–786C (5). This variant was associated with coronary vasospasm, which is also independently involved in pathogenesis of AMI (6). This polymorphism was also associated with coronary artery disease angiographically detected (7). In contrast, T-786C was not associated with venous thromboembolism (8). Also in eNOS gene, the Glu298Asp polymorphism caused by a single nucleotide substitution G894T (9) was associated with intrastent stenosis (10), therefore reduced synthesis of NO may promote the proliferation of vascular smooth muscle cells and induce the intrastent stenosis. As assessed by luciferase reporter gene assays, this mutation resulted in a significant reduction in eNOS gene promoter activity (5). This same mutation was strongly associated with AMI in the Japanese, especially those without coronary arterial stenosis (11).

During platelet aggregation, several substances are released, triggering the recruitment and activation of other surrounding platelets and formation of thrombus. Activated platelets release serotonin (5-hydroxytryptamine, 5-HT), which acts as a dilator or

constrictor of arteries. Golino *et al*. (12) and McFadden *et al*. (13) have demonstrated that intracoronary infusion of 5-HT causes an augmented constriction of coronary arteries in patients with coronary atherosclerosis. Serotonin also acts as a growth factor stimulating mitogenesis and migration of smooth muscle cells and promoting proliferation of vascular endothelial cells (14). Serotonin has been implicated in several conditions associated with abnormal vascular function such as diabetes (15), hypertension (16), and coronary vasospasm (13). Serotonin levels were associated with coronary artery disease and occurrence of ischaemic events, principally in younger patients (17). Serotonin may play an important role in pathogenesis of AMI through 5-HT_2 receptor activation. Recently one subtype of 5-HT_2A receptor has been identified, whose gene is expressed in human platelet (18), coronary artery (19), and the brain (20). A DNA polymorphism T102C has been associated with the 5-HT_2A receptor function (21) and with the risk of IAM (22).

Members of matrix metalloproteinases (MMP) family have been implicated in connective tissue remodelling during atherogenesis and weakening of the cap and subsequent rupture of the atheromatous plaque (23,24). Stromelysin-1 (a MMP-3) can play a role in rupture of atherosclerotic plaque, because it can cleave many of the extracellular matrix components as well as enhance the activity of other MMP (25). The presence of stromelysin-1 in coronary atherosclerotic plaque was originally demonstrated by in situ mRNA hibridization (26). Recently, a common polymorphism in the promoter region, at –1171 bp upstream of the start site of transcription of the human stromelysin-1 gene was described (27) in which one allele sequence has 5 adenosines (5A) and the other allele has 6 (6A). In vitro assays revealed that the 5A allele had a two-fold higher promoter activity than the 6A allele in both cultured fibroblasts and vascular smooth cells (28). Thus, the 5A allele carriers may have increased the risk of plaque rupture, leading to AMI. Ye *et al*. (27), Humphries *et al*. (29) and de Maat *et al*. (30) found an association between the angiographic progression of the disease and this genetic variant. Terrashima *et al*. (31) and Yamada *et al*. (32) supported that the 5A/5A genotype was associated with the AMI incidence.

von Willebrand factor (vWf) is a glycoprotein involved in platelet adhesion and aggregation at the site of vascular injury, and serves as the carrier for factor VIII in circulating blood. Plasma concentration of vWf are increased in disorders that affect the vascular system such as diabetes, hypertension, deep venous thrombosis, cerebrovascular, peripheral and pulmonary diseases as reviewed by Lip & Blann (33). Thus, increased concentrations of vWf may be important in pathogenesis of AMI. Several genetic and environmental factors, such ABO-blood group, age and inflammatory processes have influence in the vWf levels (34). A high concentration of vWf was an index of risk for

reinfarction and mortality in survivors of myocardial infarction (35). Sakai *et al*. (36) suggested that the vWf concentration increases in AMI, possibly in association with the hemodynamic deterioration that occurs in this condition. Keightley *et al*. (37) described three polymorphisms in the promoter region of vWf gene: C-1234T, A-1185G and G–1051A. According to the authors these polymorphisms have been associated with the circulating vWf levels. The polymorphism A-1185G in promoter region of the vWf gene have also been associated with plasma vWf concentrations by Harvey *et al*. (38). Di Bitondo *et al*. (39) investigated the combination of A-1185G and A-1021G for the risk of AMI but found no significant associations. The A-1185G polymorphism have not been associated neither with vWf levels nor with coronary artery disease in Brazilian Caucasians (40).

In the present study we examined the possible association between the T–786C and Glu298Asp variants of eNOS gene, serotonin T102C, stromelysin-1 5A/6A and vWf A–1185G polymorphisms and acute myocardial infarction in Brazilian Caucasian before the age of 60.

Methods

Two-hundred and eighty-three acute myocardial infarction Brazilian Caucasians patients who had been admitted to Instituto de Cardiologia do Rio Grande do Sul (Rio Grande do Sul, Brazil) were enrolled in this study. All patients aged ≤ 60 years at onset of myocardial infarction. Subjects who had had a myocardial infarction in the 90 days before the investigation were excluded from this study. All the patients had been submitted to previous angiography. The control subjects consisted of 96 men and women from the same ethnic group of the case group. None of the control subjects had neither history nor clinical evidence of angina or MI according to the Rose questionnaire (41). Patients and control subjects were from independent families. Participating case patients and control subjects were interviewed regarding cardiovascular risk factors. Patient's follow-up data and additional information were obtained by reviewing medical records. The acute myocardial infarction was defined if at least 2 of the following criteria were present: characteristic chest pain, elevated cardiac enzymes and ECG changes indicative of MI. The case and control groups did not differ significantly according to age (student t-test: p=0.98) or sex (chi-square test: p=0.18). This study was approved by the local Ethics Committee and all the subjects gave their written informed consent.

Smoking status was recorded as non-smoker or smoker (more than 10 cigarrette/day in last five years), diabetes was defined as present if previously diagnosed or treated. Hypertension was defined as documented increase of blood pressure (>140/90 mmHg)

requiring therapy. Family history of premature coronary artery disease if found in at least one consanguineous relative before the age of 60 years. Body mass index was measured according to the equation weight/height^2. Hyperlipidemia was defined by levels of triglycerides > 150 mg/dl, HDL-cholesterol < 40 mg/dl and LDL-cholesterol > 100 mg/dl or if the individual was receiving lipid-lowering treatment at inclusion. Blood samples were collected from the all patients after 12 hours-fasting state.

High molecular weight DNA was isolated from whole blood using a non-enzymatic technique for DNA analysis (42). The genetic variants used in this study were identified by using polymerase chain reaction (PCR) followed by restriction enzyme digestion and agarose or polyacrilamide gel electrophoresis containing ethidium bromide, and visualised under ultraviolet light. As were previously reported, the eNOS T-786C variant was identified by restriction enzyme digestion of *Msp*I (8), the Glu298Asp polymorphism was identified through digestion of *Ban*II (6), the serotonin T102C polymorphism was identified through digestion of *Msp*I (22), the stromelysin-1 5A/6A polymorphism was identified by digestion of *Xmn*I (43) and the A-1185G polymorphism was identified by digestion of *Acc*II (44).

Plasma vWf was measured by immunoelectrophoresis using a polyclonal rabbit anti-human vWf antibody (45). The assays were performed using two differents plasma dilutions. A pooled human plasma from normal individuals was considered to contain 100 U/dl of vWf. The vWf are reported as relative to this plasma reference. The skewness of the plasma vWf distribution was normalized by logarithmic transformation. In the tables the values of vWf are represented as median and interquartile range (25th and 75th). ANOVA was used to compare vWf levels between patients and controls and the Kruskall-Wallis test was used to compare the vWf levels according to the three genotypes of A-1185G.

Allele frequencies were determined by direct count of the alleles. Departures of Hardy-Weinberg equilibrium and differences between groups were evaluated by the chi-square test. A P value ≤ 0.05 was considered to indicate statistical significance. Prevalence of alleles was compared in the case and control groups using the chi-square test. The Mann-Whitney test was used for C-reactive protein comparisons. Except for analysis of genotype distribution, heterozygous and homozygous were combined and compared with non-carriers for the risk alleles. Categorical variables were compared by using Pearson chi-square test. Student t-test compared means in 2 group comparisons. Baseline characteristics are expressed as means $\pm$ standard deviations or percentages. Statistical analysis was performed with the SPSS program 11 for windows (SPSS Inc. Illinois).

Results

The clinical characteristics of patients and control subjects are shown in Table 1. There was no significance difference in age and sex between groups. As expected, recognised risk factors for coronary artery disease (CAD) (hyperlipidemia, diabetes mellitus, hypertension, and family history) were significantly more pronounced among AMI patients than among the control subjects. The non-O blood group was significantly more frequent in AMI cases than in control subjects (p=0.002).

The distribution of genotypes and the frequencies of alleles between case and control subjects are summarised in Table 2. For the T-786C polymorphism of the eNOS gene, the C allele frequencies were 0.36 in patients and 0.41 in controls (p=0.26). In the AMI group, the frequencies of C/C, C/T and T/T genotypes were: 15%, 43% and 42%, respectively. In the control group, the frequencies of C/C, C/T and T/T genotypes were: 19%, 44% and 37%, respectively. No significant difference was detected in allele or genotype frequencies between patients and controls. The genotype frequencies were in agreement with the predicted from Hardy-Weinberg equilibrium in patient and control groups. The distribution of C/C + C/T genotypes was 58% in case and 63% in control subjects (p=0.41). We did not find differences in AMI risk between the homozygosity for the C allele and those homozygous for the T allele (p=0.33).

In the analysis of Glu298Asp of the eNOS, the Asp allele frequency was 0.39 in patients and 0.31 in controls (p=0.06). In the AMI group, the frequencies of Asp/Asp, Asp/Glu and Glu/Glu genotypes were: 13%, 52% and 35%, respectively. In the control group, the frequencies of Asp/Asp, Asp/Glu and Glu/Glu genotypes were: 11%, 41% and 48%, respectively. The genotype frequencies were in agreement with those predicted by Hardy-Weinberg equilibrium for both groups. The prevalence of Asp/Asp + Asp/Glu was 65% in case and 52% in controls (p=0.03). The association of this polymorphism with AMI was not statistically significant when subjected to logistic regression analysis, controlling to other coronary risk factors. The homozygosity for the Asp allele has not conferred an increased risk of AMI when compared with those homozygous for the Glu allele (p=0.24).

For the T102C polymorphism of serotonin, the T allele frequency was 0.43 in patients and 0.36 in controls (p=0.11). In the AMI group, the frequencies of T/T, T/C and C/C genotypes were: 18%, 51% and 31%, respectively. In the control group, the frequencies of T/T, T/C and C/C genotypes were: 10%, 52% and 38%, respectively. The genotype frequencies were in agreement with the predicted from Hardy-Weinberg equilibrium in case and control groups. The distribution of T/T + T/C genotypes was 69% in case and 62% in

control subjects (p=0.30). Homozygosity for the T allele was not associated to AMI risk when compared with those homozygous for the C allele (p=0.10).

In the analysis of 5A/6A variant of stromelysin-1, the 5A allele frequency was 0.46 in patients and 0.39 in controls (p=0.08). In the AMI group, the frequencies of 5A/5A, 5A/6A and 6A/6A genotypes were: 20%, 53% and 27%, respectively. In the control group, the frequencies of 5A/5A, 5A/6A and 6A/6A genotypes were: 17%, 44% and 39%, respectively. In each group, the genotype frequencies were in agreement with the predicted from Hardy-Weinberg equilibrium. The prevalence of 5A/5A + 5A/6A genotypes was 73% in patients and 61% in control group (p=0.04). The odds ratio of 5A/5A+5A/6A versus 6A/6A between case and control subjects was 2.17 (p=0.01; 95% CI 1.19 - 3.95). The association of this polymorphism with AMI was statistically significant and independent of other coronary risk factors such as diabetes, hypertension, family history and hyperlipidemia when subjected to logistic regression analysis. However, when the homozygous for the 5A allele was compared with those homozygous for the 6A allele, the difference was not significant (p=0.17).

In the analysis of A-1185G in the von Willebrand factor, the A allele frequency was 0.48 in patients and 0.46 in controls (p=0.56). In the AMI group, the frequencies of A/A, A/G and G/G genotypes were: 23%, 51% and 26%, respectively. In the control group, the frequencies of A/A, A/G and G/G genotypes were: 24%, 43% and 33%, respectively. No significant differences were detected in allele or genotype frequencies between patients and controls. The genotype frequencies for both groups were in agreement with the predicted from Hardy-Weinberg equilibrium. The prevalence of A/A + A/G genotypes was 74% in case and 67% in control subjects (p=0.25). The homozygosity for the A allele was not associated with the risk of AMI when compared with those homozygous for the G allele (p=0.69).

No difference was found in vWf levels between patients and control subjects (Table 3). Analysis of plasma vWf levels adjusted for ABO blood groups showed no significant differences between patients and controls. In control subjects the vWf levels were higher in non O-blood groups than in O-blood groups (173.0 vs 143.0 U/dl; p=0.06) and in patients, this difference was also observed (180.5 vs 115.0 U/dl; p=0.00). In a univariate analysis, we did not find interaction between ABO blood group and vWf levels (p=0.18). There was no statistically significant association between the A–1185G genotypes and plasma vWf levels in either patients (p=0.39) or controls (p=0.53) (Table 4).

In this population, 70% of the patients with AMI have > 2 cardiovascular risk factors and 30% of these patients had $\leq$ 2 risk factors (p=0.000). This fact indicates an increase in AMI risk for subjects with > 2 risk factors. When we evaluated only subjects with > 2 risk

factors for the eNOS T-786C polymorphism, we did not find differences between case and control groups. The eNOS Glu298Asp analysis showed difference to AMI risk, the Asp allele was more prevalent in cases (67%) than in controls (39%) (p=0.001; OR=3.14, 95% IC 1.58 - 6.36). The serotonin T102C polymorphism was not associated with AMI when subjects with > 2 risk factors were evaluated (68% vs 71%, p=0.77). In analysis of stromelysin-1 5A/6A polymorphism, carriers of 5A allele were more frequent in case (73%) than in control group (57%) (p=0.04; OR=2.06; 95% IC 1.03 - 4.11). For the vWf A-1185G polymorphism, the A allele was more prevalent in cases (73%) than in controls (57%) (p=0.06; OR=2.01; 95% IC 1.1 - 4.03).

We did not find differences between AMI cases when the subjects were compared in relation to numbers of AMI suffered. When subjects who had had one AMI were compared to subjects who had had more than one AMI, we did not find differences in frequencies of polymorphisms analysed in this study.

We found no significant association between the T-786C or Glu298Asp, 5A/6A and A-1185G variants and the number of diseased vessels in the AMI group. However, in analysis of T102C variant of serotonin, among patients carriers of the T allele, 82% had > 3 affected vessels and 63% had 1-2 diseased vessels (p=0.05).

Discussion

The most important mechanism of AMI is the thrombotic oclusion of coronary artery at the site of a ruptured atherosclerotic plaque, and the endothelium may play a crucial role in this condition.

In the present study we found a significant difference in frequency of the missense Glu298Asp variant of the eNOS gene between AMI patients and control subjects. The Asp allele was present in 65% of patients and in 52% of control subjects (p=0.03). However, this significance disappeared in regression model when the other cardiovascular risk factors were included. Although Glu298Asp was not a risk factor for AMI, in subjects with > 2 risk factors this variant increased signicantly the risk of AMI. We did not find differences in frequency of the T-786C variant on the promoter region of the eNOS gene between AMI and control groups.

It has been shown that endothelium-derived NO play a key role in the regulation of vascular tone (46), supresses platelet aggregation, leucocyte adhesion and smooth muscle cell proliferation (2,3). A reduced NO production by a mutant eNOS may promote

thrombosis. The T-786C and Glu298Asp variants were described and associated with differences in the promoter activity of the eNOS gene (5). Our results about the association of Glu298Asp polymorphism and AMI are consistent with those previously reported by Shimasaki *et al*. (6), although those authors found that the association of this variant with AMI was independent of other risk factors. Hingorani *et al*. (7) found that this variant was associated with the angiographic CAD and recent AMI in the United Kingdom. This variant was also associated with the risk of CAD in the CHAOS Study, and the increased risk was confined to individuals homozygous for the Asp allele. Recent reports also support a role of this variant in intrastent stenosis (10). The frequency of the Asp allele observed in our population was similar to that reported by Rossi *et al*. (47) about Caucasian subjects, although this variant has not been associated with CAD in the GENICA Study.

Recent studies showed an association of the T-786C variant and coronary events. In the Japanese population, Nakayama *et al*. (5) associated independently the T-786C mutation with coronary vasospasm, and these authors (11) also found an association between this variant and AMI in patients without coronary stenosis at a young age. Recently, this variant has been associated with multivessel CAD in Caucasians (47). We did not find differences in frequencies of the C allele of the T-786C polymorphism in the promoter region of the eNOS gene between AMI and control groups. In the present study we found a contrast trend towards increased frequency of the C allele in the control than in AMI patients. These results are consistent with those obtained by Gonzalés-Ordóñez *et al*. (8) in the Spanish population. The C allele of T-786C variant seems to be much more frequent in Caucasian than in Japanese populations, which may explain the absence of association with the AMI risk. In the present study we observed that the frequency of C allele was 0.36 in AMI and 0.41 in control subjects. This values are similar to those reported by Rossi *et al*. (47) and by Gonzalés-Ordóñez *et al*. (8), and much more frequent than 0.03 in healthy Japanese and 0.15 in patients with coronary spasm (11). Conflicting results on association between the eNOS polymorphisms and CAD or AMI might be explained on the basis of variable distribution of the risk alleles in different populations, beyond that of the population risk factor characteristics.

The platelet activation, vasoconstriction and subsequent thombus formation is the key step for conversion of the chronic to acute coronary event, such as AMI. The serotonin 5-HT_2A receptor is expressed in platelet surface and has been associated with the receptor function and more specifically with the serotonin action. The serotonin induces vasoconstriction and stimulates the mitogenesis and migration of smooth muscle cell (12-14). The T102C variant was associated with the risk of AMI (22). The ECTIM Study (48) also

has demonstrated the association of a variant of serotonin transporter (SLC6A4) with AMI. We did not find differences in frequency of the C allele of T102C variant between AMI (0.43) and control (0.36) groups, however we found a trend towards increased frequency of this allele in AMI patients (p=0.11). Although this study has not been statistically significant, we suggest that the T102C polymorphism is associated with AMI in Caucasian subjects. We also found that patient carriers of the T allele of this variant have more affected vessels in angiographic study. Thus, we suggested that the T102C variant may also be associated with multivessel CAD in Caucasian subjects.

There was a statistically significant association between the 5A/6A polymorphism on the stromelysin-1 and AMI. The multiple logistic regression analysis revealed that the 5A/6A variant was one of the independent risk factors for AMI in the present study. The matrix metalloproteinases (MMP) are the main degrading enzymes of extracellular matrix proteins, and they play an important role in the tissue remodeling and repair in physiological and pathological processes. The stromelysin-1 is a MMP type 3 and its can play a role in aterothrombosis because it can cleave many matrix extracellular components and enhance the activity of other MMP (25). A variant of the stromelysin–1 gene called 5A/6A is associated with a promoter activity of this gene. Carriers of the 5A allele may have an increased risk of atheromatous plaque rupture leading to AMI. The 5A/6A variant was associated with the progression of angiographically determined CAD in the LOCAL Gemfibrozil Study (29) and in the REGRESS Study (30). Terashima *et al*. (31) and Yamada *et al*. (32) also found that the 5A allele was significantly more frequent in AMI patients than in control group, confirming the suggestion that patients carriers of 5A allele have lesions more prone to rupture. In conclusion, we confirm that the 5A/6A polymorphism of the stromelysin-1 was a genetic risk factor for AMI, probably through an increased susceptibillity to plaque rupture.

In the present study, there was no significant association between the A-1185G variant on the vWf gene and AMI. The vWf is involved in platelet adhesion and aggregation at the site of vascular injury. The platelet aggregation is the key event for thrombus formation in atherosclerotic sites leading to AMI. vWf concentrations have been associated with several vascular disorders as reviewed by Lip & Blann (33), including the CAD. The A-1185G located in the promoter region of the vWf gene was associated with the vWf levels (37,38). However, di Bitondo *et al*. (39) did not find association between this variant and AMI risk. In the present study, we did not find differences in vWf levels between AMI and control groups, the same was observed when the individual were evaluated separately to ABO-blood groups. There was no association of the vWf levels according to genotypes of the

polymorphism A-1185G. Montalescot *et al*. (49) and Sakai *et al*. (36) observed increases in vWf levels in AMI patients, and high vWf plasma levels were predictive of vascular events in patients with atrial fibrillation (50). Recently, we demonstrated that vWf levels and A-1185G variant were associated with neither vWf plasma levels nor with the angiographically CAD (40). Thus, the vWf levels despite acting as a marker of endothelial dysfunction were not associated with the AMI in Brazilian Caucasians and the A-1185G does not affect the vWf plasma levels. But, we found a trend towards association of this variant with the risk of AMI in patients with > 2 risk factors.

In conclusion, the present study found that the eNOS Glu298Asp variant increased the risk of AMI in subjects if further coronary risk factors were present. The stromelysin-1 5A/6A variant was an independent risk factor for AMI. However this variant was not associated with the risk of re-IAM or with the number of diseased vessels in angiographic study.

References

1. Fuster V, Badimon L, Badimon JJ and Chesebro JH. The phatogenesis of coronary artery disease and the acute coronary syndromes. New England Journal of Medicine 1992; 326(4):242-250.

2. Chiang TM, Wang YB and Kang ES. Role of the recombinat protein of the platelet receptor for type I collagen in the release of nitric oxide during platelet aggregation. Thrombosis Research 2000; 100:427-432.

3. Chiang TM, Cole F, Woo-Rasberry V and Kang ES. Role of nitric oxide synthase in collagen-platelet interaction: involvement of platelet nonintegrin collagen receptor nitrotyrosylation. Thrombosis Research 2001; 102:343-352.

4. Wang XL & Wang J. Endothelial nitric oxide synthase gene sequence variations and vascular disease. Molecular Genetics and Metabolism 2000; 70:241-251.

5. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Kiyotaka K, Ogawa H, Motoyama T, Saito Y, Ogawa Y, Miyamoto Y, Miyamamoto Y and Nakao K. T-786-Cmutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999; 99(22):2864-2870.

6. Shimasaki Y, Yasue H, Yoshimura M, Nakayama M, Kugiyama K, Ogawa H, Harada E, Masuda T, Koyama W, Saito Y, Miyamoto Y, Ogawa Y and Nakao K. Association of the missense Glu298Asp variant of the endotelial nitric oxide synthase gene with myocardial infarction. Journal of the American College of Cardiology 1998; 31:1506-10.

7. Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, Haydock S, Hooper RV, Stephens N, O'Shaughnessy KM and Brown M. A common variant of the endothelial nitric oxide synthase (Glu298Asp) is a major risk factor for coronary artery disease in the UK. Circulation 1999; 100(14):1515-1520.

8. González-Ordóñez AJ, Fernandes-Carreira JM, González-Franco A, Martín-Sanchez L, Alvarez MV and García EC. Two expressive polymorphisms on the endothelial nitric oxide synthase gene (intron 4, 27 bp repeat and –786 T/C) and the venous thromboembolism. Thrombosis Research 2000; 99:563-566.

9. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Saito Y, Miyamoto Y, Kaneshige T, Hiramatsu H, Yoshioka T, Kamitani S, Teraoka H and Nakao K. Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786C and missense Glu298Asp variants. Journal of Investigation Medicine 2000; 48(5):367-74.

10. Suzuki T, Okumura K, Sone T, Kosokabe T, Tsuboi H, Kondo J, Mukawa H, Kamiya H, Tomida T, Imai H, Matsui H and Hayakawa T. The Glu298Asp polymorphism in endothelial nitric oxide synthase gene is associated with coronary in-stent restenosis. International Journal of Cardiology 2002; 86:71-76.

11. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Ogawa H, Kugiyama K, Mizuno Y, Harada E, Nakamura S, Ito T, Saito Y, Miyamoto Y, Ogawa Y and Nakao K. T-786C Mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with myocardial infarction, especially without coronary organic stenosis. American Journal of Cardiology 2000; 86:628-34.

12. Golino P, Piscion F, Willerson JT, Cappelli-Bigazzi M, Foraccio A, Villari B, Indolfi C, Russolillo E, Condorelli M and Chiariello M. Divergent effects of serotonin on coronary artery dimensions and blood flow in patients with coronary atherosclerosis and control patients. New England Journal of Medicine 1991; 324:641-648.

13. McFadden EP, Clarke JC, Davies GJ, Kaski JG, Haider AW and Maseri A. Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. New England Journal of Medicine 1991; 324:648-54.

14. Nemeck GM, Coughlin SR, Handley DA and Moskowitz MA. Stimulation of aortic smooth muscle cell mitogenesis by serotonin. Proccedings of the National Academy Sciences of the USA 1986; 83:674-678.

15. Barradas MA, Gill DS, Fonseca VA, Mikkaihilis DP and Dandona P. Intraplatelet serotonin in patients with diabetes melllitus and peripheral vascular disease. European Journal of Clinical Investigation 1988; 18(4):399-404.

16. Kamal LA, le Quan-Bui KH and Meyer P. Decreased uptake of 3H-serotonin and endogenous content of serotonin in blood platelets in hypertensive patients. Hypertension 1984; 6(4):568-73.

17. Vikenes K, Farstad M and Nordrehaug JE. Serotonin is associated with coronary artery disease and cardiac events. Circulation 1999; 100(5):483-89.

18. Cook W, Fletcher K, Wainwright M, Marks N, Yan S and Leventhal B. Primary structure of the human platelet serotonin 5HT2A receptor: identity with frontal cortex serotonin 5HT2A receptor. Journal of Neurochemical 1994; 63:465-9.

19. Ullmer C, Schmuck K, Kalkman HO and Lübbert H. Expression of serotonin receptor mRNAs in blood vessels. FEBS Letters 1995; 370:215-21.

20. Schimidt CJ, Sorensen SM, Kehene JH, Carr AA and Palfreyman MG. The role of 5-HT receptors in antipsychotic activity. Life Science 1995; 56:2209-22.

21. Arranz M, Collier D, Sodhi M, Ball D, Roberts G and Price J. Association between clozapine response and allelic variation in 5-HT 2A receptor gene. Lancet 1995; 346:281-2.

22. Yamada S, Akita H, Kanazawa K, Ishida T, Hirata K, Ito K, Kawashima S and Yokoyama M. T102C polymorphism of the serotonin (5-HT) 2A receptor gene in patients with non-fatal acute myocardial infarction. Atherosclerosis 2000; 150:143-48.

23. Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995; 91:2844-2850.

24. Shah PK, Falk E, Badimon JJ, Ortiz AF, Mailhac A, Levy GV, Fallon JT, Regnstron J and Fuster V. Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. Circulation 1995; 92:1565-69.

25. Lu PCS, Ye H, Maeda M and Azar DT. Immunolocalization and gene expression of matrilysin during corneal wound healing. Investigative Ophthalmology Visual Science 1999; 40:20-27.

26. Henney AM, Wakeley P, Davies MJ, Foster K, Hembry R, Murphy G and Humphries SE. Localization of stromelysin gene expression in atherosclerotic plaques by in situ hybridization. Proccedings of the National Academy Sciences of the USA 1991; 88:8154.

27. Ye S, Watts GF, Mandalia S, Humphries SE and Henney AM. Genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. British Heart Journal 1995; 73:209-215.

28. Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE and Henney AM. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. Journal of Biological Chemistry 1996; 271:13055-13060.

29. Humphries SE, Luong L, Talmud PJ, Frick HM, Kesäniemi YA, Pasternack A, Taskinen MR and Syvänne M. The 5A/6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene predicts progression of angiographically determined coronary artery disease in men in the LOCAT Gemfibrozil Study. Atherosclerosis 1998; 139:49-56.

30. de Maat M, Jukema W, Ye S, Zwinderman AH, Moghaddam PH, Beekman M, Kastelein JJ, van Boven AJ, Bruschke AVG, Humphries SE, Kluft C and Henney AM. Effect of the stromelysin-1 promoter on efficacy of *Pravastatin* in coronary atherosclerosis and restenosis. American Journal of Cardiology 1999; 83:852-56.

31. Terashima M, Akita H, Kanazawa K, Inoue N, Yamada S, Ito K, Matsuda Y, Takai E, Iwai C, Kurogane H, Yoshida Y and Yokoyama M. Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction. Circulation 1999; 99:2717-19.

32. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M and Yokota M. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. New England Journal of Medicine 2002; 347(24):1916-1923.

33. Lip GY & Blann AD. von Willebrand factor and its relevance to cardiovascular disorders. British Heart Journal 1995; 74:580-583.

34. Souto JC, Almasy L, Soria JM, Buil A, Stone W, Lathrop M, Blangero J and Fontcuberta J. Genome-wide linkage analysis of von Willebrand factor plasma levels: results from the GAIT project. Thrombosis and Haemostasis 2003; 89:468-74.

35. Jansson J-H, Nilsson TK and Johnson O. von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. British Heart Journal 1991; 66:351-5.

36. Sakai H, Goto S, Kim J-Y, Aoki N, Abe S, Ichikawa N, Yoshida M, Nagaoka Y and Handa S. Plasma concentration of von Willebrand factor in acute myocardial infarction. Thrombosis and Haemostasis 2000; 84:204-9.

37. Keightley AM, Lam YM, Brady JN, Cameron CL and Lillicrap D. Variation at the von Willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. Blood 1999; 93(12):4277-83.

38. Harvey PJ, Keightley AM, Lam YM, Cameron C and Lillicrap D. A single nucleotide polymorphism at nucleotide -1793 in the von Willebrand factor (VWF) regulatory region is associated with plasma vWF:Ag levels. British Journal of Haematology 2000; 109:349-353.

39. di Bitondo R, Cameron CL, Daly ME, Croft AS, Steeds RP, Channer KS, Samani NJ, Lillicrap D and Winship PR. The –1185A/G and –1051G/A dimorphisms in the von Willebrand factor gene promoter and risk of myocardial infarction. British Journal of Haematology 2001; 115:701-706.

40. Simon D, Paludo CA, Ghisleni GC, Manfroi WC and Roisenberg I. Association studies between –1185A/G von Willebrand factor gene polymorphism and coronary artery disease. Brazilian Journal of Medical and Biological Research 2003; 36: in press.

41. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bulletin of the World Health Organization – L'Organisation Mondiale de La Santé 1962; 27:645-658.

42. Lahiri DK & Nurnberger Jr. JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 1991; 19(19):5444.

43. Dunleavey L, Beyzade S and Ye S. Rapid genotype analysis of the stromelysin gene 5A/6A polymorphism. Atherosclerosis 2000; 151:587-589.

44. Simon D, Palatinik M and Roisenberg I. Analysis of the –1185A/G von Willebrand factor (VWF) gene polymorphism in two Brazilian ethnic groups and its effect on plasma VWF levels. Thrombosis Research 2002; 105:519-522.

45. Fischer RR, Lucas EM, Pereira AMB and Roisenberg I. Preparation of a heterologous antiserum for the determination of von willebrand factor in human plasma. Brazilian Journal of Medical and Biological Research 1996; 29:1641-44.

46. Moncada S, Palmer RMJ and Riggs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews 1991; 43:109-42.

47. Rossi GP, Cesari M, Zanchetta M, Colonna S, Maiolino G, Pedon L, Cavallin M, Maiolino P and Pessina AC. The T-786C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA Study. Journal of the American College of Cardiology 2003; 41(6):930-7.

48. Fumeron F, Betoulle D, Nicaud V, Evans A, Kee F, Ruidavets JB, Arvelier D, Luc G and Cambien F. Serotonin transporter gene polymorphism and myocardial infarction: Etude Cas-Temoins de l'Infartus du Myocarde (ECTIM). Circulation 2002; 105(25):2943-5.

49. Montalescot G, Philippe F, Ankri A, Vicaut E, Bearez E, Poulard JE, Carrie D, Flammang D, Dutoit A, Carayon A, Jardel C, Chevrot M, Bastard JP, Bigonzi F and Thomas D. Early increase of von Willebrand factor predicts adverse outcome in unstable coronary artery disease. Circulation 1998; 98:294-299.

50. Conway D, Pearce LA, Chin B, Hart RG and Lip G. Prognostic value of plasma von Willebrand factor and soluble p-selectin as indices of endothelial damage and platelet activation in 994 patients with nonvalvular atrial fibrillation. Circulation 2003; 107:3141-3145.

Table 1. Clinical characteristics of AMI patients and control subjects.

Characteristic	AMI Patients (n=283)	Control Subjects (n=96)	P-value
Age	50.4 ± 6.4	50.4 ± 7.2	0.980
Male sex	82%	75%	0.180
Smoking status	18%	18%	1.000
Non-O blood group	61%	42%	0.002
Hyperlipidemia	62%	36%	<0.001
Diabetes status	26%	10%	0.004
Family history	59%	39%	0.001
Hypertension	61%	34%	<0.001
Fibrinogen (mg/dl)	299.8 ± 55.7	281.8 ± 46.1	0.006
C-reactive protein (mg/dl)	0.26 (0.13 - 0.61)	0.18 (0.08 - 0.34)	0.006

Values are represented as mean ± SD or number (%) of patients. Student-t test for continuous variables and chi-square for discrete variables were used to compare the values. For the AMI patients the value refers to the age at onset of myocardial infarction, not the current age. C-reactive protein values are expressed as median, 25th and 75th percentiles.

Table 2. Allele and genotype frequencies of eNOS T-786C and Glu298Asp, serotonin T102C, stromelysin-1 5A/6A and vWf A-1185G polymorphisms in patients and control subjects.

Polymorphisms	AMI Patients			Control Subjects			P-value
T-786C	C/C	C/T	T/T	C/C	C/T	T/T	
N (%)	40 (15%)	118 (43%)	116 (42%)	17 (19%)	40 (44%)	33 (37%)	
C allele		0.36			0.41		0.26
Glu298Asp	Asp/Asp	Asp/Glu	Glu/Glu	Asp/Asp	Asp/Glu	Glu/Glu	
N (%)	37 (13%)	143 (52%)	97 (35%)	10 (11%)	38 (41%)	45 (48%)	
Asp allele		0.39			0.31		0.06
T102C	T/T	T/C	C/C	T/T	T/C	C/C	
N (%)	49 (18%)	141 (51%)	87 (31%)	10 (10%)	49 (52%)	36 (38%)	0.11
T allele		0.43			0.36		
5A/6A	5A/5A	5A/6A	6A/6A	5A/5A	5A/6A	6A/6A	
N (%)	55 (20%)	149 (53%)	75 (27%)	16 (17%)	42 (44%)	37 (39%)	
5A allele		0.46			0.39		0.08
A-1185G	A/A	A/G	G/G	A/A	A/G	G/G	
N (%)	58 (23%)	130 (51%)	65 (26%)	23 (24%)	41 (43%)	31 (33%)	
A allele		0.48			0.46		0.56

Table 3. Plasma vWf levels in AMI patients and control subjects.

Blood group	AMI Patients		Control Subjects		P-value
All subjects	155.0	105.8 - 223.5	162.5	98.5 - 222.5	0.936
O blood group	115.0	82.0 - 177.5	143.0	86.0 - 219.5	0.234
Non-o blood group	180.5	131.1 - 246.5	173.0	133.7 - 227.8	0.511

The values of vWf are expressed as median, 25th and 75th percentiles.

Table 4. Plasma vWf levels according to the A-1185G polymorphism.

Genotypes	AMI Patients		Control Subjects	
A/A	138.0	98.0 - 213.5	158.5	135.2 - 223.7
A/G	161.5	108.5 - 223.5	171.0	94.0 - 239.0
G/G	156.5	107.5 - 234.0	134.5	90.8 - 215.2

The values of vWf are expressed as median, 25th and 75th percentiles.

capítulo 3

Genetic Variants of Fibrinogen, Factor VII, Factor V and Prothrombin: Role in Acute Myocardial Infarction

Capítulo 3

Genetic Variants of Fibrinogen, Factor VII, Factor V and Prothrombin: Role in Acute Myocardial Infarction

Crislaine A. Paludo[1], Josi Vidart[2], Vera L. Portal[2] and Israel Roisenberg[1]

[1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, [2]Serviço de Epidemiologia, Instituto de Cardiologia do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Corresponding author:

Israel Roisenberg
Departamento de Genética
Instituto de Biociências
Universidade Federal do Rio Grande do Sul
Caixa Postal 15053
91.501-970 - Porto Alegre, RS
Brazil
FAX n° + 55 51 3316 7311
Phone n° + 55 51 3316 6728
e-mail: israberg@ufrgs.br

Abstract

Several coagulation factors have been associated with the risk of acute myocardial infarction (AMI). During the last decades it has been demonstrated that mutations in genes that encode blood coagulation factors involved in thrombus formation play a crucial role in AMI risk. Fibrinogen is the clotting factor most frequently investigated in relation to arterial risk. Variants located in the α- and β-fibrinogen genes have been associated with the plasma levels and to the risk of AMI. Factor VII (fVII) is the first factor of extrinsic pathway of coagulation and high levels of fVII increases the risk of AMI. The R353Q and insertion/deletion in gene of fVII are genetic variants that affect the fVII circulating levels. The mutations factor V Leiden, located in clotting factor V, and the G20210A, located in prothrombin gene, are well established in relation to venous thrombosis, but their effects in arterial thrombosis remain controversial. We conducted a case-control study of 283 AMI cases and 96 control subjects, all aged $\leq$ 60 years to associate the contribution of polymorphisms of fibrinogen, factor VII, factor V and prothrombin genes to the risk of AMI. We also investigated the association of the G-455A and *Taq*I variants with the circulating fibrinogen levels. In the present study, the distribution of the G-455A and *Taq*I variants of fibrinogen genes and the R353Q and insertion/deletion of factor VII did not differ significantly between AMI patients and control subjects. The factor V Leiden and prothrombin G20210A mutations were found in very low frequencies in our population and were not predictive. The plasma fibrinogen levels were significantly higher in AMI patients than in control subjects, but this association was not independent of other coronary risk factors. The *Taq*I polymorphism was not affected the fibrinogen levels. The main finding of this study is that the A allele of G-455A variant is associated with increased plasma fibrinogen levels. We concluded that the variants investigated in this study did not increase the risk of acute myocardial infarction before the age of 60 years. The plasma fibrinogen levels were not independently associated with the AMI in Brazilian Caucasians in the present study.

Introduction

Genetic and environmental factors contribute to the development of acute myocardial infarction (AMI). Over the last decades it has been demonstrated that mutations in genes that encode blood coagulation factors involved in thrombus formation play a crucial role in AMI risk.

Fibrinogen is a clotting factor and an acute phase reactant, apart from being a co-factor to platelet aggregation and stimulating the smooth muscle cell migration. The first epidemiological investigation that included fibrinogen levels was the Northwick Park Heart Study (1), but in 1976 Fulton & Duckett (2) had proposed the monitoring plasma fibrinogen to reduce morbidity after AMI. Increased plasma fibrinogen level is an independent risk factor for cardiovascular events and mortality in a wide variety of studies (3-9). Fibrinogen is a glycoprotein that consists of three chains encoded by three different genes, α-, β- and γ-fibrinogen. Polymorphisms of the fibrinogen genes influence plasma fibrinogen levels (10,11). The G-455A polymorphism located in the promoter region of the β-fibrinogen gene has been associated with the elevated fibrinogen levels (12-16). Whether the G-455A polymorphism is associated with ischaemic coronary syndromes remains unclear. Previous studies have found that genotype frequencies of the G-455A polymorphism are similar between patients with a myocardial infarction and control subjects (17,18). Another variant the *Taq*I polymorphism, located in 3' region of the α-fibrinogen gene, has been investigated with relation to the role in plasma fibrinogen levels, but significant associations were not found in several studies (19,20).

The factor VII (fVII) is a coagulation factor and its plasma levels were associated with ischaemic events for the first time in Northwick Park Heart Study (1), confirmed by other studies and more pronounced in fatal events (21-23). However, this association was not observed by Folson *et al*. (24). Blood levels of factor VII are genetically determined, and previous studies have demonstrated a strong association with two polymorphisms the R353Q and the promoter insertion/deletion. The R353Q polymorphism determine the presence of a glutamine (Q) or arginine (R) at amino acid 353 of fVII gene and the insertion (I)/deletion (D) is caused by a 10 bp insertion in the –323 promoter region. Both variants are candidates for having an effect on plasma levels of fVII. The carriers of Q allele of R353Q have 20-25% lower levels of fVII (25,26). The R353Q have been investigated in relation to ischaemic events and AMI risk, and positive associations have been found in some (27,28) but not in other studies (29-31). The insertion/deletion polymorphism has been associated with plasma levels of fVII (32,33) and with ischaemic risk (34), but other studies did not find such

associations (35,36).

A mutation called factor V Leiden resultant of a single nucleotide change at position 1691 in the gene coding the coagulation factor V, leads to the replacement of an arginine by a glutamine in the activated protein C (APC) cleavage site of factor V. As a result the factor V is less efficiently inactivated by APC (37). Association of fV Leiden with venous thromboembolism is well established in the literature (38), but the role of this mutation in arterial thrombotic events is controversial. Whether fV Leiden has an influence on the incidence of arterial thrombosis and AMI has been the subject of several studies with conflicting results. Several reports have associated fV Leiden with atherosclerosis, myocardial infarction (39-41) and stroke (42). However, other studies have failed to demonstrate such an association (43,44). Some studies have documented an association between this mutation and stroke in juveniles (45). Rosendaal *et al*. (40) reported a four-fold increase in risk of AMI in young women carrying the fV Leiden, however the risk seemed to be limited to women with other major cardiovascular risk factors.

Another mutation, the prothrombin variant G20210A, a genetic variation in the 3'-unstranslated region of the prothrombin gene is associated with elevated plasma prothrombin and thombin levels, thus increasing the risk of thrombotic events (46). This variant was associated with venous thrombosis (47), coronary artery disease (48) and stroke (49). In contrast, other studies have not found an association between this polymorphism and ischaemic events (50). Doggen *et al*. (29) and Rosendaal *et al*. (40) reported an increase in risk of AMI in young women carrying the 20210A allele. However, Durante-Mangoni *et al*. (51) found that this variant confers no increased risk for MI. Two studies in Brazil found an increased prevalence of the 20210A allele in patients with arterial disease in absence of hyperlipoproteinemia, hypertension and diabetes (52,53). When both variants were evaluated jointly neither fV Leiden nor the prothrombin variant were found to be important risk factors in young women who suffered a stroke before the age of 45 years (54).

The purpose of the present study was to determine if the plasma fibrinogen levels and G-455A and *Taq*I polymorphisms of fibrinogen, R353Q and insertion/deletion of factor VII gene and the mutations fV Leiden and G20210A of prothrombin are risk factors for premature AMI.

Methods

We conducted a case-control study with 283 Brazilian Caucasians patients that had

suffered an acute myocardial infarction before the age of 60 years and 96 control subjects. The patients were enrolled from the Instituto de Cardiologia do Rio Grande do Sul (Rio Grande do Sul, Brazil) over a period of 18 months (from August 2001 to April 2003). Subjects who had had a myocardial infarction in the 90 days before the investigation were excluded from this study. All the patients had been submitted to previous angiography study.

The control subjects consisted of men and women from the same ethnic group of the case group. None of control subjects had a history of angina or MI, according to the modified ROSE questionnaire (55). Patients and control subjects were from independent families. The case and control groups did not differ significantly according to age (student t-test: p=0.98) or sex (chi-square test: p=0.18). Participating case patient and control subjects were interviewed regarding cardiovascular risk factors and additional information was obtained in clinical records. The acute myocardial infarction was defined if at least 2 of the 3 following criteria were present: characteristic chest pain, elevated cardiac enzymes and ECG changes indicative of AMI. This study was approved by the local Ethics Committee and all the subjects gave their written informed consent.

Smoking status was recorded as non-smoker or smoker (more than 10 cigarrettes/day over last five years), diabetes was defined as present if previously diagnosed or treated. Hypertension was defined as documented increase of blood pressure (>140/90 mmHg) requiring therapy. Family history of premature coronary artery disease was defined if found in at least one consanguineous relative under the age of 60 years. Body mass index was measured according to the equation weight/height^2. Dysplipidemia was defined by levels of triglycerides > 150 mg/dl, HDL-cholesterol < 40 mg/dl and LDL-cholesterol > 100 mg/dl or if the individual was receiving lipid-lowering treatment at inclusion. Blood samples were collected from all the patients after 12 hours-fasting state.

High molecular weight DNA was isolated from whole blood using a non-enzymatic technique for DNA analysis (56). The polymorphisms were identified by using polymerase chain reaction (PCR) followed by restriction enzyme digestion and polyacrilamide gel electrophoresis. As previously reported, the β-fibrinogen G-455A polymorphism was identified by restriction enzyme digestion of *Hae*III (57), the α-fibrinogen *Taq*I was identified through digestion of *Taq*I (10) and the fVII R353Q polymorphism was identified by restriction enzyme digestion of *Msp*I (58). The fVII insertion/deletion variant was identified as previously reported (59). Restriction enzyme digestion of *Hind*III was performed to identify the fV Leiden (37) and the G20210A mutation (46).

Allele frequencies were determined by direct count of the alleles. Departures of

Hardy-Weinberg equilibrium and differences between groups were evaluated by the chi-square test. A P value ≤ 0.05 was considered to indicate statistical significance. Prevalence of fV Leiden and G20210A was compared in the study and control groups using the chi-square test with Yates correction, if necessary. Except for analysis of genotype distribution, heterozygous and homozygous were combined and compared with non-carriers of risk alleles. Categorical variables were compared by using the Pearson chi-square test. Student t-test compared means in 2 group comparisons. The ANOVA test was used to compare the fibrinogen levels according to the three genotypes of G-455A and *Taq*I polymorphisms. Baseline characteristics are expressed as means $\pm$ standard deviations or percentages. Statistical analysis was performed with the SPSS program 11 for windows (SPSS Inc. Illinois).

Results

The clinical characteristics of patients and control subjects are shown in Table 1. There was no significant difference in age and sex between groups. In the comparison between AMI and control group for coronary risk factors, there were significant differences in the prevalence of hypertension (p=0.000), diabetes mellitus (p=0.004), family history (p=0.001) and non-O blood group (p=0.002). The analysis of multiple logistic regression revealed that the independent risk factors for AMI were family history (p=0.009), hypertension (p=0.000), dyslipidemia (p=0.000) and ABO blood group (p=0.002).

The plasma fibrinogen levels are showed in Table 1, AMI patients presented levels significantly more elevated than control subjects (p=0.006). But the association of fibrinogen levels with AMI was not independent of other coronary risk factors when subjected to logistic regression analysis.

The differences in fibrinogen levels between genotypes of G-455A and *Taq*I polymorphisms are presented in Table 2. There was a significant association of fibrinogen levels according to genotypes of G-455A variant of β-fibrinogen gene. Homozygous for A allele have the highest levels, GG homozygous have the lowest levels and the AG heterozygous have the intermediate levels in all subjects (F=5.99; p=0.003) and in AMI patients (F=7.59; p=0.001). For the control group this difference was not observed (F=0.27; p=0.974). There was no difference in fibrinogen levels according to genotypes of the *Taq*I polymorphism and no difference for all subjects (F=0.13; p=0.88) and no difference for AMI patients (F=0.84; p=0.43). In the control subjects we observed a trend where the A1/A1 homozygous have higher levels of fibrinogen (F=1.16; p=0.31).

The distribution of genotypes and the frequencies of alleles between case and control

subjects are summarised in Table 3. The β-fibrinogen A allele frequency was 0.19 in patients and 0.17 in controls (p=0.70). In the AMI group, the frequencies of A/A, A/G and G/G genotypes were: 3%, 32% and 65%, respectively. In the control group, the frequencies of A/A, A/G and G/G genotypes were: 4%, 26% and 70%, respectively. In both groups the genotype frequencies were in agreement with that predicted by the Hardy-Weinberg equilibrium. The distribution of A/A + A/G genotypes was 35% in case and 30% in control subjects (p=0.51). The α-fibrinogen A1 allele frequency was 0.24 in patients and 0.23 in controls (p=0.85). In the AMI group, the frequencies of A1/A1, A1/A2 and A2/A2 genotypes were: 7%, 34% and 59%, respectively. In the control group, the frequencies of A1/A1, A1/A2 and A2/A2 genotypes were: 10%, 26% and 64%, respectively. In the control subjects the genotype frequencies were in agreement with that predicted by the Hardy-Weinberg equilibrium, but there was a significant deviation in the case group. The distribution of A1/A1 + A1/A2 genotypes was 41% in case and 36% in control subjects (p=0.48).

The factor VII Q allele frequency was 0.13 in patients and 0.14 in controls (p=0.96). In the AMI group, the frequencies of Q/Q, Q/R and R/R genotypes were: 3%, 21% and 76%, respectively. In the control group, the frequencies of Q/Q, Q/R and R/R genotypes were: 2%, 23% and 75%, respectively. There was a significant deviation from the Hardy-Weinberg equilibrium in both groups. The distribution of Q/Q + Q/R genotypes was 24% in case and 25% in control subjects (p=0.88).

The factor VII insertion allele frequency was 0.18 in patients and 0.17 in controls (p=0.94). In the AMI group, the frequencies of I/I, I/D and D/D genotypes were: 5%, 26% and 69%, respectively. In the control group, the frequencies of I/I, I/D and D/D genotypes were: 2%, 30% and 68%, respectively. In the control subjects the genotype frequencies were in agreement with that predicted by the Hardy-Weinberg equilibrium, but there was a significant deviation in the case group. The distribution of I/I + I/D genotypes was 31% in case and 32% in control subjects (p=0.86).

The fV:Q506 mutation (the Gln allele) was found only in 3 patients and in no control subject. We did not find a homozygous for the fV Leiden mutation in subjects analised. The prothrombin 20210A allele was found in 5 patients and one control subject. We did not find a homozygous for the 20210A allele. The genotype frequencies for both variants in case and control groups were in agreement with that predicted by the Hardy-Weinberg equilibrium.

Discussion

The fibrinogen is the most investigated coagulation factor related to ischaemic

coronary events. The fibrinogen is the last step of the coagulation pathway. Apart from this role in coagulation, fibrinogen is an acute phase reactant, and is associated to inflammatory processes. The atherosclerotic process that composes coronary artery disease may be compared to an inflammatory process (60). Citokines produced by activated monocytes and smooth muscle cells are a stimulant for the fibrinogen synthesis (61). Kamphuisen *et al*. (7) suggested that higher fibrinogen levels observed in patients with venous thrombosis are not caused by an acute phase reaction, supporting the causal relationship between levels of fibrinogen and vascular risk. In addition, the fibrinogen acts as an antioxidant against oxidative stress arising from inflammatory conditions (62). In the present study, the levels of plasma fibrinogen were significantly higher in AMI patients than in control subjects. However, this association was not independent of other major coronary risk factors in a logistic regression model. Our results confirm those previously reported, where elevated plasma fibrinogen levels were associated with coronary disease and more specifically AMI (1,8,59). Some studies have reported an association between fibrinogen levels and other major coronary risk factors such as age, ethnic group and hypertension (14,63,64). The adjustment to these coronary risk factors substantially decreased the coronary risk associated with the higher levels of fibrinogen as previously reported (6,65).

In the present study the *Taq*I polymorphism located in 3´ region of α-fibrinogen gene was not associated with the plasma fibrinogen levels. Our results are consistent with those previously reported (15,20), however disagreeing with those obtained by Heinrich *et al*. (66). The G-455A polymorphism is located in the promoter region of the β-fibrinogen gene and the synthesis of the β-fibrinogen chain is the rate-limiting step for the fibrinogen assembly in plasma (67). According to Brown & Fuller (68), the A allele is associated with higher promoter activity through the differential binding of regulation complexes in this region. In the present study the raised levels of fibrinogen were observed in the A allele carriers, with the highest levels in AA homozygous confirming previous observations (69). In the control group there was no significant effect of β-fibrinogen polymorphism on fibrinogen levels, probably due to the smaller number of subjects. Our observations provide confirmation to previous reports (11-13,16,18,69,70) where the G-455A polymorphism is associated with differences in fibrinogen levels.

In the present study, there was no difference in frequency of the A allele of G-455A polymorphism between AMI cases and control subjects. Other studies also failed to find an association between the G-455A genotype and AMI risk (16,71). The frequency of A allele found in this study was similar to those frequencies reported in other studies (13,72). The *Taq*I polymorphism also was not associated to AMI in the present study, confirming the

results reported by Doggen *et al*. (72). The main finding of the present study is that the A allele of G-455A variant is associated with increased plasma fibrinogen levels but not with an increased risk of AMI.

In conclusion, in the present study the plasma fibrinogen level was not an independent risk factor for AMI. We suggested that the *Taq*I and G-455A variants of fibrinogen genes were not associated with the increased risk of AMI. However, as the G-455A variant was associated with the plasma fibrinogen level, and environmental factors might affect the fibrinogen levels, these factors might also affect the response to genetic predisposition to higher levels of plasma fibrinogen.

Factor VII is a clotting factor where the highest levels are commonly associated with a raised risk of AMI and other coronary syndromes (22,24). The factor VII levels are influenced by genetic and environmental factors. Previous studies have demonstrated a positive association with two variants, the R353Q and the insertion/deletion in the –323 promoter region of factor VII. The polymorphisms R353Q and insertion/deletion are associated with factor VII levels through the control of expression of fVII gene as shown in several studies (25,33,73) and consequently associated with the AMI risk. In the present study, we did not find differences in allele frequencies between AMI patients and control subjects for the R353Q and insertion/deletion polymorphisms in the factor VII gene. Our results confirm those obtained by other authors (29,30,73-76). Cai *et al*. (77) did not find an association with the R353Q polymorphism in Chinese AMI subjects, although the factor VII levels had been related to genotypes and to AMI risk. In contrast, Iacoviello *et al*. (27) and Girelli *et al*. (78) found a significant influence of the R353Q in the risk of AMI. Di Castelnuovo *et al*. (34) also found a positive association between the insertion/deletion polymorphism and the risk of AMI before the age of 45 years. We conclude that the polymorphisms R353Q and insertion/deletion in the factor VII gene despite a potential genetic predisposition to increase in factor VII levels are not associated to the risk of AMI.

Factor V Leiden is a common mutation caused by a G/A substitution that results in a factor V less efficiently inactivated by activated C protein (38). The fV Leiden is the most common risk factor to venous thrombosis but its role in arterial thrombosis remains unclear. Some studies have reported positive associations of fV Leiden and AMI (29,79) but others have not found any association (43,80,81). The G20210A variant of prothrombin is located in the 3´unstranslated region and it is associated with the plasma prothrombin levels (46) and was found a risk factor for venous thrombosis by Simioni *et al*. (47). Doggen *et al*. (29) and Rosendaal *et al*. (40) observed positive associations of this variant and the risk of AMI. Results of previous studies are controversial, some authors reported positive associations

(29,82,83) and others did not (51,84). In the present case-control study, we did not find any association between the fV Leiden and G20210A prothrombin variants and the risk of AMI. Probably, our results are not definitive because of the low frequencies of these mutations observed in our population. As these variants are not common, association studies need to be larger to detect possible relations. These variants might also be associated with the other major coronary risk factors, and thus increase the risk of AMI.

In conclusion, we suggested that the G-455A and *Taq*I of fibrinogen, R353Q and insertion/deletion of fVII, and fV Leiden and G20210A prothrombin variants were not associated with increased risk of AMI in our case-control study.

References

1. Meade TW, Brozovic M, Chakrabarti RR, Haines AP, Imeson JD, Mellows S, Miller GJ, North WRS, Stirling Y and Thompson SG. Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. Lancet 1986; ii:533-537.

2. Fulton RM & Duckett K. Plasma-fibrinogen and thromboemboli after myocardial infarction. Lancet 1976; 27:1161-1164.

3. Benderly M, Graff E, Reicher-Reiss H, Behar S, Brunner D and Gouldbourt U. Fibrinogen is a predictor of mortality in coronary heart disease patients. Arteriosclerosis, Thrombosis, and Vascular Biology 1996; 16:351-56.

4. de Maat MPM, Pietersma A, Kofflard M, Sluiter W and Kluft C. Association of plasma fibrinogen levels with coronary artery disease, smoking and inflammatory markers. Atherosclerosis 1996; 121:185-191.

5. Salonen JT, Nyssönen K, Seppänen K, Salonen R, Lynch J and Kaplan GA. Plasma fibrinogen and wall thickness in carotid and femoral arteries: findings from the KIHD Study. Fibrinolysis & Proteolysis 1997; 11(Suppl 1):35-40.

6. Woodward M, Lowe GDO, Rumley A and Tunstall-Pedoe H. Fibrinogen as a risk factor for coronary heart disease and mortality in middle-aged men and women. The Scottish Heart Health Study. European Heart Journal 1998; 19:55-62.

7. Kamphuisen PW, Eikenboom JCJ, Vos HL, Pablo R, Sturk A, Bertina RM and Rosendaal FR. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. Thrombosis and Haemostasis 1999; 81:680-3.

8. Baker IA, Pickering J, Elwood PC, Bayer A and Ebrahim S. Fibrinogen, viscosity and white blood cell count predict myocardial, but not cerebral infarction: Evidence from the Caerphilly and Speedwell cohort. Thrombosis and Haemostasis 2002; 87:421-5.

9. Reganon E, Vila V, Ferrando F, Martínez-Sales V, Fayos L, Ruano M and Aznar J. Elevated high molecular weight fibrinogen in plasma is predictive of coronary ischemic events after acute myocardial infarction. Thrombosis and Haemostasis 1999; 82:1403-5.

10. Thomas A, Lamlum H, Humphries S and Green F. Linkage disequilibrium across the fibrinogen locus as shown by five genetic polymorphisms, G/A -455 (*Hae* III), C/T -148 (*Hind* III/*Alu* I), T/G +1689 (*Ava* I), and *Bcl* I (β-Fibrinogen) and *Taq* I (α-Fibrinogen), and their detection by PCR. Human Mutation 1994; 3:79-81.

11. Humphries SE. Genetic regulation of fibrinogen. European Heart Journal 1995; 16(Suppl A):16-20.

12. Iso H, Folsom AR, Winkelmann JC, Koike K, Harada S, Greenberg B, Sato S, Shimamoto T, Iida M and Komachi Y. Polymorphisms of the beta fibrinogen gene and plasma fibrinogen concentration in Caucasian and Japanese population samples. Thrombosis and Haemostasis 1995; 73(1):106-11.

13. Tybjærg-Hansen A, Agerholm-Larsen B, Humphries SE, Abildgaard S, Schnohr P and Nordestgaard BG. A common mutation (G-455A) in the β-fibrinogen promoter is a independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9,127 individuals based on the Copenhaghen City Heart Study. Journal of Clinical Investigation 1997; 99(12):3034-3039.

14. Margaglione M, Cappucci G, Colaizzo D, Pirro L, Vecchione G, Grandone E and di Minno G. Fibrinogen plasma levels in an apparently healthy general population-relation to environmental and genetic determinants. Thrombosis and Haemostasis 1998; 80:805-10.

15. de Maat MPM, Kastelein JJP, Jukema JW, Zwinderman AH, Jansen H, Groenemeier B, Bruschke AVG and Kluft C. -455G/A polymorphism of the β-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men. Proposed role of an acute-phase reaction pattern of fibrinogen. Arteriosclerosis, Thrombosis, and Vascular Biology 1998; 18:265-271.

16. Brull DJ, Dhamrait S, Moulding R, Rumley A, Lowe GDO, World MJ, Humphries SE and Montgomery HE. The effect of fibrinogen genotype on fibrinogen levels after stenuous physical exercise. Thrombosis and Haemostasis 2002; 87:37-41.

17. Behague I, Poirier O, Nicaud V, Evans A, Arveiler D, Luc G, Cambou JP, Scarabin P-Y, Bara L, Green F and Cambien F. β Fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM Study. Circulation 1996; 93(3):440-449.

18. Leander K, Hallqvist J, Wiman B and de Faire U. The importance of the G/A-455 polymorphism of the β-fibrinogen gene in relation to myocardial infarction. Annals of XIIth International Symposium on Atherosclerosis 2000; TuP4:W15. Abstract.

19. Connor JM, Fowkes FGR, Wood J, Smith FB, Donnan PT and Lowe GDO. Genetic variation at fibrinogen loci and plasma fibrinogen levels. Journal of Medical Genetics 1992; 29:480-82.

20. Väisänen S, Rauramaa R, Penttilä I, Rankinen T, Gagnon J, Pérusse L, Chagnon M and Bouchard C. Variation in plasma fibrinogen over one year: relationship with genetic polymorphisms and non-genetic factors. Thrombosis and Haemostasis 1997; 77(5):884-9.

21. Heinrich J, Balleisen L, Schulte H, Assman G and van de Loo J. Fibrinogen and factor VII in the prediction of coronary risk: results from the PROCAM Study in healthy men. Arteriosclerosis and Thrombosis 1994; 14:54-59.

22. Junker R, Heinrich J, Schulte H, van de Loo J and Assmann G. Coagulation factor VII and the risk of coronary heart disease in healthy men. Arteriosclerosis, Thrombosis, and Vascular Biology 1997; 17:1539-1544.

23. Noto D, Barbagallo CM, Cefalu AB, Cavera G, Sapienza M, Notarbartolo A, Davi G and Averna MR. Factor VII activity is an independent predictor of cardiovascular mortality in elderly women of a Sicilian population: results of an 11-year follow-up. Thrombosis and Haemostasis 2002; 87:206-10.

24. Folsom A, Wu K, Rosamond W, Sharrett A and Chambless L. Prospective study of hemostatic factors and incidence of coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) Study. Circulation 1997; 96:1102-1108.

25. Hunault M, Arbini AA, Lopaciuk S, Carew JA and Bauer KA. The $Arg^{353}Gln$ polymorphism reduces the level of coagulation factor VII. In vivo and in vitro studies. Arteriosclerosis, Thrombosis, and Vascular Biology 1997; 17:2825-2829.

26. Heywood DM, Carter A, Catto AJ, Bamford JM and Grant PJ. Polymorphisms of the factor VII gene and circulating FVII:C levels in relation to acute cardiovascular disease and poststroke mortality. Stroke 1997; 28(4):816-821.

27. Iacoviello L, di Castelnuovo, de Knijff P, D'Orazio A, Amore C, Arboretti R, Kluft C and Donati MB. Polymorphisms in the coagulation factor VII gene and the risk of myocardial infarction. New England Journal of Medicine 1998; 338(2):79-85.

28. Holm J, Hillarp A, Zöller B, Erhardt L, Berntorp E and Dahlbäck B. Factor V Q506 (resistance to activated protein C) and prognosis after acute coronary syndrome. Thrombosis and Haemostasis 1999; 81:857-60.

29. Doggen CJM, Cats M, Bertina RM, Reitsma PH, Vandenbroucke JP and Rosendaal FR. A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. Thrombosis and Haemostasis 1998; 80:281-5.

30. Batalla A, Alvarez R, Reguero Jr, González P, Alvarez V, Cubero Gi, Cortina A and Coto E. Lack of association between polymorphisms of the coagulation factor VII and myocardial infarction in middle-aged Spanish men. International Journal of Cardiology 2001; 80:209-212.

31. Kain K, Catto AJ, Young J, Bamford J, Bavington J and Grant P. Coagulation factor VII activity, Arg/Gln353 polymorphism and features of insulin resistance in first-degree-relatives of south Asian patients with stroke. Thrombosis and Haemostasis 2002; 88:954-60.

32. Pollak ES, Hung H-L, Godin W, Overton GC and High KA. Functional characterization of the human factor VII 5'-flanking region. Journal of Biological Chemistry 1996; 271(3):1738-47.

33. di Castelnuovo A, D'Orazio A, Amore C, Falanga A, Kluft C, Donati MB and Iacoviello L. Genetic modulation of coagulation factor VII plasma levels: contribution of different polymorphisms and gender-related effects. Thrombosis and Haemostasis 1998; 80:592-7.

34. di Castelnuovo A, D'Orazio A, Amore C, Falanga A, Donati MB and Iacoviello L. Tthe decanucleotide insertion/deletion polymorphism in the promoter region of the coagulation factor VII gene and risk of familial myocardial infarction. Thrombosis Research 2000; 98:9-17.

35. Heywood DM, Mansfield MW and Grant PJ. Factor VII gene polymorphisms, factor VII:C levels and features of insulin resistance in Non-Insulin-Dependent Diabetes mellitus. Thrombosis and Haemostasis 1996; 75(3):401-6.

36. Lievers KJA, Mennen LI, Rattink AP, Zwinderman AH, Jukema JW, Schouten EG and de Maat MPM. The –323Ins10 polymorphism for factor VII is not associated with coronary atherosclerosis in symptomatic men. Thrombosis Research 2000; 97:275-280.

37. Bertina RM, Koleman BPC, Koster T, Rosendaal FR, Dirven RJ, de Ronde H, van der Velden P and Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994; 369:64-67.

38. Dahlbäck B. Resistance to activated protein C caused by the factor V R506Q mutation is a common risk factor for venous thrombosis. Thrombosis and Haemostasis 1997; 78:483-8.

39. Holm J, Zöller B, Berntorp E, Erhardt L and Dahlbäck B. Prevalence of factor V gene mutation among myocardial infarction patients and health controls is higher in Sweden than in other countries. Journal of Internal Medicine 1996; 239:221-26.

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

41. Inbal A, Freimark D, Modan B, Chetrit A, Matetzky S, Rosenberg N, Dardik R, Baron Z and Seligsohn U. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. Blood 1999; 93(7):2186-2190.

42. Navabi DG, Junker R and Wolff E. Prevalence of factor V Leiden mutation in young adults with cerebral ischaemia: a case-control study on 225 patients. Journal of Neurology 1998; 245:653-658.

43. Junker R, Heinrich J, Schulte H, Tataru M, Köhler E, Schönfeld R, Nowak-Göttl U and Assmann G. Plasminogen activator inhibitor-1 4G/5G-polymorphism and factor V Q506 mutation are not associated with myocardial infarction in young men. Blood Coagulation and Fibrinolysis 1998; 9:597-02.

44. Juul K, Tybjærg-Hansen A, Steffensen R, Kofoed S, Jensen G and Nordestgaard BG. Factor V Leiden: The Copenhagen City Heart Study and 2 meta-analyses. Blood 2002; 100(1):3-10.

45. Akar N, Akar E, Deda G, Sipahi T and Orsal A. Factor V1691 G-A, prothrombin 20210 G-A, and methylenetetrahydrofolate reductase 677 C-T variants in Turkish children with cerebral infarct. Journal of Child Neurology 1999; 14(11):749-751.

46. Poort S, Rosendaal F, Reitsma PH and Bertina R. A common genetic variation in the 3'-unstranslated region of the protrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996; 88:3698-3703.

47. Simioni P, Tormene D, Manfrin D, Gavasso S, Luni S, Stocco D and Girolami A. Prothrombin antigen levels in symptomatic and asymptomatic carriers of the 20210A prothrombin variant. British Journal of Haematology 1998; 103:1045-1050.

48. Watzke HH, Schuttrumpf J, Graf S, Huber K and Panzer S. Increased prevalence of a polymorphism in the gene coding for human prothrombin in patients with coronary artery disease. Thrombosis Research 1997; 87(6):521-6.

49. Prohaska W, Schmidt M, Mannebach H, Gleichmann U and Kleesiek K. The prevalence of the prothrombin 20210 G-A mutation is not increased in angiographically confirmed coronary artery disease. Thrombosis and Haemostasis 1999; 81:161-2.

50. Mikkelsson J & Karhunem P. Genetic variation in coagulation factors II, V, VII in fatal MI. Thrombosis and Haemostasis 2002; 87:349-50.

51. Durante-Mangoni S, Davies GJ, Ahmed N, Ruggiero G and Michaelides K. The prothrombin G20210A polymorphism in patients with myocardial infarction. Blood Coagulation and Fibrinolysis 2002; 13(7):603-8.

52. Arruda VR, Annichino-Bizzacchi JM, Gonçalves MS and Costa FF. Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. Thrombosis and Haemostasis 1997; 78:1430-3.

53. Arruda VR, Siquiera LH, Chiaparini LC, Coelho OR, Mansur AP, Ramires A and Annichino-Bizzacchi JM. Prevalence of the prothrombin gene variant 20210G-A among patients with myocardial infarction. Cardiovascular Research 1998; 37:42-5.

54. Longstreth WT, Rosendaal FR, Siscovick DS, Vos HL, Schwartz SM, Psaty BM, Raghunathan TE, Koepsell TD and Reitsma PH. Risk of stroke in young women and two prothrombotic mutations: factor V Leiden and prothrombin gene variant (G20210A). Stroke 1998; 29: 577-580.

55. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bulletin of the World Health Organization – L'Organisation Mondiale de La Santé 1962; 27:645-658.

56. Lahiri DK & Nurnberger Jr. JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 1991; 19(19):5444.

57. Thomas A, Green F, Kelleher C, Wilkes H, Brennan P, Meade T and Humphries S. Variation in the promoter region of the β fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. Thrombosis and Haemostasis 1991; 65(5):487-490.

58. Humphries S, Temple A, Lane A, Green F, Cooper J and Miller G. Low plasma levels of factor VIIc and antigen are more strongly associated with the 10 base pair promoter (-323) insertion than the glutamine 353 variant. Thrombosis and Haemostasis 1996; 75(4):567-72.

59. Luc G, Bard JM, Juhan-Vague I, Ferrieres J, Evans A, Amouyel P, Arveiler D, Frucha JC and Ducimetiere P. C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. Arteriosclerosis, Thrombosis, and Vascular Biology 2003; 23(7):1255-61.

60. Ross R. Atherosclerosis – an inflammatory disease. New England Journal of Medicine 1999; 340(2):115-126.

61. Vasse M, Paysant J, Soria J, Collet, J-P, Vannier J-P and Soria C. Regulation of fibrinogen biosynthesis by cytokines, consequences on the vascular risk. Haemostasis 1996; 26(Suppl 4):331-339.

62. Olinescu RM & Kummerow FA. Fibrinogen is an efficient antioxidant. Journal of Nutritional Biochemistry 2001; 12:162-169.

63. de Maat MPM. Effects of diet, drugs, and genes on plasma fibrinogen levels. Annals of New York Academy of Sciences 2001; 936:509-521.

64. Muscari A, Bastagli L, Poggiopollini G, Tomassetti V, Massarellli G, Cappelletti O, Platè L, Boni P and Puddu P. Different associations of C-reactive protein, fibrinogen and C3 with traditional risk factors in middle-aged men. International Journal of Cardiology 2002; 83:63-71.

65. Folsom AR, Pankow JS, Williams RR, Evans GW, Province MA and Eckfeldt JH. Fibrinogen, plasminogen activator inhibitor-1, and carotid intima-media wall thickness in the NHLBI Family Heart Study. Thrombosis and Haemostasis 1998; 79:400-4.

66. Heinrich J, Funke H, Rust S, Schulte H, Schönfeld R, Köhler E and Assmann G. Impact of polymorphisms in the alpha- and beta-fibrinogen gene on plasma fibrinogen concentrations of coronary heart disease patients. Thrombosis Research 1995; 77(3):209-215.

67. Yu S, Sher B, Kudryk B and Redman CM. Intracellular assembly of human fibrinogen. Journal of Biological Chemistry 1984, 259:10574-81.

68. Brown ET & Fuller GM. Detection of a complex that associates with the Bβ fibrinogen G-455A polymorphism. Blood 1998; 92(9):3286-3293.

69. Green F, Hamsten A, Blombäck M and Humphries S. The role of β-fibrinogen genotype in determining plasma fibrinogen levels in young survivors of myocardial infarction and healthy controls from Sweden. Thrombosis and Haemostasis 1993; 70(6):915-920.

70. van der Bom JG, de Maat MPM, Bots ML, Haverkate F, de Jong PTVM, Hofman A, Kluft C and Grobbee DK. Elevated plasma fibrinogen cause or consequence of cardiovascular disease? Arteriosclerosis, Thrombosis, and Vascular Biology 1998; 18:621-625.

71. Lee AJ, Lowe GDO, Woodward M and Tunstall-Pedoe H. Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary artery disease, and family history: Scottish Heart Health Study. British Heart Journal 1993; 69:338-342.

72. Doggen CJM, Bertina RM, Cats VM and Rosendaal FR. Fibrinogen polymorphisms are not associated with the risk of myocardial infarction. British Journal of Haematology 2000; 110:935-938.

73. Lane A, Green F, Scarabin PY, Nicaud V, Bara L, Humphries S, Evans A, Luc G, Cambou JP, Arveiler D and Cambien F. Factor VII Arg/Gln_{353} polymorphism determines factor VII coagulant 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; 119:119-127.

74. Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagliabue L, Tubaro M, Galvani M, Ottani F, Ferrario M, Corral J and Margaglione M. Prothrombotic genetic risk factors in young survivors of myocardial infarction Blood 1999; 94(1):46-51.

75. Kakko S, Elo T, Tapanaimen JM, Huikuri HV and Savolainen MJ. Polymorphisms of genes affecting thrombosis and risk of myocardial infarction. European Journal of Clinical Investigation 2002; 32(9):643-8.

76. Shimokata K, Kondo T, Ohno M, Takeshita K, Inden Y, Iino S, Saito H and Hirai M. Effects of coagulation factor VII polymorphisms on the coronary artery disease in Japanese. Thrombosis Research 2002; 105:493-498.

77. Cai Q, Chen J, Ma H, Song J and Xu M. Association of coagulation factor VII with risk of myocardial infarction in the Chinese. Chinese Medical Journal 2000; 113(12):1059-63.

78. Girelli D, Russo C, Ferraresi P, Olivieri O, Pinotti M, Friso S, Manzato F, Mazzucco A, Bernardi F and Corrocher R. Polymorphisms in the factor VII gene and risk of myocardial infarction in patients with coronary artery disease. New England Journal of Medicine 2000343:774-780.

79. Baranovskaya S, Kudinov S, Fomicheva E, Vasina V, Solovieva D, Khavinson V and Schwartz E. Age as a risk factor for myocardial infarction in Leiden mutation carriers. Molecular Genetics and Metabolism 1998; 63:155-157.

80. Roest M, Banga JD, Tempelman MJ, de Groot PG, Grobbee DE, Sixma JJ and van der Schouw YT. Factor V Arg506Gln mutation is not associated with cardiovascular mortality in older women. American Journal of Epidemiology 1999; 149(7):665-670.

81. Gowda MS, Zucker ML, Vacek JL, Carriger WL, van Laeys DL, Rachel JM and Strope BD. Incidence of factor V Leiden in patients with acute myocardial infarction. Journal of Thrombosis and Thrombolysis 2000; 9(1):43-5.

82. Gerdes VE, Cate H, de Groot E, Kwa VI, Prins MH, Reitsma PH, Buller HR and Brandjes DP. Arterial wall thickness and risk of recurrent ischemic events in carries of the prothrombin G20210A mutation with clinical manifestations of atherosclerosis. Atherosclerosis 2002; 163:135-40.

83. Burzotta F, Paciaroni K, de Stefano V, Chiusolo P, Manzoli A, Casolrelli I, Leone AM, Rossi E, Leone G, Maseri A and Andreotti F. Increased prevalence of the G20210A prothrombin gene variant in acute coronary syndromes without metabolic or acquired risk factors or with limited extent of disease. European Heart Journal 2002; 23(1):26-30.

84. Russo C, Girelli D, Olivieri O, Guarini P, Manzatto F, Pizzolo F, Zaia B, Mazzucco A and Corrocher R. G20210A prothrombin gene polymorphism and prothrombin activity in subjects with or without angiographically documented coronary artery disease. Circulation 2001; 103:2436-2440.

Table 1. Characteristics of AMI patients and control subjects.

Characteristic	AMI Patients (n=283)	Control Subjects (n=96)	P-value
Age	50.4 ± 6.4	50.4 ± 7.2	0.980
Male sex	82%	75%	0.180
Smoking status	18%	18%	1.000
Non-O blood group	61%	42%	0.002
Hyperlipidemia	62%	36.5%	<0.001
Diabetes status	26%	10.5%	0.004
Hypertension	61%	34%	<0.001
Family history	59%	40%	0.001
Fibrinogen (mg/dl)	299.8 ± 55.7	281.8 ± 46.1	0.006
C-reactive protein	0.26 (0.13 - 0.61)	0.18 (0.08 - 0.34)	0.006

Student-t test for continuous variables and qui-square for discrete variables were used to compare the values. Ages are presented as mean ± SD. For the AMI patients the value refers to the age at onset of myocardial infarction, not the current age. C-reactive protein values are expressed as median, 25th and 75th percentiles.

Table 2. Plasma fibrinogen levels according to G-455A and *Taq*I polymorphisms.

Genotypes	All Subjects	AMI Patients	Control Subjects
G-455A			
A/A	316.4 ± 54.7	331.3 ± 56.4	281.6 ± 36.9
A/G	307.4 ± 58.5	314.8 ± 59.8	279.9 ± 44.8
G/G	287.6 ± 49.9	289.4 ± 50.7	282.5 ± 47.6
	P=0.003	P=0.001	P=0.974
*Taq*I			
A1/A1	291.4 ± 54.4	285.0 ± 54.2	304.1 ± 55.6
A1/A2	295.6 ± 57.3	299.3 ± 57.0	281.3 ± 57.3
A2/A2	296.9 ± 52.5	302.7 ± 55.2	278.1 ± 37.1
	P= 0.880	P=0.432	P=0.318

P-value of ANOVA comparing fibrinogen levels between three genotypes of G-455A and *Taq*I polymorphisms.

Table 3. Allele and genotype frequencies of G-455A and *Taq*I of fibrinogen gene and R353Q and I/D polymorphisms of factor VII in patient and control subjects.

Polymorphism	AMI Patients			Control Subjects			P-value
G-455A	A/A	A/G	G/G	A/A	A/G	G/G	
N (%)	8 (3%)	85 (32%)	173 (65%)	4 (4%)	25 (26%)	66 (70%)	
A allele		0.19			0.17		0.70
TaqI	A1/A1	A1/A2	A2/A2	A1/A1	A1/A2	A2/A2	
N (%)	19 (7%)	95 (34%)	164 (59%)	9 (10%)	25 (26%)	60 (64%)	
A1 allele		0.24			0.23		0.85
R353Q	Q/Q	Q/R	R/R	Q/Q	Q/R	R/R	
N (%)	7 (3%)	59 (21%)	211 (76%)	2 (2%)	22 (23%)	71 (75%)	
353Q allele		0.13			0.14		0.96
I/D	I/I	I/D	D/D	I/I	I/D	D/D	
N (%)	14 (5%)	71 (26%)	193 (69%)	2 (2%)	28 (30%)	63 (68%)	
I allele		0.18			0.17		0.94

capítulo 4

Association Studies of Tissue-Type Plasminogen Activator Insertion/ Deletion and Plasminogen Activator Inhibitor-1 4G/5G Polymorphisms with Premature Acute Myocardial Infarction

Capítulo 4

Associations Studies of Tissue-Type Plasminogen Activator Insertion/ Deletion and Plasminogen Activator Inhibitor-1 4G/5G Polymorphisms with Premature Acute Myocardial Infarction

Crislaine A. Paludo[1], Josi Vidart[2], Vera L. Portal[2] and Israel Roisenberg[1]

[1]Departamento de Genética, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, [2]Serviço de Epidemiologia, Instituto de Cardiologia do Rio Grande do Sul, Porto Alegre, RS, Brazil.

Corresponding author:

Israel Roisenberg
Departamento de Genética
Instituto de Biociências
Universidade Federal do Rio Grande do Sul
Caixa Postal 15053
91.501-970 Porto Alegre, RS
Brazil
FAX n° + 55 51 3316 7311
Phone n° + 55 51 3316 6728
e-mail: israberg@ufrgs.br

Abstract

Pathophysiological alterations of proteins participating in the fibrinolytic pathway have been investigated in etiological studies related to acute myocardial infarction (AMI) risk. The tissue-type plasminogen activator (t-PA) is the main endogenous fibrinolytic enzyme. Prospective studies indicate that elevated endogenous t-PA levels predict the risk of AMI. An insertion (I)/deletion (D) polymorphism in the t-PA gene has been associated with the risk of AMI. Plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of t-PA, and high plasma levels of PAI-1 could be associated to AMI by an impaired fibrinolysis. The 4G/5G polymorphism has been associated with the plasma levels of PAI-1 by a regulation of expression of the PAI-1 gene. We conducted a case-control study of 283 AMI cases and 96 control subjects, all aged ≤ 60 years to investigate the association of t-PA I/D and PAI-1 4G/5G polymorphisms with the risk of AMI. In the present study, we did not find difference in 4G allele frequency of the 4G/5G variant of PAI-1 between AMI patients and control subjects. The I allele frequency of the t-PA I/D variant was not different between patients and control subjects. However, we detected a significant increase in the I allele frequency among the AMI patients under the age of 45 years, when compared to control subjects with the same age. We did not find difference in frequencies of the t-PA I and PAI-1 4G alleles according to the number of diseased vessels or number of AMI suffered. We conclude that in this study the PAI-1 4G/5G polymorphism was not associated with the risk of AMI and we suggested that the t-PA insertion/deletion variant contributes to AMI only in subjects under the age of 45 years. Thus, at a younger age the t-PA I/D might be of importance in AMI.

Introduction

Epidemiological studies have shown that a decreased fibrinolytic activity has been associated with first and recurring acute myocardial infarction (AMI), and the use of fibrinolytic agents has become standard treatment for MI. The fibrinolytic system is responsible for the dissolution of a fibrin clot through the conversion of proenzyme plasminogen into the active protease plasmin by the endogenous tissue-type plasminogen activator (t-PA), which is synthesised by endothelial cells. The plasminogen activator inhibitor-1 (PAI-1) is the major inhibitor of t-PA and data indicated that reduced fibrinolytic activity due to increased PAI-1 plasma levels predispose to recurrent myocardial infarction (1).

The t-PA is the main endogenous fibrinolytic enzyme (2,3). High levels of t-PA are indicative of an activation of the fibrinolytic system. Some studies have shown an association between t-PA activity and risk of AMI (4,5). Other studies have found similar associations with stroke (6,7) and venous thromboembolism (8). Prospective data suggested that t-PA concentration may be a marker of risk for future AMI (5).

An insertion (I)/deletion (D) type polymorphism due an *Alu* repeat sequence in intron h of the human t-PA gene has been associated with the plasma levels of t-PA (9). Thus, this polymorphism would represent a risk factor for AMI with potentially important pathophysiological and therapeutic implications. Both I/I genotype of t-PA I/D polymorphism and increased t-PA levels were considered risk factor for AMI (10), however no association between the t-PA levels and genotype was observed. In contrast, other studies found no association between the t-PA I/D polymorphism and AMI (8,11,12).

The PAI-1 acts in the regulation of fibrinolysis and is an acute phase reactant (13). PAI-1 plasma levels are associated with the 4G/5G insertion/deletion polymorphism located in the promoter region roughly 675bp upstream from the transcriptional start site of the PAI-1 gene (14). Reports have suggested that the 5G allele but not the 4G allele contain an additional binding site for a DNA-binding protein that could be important as a transcriptional repressor. An association between deep vein thrombosis and the 4G/5G polymorphism has been reported (15). The 4G allele of 4G/5G polymorphism was associated with a family history of coronary artery disease (16), with CAD (17), with progression of coronary syndromes in patients with atherosclerosis (18), coronary thrombosis and AMI among middle-aged men who had died suddenly (19). However, the 4G/5G polymorphism was not associated with an increased risk of stroke (6,20,21).

In this study we investigated the polymorphisms of insertion/deletion of t-PA gene

and 4G/5G of PAI-1 gene in Brazilian Caucasians with AMI below the age of 60 years compared to control subjects.

Methods

We conducted a case-control study of 283 Brazilian Caucasian patients that suffered acute myocardial infarction before the age of 60 years and 96 control subjects. The patients were recruited from the Instituto de Cardiologia do Rio Grande do Sul (Rio Grande do Sul, Brazil) over a period of 18 months (from August of 2001 to April of 2003). Subjects who had had an AMI in the 90 days before the investigation were excluded from this study. The patients were submitted to previous angiography. The control subjects consisted of men and women from the same ethnic group as the group of cases, participants in preventive campaigns from the above mentioned Institution.

The control subjects were availed by the modified ROSE questionnaire (22). None of the control subjects had a history or clinical evidence of angina pectoris or AMI. Patients and control subjects were from independent families. Data of personal and family history for cardiovascular disease and acquired risk factors were obtained from all patients, and additional information was obtained from medical records. The acute myocardial infarction was defined if at least two of the following criteria were present: characteristic chest pain, elevated cardiac enzymes and ECG changes indicative of AMI. The case and control groups did not differ significantly according to age (student t-test: $p=0.98$) or sex (chi-square test: $p=0.18$). This study was approved by the local Ethics Committee and all the subjects gave their written informed consent.

Smoking status was recorded as non-smoker or smoker (more than 10 cigarrette/day in last five years), diabetes mellitus was defined as present if previously diagnosed or treated. Hypertension was defined as documented increase of blood pressure (>140/90 mmHg) requiring therapy. Family history of coronary artery disease was defined if found in at least one consanguineous relative. Body mass index was measured according to the equation weight/height^2. Dysplipidemia was defined by levels of triglycerides > 150 mg/dl, HDL-cholesterol < 40 mg/dl and LDL-cholesterol > 100 mg/dl or if the individual was receiving lipid-lowering treatment at inclusion. Blood samples were collected from all the patients after 12 hours-fasting state.

High molecular weight DNA was isolated from whole blood using a non-enzymatic technique (23) for DNA analysis. The polymorphisms were identified by using polymerase chain reaction (PCR) and direct agarose gel electrophoresis containing ethidium bromide,

and visualised under ultraviolet light. The t-PA I/D polymorphism was identified as previously reported (9). Specific primers for the 4G allele or the 5G allele were used for the identification of PAI-1 4G/5G polymorphism (24).

Allele frequencies were determined by direct count of the alleles. Departures from the Hardy-Weinberg equilibrium and differences between groups were evaluated by the chi-square test. A P value ≤ 0.05 was considered to indicate statistical significance. Except for analysis of genotype distribution, heterozygous and homozygous were combined and compared with non-carriers of the risk alleles. Categorical variables were compared by using the Pearson chi-square test. The Student t-test compared means in the comparisons between 2 groups. Baseline characteristics were expressed as means $\pm$ standard deviations or percentages. A logistic regression model was used to control metabolic risk factors. Statistical analysis was performed with the SPSS program 11 for windows (SPSS Inc. Illinois).

Results

The clinical characteristics of patients and control subjects are shown in Table 1. There was no significant difference in age and sex between groups. As expected, recognised risk factors for CAD (hyperlipidemia, diabetes mellitus, hypertension, and family history) were significantly more frequent among AMI patients than among the control subjects. The non-O blood group was significantly more prevalent in AMI cases than in control subjects (p=0.002).

The distribution of genotypes and frequencies of alleles between case and control subjects are summarised in Table 2. The t-PA I allele frequency was 0.54 in patients and 0.47 in controls (p=0.10). In the AMI group, the frequencies of I/I, I/D and D/D genotypes were: 31%, 47% and 22%, respectively. In the control group, the frequencies of I/I, I/D and D/D genotypes were: 23%, 48% and 29%, respectively. In each group, the genotype frequencies were in agreement with that predicted by the Hardy-Weinberg equilibrium. The I/I + I/D genotypes were more prevalent in patients (78%) than in control subjects (72%) (p=0.21). The homozygosity for the I allele was not associated to AMI risk when compared with those homozygous for the D allele (p=0.13).

The 4G allele frequency was 0.44 in patients and 0.48 in controls (p=0.39). In the AMI group, the frequencies of 4G/4G, 4G/5G and 5G/5G genotypes were: 19%, 51% and 30%, respectively. In the control group, the frequencies of 4G/4G, 4G/5G and 5G/5G genotypes were: 21%, 54% and 25%, respectively. For both groups, the genotype frequencies were in agreement with that predicted by the Hardy-Weinberg equilibrium. The

distribution of 4G/4G + 4G/5G genotypes was 69% in AMI patients and 73% in controls (p=0.44). The homozygosity for the 4G allele was not associated with an increased risk of AMI when compared with those homozygous for the 5G allele (p=0.45).

We found no significant association between the t-PA and PAI-1 polymorphisms and the number of vessels affected in the AMI group: 82% of t-PA I allele carriers had ≥ 3 diseased vessels and 78% had ≤ 2 diseased vessels (p=0.64). When carriers of 4G allele of 4G/5G polymorphism were availed for the number of diseased vessels, 60% of the subjects had ≥ 3 and 73% had ≤ 2 diseased vessels (p=0.14). We did not find differences between cases suffering one AMI when compared with subjects with more than one AMI for the t-PA polymorphism: 79% of carriers of I allele had one AMI and 78% of carriers had more than one AMI (p=1.00). In analysis of PAI-1 polymorphism, 69% of carriers of 4G allele had one AMI and 72% of carriers had more than one AMI (p=0.81).

The prevalence of carriers of the PAI-1 4G allele was similar between case and control subjects indifferent to number of risk factors. When individuals were divided because of the number of risk factors, the frequency of carriers of t-PA I allele was not significantly different between case and control subjects (73% vs 73%, p=0.81) in carriers of more than 2 risk factors. Also for the analysis of 4G/5G variant we did not find difference between cases and controls for carriers of 4G allele (70% vs 76%, p=0.62) for subjects with more than 2 risk factors.

We investigated the association of t-PA and PAI-1 polymorphisms in relation to AMI risk in a subgroup of individuals with age ≤ 45 years. We found a significant association between t-PA polymorphism in relation to risk of AMI. The t-PA I allele frequency was 0.55 in AMI patients and 0.37 in control subjects (p=0.04). However, for the PAI-1 variant, the 4G allele frequency was 0.48 in case and 0.45 in control subjects (p=0.25).

Discussion

The present study indicates that the t-PA I/D and PAI-1 4G/5G polymorphisms were not associated with the risk of acute myocardial infarction in Brazilian Caucasians before the age of 60 years. However, we found a trend towards a higher prevalence of the t-PA I/I genotype in AMI cases than in control subjects. When subjects ≤ 45 years were evaluated, the I allele of t-PA I/D variant was significantly associated with AMI.

Tissue plasminogen activator (t-PA) is the major endogenous activator of the fibrinolytic system and high levels of t-PA have been associated with increased risk of AMI

(4,5). Plasminogen activator inhibitor-1 (PAI-1) is the main inhibitor of t-PA and high levels of PAI-1 have been associated with a decreased fibrinolytic activity. Patients with previous episodes of thrombosis have low systemic fibrinolytic activity and elevated protein concentrations of t-PA and PAI-1 (1,25). The *Alu* repeat insertion/deletion polymorphism has been associated with the risk of venous and arterial thrombosis. There is evidence that circulating levels of PAI-1 may be under genetic control and the 4G/5G is the most investigated polymorphism because it is located in the promoter region of the PAI-1 gene, and it has been associated with the PAI-1 levels in several studies (16,26-28).

In the Physicians' Health Study, the t-PA I/D polymorphism has not been associated with the risk of myocardial infarction (11). In addition, this polymorphism is not a risk factor for MI as reported by Steeds *et al*. (12) neither in United Kingdom patients, nor in African-Americans (8). In this latter study, the genotypes were also unrelated to t-PA plasma levels.

In contrast, studies reported the association of t-PA I allele with an increased risk of coronary thrombosis and AMI (10). In the latter study an increased t-PA level was associated with an increased risk of AMI, however this association was not independent of other cardiovascular risk factors. Ridker *et al*. (5) reported that high levels of t-PA were associated with the three-fold risk of further AMI, but without evidence of association between the t-PA I/D polymorphism. Gram *et al*. (29) followed-up 29 patients with AMI for 4 years and found the highest concentrations of t-PA in plasma of patients who had had a reinfarction. In most of the studies the circulating levels of t-PA were measured in an asymptomatic period, this fact might not reflect the fibrinolytic capacity at the moment and site of thrombus formation. Differences might be due to the design of studies. Our study restricted the analysis to subjects under the age of 60 and a subgroup with $\leq$ 45 years, to search for the effects of genetic factors. Caucasian subjects were studied in order to control the ethnic differences in t-PA I/D distributions. The studies of Steeds *et al*. (12) used patients under 75 years and the study of van der Bom *et al*. (10), which found an independent association between the I allele and AMI, analysed patients with an mean age of 72 years, much higher than defined in our population. The I allele frequency found in our AMI patients was similar to those found in AMI cases by Ridker *et al*. (11) and Steeds *et al*. (12). However, the I allele frequency was lower in our control group (0.47) than among the controls studied by Steeds *et al*. (12) (0.58). This frequency found in control group was reduced when we avaliated only subjects younger than 45 years. When analysing a subgroup with the same age as our group, a trend towards association was observed (11). Population-specific factors may be responsible to the differences in results between our study and that by van der Bom *et al*. (10) which found a positive association between I allele and MI.

In our study, the PAI-1 4G allele was not associated with AMI independently of age at onset of AMI (≤ 60 or ≤ 45 years), which is confirmed by other studies (10,27,30,31). There were only few studies available concerning the effect of PAI-1 4G/5G in young AMI survivors, Eriksson *et al*. (14) included MI patients under 45 years and the frequency of 4G allele was 0.63 compared to 0.53 among control subjects. These frequencies were more elevated than those found in our population, even when only the subjects under the age of 45 years were avaliated. But in the Eriksson *et al*. (14) study the number of patients was rather small in comparison to our study. Contrasting results to previous studies were obtained by Hindorff *et al*. (32), which suggest a decreased risk of MI among young women carrying the 4G allele.

Various studies have demonstrated that the PAI-1 activity has an association with genotype of 4G/5G variant. The 4G/4G homozygous has higher PAI-1 activity in comparison with the 5G homozygous (27,28). Panahloo *et al*. (33) studied the 4G/5G and PAI-1 levels in AMI patients and found no evidence that 4G/4G genotype carriers have higher PAI-1 levels on admission to hospital or 6 months after AMI. The 4G/4G genotype was not associated with stroke before the age of 60 years (21). Other studies (5,19,34) have found an association between the 4G/4G genotype with myocardial infarction, in comparison with carriers of 5G allele. The genotype of the PAI-1 was related to the presence of CAD (17), and CAD in subjects with non-insulin-dependent diabetes mellitus (24), supporting the hypothesis that elevated levels of PAI-1 may play a role in the development of CAD.

Studies have supported that PAI-1 is produced at sites of atheromatous plaques (35) and the high PAI-1 levels in patients with coronary disease might be a result of the established disease and it does not have a causal role in atherosclerosis or AMI. The raised PAI-1 levels might act as a marker of a suppression of fibrinolytic system. Catto *et al*. (20) reported no association between 4G/5G and risk of stroke, but the PAI-1 levels were elevated at the time of acute stroke, probably as a result of an acute phase response. Elevated plasma PAI-1 levels have been shown to be associated with AMI (25) and a higher risk of reinfarction amongst survivors of AMI under 45 years of age (1). Also it is possible that the PAI-1 levels is a marker of the severity of the acute event. The PAI-1 4G/5G polymorphism was analysed in African-Americans for the risk of AMI and venous thromboembolism (8) and no association was found. Geographic differences in genotype distribution as well as prevalence of risk factors might contribute to variation in risk of MI in different populations. Recently, Yamada *et al*. (36) in an extensive study of gene candidates for AMI risk, found an association between 4G/5G polymorphism with AMI in women, but not in men.

In conclusion, in the present study, the PAI-1 4G/5G polymorphism was not associated with risk of AMI and the t-PA insertion/deletion variant was associated with AMI only in individuals under the age of 45 years. Thus, at a younger age the t-PA I/D might to be of importance in AMI.

References

1. Hamsten A, Walldius G, Szamosi M, Faire U, Dahlén G, Landou C and Wiman B. Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. Lancet 1987; vii(8549):3-8.

2. Vassali J-D, Sappino A-P and Belin D. The plasminogen activator/plasmin system. Journal of Clinical Investigation 1991; 88:1067-1072.

3. Collen D. The plasminogen (fibrinolytic) system. Thrombosis and Haemostasis 1999; 82(2):259-70.

4. Jansson JH, Nilsson TK and Olofsson BO. Tissue plasminogen activator and other risk factors as predictors of cardiovascular events in patients with severe angina pectoris. European Heart Journal 1991; 12:157-161.

5. Ridker PM, Vauguan DE, Stampfer MJ, Manson JE and Henneckens CH. Endogenous tissue-type plasminogen activator and risk of myocardial infarction. Lancet 1993; 341(8854):1165-68.

6. Lindgren A, Lindoff C, Norrving B, Astedt B and Johansson BB. Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. Stroke 1996; 27:1066-71.

7. Johansson L, Jansson J-H, Boman K, Nilsson TK, Stegmayr B and Hallmans G. Tissue plasminogen activator, plasminogen activator inhibitor-1, and tissue plasminogen activator/plasminogen activator inhibitor-1 complex as risk factors for the development of a first stroke. Stroke 2000; 31:26-32.

8. Hooper WC, Lally C, Austin H, Renshaw M, Dilley A, Wenger NK, Phillips DJ, Whitsett C, Rawlins P and Evatt BL. The role of the t-PA I/D and PAI-1 4G/5G polymorphisms in African-American adults with a diagnosis of myocardial infarction or venous thromboembolism. Thrombosis Research 2000; 99:223-230.

9. Ludwig M, Wohn KD, Scheuning WD and Olek K. Allelic dimorphism in the human tissue-type plasminogen activator (t-PA) gene as a result of an Alu insertion/deletion event. Human Genetics 1992; 88:288-392.

10. van der Bom J, de Knijff P, Haverkate F, Bots M, Meijer P, de Jong P, Hofman A, Kluft C and Grobbee D. Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. Circulation 1997; 95:2623-2627.

11. Ridker P, Hennekens C, Lindpaintner K, Stampfer M and Miletich J. Arterial and venous thrombosis in not associated with the 4G/5G polymorphism in the promoter of the plasminogen activator inhibitor gene in a large cohort of UK men. Circulation 1997; 95:59-62.

12. Steeds R, Adams M, Smith P, Channer K and Samani NJ. Distribution of tissue plasminogen activator insertion/deletion polymorphism in myocardial infarction and control subjects. Thrombosis and Haemostasis 1998; 79:980-4.

13. Páramo JA, Fernández FJ, Cuesta B, Hernández M, Paloma MJ, Rifón J and Roscha E. Concentraciones de la forma latente y activa del inhibidor del activator del plasminógeno en diversas situaciones clínicas. Revista Iberoamericana de Trombosis e Hemostasia 1989; 2:87-89.

14. Eriksson P, Kallin B, van't Hoof FM, Bavenholm P and Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. Proccedings of the National Academy Sciences of the USA 1995; 92:1851-55.

15. Stegnar M, Uhrin P, Peternal P, Mauri A, Salobir-Pajnic B, Stare J and Binder BR. The 4G/5G sequence polymorphism in the promoter of plasminogen activator inhibitor-1 (PAI-1) gene: relationship to plasma PAI-1 level in venous thromboembolism. Thrombosis and Haemostasis 1998; 79:975-9.

16. Margaglione M, Cappucci G, Colaizzo D, Giuliani N, Vecchione G, Grandone E, Pennelli O and di Minno G. The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. Arteriosclerosis, Thrombosis, and Vascular Biology 1998; 18: 152-156.

17. Gardemann A, Lohre J, Katz N, Tillmanns H, Hehrlein FW and Haberbosch W. The 4G/5G genotype of the plasminogen activator inhibitor-4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk fot this disease. Thrombosis and Haemostasis 1999; 82:1121-6.

18. Iwai N, Shimoike H, Nakamura Y, Tamaki S and 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; 136:109-114.

19. Mikkelson J, Perola M, Wartiovaara U, Peltonen L, Palotie A, Pentiilä A and Karhunen PJ. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G polymorphism, coronary thrombosis, and myocardial infarction in middle-aged Finnish men who died suddently. Thrombosis and Haemostasis 2000; 84:78-83.

20. Catto AJ, Carter AM, Stickland M, Bamford JM, Davies JA and Grant PJ. Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. Thrombosis and Haemostasis 1997; 77(4):730-4.

21. Endler G, Lalouschek W, Exner M, Mitterbauer G, Häring D and Mannhalter C. The 4G/5G genotype at nucleotide position –675 in the promotor region of the plasminogen activator inhibitor 1 (PAI-1) gene is less frequent in young patients with minor stroke than in controls. British Journal of Haematology 2000; 110:469-471.

22. Rose GA. The diagnosis of ischaemic heart pain and intermittent claudication in field surveys. Bulletin of the World Health Organization – L'Organisation Mondiale de La Santé 1962; 27:645-658.

23. Lahiri DK & Nurnberger Jr JI. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. Nucleic Acids Research 1991; 19(19):5444.

24. Mansfield MW, Stickland MH and Grant PJ. Environmental and genetic factors in relation to elevated circulating levels of plasminogen activator inhibitor-1 in Causasian patients with Non-Insulin-Dependent Diabetes Mellitus. Thrombosis and Haemostasis 1995; 74:842-7.

25. Hamsten A, Wiman B, de Faire U and Blombäck M. Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. New England Journal of Medicine 1985; 19:1557-1563.

26. Margaglione M, Grandone E, Vecchione G, Cappucci G, Giuliani N, Colaizzo D, Celentano E, Panico S and di Mino G. Plasminogen activator inhibitor (PAI-1) antigen plasma levels in subjects attending a metabolic ward: relation to polymorphisms of PAI-1 and angiotensinogen converting enzyme (ACE) genes. Arterioclerosis, Thrombosis, and Vascular Biology 1997; 17:2082-87.

27. Junker R, Heinrich J, Schulte H, Tataru M, Köhler E, Schönfeld R, Nowak-Göttl U and Assmann G. Plasminogen activator inhibitor-1 4G/5G-polymorphism and Factor V Q506 mutation are not associated with myocardial infarction in young men. Blood Coagulation and Fibrinolysis 1998; 9:597-02.

28. Burzotta F, di Castelnuovo A, Amore C, D'Orazio A, Donati MB and Iacoviello L. The role of 4G/5G polymorphism in the regulation of plasma levels of PAI-1: a model of interaction between genetic and environmental factors. Cardiologia 1998; 43(1):83-8.

29. Gram J, Kluft C and Jespersen J. Depression of tissue plasminogen activator (t-PA) activity and rise of t-PA inhibition and acute phase reactants in blood of patients with acute myocardial infarction. Thrombosis and Haemostasis 1987; 58(3):817-21.

30. Doggen CJM, Bertina RM, Cats VM, Reitsma PH and Rosendaal FR. The 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is not associated with myocardial infarction. Thrombosis and Haemostasis 1999; 82:115-20.

31. Ye S, Green FR, Scarabin PY, Nicaud V, Bara L, Dawson SJ, Humphries SE, Evans A, Luc G, Camou JP, Arveiler I, Henney AM and Cambien F. 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. Thrombosis and Haemostasis 1995; 74:837-841.

32. Hindorff LA, Schwartz SM, Siscovick DS, Psaty BM, Longstreth Jr. WT and Reiner AP. The association of PAI-1 promoter 4G/5G insertion/deletion polymorphism with myocardial infarction and stroke in young women. Journal of Cardiovascular Risk 2002; 9(2):131-7.

33. Panahloo A, Mohamed-Ali V, Gray RP, Humphries SE and Yudkin JS. Plasminogen activator inhibitor (PAI-1) activity post myocardial infarction: the role of acute phase reactants, insulin-like molecules and promoter (4G/5G) polymorphism in the PAI-1 gene. Atherosclerosis 2003; 168:297-304.

34. Ossei-Gerning N, Mansfield M, Stikland M, Wilson IJ and 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. Arteriosclerosis, Thrombosis, and Vascular Biology 1997; 17:33-37.

35. Schneidermann J, Sawdey MS, Keeton MR, Bordin GM, Bernstein EF, Dilley RB and Loskutoff DJ. Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. Proccedings of the National Academy Sciences of the USA 1992; 89:6998-7002.

36. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, Sone T, Tanaka M and Yokota M. Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. New England Journal of Medicine 2002; 347(24):1916-1923.

Table 1. Clinical characteristics of AMI patients and control subjects.

Characteristic	AMI Patients (n=283)	Control Subjects (n=96)	P-value
Age	50.4 ± 6.4	50.4 ± 7.2	0.980
Male sex	82%	75%	0.180
Smoking status	18%	18%	1.000
Non-O blood group	61%	42%	0.002
Hyperlipidemia	62%	36.5%	<0.001
Diabetes status	26%	10.5%	0.004
Family history	59%	40%	0.001
Hypertension	61%	34%	<0.001
Fibrinogen (mg/dl)	299.8 ± 55.7	281.8 ± 46.1	0.006
C-reactive protein (mg/dl)	0.26 (0.13 - 0.61)	0.18 (0.08 - 0.34)	0.006

Values are expressed as mean ± SD or number (%) of patients. Student-t test for continuous variables and chi-square for discrete variables were used to compare the values. For the AMI patients the value refers to the age at onset of myocardial infarction, not the current age. C-reactive protein values are expressed as median, 25th and 75th percentiles.

Table 2. Allele and genotype frequencies of t-PA and PAI-1 polymorphisms in patients and control subjects.

Polymorphism	AMI Patients			Control Subjects			P-value
t-PA	I/I	I/D	D/D	I/I	I/D	D/D	
N (%)	85 (31%)	132 (47%)	60 (22%)	22 (23%)	45 (48%)	27 (29%)	
I allele		0.54			0.47		0.10
PAI-1	4G/4G	4G/5G	5G/5G	4G/4G	4G/5G	5G/5G	
N (%)	52 (19%)	143 (51%)	83 (30%)	20 (21%)	51 (54%)	23 (25%)	
4G allele		0.44			0.48		0.39

Capítulo 6

DISCUSSÃO

O infarto agudo do miocárdio é uma das principais causas de morte e diminuição da qualidade de vida no Brasil, por isso a prevenção do IAM é um dos mais importantes focos de interesse em programas de saúde pública. O IAM é uma doença complexa e multifatorial que resulta da interação de fatores genéticos e ambientais. Aparentemente, a incidência do IAM aumenta proporcionalmente ao número de fatores de risco, entre os quais podemos citar: hipercolesterolemia, diabetes mellitus, tabagismo e hipertensão. Uma história familiar positiva é considerada um fator de risco, indicando a existência de genes de suscetibilidade, muito embora os fatores de risco convencionais também tenham seu componente genético. O interesse em identificar o componente genético na predição do risco coronariano é justificado pelo fato que indivíduos saudáveis, portanto sem fatores de risco conhecidos, podem apresentar um IAM, principalmente em idade precoce.

Diversos estudos genéticos têm identificado genes candidatos como fatores de risco para IAM e doença arterial coronariana. Embora diversos trabalhos tenham relatado resultados controversos ao estudarem populações diferentes em relação a um polimorfismo em um único gene, o tipo de estudo mais indicado seria a avaliação simultânea de diversos polimorfismos, em diversos genes, além de um delineamento adequado. Um fator que deve ser levado em consideração na escolha das variantes genéticas a serem analisadas é o significado biológico dos produtos protéicos dos genes candidatos. Como geralmente o IAM resulta da ruptura de uma placa aterosclerótica e posterior trombose, polimorfismos envolvidos com a formação e progressão da aterosclerose podem ser úteis na predição do risco. Tendo em vista os processos que controlam o tônus vascular, a disfunção endotelial, a adesão e agregação plaquetárias, a coagulação sangüínea e a fibrinólise, o presente trabalho se propõe a elucidar quais das variantes genéticas analisadas são preditivas para o IAM em idade precoce.

Os Fatores Endoteliais

Uma das funções mais importantes do endotélio é fornecer uma superfície anti-trombótica. A indução e posterior liberação do fator tissular transforma o endotélio em uma

superfície procoagulante. Oscilações no fluxo sangüíneo estimulam a expressão e liberação do fator tissular pelas células endoteliais e forças hemodinâmicas, localizadas principalmente em regiões de bifurcação dos vasos, podendo favorecer a trombogênese (MAZZOLAI *et al.*, 2002). O fator tissular também atua na proliferação de células musculares lisas e na ativação de metaloproteinases de matriz, que degradam a matriz extracelular que recobre a placa aterosclerótica. Assim, a aterosclerose é um processo sistêmico que resulta da interação de fatores de risco e condições locais do endotélio vascular.

O óxido nítrico (ON) é um subproduto da conversão do aminoácido L-arginina em citrulina, através da ação da enzima óxido nítrico sintetase (NOS). A forma endotelial da NOS (eNOS) é encontrada no endotélio e plaquetas, e seu produto atua no controle do tônus vascular, inibindo a adesão e agregação palquetárias e a proliferação de células musculares lisas (ROSS, 1999; CHIANG, 2000; 2001). Na última década, diversos estudos têm investigado a associação entre os níveis de ON e diversas doenças do sistema cardiovascular. Devido ao seu efeito sobre o endotélio vascular, surgiu o interesse na identificação de variantes genéticas determinantes dos níveis de ON, e portanto associadas ao risco de eventos agudos, como o IAM. HIBI *et al*. (1998) foram os primeiros autores a propor a associação entre o polimorfismo Glu298Asp presente no gene da eNOS com o risco de IAM. Esse polimorfismo também foi associado à hipertensão (MIYAMAMOTO *et al*., 1998; SHOJI *et al*., 2000) e IAM (SHIMASAKI *et al*., 1998; HINGORAMI *et al*., 1999) em japoneses, através de diferenças nas concentrações de ON e seu efeito sobre as artérias. Apesar da associação clara em japoneses, diferentes populações apresentam resultados contraditórios, como revisto em ARAS *et al*. (2002). O polimorfismo T-786C, por estar localizado na região promotora do gene da eNOS, foi associado aos níveis de ON, à ocorrência de espasmo coronariano (NAKAIAMA *et al*., 1999; YOSHIMURA *et al*., 2000) e IAM (NAKAIAMA *et al*., 2000).

AKAR *et al*. (1999a) associaram um polimorfismo localizado no intron 4 do gene da eNOS ao risco de acidente vascular cerebral, porém sem asociação com trombose venosa. Os polimorfismos T-786C e Glu298Asp foram avaliados por ROSSI *et al.* (2003), no GENICA Study, quanto ao risco de CAD em caucasóides. Os resultados indicaram que a variante T-786C foi preditiva para CAD, independentemente de outros fatores de risco, inclusive relacionada à severidade da doença, entretanto o polimorfismo Glu298Asp não se mostrou associado.

Nosso estudo demonstrou a existência de associação entre a ocorrência de IAM e o polimorfismo Glu298Asp. Entretanto, quanto ao polimorfismo T-786C não encontramos essa mesma associação. SHIMASAKI *et al*. (1998) e HINGORANI *et al*. (1999) também

encontraram associação entre o polimorfismo Glu298Asp e IAM em japoneses. Quanto ao polimorfismo T-786C, este foi associado ao risco de IAM no trabalho de NAKAYAMA *et al*. (2000) e com a severidade de CAD por ROSSI *et al*. (2003), esse último em caucasóides. O alelo C da variante T-786C é muito mais freqüente em caucasóides, como pôde ser observado em nosso trabalho e no de ROSSI *et al*. (2003). Em japoneses sem sintomas de doença cardiovascular a freqüência do alelo C é de aproximadamente 0,03, enquanto em pacientes com espasmo coronariano é de 0,15. A freqüência do alelo C em japoneses é muito menor do que a observada em indivíduos controles utilizados em nosso estudo (0,41) e das relatadas por GONZÁLES-ORDÓÑEZ *et al*. (2000) e ROSSI *et al*. (2003), de 0,41 e de 0,38, respectivamente.

O fator von Willebrand tem sido considerado um marcador de lesão endotelial e associado à ocorrência de IAM (JANSSON *et al*., 1991; CORTELLARO *et al*., 1992; THOMPSON *et al*., 1995), presença de CAD (OSSEI-GERNING *et al*., 1998) e disfunção endotelial pulmonar (LOPES *et al*., 1998). Entretanto, em nosso estudo não observamos diferenças nos níveis de fvW em indivíduos infartados quando comparados aos indivíduos controles. Provavelmente, os maiores níveis de fvW estejam associados à fase aguda do IAM (primeiros 3 meses) e como indivíduos em fase aguda não foram incluídos em nosso estudo, esse poderia ser o motivo da falta de associação. Quando avaliamos os níveis de fvW em indivíduos com e sem lesão aparente na angiografia também não observamos diferenças (179 vs. 193 U/dl; p=0,31). Nós também não encontramos diferenças nos níveis de fvW quando indivíduos com e sem lesão coronariana foram comparados em um estudo anterior (SIMON *et al*., 2003). Os níveis de fvW também não foram preditivos da extensão da aterosclerose no trabalho de NILSSON *et al*. (2002), porém foram associados com a doença coronariana de acordo com SCHUMACHER *et al*. (2002). Recentemente, foram descritos 3 polimorfismos de DNA (C-1234T, A-1185G e G-1051A) localizados na região promotora do gene do fvW que de acordo com KEIGHTLEY *et al*. (1999) estariam associados aos níveis circulantes desse fator. Em nosso trabalho nós não observamos associação entre os níveis de fvW com os genótipos do polimorfismo A-1185G, assim como também não foi associado no trabalho de SIMON *et al*. (2003), confirmando os dados de di BITONDO *et al*. (2001) que também não observaram associação entre a variante A-1185G e o risco de AMI.

Alguns trabalhos, como o de THOMPSON *et al*. (1995) observaram que os níveis elevados de fvW podem ser usados como marcadores de risco para eventos agudos subseqüentes. É difícil determinar se os níveis elevados de fvW são realmente preditivos para eventos agudos subseqüentes: se são causais para esses eventos (na formação de novos trombos) ou meramente conseqüência do dano celular pós-IAM ou pós-angina.

Fatores genéticos e ambientais parecem afetar os níveis de fator von Willebrand, o grupo sangüíneo ABO parece explicar de 30% a 40% da variabilidade genética (SOUTO *et al.*, 2003). Níveis de fvW são mais elevados em indivíduos do grupo sangüíneo não-O. Entretanto, em nosso estudo os níveis de fvW não diferiram entre pacientes e controles apesar do controle para o tipo sangüíneo ABO. Verificamos que indivíduos do grupo não-O apresentaram níveis de fvW mais elevados que os indivíduos do grupo O. É possível que os níveis do fvW também possam ser afetados por outros fatores de risco ou variáveis não controladas no presente estudo.

A ativação plaquetária e a conseqüente formação do trombo, aliados à vasoconstrição são os eventos que diferenciam um estado crônico de um evento coronariano agudo. As plaquetas interagem com diversos fatores de coagulação, aderindo ao colágeno exposto na lesão endotelial e fornecendo sítios de ligação para protrombina e fator XI (HEEMSKERK *et al.* 2002). A ativação plaquetária e a cascata da coagulação são processos mutuamente dependentes para a hemostasia e trombose. Durante a ativação plaquetária diversas substâncias são liberadas, uma delas é a serotonina, que exerce diversos efeitos sobre a parede vascular, favorecendo a trombogênese, a mitogênese e a vasoconstrição (McFADDEN *et al.*, 1991; VANHOUTTE *et al.*, 1991; KAUMANN *et al.*, 1994). Plaquetas são ricas em serotonina, e os receptores para serotonina estão localizados nas membranas celulares de plaquetas e células musculares lisas. A ligação dos receptores plaquetários à serotonina liberada, induz a agregação plaquetária e a contração de células musculares lisas, o que por conseguinte estimula a liberação de mais serotonina. Dessa forma, a serotonina estimula a ação dos receptores e a ativação de outras plaquetas. A proliferação de células musculares lisas é um importante componente da aterosclerose e reestenose pós-"stent".

Até recentemente, a serotonina vinha sendo associada a doenças como depressão, esquizofrenia e transtornos afetivos, sendo considerada um fator de risco para IAM pela primeira vez por YAMADA *et al.* (2000). Desde então, diversos estudos têm relacionado os níveis de serotonina, assim como a depressão, ao risco de IAM. O uso de agentes antidepresssivos (tricíclicos e bloqueadores seletivos de recaptação da serotonina) tem sido considerado cardioprotetor (COHEN *et al.*, 2000; STRIK *et al.*, 2001). Inclusive no período pós-IAM, a depressão é um sintoma comum e o uso de bloqueadores de recaptação da serotonina inibe a adesão plaquetária. Modelos animais mostram que durante episódios isquêmicos o nível de serotonina aumenta, e o bloqueio do receptor 5-HT_2A aumenta o fluxo sangüíneo e diminui o tamanho da lesão resultante do infarto, pela inibição da liberação de serotonina (SANADA *et al.*, 2002; SHIMIZU *et al.*, 2002b). O polimorfismo T102C no gene do receptor 5-HT_2A, parece afetar a função receptora. Indivíduos com genótipo T/T mostram

picos de agregação plaquetária induzida mais elevados que indivíduos T/C ou C/C (SHIMIZU et *al.* 2002a), sugerindo um significado funcional a esse polimorfismo. Em nosso trabalho, observamos diferenças nas freqüências do alelo T da variante T102C associada ao IAM, entretanto os valores não alcançaram significância estatística. Mesmo assim, sugerimos uma associação entre a variante T102C do receptor da serotonina ao risco de IAM, provavelmente através de diferenças na adesão e agregação plaquetárias que são inerentes ao desenvolvimento do IAM.

A estromelisina-1 é uma metaloproteinase de matriz do tipo 3, envolvida nos processos de degradação da matriz extracelular de processos fisiológicos (crescimento, remodelamento e diferenciação) e patológicos (artrite, úlcera e metástase de tumores) (revisto em VISSE & NAGASE, 2003). A estromelisina-1 parece estar envolvida na progressão de lesões ateroscleróticas e posterior trombose, através do enfraquecimento da capa fibrosa que protege a placa aterosclerótica, tornando-a instável e mais propensa à ruptura, o que freqüentemente leva ao IAM. O polimorfismo 5A/6A na região promotora do gene da estromelisina-1 está associado a diferenças na atividade promotora do gene, e assim à expressão e atividade da estromelisina-1. Como foi demonstrado em nosso trabalho, portadores do alelo 5A foram significativamente mais freqüentes no grupo de pacientes com IAM do que no grupo controle, confirmando essa hipótese. Nossos resultados confirmam aqueles obtidos por TERASHIMA *et al*. (1999) e YAMADA *et al*. (2002). BEYZADE *et al*. (2003) avaliaram indivíduos submetidos à angiografia coronariana e observaram que indivíduos com genótipo 6A/6A apresentaram um maior número de artérias coronárias comprometidas por estenoses, enquanto que indivíduos com genótipos 5A/5A e 5A/6A apresentaram um risco elevado de IAM decorrente, provavelmente, da presença de placas mais propensas à ruptura.

Os Fatores de Coagulação

A associação entre concentrações elevadas de fatores da coagulação sangüínea e o risco cardiovascular têm sido o objeto de inúmeras investigações epidemiológicas, na tentativa de predizer o risco de IAM. Entretanto, diversos fatores de coagulação não agem somente na formação do coágulo sangüíneo, mas também interagem com outros fatores da coagulação e substâncias inflamatórias, o que dificulta a interpretação da causalidade da relação. Como a aterosclerose é um processo inflamatório (ROSS & GLOMSET, 1973; ROSS, 1993; 1999), a participação dos fatores de coagulação pode se dar no componente inflamatório do processo aterosclerótico ou no componente trombótico.

O fibrinogênio é o fator de coagulação mais estudado em relação ao risco vascular, por ser o precursor imediato do coágulo sangüíneo. Além de ser um fator da coagulação, o fibrinogênio também é uma proteína de fase aguda, sendo um marcador de processos inflamatórios. O fibrinogênio aumenta a viscosidade sangüínea por ser a proteína de maior concentração no plasma sangüíneo e exerce um efeito anti-oxidante (ERNST, 1993; ERNST & RESCH, 1993; OLINESCU & KUMMEROW, 2001). A associação de níveis elevados de fibrinogênio com o risco de IAM, inclusive de IAM recurrente, foi observada em diversos trabalhos (THOMPSON *et al*., 1995; LUC *et al*., 2003). Os níveis de fibrinogênio também foram associados ao risco de mortalidade pós-IAM (WOODWARD *et al*., 1998; BAKER *et al*., 2002). Diversas variantes genéticas parecem influenciar os níveis plasmáticos de fibrinogênio, os polimorfismos mais estudados são G-455A no gene β-fibrinogênio e *Taq*I no gene α-fibrinogênio (de MAAT *et al*., 1998; MARGAGLIONE *et al*., 1998b; VÄISÄNEN *et al*., 1997; BRULL *et al*., 2002).

Apesar de diversos estudos terem associado essas duas variantes genéticas aos níveis de fibrinogênio, e os níveis de fibrinogênio estarem associados ao risco de IAM, a associação entre as variantes e o risco de IAM permanece controversa. No presente estudo nós relatamos que nos pacientes com IAM, os níveis de fibrinogênio foram significativamente mais elevados que nos indivíduos controles, entretanto essa associação foi perdida quando outros fatores de risco foram considerados. Nós também observamos que o polimorfismo G-455A teve efeito sobre os níveis de fibrinogênio. Indivíduos com genótipo A/A apresentaram os níveis mais elevados e indivíduos com genótipo G/G os menores níveis, tendo os heterozigotos A/G níveis intermediários, estando de acordo com a literatura (van der BOM *et al*., 1998). Porém, nem o polimorfismo G-455A nem *Taq*I foram associados ao risco de IAM, concordando com os trabalhos de LEE *et al*. (1993) e BRULL *et al*. (2002).

Dessa forma, podemos concluir que o polimorfismo G-455A está associado com os níveis de fibrinogênio, porém não mostrou associação com o risco de IAM. Por outro lado, os níveis elevados de fibrinogênio plasmático estão associados ao risco de IAM, porém essa associação parece não ser independente de outros fatores de risco. Portanto, se existir associação entre o polimorfismo G-455A e IAM, essa inferência ainda deverá ser esclarecida.

O fator VII (fVII) é o primerio fator plasmático da via extrínseca da coagulação, desencadeada pela liberação do fator tissular. E é exatamente por ser ativado na presença do fator tissular, que o fVII têm sido investigado em relação à sua associação com o IAM. Níveis elevados de fVII foram associados ao risco de IAM nos trabalhos de JUNKER *et al*. (1997) e NOTO *et al*. (2002). Duas variantes genéticas localizadas no gene do fVII, - R353Q e inserção/deleção - foram associadas aos níveis plasmáticos de fVII em diversos trabalhos

(POLLAK *et al.*, 1996; HUNALT *et al.*, 1997; HEYWOOD *et al.*, 1997; di CASTELNUOVO *et al.*, 1998). Apesar da associação entre esses polimorfismos e os níveis de fVII, resultados conflitantes quanto ao efeito das variantes no risco do IAM ainda permanecem.

Nossos resultados não mostraram diferenças nas freqüências alélicas dos polimorfismos R353Q e I/D no gene do fVII entre pacientes com IAM e controles. Apesar de não termos investigado os níveis de fVII, e por isso não podermos avaliar seu efeito fisiológico, esses polimorfismos não foram associados ao risco de IAM. Outros estudos também não encontraram associação entre os genótipos dos polimorfimos R353Q e I/D e o risco de IAM (LANE *et al.*, 1996; DOGGEN *et al.*, 1998a; ARDISSINO *et al.*, 1999; BATALLA *et al.*, 2001; KAKKO *et al.*, 2002; SHIMOKATA *et al.*, 2002). No trabalho de CAI *et al.* (2000), embora os níveis de fVII tenham sido associados aos genótipos do polimorfismo R353Q, não foi observada associação deste com IAM. Por outro lado, no estudo de LI *et al.* (2002), entre diversos polimorfismos estudados, o polimorfismo R353Q foi o único associado ao risco de IAM em pacientes com menos de 45 anos.

Como os níveis de fibrinogênio e fVII são influenciados por diversos fatores, a associação entre os níveis desses fatores da coagulação e o risco de IAM é difícil de ser determinada. A maior limitação dos estudos que avaliam a dosagem dos fatores hemostáticos como o fibrinogênio e fvW, analisados no presente estudo, é a dosagem plasmática no período pós-IAM.

O fator V Leiden é uma mutação causada pela substituição de uma arginina por uma glutamina na proteína resultante, o que torna o fator V resistente à ação da proteína C ativada, não sendo apropriadamente inativado (BERTINA *et al.*, 1994). O fV Leiden é a causa genética mais comum de trombose venosa entre caucasóides (BURICK *et al.*, 1997). Embora a relação do fV Leiden com trombose venosa seja clara, ainda que não seja independente de outros fatores de risco (ESPINOZA et al., 2001), sua associação com trombose arterial é bastante controversa. Os trabalhos de RIDKER *et al.* (1995), JUNKER *et al.* (1998) e REDONDO *et al.* (1999) não observaram associação entre essa mutação e o risco de IAM, já HOLM *et al.* (1996) e ROSENDAAL *et al.* (1997a) observaram um aumento significativo no risco de IAM em portadores do fV Leiden. Em nosso trabalho, a freqüência do fV Leiden foi muito reduzida, ficando abaixo do esperado para populações caucasóides. Assim sendo, qualquer inferência fica prejudicada pelo tamanho amostral reduzido.

A protrombina ou fator II além de ser responsável pela transformação do fibrinogênio circulante em polímeros de fibrina, tem diversas funções celulares. Um polimorfismo denominado G20210A localizado na região 3' não-traduzida foi associado aos níveis de

protrombina (POORT *et al.*, 1996). Essa mutação tem sido associada ao risco de trombose venosa (ROSENDAAL *et al.*, 1997b; SIMIONI *et al.*, 1998; DOGGEN *et al.*, 1998b) entretanto, os resultados relativos à trombose arterial são controversos. Em nosso trabalho, não observamos associação da variante da protrombina e o risco de IAM, provavelmente devido à baixa freqüência de portadores. No trabalho de AKAR *et al.* (1999b) tanto o fator V Leiden quanto a mutação da protrombina foram independentemente associados a infarto cerebral em crianças. Como as variantes do fV Leiden e da protrombina mostram diferenças populacionais, em nosso estudo a associação dessas variantes com o risco de IAM não foram evidenciadas.

Os Fatores Fibrinolíticos

Uma capacidade fibrinolítica reduzida pode estar associada à manutenção da rede de fibrina que compõe o trombo intravascular e indivíduos portadores dessa condição estariam mais propensos a desenvolver um IAM. Diversos métodos para acessar a capacidade fibrinolítica têm sido propostos, mas as medidas das concentrações do antígeno do ativador tipo tissular do plasminogênio (t-PA) e do complexo t-PA/PAI-1 (t-PA complexado a seu inibidor) parecem ser as mais utilizadas. A concentração plasmática do inibidor tipo 1 do ativador do plasminogênio (PAI-1) é muito mais elevada do que a concentração do t-PA, assim medidas aumentadas de t-PA livre e complexado são indicativas de atividade fibrinolítica reduzida. JOHANSSON *et al.* (2000) estudaram os níveis plasmáticos de t-PA, PAI-1 e do complexo t-PA/PAI-1, observando que níveis elevados de t-PA livre e complexado foram associados independentemente com o risco de AVC hemorrágico ou trombótico. Níveis de t-PA:Ag foram elevados em pacientes com doença coronariana (SCHUMACHER *et al.*, 2002). Níveis plasmáticos de PAI-1 foram um fator de risco para eventos isquêmicos no PRIME Study (SCARABIN *et al.*, 1998). LOWE *et al.* (1998) acompanharam durante 5 anos indivíduos entre 45 e 65 anos, e observaram que os níveis mais elevados de t-PA e PAI-1 no início do estudo foram observados em homens que posteriormente desenvolveram IAM ou morreram por causa isquêmica. CORTELARO *et al.* (1992) no PLAT Study associaram os altos níveis de PAI-1 com eventos trombóticos isquêmicos. THOMPSON *et al.* (1995) no ECAT Study também verificaram que altas concentrações de PAI-1 foram associadas a eventos isquêmicos em pacientes com angina pectoris.

Embora o polimorfismo de inserção/deleção de uma seqüência *Alu* no gene do t-PA seja associado ao risco de IAM em alguns trabalhos, por estar localizado em um íntron, um papel funcional seria improvável. Tem sido sugerido que a variante I/D poderia alterar a

estabilidade ou o "splincing" do mRNA e van der EIJNDEN-SCHRAUWEN *et al*. (1995) reporta que os níveis basais de t-PA não seriam influenciados pelo polimorfismo I/D. Recentemente, 8 novos polimorfismos localizados no locus do t-PA foram descritos por LADENVALL *et al*. (2000) e a variante denominada C–7351T parece estar associada ao risco de IAM (LADENVALL *et al*., 2002) pelo seu efeito na liberação de t-PA. Aparentemente, existem diferenças nas taxas de liberação local e sistêmica do t-PA, e essas diferenças podem influenciar o risco de eventos isquêmicos. De acordo com GUNADSON *et al*. (2003) indivíduos em idade avançada apresentam um aumento na liberação de t-PA, numa tentativa de compensar o risco aterotrombótico, entretanto esse processo é influenciado pela presença de outros fatores de risco.

O polimorfismo 4G/5G tem sido associado aos níveis de PAI-1 e estudos "in vitro" têm demonstrado que ambos os alelos do polimorfismo apresentam sítios de ligação para ativadores de transcrição, porém o alelo 5G apresenta um sítio adicional para uma proteína repressora (ERIKSSON *et al*., 1995). Dessa forma, na presença do repressor o alelo 4G estaria associado a um aumento nos níveis basais de transcrição. Aparentemente, pacientes diabéticos com genótipo 4G/4G têm níveis de PAI-1 muito mais elevados quando também apresentam altos níveis de triglicerídeos (MANSFIELD *et al*., 1995). Entretanto, não é sabido como os níveis de triglicerídeos influenciam os níveis plasmáticos de PAI-1. Uma associação negativa entre os níveis de PAI-1 e HDL-colesterol também foi descrita em pacientes com angina pectoris (JUHAN-VAGUE *et al*., 1996). A associação dos níveis de PAI-1 com os fatores de risco cardiovascular poderia explicar porque diversos estudos encontram uma relação entre os níveis de PAI-1 e o risco de IAM, enquanto outros estudos, após o ajuste para fatores de risco, perdem a associação. Em nosso estudo, nem o polimorfismo I/D do t-PA nem 4G/5G do PAI-1 foram associados ao risco de infarto antes dos 60 anos. No entanto, quando avaliamos um subgrupo com idade inferior aos 45 anos, observamos uma associação positiva com o polimorfismo I/D do gene do t-PA.

A idade de ocorrência do IAM pode ser determinante para a suscetibilidade de portadores de determinados fatores de risco genéticos, a identificação desses fatores não só aumenta o entendimento da fisiopatologia das síndromes isquêmicas agudas, como também oferece uma perspectiva futura, podendo (re)direcionar os esforços em terapêutica e prevenção. Nossos resultados sugerem a investigação de genótipos dos polimorfismos do t-PA, da serotonina e da estromelisina, pois esses foram associados ao risco de IAM.

Talvez os polimorfismos que se mostram preditivos para o risco de IAM em homens não o sejam em mulheres. A incidência de IAM em mulheres é menor do que em homens, e a idade difere em ambos os grupos. Também são observadas diferenças nas taxas de

sobrevivência pós-IAM entre os sexos (PELTONEN *et al.*, 2000). YAMADA *et al.* (2002) realizaram um grande estudo caso-controle em japoneses avaliando 112 polimorfismos genéticos localizados em 71 genes candidatos à predição do risco de IAM. De todos os polimorfismos analisados, somente 4G/5G do PAI-1 e 5A/6A da estromelisina-1 foram preditivos do risco de IAM em mulheres, e em homens apenas o polimorfismo da conexina 37, uma proteína vascular.

Em virtude do delineamento desse estudo, a investigação da associação com IAM é limitada a casos não-fatais, e somente um estudo longitudinal poderia esclarecer se esses polimorfismos são marcadores para casos fatais. Uma fonte de viés que não pode ser controlada é a modificação de hábitos de vida nos pacientes pós-IAM, conforme foi observado em nosso trabalho quanto à freqüência de fumantes no grupo de pacientes. Como o IAM é multifatorial e influenciado por diversos fatores que podem interagir entre si de forma sinergística ou competitiva, a interação entre fatores de risco conhecidos e os ainda não-descobertos pode influenciar o impacto destes no curso da doença.

Capítulo 6

Resumo e Conclusões

O mecanismo patogênico mais importante no IAM é a oclusão trombótica de uma artéria coronariária no local de ruptura de uma placa aterosclerótica. Recentemente, diversos estudos têm investigado a associação entre o IAM e fatores genéticos protrombóticos. O presentetrabalho é um estudo tipo caso-controle a fim de avaliar o efeito de diversos polimorfismos genéticos em um grupo de pacientes com IAM antes dos 60 anos de idade. Foram investigados 283 pacientes e 93 indivíduos controles, todos caucasóides e sem diferenças quanto à proporção sexual e idade média entre os grupos.

As principais conclusões estão resumidas abaixo:

1. O polimorfismo T-786C, localizado na região promotora do gene da eNOS, não foi associado ao risco de IAM.
2. A variante Glu298Asp, localizada no gene da eNOS, foi associada ao risco de IAM. Indivíduos portadores do alelo Asp foram mais freqüentes no grupo caso que no grupo controle. Entretanto, essa associação não foi independente de outros fatores de risco coronarianos. Essa variante aumentou significativamente o risco de IAM quando associada a outros fatores metabólicos.
3. O polimorfismo T102C, localizado na região promotora do gene do receptor da serotonina não foi associado ao risco de IAM no presente estudo. Entretanto, essa variante parece estar associada à severidade de CAD, pois indivíduos portadores do alelo T dessa variante apresentaram mais vasos afetados no estudo angiográfico.
4. A variante 5A/6A no gene da estromelisina-1, uma metaloproteinase de matriz, foi significativamente associada ao risco de IAM no presente estudo. Essa variante não foi associada ao risco de re-IAM ou ao número de vasos afetados. Esse polimorfismo foi um fator de risco genético independente para IAM.
5. A variante A-1185G no gene do fator von Willebrand não foi associada ao risco de IAM e não afetou os níveis plasmáticos de fvW. Os níveis plasmáticos de fvW também não

foram associados ao risco de IAM no presente estudo.

6. Os níveis de fibrinogênio foram significativamente mais elevados nos pacientes com IAM comparado ao grupo controle, porém essa associação não foi independente de outros fatores de risco coronarianos.
7. O polimorfismo *Taq*I não foi associado aos níveis de fibrinogênio plasmático. Entretanto, a variante G-455A teve efeito significativo sobre os níveis plasmáticos de fibrinogênio.
8. A variante G-455A, localizada no gene β-fibrinogênio e a variante *Taq*I, localizada no gene α-fibrinogênio não foram associadas ao risco de IAM.
9. Os polimorfismos R353Q and inserção/deleção no gene do fator VII não foram associados ao risco de IAM.
10. As mutações do fator V Leiden e G20210A da protrombina foram encontradas em freqüências muito baixas na população estudada e assim, não foram preditivas.
11. O polimorfismo de inserção/deleção no gene do t-PA não foi associado ao risco de IAM em pacientes com idade inferior a 60 anos. Entretanto, nós detectamos um aumento significativo na freqüência do alelo I entre pacientes com IAM antes dos 45 anos de idade. Assim, em uma idade precoce o polimorfismos I/D do gene do t-PA poderia ser um importante fator preditivo para o IAM.
12. O polimorfismo 4G/5G no gene do PAI-1 não foi associado ao risco de IAM no presente estudo. Além disso, também não encontramos associações dessa variante com o número de vasos afetados ou a recurrência de IAM.

Summary

The most important mechanism of AMI is the thrombotic oclusion of coronary artery at the site of a ruptured atherosclerotic plaque. Recently, several studies have been investigated the association between the AMI risk and prothrombotic genetic factors. The present study is a case-control study to evaluate the effect of several genetic polymorphisms in a case group with AMI before the age of 60 years. 283 patients and 93 control subjects were investigated, all caucasian and without sex and age differences between groups.

The main concludions are the following:

1. The T-786C polymorphism, located in the promoter region of the eNOS gene, was not associated to AMI.
2. The Glu298Asp variant, in the gene of eNOS, was associated with the risk of AMI. Carriers of the Asp allele were more frequent in cases than in controls. However, this association was not independent of other major risk factors. This variant significantly increased the risk of AMI when associated with other metabolic risk factors.
3. The T102C polymorphism, located in the serotonin receptor gene, was not associated with the risk of AMI in the present study. However, this variant appear be related to severity of CAD, because carriers of T allele shown more diseased vessels in angiographic study.
4. The 5A/6A variant of stromelysin-1, a matrix metalloproteinase enzyme, was significantly asssociated to AMI risk in the present study. This variant was not associated with the risk of re-IAM or with the number of diseased vessels. This polymorphism was an independet genetic risk factor for AMI.
5. The A-1185G variant of the von Willebrand factor gene was not associated with the risk of AMI and does not affect the vWf plasma levels. The vWf plasma levels also was not associated with the risk of AMI.
6. The plasma levels of fibrinogen was significantly higher in AMI patients than in control subjects, but this association was not independent of other major coronary risk factors.
7. The *Taq*I polymorphism was not associated with the fibrinogen levels. However, the G-455A variant affected significantly the fibrinogen plasma levels.
8. The G-455A variant located in the β-fibrinogen gene and the *Taq*I located in the α-

fibrinogen gene were not associated with the risk of AMI.

9. The R353Q and insertion/deletion variants of the factor VII gene were not associated with the risk of AMI.
10. The factor V Leiden and prothrombin G20210A mutations were found in very low frequencies in our population an were not predictive.
11. The insertion/deletion polymorphism of the t-PA gene was not associated with the risk of AMI in patients before the age of 60 years. However, we detect a significant increase in the I allele frequency among the AMI patients under the age of 45 years. Thus, at a younger age the t-PA I/D variant might be an importat predictive factor to the AMI.
12. The 4G/5G polymorphism of the PAI-1 gene was not associated with the risk of AMI in the present study. Also, we did not find associations of this variant according to the number of diseased vessels or number of AMI suffered.

Capítulo 7

Referências Bibliográficas

AKAR N, AKAR E, CIN S, DEDA G, AVCU F and YALÇM A (1999a). Endothelial nitric oxide synthase intron 4, 27 bp repeat polymorphism in Turkish patients with deep vein thrombosis and cerebrovascular accidents. **Thrombosis Research 94:**63-64.

AKAR N, AKAR E, DEDA G, SIPAHI T and ORSAL A (1999b). Factor V1691 G-A, prothrombin 20210 G-A, and methylenetetrahydrofolate reductase 677 C-T variants in Turkish children with cerebral infarct. **Journal of Child Neurology 14**(11):749-751.

AKHAVAN S, LAVORETANO S, CHANTARANGKUL V and MANNUCCI PM (2002). Plasma prothrombin levels are not associated with mutations in the promoter region of the prothrombin gene. **Thrombosis and Haemostasis 88**(5):877-8.

von AHSEN N, LEWCZUK P, SCHÜTZ E, OELLERICH M and EHRENREICH H (2000). Prothrombin activity in healthy subjects with and without the prothrombin G20210A mutation. **Thrombosis and Haemostasis 99**(6):549-556.

ALHENC-GELAS M, ARNAUD E, NICAUD V, AUBRY M-L, FIESSINGER J-N, AIACH M and EMMERICH J (1999). Venous thromboembolism disease and the prothrombin, methylenetetrahydrofolate reductase and factor V genes. **Thrombosis and Haemostasis 81:**506-10.

ARAS O, HANSON NQ, BAKANAY SM, TSAI MY and GULEC S (2002). Endothelial nitric oxide gene polymorphism (Glu298Asp) is not associated with coronary artery disease in Turkish population. **Thrombosis and Haemostasis 87:**347-9.

ARBINI AA & BAUER KA (1994). Reduced plasma factor VII coagulant activity due to the Arg 353Gln polymorphism in the factor VII gene results from defective secretion. **Blood 86**(Suppl 1):86.

ARDISSINO D, MANNUCCI PM, MERLINI PA, DUCA F, FETIVEAU R, TAGLIABUE L, TUBARO M, GALVANI M, OTTANI F, FERRARIO M, CORRAL J and MARGAGLIONE M (1999). Prothrombotic genetic risk factors in young survivors of myocardial infarction **Blood 94**(1):46-51.

ARRUDA VR, ANNICHINO-BIZZACCHI JM, COSTA FF and REITSMA PH (1995). Factor V Leiden (FVQ 506) is common in a Brazilian population. **American Journal of Haematology 49:**242-3.

ARRUDA VR, ANNICHINO-BIZZACCHI JM, GONÇALVES MS and COSTA FF (1997). Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. **Thrombosis and Haemostasis 78:**1430-3.

BAKER IA, PICKERING J, ELWOOD PC, BAYER A and EBRAHIM S (2002). Fibrinogen, viscosity and white blood cell count predict myocardial, but not cerebral infarction: evidence from the Caerphilly and Speedwell cohort. **Thrombosis and Haemostasis 87:**421-5.

BATALLA A, ALVAREZ R, REGUERO JR, GONZÁLEZ P, ALVAREZ V, CUBERO GI, CORTINA A and COTO E (2001). Lack of association between polymorphisms of the coagulation factor VII and myocardial infarction in middle-aged Spanish men. **International Journal of Cardiology 80:**209-212.

BEAUCHAMP NJ, DALY ME, HAMPTON KK, COOPER PC, PRESTON FR and PEAKE IR (1994). High prevalence of a mutation in the factor V gene within the UK population: relationship to activated protein C resistance and familial thrombosis. **British Journal of Haematology 88:**219-22.

BEHAGUE I, POIRIER O, NICAUD V, EVANS A, ARVEILER D, LUC G, CAMBOU JP, SCARABIN P-Y, BARA L, GREEN F and CAMBIEN F (1996). β Fibrinogen gene polymorphisms are associated with plasma fibrinogen and coronary artery disease in patients with myocardial infarction. The ECTIM Study. **Circulation 93**(3):440-449.

BENDERLY M, GRAFF E, REICHER-REISS H, BEHAR S, BRUNNER D and GOULDBOURT U (1996). Fibrinogen is a predictor of mortality in coronary heart disease patients. **Arteriosclerosis, Thrombosis, and Vascular Biology 16:**351-56.

BERNARDI F, ARCIERI P, BERTINA RM, CHIAROTTI F, CORRAL J, PINOTTI M, PRYDZ H, SAMAMA M, SANDSET PM, STROM R, VICENTE GARCIA V and MARIANI G (1997). Contribution of factor VII genotype to activated FVII levels. differences in genotype frequencies between northern and southern European populations. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**2548-53.

BERTINA RM, KOLEMAN BPC, KOSTER T, ROSENDAAL FR, DIRVEN RJ, de RONDE H, van der VELDEN P and REITSMA PH (1994). Mutation in blood coagulation factor V associated with resistance to activated protein C. **Nature 369:**64-67.

BEYZADE S, ZHANG S, WONG YK, DAY IN, ERIKSSON P and YE S (2003). Influences of matrix metalloproteinase-3 gene variation on extent of coronary atherosclerosis and risk of myocardial infarction. **Journal of the American College of Cardiology 41**(12):2130-7.

di BITONDO R, CAMERON CL, DALY ME, CROFT AS, STEEDS RP, CHANNER KS, SAMANI NJ, LILLICRAP D and WINSHIP PR (2001). The –1185A/G and –1051G/A dimorphisms in the von Willebrand factor gene promoter and risk of myocardial infarction. **British Journal of Haematology 115:**701-706.

BLOEM BR, van PUTTEN MJ, van der MEER FJ, van HILTEN JJ and BERTINA RM (1998). Superior sagittal sinus thrombosis in a patient heterozygous for the novel 20210 A allele of the prothrombin gene. **Thrombosis and Haemostasis 79**(1):235.

van der BOM J, de KNIJFF P, HAVERKATE F, BOTS M, MEIJER P, de JONG P, HOFMAN A, KLUFT C and GROBBEE D (1997). Tissue plasminogen activator and risk of myocardial infarction. The Rotterdam Study. **Circulation 95:**2623-2627.

van der BOM JG, de MAAT MPM, BOTS ML, HAVERKATE F, de JONG PTVM, HOFMAN A, KLUFT C and GROBBEE DK (1998). Elevated plasma fibrinogen cause or consequence of cardiovascular disease? **Arteriosclerosis, Thrombosis, and Vascular Biology 18:**621-625.

BOUNDEL M, HEPNER M, SCIUCCATI G, PIERONI G, FELIÚ-TORRES A, MARDARAZ C and FRONTROTH JP (2002). Factor V Leiden and prothrombin gene G20210A mutation in children with venous thromboembolism. **Thrombosis and Haemostasis 87:**972-7.

BROWN K, LUDDINGTON R, WILLIANSON D, BAKER P and BAGLIN T (1997). Risk of venous thromboembolism associated with a G to A transition at position 20210 in the 3'-untranslated region of he prothrombin gene. **British Journal of Haematology 98:**907-9.

BRULL DJ, DHAMRAIT S, MOULDING R, RUMLEY A, LOWE GDO, WORLD MJ, HUMPHRIES SE and MONTGOMERY HE (2002). The effect of fibrinogen genotype on fibrinogen levels after stenuous physical exercise. **Thrombosis and Haemostasis 87:**37-41

BUCCIARELLI P, FRANCHI F, ALATRI A, BETTINI P and MOIA M (1998). Budd-Chiari syndrome in a patient heterozygous for the G20210A mutation of the prothrombin gene. **Thrombosis and Haemostasis 79(**2):445-6.

BURICK A, WISOTZKEY JD, NAJARIAN MP, MONK Jr. JS and RHOADS Jr. JE (1997). The role of preoperative factor V Leiden screening in different geographic populations. **The American Surgeon 63:**547-550.

CAI Q, CHEN J, MA H, SONG J and XU M (2000). Association of coagulation factor VII with risk of myocardial infarction in the Chinese. **Chinese Medical Journal 113(12):**1059-63.

di CASTELNUOVO A, D'ORAZIO A, AMORE C, FALANGA A, DONATI MB and IACOVIELLO L (2000). The decanucleotide insertion/deletion polymorphism in the promoter region of the coagulation factor VII gene and risk of familial myocardial infarction. **Thrombosis Research 98:**9-17.

di CASTELNUOVO A, D'ORAZIO A, AMORE C, FALANGA A, KLUFT C, DONATI MB and IACOVIELLO L (1998). Genetic modulation of coagulation factor VII plasma levels: contribution of different polymorphisms and gender-related effects. **Thrombosis and Haemostasis 80:**592-7.

CARTER AM, MANSFIELD MW, STICKLAND MH and GRANT PJ (1996). β-fibrinogen -455 G/A polymorphism and fibrinogen levels. Risk factors for coronary artery disease in subjects with NIDDM. **Diabetes Care 19**(1):1265-1268.

CARTER AM, OSSEI-GERNING N, WILSON IJ and GRANT PJ (1997). Association of the platelet PI^A polymorphism of glicoprotein IIb/IIIa and the fibrinogen Bβ 448 polymorphism with myocardial infarction and extent of coronary artery disease. **Circulation 96:**1424-1431.

CATTO AJ, CARTER AM, STICKLAND M, BAMFORD JM, DAVIES JA and GRANT PJ (1997). Plasminogen activator inhibitor-1 (PAI-1) 4G/5G promoter polymorphism and levels in subjects with cerebrovascular disease. **Thrombosis and Haemostasis 77(4):**730-4.

CHENG K, YANG W, GRIMSBY J and SHIH JC (1992). The human 5 HT2 receptor is encoded by a multiple intron-exon gene. **Brain Research Molecular Brain Research 14:**20-26.

CHIANG TM, COLE F, WOO-RASBERRY V and KANG ES (2001). Role of nitric oxide synthase in collagen-platelet interaction: involvement of platelet nonintegrin collagen receptor nitrotyrosylation. **Thrombosis Research 102:**343-352.

CHIANG TM, WANG YB and KANG ES (2000). Role of the recombinant protein of the platelet receptor for type I collagen in the release of nitric oxide during platelet aggregation. **Thrombosis Research 100:**427-432.

COHEN HW, GIBSON G and ALDERMAN MH (2000). Excess risk of myocardial infarction in patients treated with antidepressant medications: association with use of tricyclic agents. **The American Journal of Medicine 108:**2-8.

COLLEN D (1999). The plasminogen (fibrinolytic) system. **Thrombosis and Haemostasis 82**(2):259-70.

CONNOR JM, FOWKES FGR, WOOD J, SMITH FB, DONNAN PT and LOWE GDO (1992). Genetic variation at fibrinogen loci and plasma fibrinogen levels. **Journal of Medical Genetics 29:**480-82.

CORRAL J, GONZALEZ-CONEJERO R, LOZANO ML, RIVERA J, HERAS I and VICENTE V (1997). The venous thrombosis risk factor 20210 A allele of prothrombin gene is not a major risk factor for arterial thrombotic disease. **British Journal of Haematology 99:**304-307.

CORTELLARO M, BOSCHETTI C, COFRANCESCO E, ZANUSSI C, CATALANO M, de GAETANO G, GABRIELLI L, LOMBARDI B, SPECCHIA G, TAVAZZI L, TREMOLI E, della VOLPE A, POLLI E and The PLAT Study Group (1992). The PLAT Study: hemostatic function in relation to atherothrombotic ischemic events in vascular disease patients. Principal results. **Arterioclerosis and Thrombosis 12:**1063-1070.

CROFT SA, DALY ME, STEEDS RP, CHANNER KS, SAMANI NJ and HAMPTON KK (1999). The prothrombin 20210A allele and its association with myocardial infarction. **Thrombosis and Haemostasis 81:**861-4.

CUMMING AM, KEENEY S, SALDEN A, BHAVNANI M, SHWE KH and HAY CRM (1997). The prothrombin gene G20210A variant: prevalence in a UK anticoagulant clinic population. **British Journal of Haematology 98:**353-5.

DAMIN DC, ROSITO MA, GUS P, ROISENBERG I, BANDINELLI E and SCHWARTZMANN G (2002). von Willebrand factor in colorectal cancer. **International Journal of Colorectal Disease 17:**42-5.

DAVIES MJ (1996). The contribution of thrombosis to the clinical expression of coronary atherosclerosis. **Thrombosis Research 82**(1):1-32.

DESCHIENS MA, CONARD J, HORELLOU MH, AMERI M, PRETER M, CHEDRU F, SAMANA MM and BOUSSER MG (1996). Coagulation studies, factor V Leiden, and anticardiolipin antibodies in 40 cases of cerebral venous thrombosis. **Stroke 27**(10):1724-1730.

DILLEY A, AUSTIN H, HOOPER AW, EL-JAMIL M, WHITSETT C, WENGER N, BENSON J and EVATT B (1998). Prevalence of the prothrombin 20210G-to-A variant in Blacks: infants, patients with venous thrombosis, patients with myocardial infarction, and control subjects. **Journal of Laboratory and Clinical Medicine 132:**425-5.

DOGGEN CJM, BERTINA RM, CATS VM, REITSMA PH and ROSENDAAL FR (1999). The 4G/5G polymorphism in the plasminogen activator inhibitor-1 gene is not associated with myocardial infarction. **Thrombosis and Haemostasis 82:**115-20.

DOGGEN CJM, CATS M, BERTINA RM, REITSMA PH, VANDENBROUCKE JP and ROSENDAAL FR (1998a). A genetic propensity to high factor VII is not associated with the risk of myocardial infarction in men. **Thrombosis and Haemostasis 80:**281-5.

DOGGEN CJM, CATS V, BERTINA RM and ROSENDAAL FR (1998b). Interaction of coagulation defects and cardiovascular risk factors. Increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. **Circulation 97:**1037-1041.

DUNN TS, ROBERTS CR, SCHECHTER E, MOORE WE, LEE ET and EICHNER JE (1998). Role of factor V Leiden mutation in patients with angiographically demonstrated coronary artery disease. **Thrombosis Research 91:**91-99.

DURANTE-MANGONI S, DAVIES GJ, AHMED N, RUGGIERO G and MICHAELIDES K (2002). The prothrombin G20210A polymorphism in patients with myocardial infarction. **Blood Coagulation and Fibrinolysis 13**(7):603-8.

EICHNER JE, MOORE WE, McKEE PA, SCHECHTER E, REYNOLDS DW, QI H and COMP PC (1996). Fibrinogen levels in women having coronary angiography. **American Journal of Cardiology 78**(1):15-8.

van den EIJNDEN-SCHRAUWEN Y, LAKENBERG N, EMEIS JJ and de KNIJFF P (1995). Alu-repeat polymorphism in the tissue-type plasminogen activator (t-PA) gene does not affect basal endothelial t-PA synthesis. **Thrombosis and Haemostasis 74**(4):1202.

ERIKSSON P, KALLIN B, van't HOOF FM, BAVENHOLM P and HAMSTEN A (1995). Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. **Proccedings of the National Academy Sciences of the USA 92:**1851-55.

ERNST E (1993). The role of fibrinogen as a cardiovascular risk factor. **Atherosclerosis 100:**1-12.

ERNST E & RESCH KL (1993). Fibrinogen as a cardiovascular risk factor: a meta-analysis and review of the literature. **Annals of Internal Medicine 118:**956-963.

ESPINOZA J, LLAMAS P, de VELASCO JF, SOTO C, ROMÁN A and TOMÁS JF (2001). Prothrombin 20210A-Associated thrombosis may need concurrency of another prothrombotic factor. **Thrombosis Research 102:**381-383.

FERRARESI P, MARCHETTI G, LEGNANI C, CAVALLARI E, CASTOLDI E, MASCOLI F, ARDISSINO D, PALARETI G and BERNARDI F (1997). The heterozygous 20210 G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increased in frequency in artery disease. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**2418-2422.

FOLSOM AR, PANKOW JS, WILLIAMS RR, EVANS GW, PROVINCE MA and ECKFELDT JH (1998). Fibrinogen, plasminogen activator inhibitor-1, and carotid intima-media wall thickness in the NHLBI Family Heart Study. **Thrombosis and Haemostasis 79:**400-4.

FOLSOM AR, WU KK, DAVIS CE, CONLAN MG, SORLIE PD and SZKLO M (1991). Population correlates of plasma fibrinogen and factor VII, putative cardiovascular risk factors. **Atherosclerosis 91:**191-205.

FOLSOM A, WU K, ROSAMOND W, SHARRETT A and CHAMBLESS L (1997) Prospective study of hemostatic factors and incidence of coronary heart disease. The Atherosclerosis Risk in Communities (ARIC) Study. **Circulation 96:**1102-1108.

FORMSTONE CJ, BYRD PJ, AMBROSE HJ, RILEY JH, HERNANDEZ D, McCONVILLE CM and TAYLOR AMR (1993). The order and orientation of a cluster of metalloproteinase genes, stromelysin 2, collagenase, and stromelysin, together with D11S385, on chromosome 11q22-q23. **Genomics 16:**289-291.

FRANCO RF, SANTOS SEB, ELION J, TAVELLA MH and ZAGO MA (1998). Prevalence of the G20210A polymorphism in the 3'-unstranslated region of the prothrombin gene in different human populations. **Acta Haematologica 100:**9-12.

FRANCO RF, TRIP MD, CATE HT, van der ENDE A, PRINS MH, KASTELEIN JJP and REITSMA PH (1999). The 20210-A mutation in the 3' unstranslated region of the prothrombin gene and the risk for arterial thrombotic disease. **British Journal of Haematology 104:**50-54.

FUSTER V (1994). Lewis A. Conner Memorial Lecture. Mechanisms leading to myocardial infarction: insights from studies of vascular biology. **Circulation 90**(4):2126-46.

FUSTER V, BADIMON L, BADIMON JJ and CHESEBRO JH (1992). The pathogenesis of coronary artery disease and the acute coronary syndromes. **New England Journal of Medicine 326**(4):242-250.

FUSTER V, STEIN B, AMBROSE JA, BADIMON L, BADIMON JJ and CHESEBRO JH (1990). Atherosclerotic plaque rupture and thrombosis. Evolving concepts. **Circulation 82**(Suppl II):47-59.

GARDEMANN A, LOHRE J, KATZ N, TILLMANNS H, HEHRLEIN FW and HABERBOSCH W (1999). The 4G/5G genotype of the plasminogen activator inhibitor-4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk fot this disease. **Thrombosis and Haemostasis 82:**1121-6.

GARDEMANN A, SCHWARTZ O, HABERBOSCH W, KATZ N, WEIß T, TILLMANNS H, HEHRLEIN FW, WAAS W and EBERBACH A (1997). Positive association of the β fibrinogen H1/H2 gene 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. **Thrombosis and Haemostasis 77:**1120-6.

GIANINI SD (1998). Aterosclerose e dislipidemias. São Paulo. **BG Cultural**.

GIDDINGS JC (1988). **Molecular genetics and immunoanalysis in blood coagulation.** Ellis Horwood & VCH.

GONZÁLEZ-ORDÓÑEZ AJ, FERNADES-CARREIRA JM, GONZÁLEZ-FRANCO A, MARTÍN-SANCHEZ L, ALVAREZ MV and GARCÍA EC (2000). Two expressive polymorphisms on the endothelial nitric oxide synthase gene (intron4, 27 bp repeat and –786 T/C) and the venous thromboembolism. **Thrombosis Research 99:**563-566.

GRAM J, SIDELMANN J and JESPERSEN J (1995). Does low protein concentration of tissue-type plasminogen activator predict a low risk of spontaneous deep vein thrombosis? **Thrombosis and Haemostasis 74**(2):718-21.

GREEN F & HUMPHRIES S (1989). Control of plasma fibrinogen levels. **Baillière's Clinical Haematology 2**(4):945-959.

GREEN F & HUMPHRIES S (1994). Genetic determinants of arterial thrombosis. **Ballière's Clinical Haematology 7:**675-692.

GREGG JP, YAMANE AJ and GRODY WW (1997). Prevalence of factor V-Leiden mutation in four distinct American ethnic populations. **American Journal of Medical Genetics 73:**334-336.

GUNADSON T, HTAFNKELSDÓTTIR T, WALL U, SWEDBERG K and JERN S (2003). Fibrinlytic capacity increases with age in healthy humans, while endothelium-dependent vasodilation is unaffected. **Thrombosis and Haemostasis 89:**374-81.

HALLAK M, SENDEROWICZ J, CASSEL A, SHAPIRA C, AGHAI E, AUSLENDER R and ABRAMOVIVI H (1997). Activated protein C resitance (factot V Leiden) associated with thrombosis in pregnancy. **American Journal of Obstretics and Gynecology 176:**889-893.

HAMSTEN A, WALLDIUS G, SZAMOSI M, FAIRE U, DAHLÉN G, LANDOU C and WIMAN B (1987). Plasminogen activator inhibitor in plasma: risk factor for recurrent myocardial infarction. **Lancet VII**(8549):3-8.

HAMSTEN A, WIMAN B, de FAIRE U and BLOMBÄCK M (1985). Increased plasma levels of a rapid inhibitor of tissue plasminogen activator in young survivors of myocardial infarction. **New England Journal of Medicine 19:**1557-1563.

HARVEY PJ, KEIGHTLEY AM, LAM YM, CAMERON C and LILLICRAP D (2000). A single nucleotide polymorphism at nucleotide -1793 in the von Willebrand factor (VWF) regulatory region is associated with plasma VWF:Ag levels. **British Journal of Haematology 109:**349-353.

HEEMSKERK JWM, BEVERS EM and LINDHOUT T (2002). Platelet activation and blood coagulation. **Thrombosis and Haemostasis 88:**186-93.

HEINRICH J, FUNKE H, RUST S, SCHULTE H, SCHÖNFELD R, KÖHLER E and ASSMANN G (1995). Impact of polymorphisms in the alpha- and beta-fibrinogen gene on plasma fibrinogen concentrations of coronary heart disease patients. **Thrombosis Research 77**(3):209-215.

HELLER RF, CHIN S, PEDOE HD and ROSE G (1984). How well can we predict coronary heart disease?: Findings in the United Kingdom Heart Disease Prevention Project. **British Medical Journal 288:**1409-1411.

HELLGREN M, SVENSSON PJ and DAHLBÄCK B (1995). Resitance to activated protein C as a basis for venous thromboembolism associate with pregnancy and oral contraceptives. **American Journal of Obstetrics and Gynecology 173:**210-3.

HEYWOOD DM, CARTER A, CATTO AJ, BAMFORD JM and GRANT PJ (1997). Polymorphisms of the factor VII gene and circulating FVII:C levels in relation to acute cardiovascular disease and poststroke mortality. **Stroke 28(4):**816-821.

HEYWOOD DM, MANSFIELD MW and GRANT PJ (1996a). Factor VII gene polymorphisms, factor VII:C levels and features of insulin resistance in non-insulin-dependent diabetes mellitus. **Thrombosis and Haemostasis 75**(3):401-6.

HEYWOOD DM, OSSEI-GERNING N and GRANT PJ (1996b). Association of factor VII:C levels with environmental and genetic factors in patients with ischaemic heart disease and coronary atheroma characterised by angiography. **Thrombosis and Haemostasis 76**(2):161-5.

HIBI K, KOUICHI T, MIZUSHIMA S, NYUI N, FUJITA T, OCHIAI H, KOSUGE M, WATANABE Y, YOSHII Y, KIHARA M, KIMURA K, ISHII M and UMEMURA S (1998). Endothelial nitric oxide synthase gene polymorphism and acute myocardial infarction. **Hypertension 32:**521-6.

HILLARP A, ZÖLLER B, SVENSSON PJ and DAHLBÄCK B (1997). The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis**. Thrombosis and Haemostasis 78**:990-2.

HINGORANI AD, LIANG CF, FATIBENE J, LYON A, MONTEITH S, PARSONS A, HAYDOCK S, HOOPER RV, STEPHENS N, O'SHAUGHNESSY KM and BROWN M (1999). A common variant of the endothelial nitric oxide synthase (Glu298Asp) is a major risk factor for coronary artery disease in the UK. **Circulation 100**(14):1515-1520.

HOLM J, HILLARP A, ZÖLLER B, ERHARDT L, BERNTORP E and DAHLBÄCK B (1999). Factor V Q506 (resistance to activated protein C) and prognosis after acute coronary syndrome. **Thrombosis and Haemostasis 81:**857-60.

HOLM J, ZÖLLER B, BERNTORP E, ERHARDT L and DAHLBÄCK B (1996). Prevalence of factor V gene mutation amongst myocardial infarction patients and health controls is higher in Sweden than in other countries. **Journal of Internal Medicine 239:**221-26.

HOWARD TE, MARUSA M, CHANNEL C and DUNCAN A (1997). A patient homozygous for a mutation in the prothrombin gene 3'-unstranslated region associated with massive thrombosis. **Blood Coagulation and Fibrinolysis 8:**316-9.

HUMPHREY PP, HARTIG P and HOYER D (1993). A proposed new nomenclature for 5-HT receptors. **Trends in Pharmacology Sciences 14**(6):233-6.

HUMPHRIES SE (1995). Genetic regulation of fibrinogen. **European Heart Journal 16**(Suppl A):16-20.

HUMPHRIES S, BAUTERS C, MEIRHAEGHE A, LUONG L, BERTRAND M and AMOUYEL P (2002a). The 5A/6A polymorphism in the promoter of the stromelysin-1 (MMP3) gene as a risk factor for restenosis. **European Heart Journal 23(9):**721-725.

HUMPHRIES SE, COOK M, DUBOWITZ M, STIRLING Y and MEADE TW (1987). Role of genetic variation at the fibrinogen locus in determination of plasma fibrinogen concentrations. **Lancet 1:**1452-1455.

HUMPHRIES SE, LUONG L, TALMUD PJ, FRICK HM, KESÄNIEMI YA, PASTERNACK A, TASKINEN MR and SYVÄNNE M (1998). The 5A/6A polymorphism in the promoter of the stromelysin-1 (MMP-3) gene predicts progression of angiographically determined coronary artery disease in men in the LOCAT Gemfibrozil study. **Atherosclerosis 139:**49-56.

HUMPHRIES SE, MARTIN S, COOPER J and MILLER G (2002b). Interaction between smoking and the stromelysin-1 (MMP3) gene 5A/6A promoter polymorphism and risk of coronary heart other matrix metalloproteinases and enzymic properties. **Annals of Human Genetics 66:**343-352.

HUMPHRIES S, TEMPLE A, LANE A, GREEN F, COOPER J and MILLER G (1996). Low plasma levels of factor VIIc and antigen are more strongly associated with the 10 base pair promoter (-323) insertion than the glutamine 353 variant. **Thrombosis and Haemostasis 75**(4):567-72.

HUNAULT M, ARBINI AA, LOPACIUK S, CAREW JA and BAUER KA (1997). The Arg^{353}Gln polymorphism reduces the level of coagulation factor VII. In vivo and in vitro studies. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**2825-2829.

INAYAMA Y, YONEDA H, SAKAI T, ISHIDA T, NONOMURA Y, KONO Y, TAKANATA R, KOH J, SAKAI J, TAKAI A, INADA Y and ASABA H (1996). Positive association between a DNA sequence variant in the serotonin 2A receptor gene and schizophrenia. **American Journal of Medical Genetics 67:**103-105.

INIESTA JA, CORRAL J, FÉRNANDEZ-PARDO, GONZÁLEZ-CONEJERO and VICENTE V (1997). Factor-V ($Arg^{506}\rightarrow$Gin) mutation in ischemic cerebrovascular disease. **Haemostasis 27:**105-111.

IWAI N, SHIMOIKE H, NAKAMURA Y, TAMAKI S and KINOSHITA M (1998). The 4G/5G polymorphism of the plasminogen activator inhibitor gene is associated with the time course of progression to acute coronary syndromes. **Atherosclerosis 136:**109-114.

JANSSON JH, NILSSON TK and JOHNSON O (1991). Von Willebrand factor in plasma: a novel risk factor for recurrent myocardial infarction and death. **British Heart Journal 66:**351-5.

JEFFERY S, LEATHAM E, ZHANG Y, CARTER J, PRATEL P and KASKI JC (1996). Factor V Leiden polymorphism (FV Q506) in patients with ischaemic heart disease, and in different populations groups. **Journal of Human Hypertension 10:**433-434.

JENKINS RG, SEIFFERT D, PARMER RJ and MILES LA (1997). Regulation of plasminogen gene expression by interleukin-6. **Blood 89**(7):2394-2403.

JOHANSSON L, JANSSON J-H, BOMAN K, NILSSON TK, STEGMAYR B and HALLMANS G (2000). Tissue plasminogen activator, plasminogen activator inhibitor-1, and tissue plasminogen activator/plasminogen activator inhibitor-1 complex as risk factors for the development of a first stroke. **Stroke 31:**26-32.

The Joint European Society of Cardiology/American College of Cardiology Committee (2000). Myocardial infarction redefined – A consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of myocardial infarction. **European Heart Journal 21:**1502-1513.

JUHAN-VAGUE I (1996). Haemostatic parameters and vascular risk. **Atherosclerosis 124**(Suppl S):49-55.

JUHAN-VAGUE I, PYKE SDM, ALESSI MC, JESPERSEN J, HAVERKATE F and THOMPSON SG (1996). Fibrinolytic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. **Circulation 94:**2057-63.

JUNKER R, HEINRICH J, SCHULTE H, van de LOO J and ASSMANN G (1997). Coagulation factor VII and the risk of coronary heart disease in healthy men. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**1539-1544.

JUNKER R, HEINRICH J, SCHULTE H, TATARU M, KÖHLER E, SCHÖNFELD R, NOWAK-GÖTTL U and ASSMANN G (1998). Plasminogen activator inhibitor-1 4G/5G-polymorphism and factor V Q506 mutation are not associated with myocardial infarction in young men. **Blood Coagulation and Fibrinolysis 9:**597-02.

JUUL K, TYBJÆRG-HANSEN A, STEFFENSEN R, KOFOED S, JENSEN G and NORDESTGAARD BG (2002). Factor V Leiden: the Copenhagen City Heart Study and 2 meta-analyses. **Blood 100**(1):3-10.

KAKKO S, ELO T, TAPANAIMEN JM, HUIKURI HV and SAVOLAINEN MJ (2002). Polymorphisms of genes affecting thrombosis and risk of myocardial infarction. **European Journal of Clinical Investigation 32**(9):643-8.

KANNEL WB, WOLF PA, CASTELLI WP and D'AGOSTINO RB (1987). Fibrinogen and risk of cardiovascular disease. **Journal of the American Medical Association 258**(9):1183-1186.

KAPUR RK, MILLS LA, SPITZER SG and HULTIN MB (1997). A prothrombin gene mutation is significantly associated with venous thrombosis. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**2875-2879.

KAUMANN AJ, FRENKEN M, POSIVEAL H and BROWN AM (1994). Variable participation of 5-HT 1-like receptors and 5-HT2 receptors in serotonin-induced contraction of human isolated coronary arteries. 5-HT1-like receptors resemble cloned 5-HT1D beta receptors. **Circulation 90**(3):1141-53.

KEIGHTLEY AM, LAM YM, BRADY JN, CAMERON CL and LILLICRAP D (1999). Variation at the von Willebrand factor (vWF) gene locus is associated with plasma vWF:Ag levels: identification of three novel single nucleotide polymorphisms in the vWF gene promoter. **Blood 93**(12):4277-83.

KIM JS, PARK HY, KWON JH, IM EK, CHOI D, JANG Y and CHO SY (2002). The roles of stromelysin-1 and the gelatinase B gene polymorphism in stable angina. **Yonsei Medical Journal 43**(4):473-481.

KOBA S, PAKALA R, WATANABE T, KATAGIRI T and BENEDICT CR (1999). Vascular smooth muscle proliferation synergistic interaction between serotonin and low density lipoproteins. **Journal of the American College of Cardiology 34:**1644-51.

KOROVESIS S, FREDERICKS S, HOLT D, TOUTOUZAS P, KASKI JC, WEBB-PEPLOE MM and KATRITSIS D (2000). Release of platelet activation markers during coronary angioplasty. **Coronary Artery Disease 11**(5):391-98.

LADENVALL P, WALL U and JERN S (2000). Identification of eight novel single-nucleotide polymorphisms at human tissue-type plasminogen activator (t-PA) locus: association with vascular t-PA release. **Thrombosis and Haemostasis 84:**150-5.

LADENVALL P, JOHANSSON L, JANSSON JH, JERN S, NILSSON TK, TJÅRNLUND, JERN C and BOMAN K (2002). Tissue-type plasminogen activator 7,351C/T enhancer polymorphism is associated with a first myocardial infarction. **Thrombosis and Haemostasis 87:**105-9.

LAMBLIN N, BAUTERS C, HERMANT X, LABLANCHE J-M, HELBECQUE N and AMOUYEL P (2002). Polymorphisms in the promoter regions of MMP-2, MMP-3, MMP-9 and MMP-12 genes as determinants of aneurismal coronary artery disease. **Journal of the American College of Cardiology 40**(1):43-8.

LANE A, GREEN F, SCARABIN PY, NICAUD V, BARA L, HUMPHRIES S, EVANS A, LUC G, CAMBOU JP, ARVEILER D and CAMBIEN F (1996). Factor VII Arg/Gln_{353} polymorphism determines factor VII coagulant 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 119:**119-127.

LEANDER K, HALLQVIST J, WIMAN B and de FAIRE U (2000). The importance of the G/A-455 polymorphism of the β-fibrinogen gene in relation to myocardial infarction. Annals of XIIth International Symposium on Atherosclerosis TuP4:W15. Abstract.

LEE AJ, LOWE GDO, WOODWARD M and TUNSTALL-PEDOE H (1993). Fibrinogen in relation to personal history of prevalent hypertension, diabetes, stroke, intermittent claudication, coronary artery disease, and family history: Scottish Heart Health Study. **British Heart Journal 69:**338-342.

LEE DH, HENDERSON PA and BLAJCHMAN MA (1996). Prevalence of factor V Leiden in a Canadian blood donor population. **Canadian Medical Association Journal 155**(3):285-289.

LI Y-H, CHEN J-H, GUO H-R, TSAI W-C and CHAO T-H (2002). Genetic risk factors associated with the prognosis of myocardial infarction in young patients. **Thrombosis and Haemostasis 88:**694-7.

LIEVERS KJA, MENNEN LI, RATTINK AP, ZWINDERMAN AH, JUKEMA JW, SCHOUTEN EG and de MAAT MPM (2000). The –323Ins10 polymorphism for factor VII is not associated with coronary atherosclerosis in symptomatic men. **Thrombosis Research 97:**275-280.

LINDGREN A, LINDOFF C, NORRVING B, ASTEDT B and JOHANSSON BB (1996). Tissue plasminogen activator and plasminogen activator inhibitor-1 in stroke patients. **Stroke 27:**1066-71.

LOHMUELLER KE, PEARCE CL, PIKE M, LANDER ES and HIRSCHORN JN (2003). Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. **Nature Genetics 33:**177-182.

LOPES AAB, MAEDA NY e BYDLOWSKI SP (1998). Fator von Willebrand e disfunção endotelial pulmonar. Implicações prognósticas. **Arquivos Brasileiros de Cardiologia 70**(3):141-145.

LOWE GD, YARNELL JW, SWEETNAM PM, RUMLEY A, THOMAS HF and ELWOOD PC (1998). Fibrin D-dimer, tissue plasminogen activator, plasminogen activator inhibitor, and the risk of major ischaemic heart disease in the Caerphilly Study. **Thrombosis and Haemostasis 79**(1):129-33.

LU PCS, YE H, MAEDA M and AZAR DT (1999). Immunolocalization and gene expression of matrilysin during corneal wound healing. **Investigative Ophthalmology Visual Science 40:**20-27.

LUC G, BARD JM, JUHAN-VAGUE I, FERRIERES J, EVANS A, AMOUYEL P, ARVEILER D, FRUCHA JC and DUCIMETIERE P (2003). C-reactive protein, interleukin-6, and fibrinogen as predictors of coronary heart disease: the PRIME Study. **Arteriosclerosis, Thrombosis, and Vascular Biology 23**(7):1255-61.

de MAAT M, JUKEMA W, YE S, ZWINDERMAN AH, MOGHADDAM PH, BEEKMAN M, KASTELEIN JJ, van BOVEN AJ, BRUSCHKE AVG, HUMPHRIES SE, KLUFT C and HENNEY AM (1999). Effect of the stromelysin-1 promoter on efficacy of *Pravastatin* in coronary atherosclerosis and restenosis. **American Journal of Cardiology 83:**852-56.

de MAAT MPM, KASTELEIN JJP, JUKEMA JW, ZWINDERMAN AH, JANSEN H, GROENEMEIER B, BRUSCHKE AVG and KLUFT C (1998). -455G/A polymorphism of the β-fibrinogen gene is associated with the progression of coronary atherosclerosis in symptomatic men. Proposed role of an acute-phase reaction pattern of fibrinogen. **Arteriosclerosis, Thrombosis, and Vascular Biology 18:**265-271.

de MAAT MPM, de BART ACW, MEIJER HP, HAVELAAR AC, MULDER PGH and KLUFT C (1996a). Interindividual and intraindividual variability in plasma fibrinogen, t-PA antigen, PAI-1 activity, and CRP in healthy, young volunteers and patients with angina pectoris. **Arteriosclerosis, Thrombosis, and Vascular Biology 16:**1156-1162.

de MAAT MPM, PIETERSMA A, KOFFLARD M, SLUITER W and KLUFT C (1996b). Association of plasma fibrinogen levels with coronary artery disease, smoking and inflammatory markers. **Atherosclerosis 121:**185-191.

MacALLISTER RJ (1998). Nitric oxide in human cardiovascular disease. **Journal of Hypertension 16**(Suppl 8):3-6.

MACCARRONE M, BARI M, del PRINCIPE D and FINAZZI-AGRO A (2003). Activation of human platelets by 2-arachidonoyglycerol is enhanced by serotonin. **Thrombosis and Haemostasis 89:**340-7.

MACH F, SCHONBECK U, BONNEFOY J-Y, POBER J and LIBBY P (1997). Activation of monocyte/macrophage functions related to acute atheroma complication by ligation of CD40: induction of collagenase, stromelysin, and tissue factor. **Circulation 96**(2):396-399.

MANDEL H, BRENNER B, BERANT M, ROSENBERG N, LANIR N, JAKOBS C, FOWLER B and SELIGSOHN U (1996). Coexistence of hereditary homocistinuria and factor V Leiden. Effect on thrombosis. **New England Journal of Medicine 334:**763-8.

MANNUCCI PM (1995). Recent progress in the pathophysiology of fibrinogen. **European Heart Journal 16**(Suppl A):25-30.

MANNUCCI PM (1998). Von Willebrand factor. A marker of endothelial damage? **Arteriosclerosis, Thrombosis, and Vascular Biology 18:**1359-1362.

MANNUCCI PM, MARI D, MERATI G, PEYVANDI F, TAGLIABUE L, SACCHI E, TAIOLI E, SANSONI P, BERTOLINI S and FRANCESCHI C (1997). Gene polymorphisms predicting high plasma levels of coagulation and fibrinolysis proteins. A study in centenarians. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**755-759.

MANSFIELD MW, STICKLAND MH and GRANT PJ (1995). Environmental and genetic factors in relation to elevated circulating levels of plasminogen activator inhibitor-1 in Causasian patients with Non-Insulin-Dependent Diabetes mellitus. **Thrombosis and Haemostasis 74:**842-7.

MANSOURATI J, da COSTA A, MUNIER S, MERCIER B, TARDY B, FEREC C, ISSAZ K and BLANC J-J (2000). Prevelence of factor V Leiden in patients with myocardial infarction and normal coronary angiography. **Thrombosis and Haemostasis 83:**822-5.

MAQUOI E, DEMELEMEESTER D, VÖRÖS G, COLLEN D and LIJNEN HR (2003). Enhanced nutritionally induced adipose tissue development in mice with stromelysin-1 gene inactivation. **Thrombosis and Haemostais 89:**696-704.

MARAGANORE JM (1993). Thrombin, thrombin inhibitors, and the arterial thrombotic process. **Thrombosis and Haemostasis 70:**208-211.

MARDER VJ, FRANCIS CW and DOOLITTLE RF (1982). Fibrinogen structure and physiology. pp. 145-160. **In.** R.W. COLMAN J, HIRSH VJ, MARDER and SALZMAN EW (Eds.). **Hemostasis and thrombosis. Basic principles and clinical practice.** J.B. Lippincott Co.

MARGAGLIONE M, D'ANDREA G, GIULKIANI N, BRANCACCIO V, de LUCIA D, GRANDONE E, de STEFANO V, TONALI PA and di MINNO G (1999). Inherited prothrombotic conditions and premature ischemic stroke: sex difference in the association with factor V Leiden. **Arteriosclerosis, Thrombosis, and Vascular Biology 19:**1750-59.

MARGAGLIONE M, CAPPUCCI G, COLAIZZO D, GIULIANI N, VECCHIONE G, GRANDONE E, PENNELLI O and di MINNO G (1998a). The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. **Arteriosclerosis, Thrombosis, and Vascular Biology 18:**152-156.

MARGAGLIONE M, CAPPUCCI G, COLAIZZO D, PIRRO L, VECCHIONE G, GRANDONE E and di MINNO G (1998b). Fibrinogen plasma levels in an apparently healthy general population-relation to environmental and genetic determinants. **Thrombosis and Haemostasis 80:**805-10.

MARGAGLIONE M, GRANDONE E, VECCHIONE G, CAPPUCCI G, GIULIANI N, COLAIZZO D, CELENTANO E, PANICO S and di MINO G (1997). Plasminogen activator inhibitor (PAI-1) antigen plasma levels in subjects attending a metabolic ward: relation to polymorphisms of PAI-1 and angiotensinogen converting enzyme (ACE) genes. **Arterioclerosis, Thrombosis, and Vascular Biology 17:**2082-87.

MARSDEN PA, HENG HH, SHERER SW, STEWART RJ, HALL AV, SHI XM, TSUI LC and SCHAPPER KT (1993). Structure and chromossomal localization of the human constitutive endothelial nitric oxide synthase gene**. Journal of Biological Chemistry 268:**17478-17488.

MARTINELLI I, LANDI G, MERATI G, CELLA R, TOSETTO A and MANNUCCI PM (1996). Factor V gene mutation is a risk factor for cerebral venous thrombosis. **Thrombosis and Haemostasis 75**(3):393-4.

MATHONNET F, NADIFI S, SERAZIN-LEROY V, DAKOUANE A and GIUDICELLI Y (2002). Absence of factor V Leiden mutation and low prothrombin G20210A mutation prevalence in a healthy Moroccan population. **Thrombosis and Haemostasis 88:**1073-4.

MAZZOLAI L, SILACCI P, BOUZOURENE K, DANIEL F, BRUNNER H and HAYOZ D (2002). Tissue factor activity is upregulated in human endothelial cells exposed to oscillatory shear stress. **Thrombosis and Haemostasis 87:**1062-8.

McFADDEN EP, CLARKE JC, DAVIES GJ, KASKI JG, HAIDER AW and MASERI A (1991). Effect of intracoronary serotonin on coronary vessels in patients with stable angina and patients with variant angina. **New England Journal of Medicine 324:**648-54.

McCAUWLEY LJ & MATRISLAN LM (2000). Matrix metalloproteinases: multifuntional contributors to tumor progression. **Molecular Medicine Today 6:**149-156.

MEADE TW, CHAKRABARTI R, HAINES AP, NORTH WRS, STIRLING Y and THOMPSON SG (1980). Haemostatic function and cardiovascular death: early results of a prospective study. **Lancet 1:**1050-4.

MEADE TW, BROZOVIC M, CHAKRABARTI RR, HAINES AP, IMESON JD, MELLOWS S, MILLER GJ, NORTH WRS, STIRLING Y and THOMPSON SG (1986). Haemostatic function and ischaemic heart disease: principal results of the Northwick Park Heart Study. **Lancet ii:**533-537.

MEADE TW, RUDDOCK V, STIRLING Y, CHAKRABARTI R and MILLER GJ (1993). Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study. **Lancet 342:**1076-79.

MEDVED L & NIEUWENHUIZEN W (2003). Molecular mechanisms of initiation of fibrinolysis by fibrin. **Thrombosis and Haemostasis 89:**409-19.

MIKKELSSON J & KARHUNEM P (2002). Genetic variation in coagulation factors II, V, VII in fatal MI. **Thrombosis and Haemostasis 87:**349-50.

MIYAMOTO Y, SAITO Y, KAJIYAMA N, YOSHIMURA M, SHIMASAKI Y, NAKAYAMA M, KAMITANI S, HARADA M, ISHIKAWA M, KUWAHARA K, OGAWA E, HAMANAKA I, TAKAHASHI N, KANESHIGE T, TERAOKA H, AKAMIZU T, AZUMA N, YOSHIMASA Y, YOSHIMASA T, ITO H, MASUDA I, YASUE H and NAKAO K (1998). Endothelial nitric oxide synthase gene is positively associated with essential hypertension. **Hypertension 32**(1):3-8.

MURPHY RP, DONOGHUE C, NALLEN RJ, D´ELLO M, REGAN C, WHITEHEAD AS and FITZGERALD DJ (2000). Prospective evaluation of the risk conferred by factor V Leiden and thermolabile methylenetetrahydrofolate reductase polymorphisms in pregnancy. **Arteriosclerosis, Thrombosis, and Vascular Biology 20:**266-270.

NAKAYAMA M, YASUE H, YOSHIMURA M, SHIMASAKI Y, KUGIYAMA K, KIYOTAKA K, OGAWA H, MOTOYAMA T, SAITO Y, OGAWA Y, MIYAMOTO Y, MIYAMAMOTO Y and NAKAO K (1999). T-786-C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. **Circulation 99**(22):2864-2870.

NAKAYAMA M, YASUE H, YOSHIMURA M, SHIMASAKI Y, OGAWA H, KUGIYAMA K, MIZUNO Y, HARADA E, NAKAMURA S, ITO T, SAITO Y, MIYAMOTO Y, OGAWA Y and NAKAO K (2000). T-786C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with myocardial infarction, especially without coronary organic stenosis. **American Journal of Cardiology 86:**628-34.

NICHOLS TC, BELLINGER DA, REDDICK RL, READ MS, KOCH GG, BRINKHOUS KM and GRIGGS TR (1991). Role of von Willebrand factor in arterial thrombosis. Studies in normal and von Willebrand disease pigs. **Circulation 83**(Suppl IV):56-64.

NILSSON TK, SPENCE JD, NILSSON PM, ELIASSON M, JANSSON J-H and BOMAN K (2002). Quantitative measurement of carotid atherosclerosis in relation to levels of von Willebrand factor and fibrinolytic variables in plasma – a 2-year follow-up study. **Journal of Cardiovascular Risk 9**(4):215-221.

NOTO D, BARBAGALLO CM, CEFALU AB, CAVERA G, SAPIENZA M, NOTARBARTOLO A, DAVI G and AVERNA MR (2002). Factor VII activity is an independent predictor of cardiovascular mortality in elderly women of a Sicilian population: results of an 11-year follow-up. **Thrombosis and Haemostasis 87:**206-10.

OGER E, LACUT K, le GAL G, van DREDEN P, BRESSOLLETTE L, SCARABIN PY, LEROYER C and MOTTIER D (2002). Is APC resistance a risk factor for venous thromboembolism in patients over 70 years? **Thrombosis and Haemostasis 88:**587-91.

OLINESCU RM & KUMMEROW FA (2001). Fibrinogen is an efficient antioxidant. **Journal of Nutritional Biochemistry 12:**162-169.

OSSEI-GERNING N, MANSFIELD M, STIKLAND M, WILSON IJ and GRANT PJ (1997). 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. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**33-37.

OSSEI-GERNING N, WILSON IJ and GRANT PJ (1998). Sex differences in coagulation and fibrinolysis in subjects with coronary artery disease. **Thrombosis and Haemostasis 79:**736-40.

PALUDO CA (1999). Estudo de polimorfismos de DNA dos genes do fibrinogênio e fator VII, níveis de fator von Willebrand e fibrinogênio, e outros fatores de risco no perfil da doença aterosclerótica coronariana no Rio Grande do Sul. Dissertação de mestrado em Genética e Biologia Molecular. Curso de Pós-graduação em Genética e Biologia Molecular. UFRGS. 119p.

PELTONEN M, LUNDBERG V, HUHTASAARI F and ASPLUND K (2000). Marked improvement in survival after acute myocardial infarction in middle-aged men but not in women. The Northen Sweden MONICA Study 1985-94. **Journal of Internal Medicine 247:**579-587.

PETROVICK D, ZORC M, KEBER I and PETERLIN B (2001). Joint effect of G1691A factor V point mutation and factor VII Arg/Gnl353 gene polymorphism on the risk of premature coronary artery disease. **Annales de Génétique 44:**33-36.

POLLAK ES, HUNG H-L, GODIN W, OVERTON GC and HIGH KA (1996). Functional characterization of the human factor VII 5´flanking region. **Journal of Biological Chemistry 271(3):**1738-47.

POORT S, ROSENDAAL F, REITSMA P and BERTINA R (1996). A common genetic variation in the 3'-unstranslated region of the protrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. **Blood 88:**3698-3703.

POTRON G, N'GUYEN P and PIGNON B (1996). Le fibrinogène: facteur de risque. **Journal des Maladies Vasculaires 21**(3):126-132.

PROHASKA W, SCHMIDT M, MANNEBACH H, GLEICHMANN U and KLEESIEK K (1999). The prevalence of the prothrombin 20210 G-A mutation is not increased in angiographically confirmed coronary artery disease. **Thrombosis and Haemostasis 81:**161-2.

QUINONES S, BITTICE G and KURKINEN M (1994). Promoter elements in the transcriptional activation of the human stromelysin-1 gene by the inflammatory cytokine, interleukin 1. **Biochemical Journal 302:**471-477.

QUINONES S, SAUS J, OTANI Y, HARRIS Jr. ED and KURKINEN M (1989). Transcriptional regulation of human stromelysin. **Journal of Biological Chemistry 26:**8339-8344.

REDONDO M, WATZKE HH and STUCKI B (1999). Coagulation factors II, V, VII and X, prothrombin gene 20210G-A transition, and factor V Leiden in coronary artery disease: high factor V clotting activity is an independent risk factor for myocardial infarction. **Arteriosclerosis, Thrombosis, and Vascular Biology 19:**1020-1025.

RIDKER PM, HENNECKENS CH, LINDPAINTER K, STAMFER MJ, EISENBERG PR and MILETICH JP (1995). Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and thrombosis in apparently healthy men. **New England Journal of Medicine 332:**912-17.

RIDKER P, HENNEKENS C, LINDPAINTNER K, STAMPFER M and MILETICH J (1997). 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 95:**59-62.

RIDKER PM, HENNEKENS CH and MILETICH JP (1999). G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. **Circulation 99:**999-1004.

RIDKER PM, VAUGUAN DE, STAMPFER MJ, MANSON JE and HENNECKENS CH (1993). Endogenous tissue-type plasminogen activator and risk of myocardial infarction. **Lancet 341**(8854):1165-68.

RÖHSIG LM, DAMIN DC, STEFANI SD, CASTRO Jr. CG, ROISENBERG I and SCHWARTZMANN G (2001). Von Willebrand factor antigen levels in plasma of patients with malignant breast disease. **Brazilian Journal of Medical and Biological Research 34:**1125-9.

ROSENDAAL FR, DOGGEN CJM, ZIVELIN A, ARRUDA VR, AIACH M, SISCOVICK DS, HILLARP A, WATZKE HH, BERNARDI F, CUMMING AM, PRESTON FE and REITSMA PH (1998). Geographic distribution of the 20210 G to A prothrombin variant. **Thrombosis and Haemostasis 79:**706-8.

ROSENDAAL FFR, SISCOVICK DS, SCHWARTZ SM, BEVERLY RK, PSATY BM, LONGSTRETH WT, RAGHUNATHAN TE, KOEPSEL L and REITSMA PH (1997a). Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. **Blood 89**(8):2817-2821.

ROSENDAAL FFR, SISCOVICK DS, SCHWARTZ SM, PASTY BM, RAGHUNATHAN TE and VOS HL (1997b). A common prothrombin variant (20210G to A) increases the risk of myocardial infarction in young women. **Blood 90**(5):1747-1750.

ROSENGREN A & WILHELMSEN L (1996). Fibrinogen, coronary heart disease and mortality from all causes in smokers and nonsmokers. The study of men born in 1933. **Journal of Internal Medicine 239:**499-507.

ROSS R (1993). The pathogenesis of atherosclerosis: a perspective for the 1990s. **Nature 362:**801-09.

ROSS R (1999). Atherosclerosis – an inflammatory disease. **New England Journal of Medicine 340(2):**115-126.

ROSS R & GLOMSET JA (1973). Atherosclerosis and the arterial smooth cell. **Science 180:** 1332-9.

ROSSI GP, CESARI M, ZANCHETTA M, COLONNA S, MAIOLINO G, PEDON L, CAVALLIN M, MAIOLINO P and PESSINA AC (2003). The T-786C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA Study. **Journal of the American College of Cardiology 41**(6):930-7.

SADLER JE (1998). Biochemistry and genetics of von Willebrand factor. **Annual Review of Biochemistry 67:**395-424.

SANADA S, KITAKAZE M, NODE K, ASANUMA H, OGITA H, TAKASHIMA S, ASAKURA M, MINAMINO T and HORI M (2002). Selective serotonin 5-HT2A receptor inhibition attenuates myocardial ischemia through the increase in nitric oxide production: the alternative role of 5Ht1B receptor. **Journal of the American College of Cardiology 6:**292.

SCARABIN P-Y, AILLAUD M-F, AMOUYEL P, EVANS A, LUC G, FERRIÈRES J, ARVEILER D and JUHAN-VAGUE I (1998). Associations of fibrinogen, factor VII and PAI-1 with baseline findings among 10,500 male participants in a prospective study of myocardial infarction. **Thrombosis and Haemostasis 80:**749-56.

SCARABIN P-Y, BARA L, RICARD S, POIRIER O, CAMBOU JP, ARVEILER D, LUC G, EVANS AE, SAMAMA MM and CAMBIEN F (1993). Genetic variation at the β-fibrinogen locus in relation to plasma fibrinogen concentrations and risk of myocardial infarction. The ECTIM Study. **Arteriosclerosis and Thrombosis 13:**886-891.

SCHMITZ-HUEBNER U, THOMPSON SG, BALLEISEN L, FECHTRUP C, GROSSE-HEITMEYER W, KIRCHHOF B, MOST E, MÜLLER U-S, SEIFFERT C, SEIFFERT D and van de LOO J (1988). Lack of Association between haemostatic variables and the presence or the extent of coronary atherosclerosis. **British Heart Journal 59:**287-91.

SCHUMACHER A, SELJEFLOT I, SOMMERVOLL L, CHRISTENSEN B, OTTERSTAD JE and ARNESEN H (2002). Increased levels of endothelial haemostatic markers in patients with coronary artery disease. **Thrombosis Research 105:**25-31.

SCHWARTZMANN G, DAMIN DC and ROISENBERG I (2001). Malignant disease and von Willebrand factor. **The Lancet Oncology 2:**716-7.

SELLERS A & MURPHY G (1981). Collagenolytic enzymes and their naturally occurring inhibitors. **International Review of Connective Tissue Research 9:**151-90.

SHAH PK, FALK E, BADIMON JJ, ORTIZ AF, MAILHAC A, LEVY GV, FALLON JT, REGNSTRON J and FUSTER V (1995). Human monocyte-derived macrophages induce collagen breakdown in fibrous caps of atherosclerotic plaques. **Circulation 92:**1565-69.

SHIMASAKI Y, YASUE H, YOSHIMURA M, NAKAYAMA M, KUGIYAMA K, OGAWA H, HARADA E, MASUDA T, KOYAMA W, SAITO Y, MIYAMOTO Y, OGAWA Y and NAKAO K (1998). Association of the missense Glu298Asp variant of the endotelial nitric oxide synthase gene with myocardial infarction. **Journal of the American College of Cardiology 31:**1506-10.

SHIMIZU M, AKITA H, SHIGA N, TAKAI E, IWAI C, MIYAMOTO Y, JYOUKAI Y, KUMAGAI S, HASHIMOTO M and YOKOYAMA M (2002a). The relationship between serotonin 2A receptor gene polymorphism and serotonin induced platelet aggregation **Journal of the American College of Cardiology 259** (Supll A):89. Abstract.

SHIMIZU Y, MINATOGUCHI S, HASHIMOTO K, UNO Y, ARAI M, WANG N, CHEN X, LU C, TAKEMURA G, SHIMOMURA M, FUJIWARA T and FUJIWARA H (2002b). The role of serotonin in ischemic cellular damage and the infarct size-reduced effect of sarpogrelate, a 5-hydroxytryptamine-2 receptor blocker, in rabbit hearts. **Journal of the American College of Cardiology 40**(7):1347-55.

SHIMOKATA K, KONDO T, OHNO M, TAKESHITA K, INDEN Y, IINO S, SAITO H and HIRAI M (2002). Effects of coagulation factor VII polymorphisms on the coronary artery disease in Japanese. **Thrombosis Research 105:**493-498.

SHOJI M, TSUTAYA S, SAITO R, TAKAMATU H and YASUJIMA M (2000). Positive association of endothelial nitric oxide synthase gene polymorphism with hypertension in northen Japan. **Life Sciences 66**(26):2557-2562.

SIMIONI P, PRADONI P, LENSING AWA, SCUDELLER A, SARDELLA C, PRINS MH, VILLALTA S, DAZZI F and GIROLAMI A (1997). The risk of recurrent venous thromboembolism in patients with an Arg506-Gln mutation in the gene for factor V (factor V Leiden). **New England Journal of Medicine 336:**399-03.

SIMIONI P, TORMENE D, MANFRIN D, GAVASSO S, LUNI S, STOCCO D and GIROLAMI A (1998). Prothrombin antigen levels in symptomatic and asymptomatic carriers of the 20210A prothrombin variant. **British Journal of Haematology 103:**1045-1050.

SIMON D, PALATINIK M and ROISENBERG I (2002). Analysis of the –1185A/G von Willebrand factor (VWF) gene polymorphism in two Brazilian ethnic groups and its effect on plasma VWF levels. **Thrombosis Research 105:**519-522.

SIMON D, PALUDO CA, GHISLENI, GC, MANFROI WC and ROISENBERG I (2003). Association studies between –1185A/G von Willebrand factor gene polymorphism and coronary artery disease. **Brazilian Journal of Medical and Biological Research 36:**in press.

SMILES AM, JENNY NS, TANG Z, ARNOLS A, CUSHMAN M and TRACY RP (2002). No association of plasma prothrombin concentration or the G20210A mutation with incident cardiovascular disease: results from the Cardiovascular Health Study. **Thrombosis and Haemostasis 87**(4):614-21.

SMITH CCT & BETTERIDGE DJ (1997). Reduced platelet serotonin content and release in familial hypercholesterolemia. **Atherosclerosis 130:**87-92.

SPARKES RS, LAN N, KLISAK I, MOHANDAS T, DIEP A, KOJIS T, HEINZMANN C and SHIH JC (1991). Assignment of a serotonin 5HT-2 receptor gene (HTR2) to human chromossome 13q14-q21 and mouse chromossome 14. **Genomics 9**(3):461-5.

SPURR NK, GOUGH AC, GOSDEN J, ROUT D, PORTEUS DJ, van HEYNINGEN V and DOCHERTY AJP (1988). Restriction fragment length polymorphism analysis and assignment of the metalloproteinases stromelysin and collagenase to the long arm of chromossome 11. **Genomics 2:**119-127.

SOSEF MN, BOSCH JG, van OOSTAYEN J, VISSER I, REIBER JH and ROSENDAAL FR (1994). Relation of plasma coagulation factor VII and fibrinogen to carotid artery intima-media thickness. **Thrombosis and Haemostasis 72:**250-254.

SOUTO JC, ALMASY L, SORIA JM, BUIL A, STONE W, LATHROP M, BLANGERO J and FONTCUBERTA J (2003). Genome-wide linkage analysis of von Willebrand factor plasma levels: results from the GAIT project. **Thrombosis and Haemostasis 89:**468-74.

STEEDS R, ADAMS M, SMITH P, CHANNER K and SAMANI NJ (1998). Distribution of tissue plasminogen activator insertion/deletion polymorphism in myocardial infarction and control subjects. **Thrombosis and Haemostasis 79:** 980-4.

de STEFANO V, CHIUSOLO P, PACIARONI K, CASORELLI I, ROSSI E, MOLINARI M, SERVIDEI S, TONALI PA and LEONE G (1998). Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. **Blood 91**(10):3562-65.

STERNLICHT MD, LOCHTER A, SYMPSON CJ, HUEY B, ROUGIER JP, GRAY JW, PINKEL D, BISSELL MJ and WERB Z (1999). The stromal proteinase MMP3/stromelysisn-1 promotes mammary carcinogenesis. **Cell 98**(2):137-46.

STRIK J, HONIG A and MAES M (2001). Depression and myocardial infarction: relationship between heart and mind. **Progress in Neuro-Psychopharmacology and Biological Psychiatry 25:**879-892.

SUZUKI T, OKUMURA K, SONE T, KOSOKABE T, TSUBOI H, KONDO J, MUKAWA H, KAMIYA H, TOMIDA T, IMAI H, MATSUI H and HAYAKAWA T (2002). The Glu298Asp polymorphism in endothelial nitric oxide synthase gene is associated with coronary in-stent restenosis. **International Journal of Cardiology 86:**71-76.

SVENSSON PJ, ZÖLLER B, MATTIASSON I and DAHLBÄCK B (1997). The factor V R506Q mutation causing APC resistance is highly prevalent amongst unselected outpatients with clinically suspected deep venous thrombosis. **Journal of Internal Medicine 241**(5):379-85.

TAMIN H, FINAN RR and ALMAWI Y (2002). Prevalence of two thrombophilia predisposing mutations: factor V G1691A (R506Q, Leiden) and prothrombin G20210A, among healthy Lebanese. **Thrombosis and Haemostasis 88:**691-2.

TERASHIMA M, AKITA H, KANAZAWA K, INOUE N, YAMADA S, ITO K, MATSUDA Y, TAKAI E, IWAI C, KUROGANE H, YOSHIDA Y and YOKOYAMA M (1999). Stromelysin promoter 5A/6A polymorphism is associated with acute myocardial infarction. **Circulation 99:**2717-19.

THOMAS AE, GREEN FR and HUMPHRIES SE (1996). Association of genetic variation at the β-fibrinogen gene locus and plasma fibrinogen levels; interaction between allele frequency of the G/A^{-455} polymorphism, age and smoking. **Clinical Genetics 50:**184-190.

THOMAS A, GREEN F, KELLEHER C, WILKES H, BRENNAN P, MEADE T and HUMPHRIES S (1991). Variation in the promoter region of the β fibrinogen gene is associated with plasma fibrinogen levels in smokers and non-smokers. **Thrombosis and Haemostasis 65**(5):487-490.

THOMAS AE, GREEN FR, LAMLUM H and HUMPHRIES SE (1995). The association of combined α and β fibrinogen genotype on plasma fibrinogen levels in smokers and non-smokers. **Journal of Medical Genetics 32:**585-589.

THOMAS DP & ROBERTS HR (1997). Hypercoagulability in venous and arterial thrombosis. **Annals of Internal Medicine 126:**638-644.

THOMPSON SG, KIENAST J, PYKE SDM, HAVERKATE F and van de LOO JCW (1995). Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. **New England Journal of Medicine 332:**635-41.

TUDDENHAM EGD & COOPER DN (1994). **The molecular genetics of haemostasis and its inherited disorders.** Oxford University Press.

TYBJÆRG-HANSEN A, AGERHOLM-LARSEN B, HUMPHRIES SE, ABILDGAARD S, SCHNOHR P and NORDESTGAARD BG (1997). A common mutation (G-455A) in the β-fibrinogen promoter is an independent predictor of plasma fibrinogen, but not of ischemic heart disease. A study of 9,127 individuals based on the Copenhagen City Heart Study. **Journal of Clinical Investigation 99**(12):3034-3039.

ULLMER C, SCHMUCK K, KALKMAN HO and LÜBBERT H (1995). Expression of serotonin receptor mRNAs in blood vessels. **FEBS Letters 370:**215-21.

VÄISÄNEN S, RAURAMAA R, PENTTILÄ I, RANKINEN T, GAGNON J, PÉRUSSE L, CHAGNON M and BOUCHARD C (1997). Variation in plasma fibrinogen over one year: relationship with genetic polymorphisms and non-genetic factors. **Thrombosis and Haemostasis 77**(5):884-9.

VANHOUTTE PM (1991). Platelet-derived serotonin, the endothelium and cardiovascular disease. **Journal of Cardiovascular Pharmacology 17**(Suppl 5):6-12.

VASSE M, PAYSANT J, SORIA J, COLLET JP, VANNIER J-P and SORIA C (1996). Regulation of fibrinogen biosynthesis by cytokines, consequences on the vascular risk. **Haemostasis 26**(Suppl 4):331-339.

VIKENES K, FARSTAD M and NORDREHAUG JE (1999). Serotonin is associated with coronary artery disease and cardiac events. **Circulation 100**(5):483-89.

VISSE R & NAGASE H (2003). Matrix metalloproteinases and tissue inhibitors of metalloproteinases. Structure, function, and biochemistry. **Circulation Research 92:**827-839.

WÅHLANDER K, LARSON G, LINDAHL TL, ANDERSSON C, FRISON L, GUSTAFSSON D, BYLOCK A and ERIKSSON BI (2002). Factor V Leiden (G1691A) and prothrombin gene G20210A mutations as potential risk factors for venous thromboembolism after total hip or total knee replacement surgery. **Thrombosis and Haemostasis 87:**580-5.

WANG XL & WANG J (2000). Endothelial nitric oxide synthase gene sequence variations and vascular disease. **Molecular Genetics and Metabolism 70:**241-251.

WANG XL, WANG J, McCREDIE RM and WILCKEN DEL (1997). Polymorphisms of factor V, factor VII, and fibrinogen genes. Relevance to severity of coronary artery disease. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**246-251.

WARREN JT, PEACOCK ML, RODRIGUEZ LC and FINK JK (1993). An Msp I polymorphism in the human serotonin receptor gene (HTR2): detection by DGGE and RFLP analysis. **Human Molecular Genetics 2:**338.

van der WATER NS, FRENCH JK, LUND M, HYDE TA, WHITE HD and BROWETT PJ (2000). Prevalence of factor V Leiden and prothrombin variant G20210A in patients age < 50 years with no significant stenoses at angiography three to four weeks after myocardial infarction. **Journal of the American College of Cardiology 26**(3):717-22.

WILHELMSEN L, SVÄRDSUDD K, KORSAN-BENGTSEN K, LARSSON B, WELIN L and TIBBLIN G (1984). Fibrinogen as a risk factor for stroke and myocardial infarction. **New England Journal of Medicine 311:**501-5.

WILLIAMS J, McGUFFIN P, NOTHEN M, OWEN MJ, and EMASS Collaborative Group (1997). Meta-analysis of association between the 5-HT (2A) receptor T102C polymorphism and schizophrenia. **Lancet 349:**1221.

WILLIAMS J, SPURLOCK G, McGUFFIN P, MALLET J, NOTHEN M, GILL M, NYLANDER PO, MACCIARDI F and OWEN MJ (1996). Association between schizophrenia and T102C polymorphism of the 5-hydroxytryptophan type 2A receptor gene. European Multicentre Association Study of Schizophrenia (EMASS) Group. **Lancet 347:**1294-1296.

WOODWARD M, LOWE GDO, RUMLEY A and TUNSTALL-PEDOE H (1998). Fibrinogen as a risk factor for coronary heart disease and mortality in middle-aged men and women. The Scottish Heart Health Study. **European Heart Journal 19:**55-62.

WU KK & THIAGARAJAN P (1996). Role of endothelium in thrombosis and hemostasis. **Annual Review of Medicine 47:**315-31.

YAMADA S, AKITA H, KANAZAWA K, ISHIDA T, HIRATA K, ITO K, KAWASHIMA S and YOKOYAMA M (2000). T102C polymorphism of the serotonin (5-HT) 2A receptor gene in patients with non-fatal acute myocardial infarction. **Atherosclerosis 150:**143-48.

YAMADA Y, IZAWA H, ICHIHARA S, TAKATSU F, ISHIHARA H, HIRAYAMA H, SONE T, TANAKA M and YOKOTA M (2002). Prediction of the risk of myocardial infarction from polymorphisms in candidate genes. **New England Journal of Medicine 347**(24):1916-1923.

YANQING H, FANGPING C, QINZHI X, ZAIFU J, GUANGPING W, XIAOXIA Z, XIAOQUN P and XIAOBO (2003). No association between thrombosis and factor V gene polymorphism in Chinese Han population. **Thrombosis and Haemostasis 89:**446-51.

YARNELL JWG, BAKER IA, SWEETNAM PM, BAINTON D, O'BRIEN JR, WHITEHEAD PJ and ELWOOD PC (1991). Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell Collaborative Heart Disease Studies. **Circulation 83:**836-844.

YE S, WATTS GF, MANDALIA S, HUMPHRIES SE and HENNEY AM (1995). Genetic variation in the human stromelysin promoter is associated with progression of coronary atherosclerosis. **British Heart Journal 73:**209-215.

YE S, WHATLING C, WATKINS H and HENNEY A (1999). Human stromelysin gene promoter activity is modulated by transcription factor ZBP-89. **FEBS Letters 450:**268-272.

YOKOYAMA M, AKITA H, MIZUTANI T, FUZUZAKI H and WATANABE Y (1983). Hyperreactivity of coronary arterial smooth muscles in response to ergonovine from rabbits with hereditary hiperlipidemia. **Circulation Research 53:**63-71.

YOON S, TROMP G, VONGPUNSAWAD S, RONKAINEN A, JUVONEN T and KUIVANIEMI H (1999). Genetic analysis of MMP3, MMP9, and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms. **Biochemical and Biophysical Research Communications 265:**563-568.

YOSHIMURA M, YASUE H, NAKAYAMA M, SHIMASAKI Y, OGAWA H, KUGIYAMA K, SAITO Y, MIYAMOTO Y, KANESHIGE T, HIRAMATSU H, YOSHIOKA T, KAMITANI S, TERAOKA H and NAKAO K (2000). Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786C and missense Glu298Asp variants. **Journal of Investigation Medicine 48**(5):367-74.

YOSHIMURA M, YASUE H, NAKAYAMA M, SHIMASAKI Y, SUMIDA H, SUGIYAMA S, KUGIYAMA K, OGAWA Y, SAITO Y, MIYAMOTO Y and NAKAO K (1998). A missense Glu298Asp variant in the endothelial nitric oxide synthase gene is associated with coronary spasm in the Japanese. **Human Genetics 103:**65-69.

ZIMMERMAN TS & MEYER D (1982). Factor VIII-von Willebrand factor and the molecular basis of von Willebrand's disease. pp. 54-90. **In.** RW COLMAN, J HIRSH, VJ MARDER and EW SALZMAN (Eds.). **Hemostasis and thrombosis. Basic principles and clinical practice.** J.B. Linpcott Co.

ZITO F, di CASTELNUOVO A, AMORE C, D´ORAZIO A, DONATI MB and IACOVIELLO L (1997). Bcl I polymorphism in the fibrinogen β-chain gene is associated with the risk of familial myocardial infarction by increasing plasma fibrinogen levels. A case-control study in a sample of GISSI-2 patients. **Arteriosclerosis, Thrombosis, and Vascular Biology 17:**3489-3494.

ZUCKER DK & BRANCHEY L (1985). Lithium, CNS serotonergic tone, and intoxication. **American Journal of Psychiatry 142**(7):886-7.

Capítulo 8

Anexos

Anexo 1. Termo de consentimento informado.

Anexo 2. Protocolo aplicado durante a entrevista com os pacientes.

Anexo 3. Protocolo aplicado durante a entrevista com os indivíduos controles.

Anexo 4. Questionário ROSE modificado, aplicado durante a entrevista com os indivíduos controles.

Anexo 5. Comparações das freqüências de indivíduos portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes que apresentaram intercorrências durante o período hospitalar.

Anexo 6. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes, de acordo com o tipo de intercorrência hospitalar:

6a. Angina pós-IAM.

6b. Re-IAM.

6c. Arritmias.

6d. Insuficiência ventricular esquerda.

Anexo 7. Comparações das freqüências de indivíduos portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes que tiveram intercorrências durante o período pós-hospitalar.

Anexo 8. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes, de acordo com o tipo de intercorrência pós-hospitalar:

8a. Angina pós-IAM.

8b. Re-IAM.

8c. Insuficiência ventricular esquerda.

Anexo 9. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes e controles que apresentam mais de 2 fatores de risco cardiovasculares.

Anexo 10. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes que tiveram um evento de IAM e pacientes com re-IAM (de 2 a 4 IAMs).

Anexo 11. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes sem estenose angiográfica e pacientes com estenoses evidente.

Anexo 12. Comparações das freqüências de portadores dos alelos de risco (homozigotos e heterozigotos) entre pacientes com 1 ou 2 estenoses e pacientes com $\geq$ 3 estenoses.

Anexo 1

TERMO DE CONSENTIMENTO INFORMADO

Eu,…….. declaro, sob a responsabilidade do médico que assina esse documento, que concordo em participar do projeto de pesquisa ESTUDO DOS FATORES GENÉTICOS DE RISCO PARA O INFARTO DO MIOCÁRDIO. Recebi explicação clara e detalhada sobre a pesquisa acima mencionada, a qual submeto-me de livre e espontânea vontade, reconhecendo que:

1) Foi explicado que o objetivo do estudo é possibilitar uma melhor compreensão do mecanismo do infarto agudo do miocárdio em idade precoce. Esse é um estudo genético que avaliará dosagens protéicas e polimorfismos de DNA em genes de fatores hemostáticos, que estão envolvidos na patogênese dessa doença.

2) Minha participação envolve a retirada de 10 ml de sangue periférico para análise e uma entrevista. O desconforto que poderei sentir é o da picada da agulha e a formação de um pequeno hematoma. A amostra de sangue coletada será utilizada estritamente para os exames laboratoriais descritos no presente projeto. O DNA genômico extraído da minha amostra de sangue será armazenado apropriadamente e identificado por um código, garantindo o sigilo da minha identidade. O DNA extraído somente será utilizado para a genotipagem dos polimorfismos genéticos descritos no presente projeto, não sendo utilizado para nenhum outro fim. Foi garantido que nenhuma outra pessoa, além da doutoranda e seu orientador, terá acesso ao material proveniente da minha amostra. Foi explicado que todos os restos celulares resultantes da coleta de sangue, não utilizados no presente trabalho, serão desprezados.

3) Me foi dada a liberdade de retirar meu consentimento a qualquer momento e deixar de participar do estudo, sem que isso traga prejuízo à minha pessoa.

4) Foi dada a garantia de receber resposta a qualquer pergunta ou dúvida acerca dos benefícios e riscos da pesquisa. Os dados referentes a este estudo poderão ser acessados por mim, pelos pesquisadores envolvidos ou pelo médico responsável.

5) Foi dada a garantia de não ser identificado e de ser mantido o caráter confidencial da informação em relação à minha privacidade.

6) Foi explicado que não receberei medicação e foi garantido que não terei gastos em

participar desse estudo.

……

assinatura do paciente

.....

assinatura do médico responsável

.....

assinatura da doutoranda

Declaro que esse formulário foi lido para o paciente……em……/……/……, pela Dra……enquanto eu……estava presente.

……

assinatura da testemunha

Anexo 2

PROTOCOLO DO PACIENTE IAM ____

nº prontuário:_____ Data:_____

Nome:_____ ABO:_____

Raça: □ caucasóide □ negróide □ outro_____ Sexo: □ masculino □ feminino

Data de Nascimento: _____/_____/_____ Idade: _____anos

Altura: _____ m Peso: _____Kg IMC:_____

Endereço:_____n°_____

apto_____bairro_____CEP_____Cidade:_____

Telefone:_____Ocupação:_____

Proveniente de: _____

Infarto do Miocárdio (número):_____Data e localização do primeiro IAM: _____

Ocorrência de outros IAMs entre o primeiro IAM e o IAM em estudo: _____

Data do IAM em estudo:_____

Modo de tratamento hospitalar do IAM em estudo:

( ) conservador ( ) trombolítico ( ) ACTP primária

Evolução hospitalar:

( ) Angina pós IAM ( ) Re–IAM relacionado ao último infarto

( ) Arritmias Ventriculares ( ) IVE ( ) Morte relacionada ao IAM

( ) nenhuma intercorrência

Procedimentos de Revascularização utilizados no caso:

1) ACTP primária (data) citar o tipo (balão, stent ou ambos)_____

_____

2) ACTP com balão (data e vaso dilatado)_____

_____

3) Implante de Stent (data e vaso dilatado)_____

_____

4) CRM (data e descrição) _____

_____

Complicações do(s) procedimento(s): _____

% de lesão residual da ACTP: _____

Evolução pós hospitalar: _____

AVC pré-IAM em estudo: □ sim (data) (_____) □ não

AVC pós IAM em estudo: _____trombose: _____

Outra doença aterosclerótica presente: Qual? _____

Data do diagnóstico de outra doença aterosclerótica: _____

FATORES DE RISCO

Doenças crônicas: _____

HAS: □ sim (□ tratada_____) □ não □ não sabe _____

Tabagismo: □ sim (tempo de fumo e quantidade de cig/dia _____) □ não □ ex-fumante (_____) _____

Hiperlipidemia: □ sim Há quanto tempo? _____ Tratamento (□ dieta_____ □ droga_____) □ não □ não sabe _____

Atividade Física: □ sedentária □ não-sedentária freqüência:_____

Diabete: □ sim (tipo_____ □ tratada_____) □ não □ não sabe

Etilismo: □sim □ não quantidade: _____/_____ /_____

Gestações: □ sim (_____) □ nãoACO: □ sim (tempo:_____) □ não

Menopausa: □ sim (tempo:_____) □ não □ HTR _____

HISTÓRIA FAMILIAR

grau de parentesco e idade do diagnóstico

□ Angina (_____)

□ Infarto do Miocárdio (_____)

□ Diabete (_____)

□ HAS (_____)

□ Morte Súbita (_____)

□ Outra doença aterosclerótica(carotídea e/ou doença vascular periférica (_____)

□ HF de outras doenças(_____)

Medicamentos em uso (descrever) _____

_____

_____

Cineangiocoronariografia relacionada ao IAM em estudo (data e descrição) _____/_____/_____

_____

_____

Observações:_____

_____

_____

Anexo 3

PROTOCOLO DO CONTROLE CO _____

data _____

Nome: _____ ABO: _____

raça: □ caucasóide □ negróide □ outra _____sexo: □ feminino □ masculino

idade: _____ data de nascimento: _____/_____/_____

altura: _____ peso: _____ IMC: _____

endereço: _____ n°_____apto_____

bairro_____ cidade_____ CEP_____

telefone _____ ocupação _____

estado civil _____ proveniente de _____

doenças crônicas: _____

HAS: □ sim (desde quando? _____tratada? _____desde quando? _____)

□ não □ não sabe

tabagismo: □ sim (quantos cigarros/dia? _____ quantos anos? _____

□ não □ ex-fumante/tempo de abandono? _____

atividade física: □ sedentário □ não sedentário freqüência por semana:_____

diabete: □ sim (desde quando? _____tratada? _____) □ não □ não sabe

etilismo: □ sim/quantidade por semana: _____ □ não

gestações: □ sim ____ □ não ACO: □ sim □ não

menopausa: □ sim desde quando? _____ □ não HTR: □ sim □ não

HISTÓRIA FAMILIAR

(grau de parentesco e idade do diagnóstico):

Angina: _____

Infarto do miocárdio: _____

Diabete: _____

HAS: _____

Morte súbita: _____

Outra doença aterosclerótica (carotídea e/ou vascular periférica): _____

Outras doenças: _____

Medicação:_____
_____
_____

Observações:_____
_____
_____

Anexo 4

QUESTIONÁRIO ROSE MODIFICADO

É necessário que a resposta seja SIM para todos os itens de cada diagnóstico

ANGINA

1. Você tem dor no peito quando sobe lomba/escada ou caminha rápido?
2. Se você ficar imóvel a dor alivia?
3. Em menos de 10 minutos?

INFARTO

1. Você já teve dor forte no peito por meia hora ou mais na região anterior/frontal do peito?
2. Você recebeu atendimento médico nesta situação?
3. O que foi dito?

CLAUDICAÇÃO

1. Você já teve dor nas pernas ao caminhar?
2. Se você parar a dor alivia em menos de 10 minutos?
3. A dor inclui a panturrilha?

ISQUEMIA CEREBRAL

1. Você, alguma vez, já ficou com o braço, a perna, ou todo o lado do corpo dormente ou com perda da força?
2. Você já teve alteração súbita da fala, da visão e/ou “repuxamento” em um lado da face (“boca torta”)?

Anexo 5

INTERCORRÊNCIAS HOSPITALARES

	Com Intercorrências	Sem Intercorrências	P	odds ratio	I.C.
Alelo C eNOS / T-786C	59 (56,2%)	99 (58,6%)	0,792	0,90	0,55 - 1,48
Alelo Asp eNOS / Glu298Asp	60 (76%)	110 (64,3%)	0,873	1,07	0,64 - 1,79
Alelo T 5-HT / T102C	79 (73,8%)	111 (65,3%)	0,175	1,50	0,87 - 2,55
Alelo 5A 5A/6A	81 (75,7%)	123 (71,5%)	0,530	1,24	0,71 - 2,15
Alelo A fvW / -1185G	74 (75,5%)	114 (73,5%)	0,841	1,10	0,61 - 1,98
Alelo Q fVII / R353Q	26 (24,3%)	40 (23,5%)	0,999	1,04	0,59 - 1,83
Alelo I fVII / I/D	33 (30,8%)	52 (30,4%)	1,00	1,02	0,60 - 1,72
Alelo A β-fibrinogênio	28 (37,6%)	55 (33,3%)	0,562	1,20	0,72 - 2,02
Alelo A1 α-fibrinogênio	46 (43%)	68 (39,8%)	0,684	1,14	0,70 - 1,86
Alelo Q fV Leiden	1 (0,9%)	2 (1,1%)	1,00	0,79	0,07 - 8,88
Alelo A G20210A	5 (4,6%)	-	0,017	-	-
Alelo I t-PA / I/D	87 (81,3%)	130 (76,5%)	0,423	1,33	0,73 - 2,44
Alelo 4G PAI-1 / 4G/5G	80 (74,8%)	115 (67,3%)	0,231	1,44	0,84 - 2,47

Os valores estão apresentados como número de indivíduos (%).

Anexo 6a

Angina pós-IAM

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	29 (61,7%)	129 (56,8%)	0,627	1,22	0,64 - 2,33
Alelo Asp eNOS / Glu298Asp	32 (68,1%)	148 (64,3%)	0,738	1,18	0,60 - 2,31
Alelo T 5-HT / T102C	32 (68,1%)	158 (68,7%)	1,00	0,97	0,49 - 1,90
Alelo 5A 5A/6A	32 (66,7%)	172 (74,5%)	0,285	0,68	0,35 - 1,33
Alelo A fvW / -1185G	32 (71,1%)	156 (75%)	0,577	0,82	0,40 - 1,68
Alelo Q fVII / R353Q	10 (20,8%)	56 (24,5%)	0,710	0,81	0,38 - 1,73
Alelo I fVII / I/D	14 (29,2%)	71 (30,9%)	0,865	0,96	0,46 - 1,82
Alelo A β-fibrinogênio	20 (43,5%)	73 (33,2%)	0,234	1,54	0,81 - 2,95
Alelo A1 α-fibrinogênio	22 (45,8%)	92 (40%)	0,519	1,26	0,67 - 2,37
Alelo Q fV Leiden	-	3 (1,3%)	1,00	-	-
Alelo A G20210A	2 (4,1%)	3 (1,3%)	0,208	3,27	0,53 - 20,10
Alelo I t-PA / I/D	36 (75%)	181 (79%)	0,565	0,79	0,38 - 1,64
Alelo 4G PAI-1 / 4G/5G	33 (68,8%)	162 (70,4%)	0,863	0,92	0,47 - 1,81

Os valores estão apresentados como número de indivíduos (%).

Anexo 6b

Re-IAM

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	6 (66,7%)	152 (57,4%)	0,738	1,48	0,36 - 6,07
Alelo Asp eNOS / Glu298Asp	7 (77,8%)	173 (64,6%)	0,502	1,92	0,39 - 9,43
Alelo T 5-HT / T102C	8 (88,9%)	182 (67,9%)	0,281	3,78	0,46 - 30,70
Alelo 5A 5A/6A	8 (88,9%)	196 (72,6%)	0,452	3,02	0,37 - 24,50
Alelo A fvW / -1185G	7 (77,8%)	181 (74,2%)	1,00	1,21	0,24 - 6,01
Alelo Q fVII / R353Q	4 (44,4%)	62 (23,1%)	0,224	2,65	0,69 - 10,20
Alelo I fVII / I/D	6 (66,7%)	79 (29,4%)	0,026	4,80	1,17 - 19,70
Alelo A β-fibrinogênio	1 (12,5%)	92 (35,7%)	0,268	0,25	0,30 - 2,12
Alelo A1 α-fibrinogênio	4 (44,4%)	110 (40,9%)	1,00	1,15	0,30 - 4,40
Alelo Q fV Leiden	1 (11,1%)	2 (0,7%)	0,093	17,0	1,39 - 207,30
Alelo A G20210A	-	5 (1,8%)	1,00	-	-
Alelo I t-PA / I/D	8 (88,9%)	209 (78%)	0,689	2,25	0,27 - 18,40
Alelo 4G PAI-1 / 4G/5G	9 (100%)	186 (69,1%)	0,062	-	-

Os valores estão apresentados como número de indivíduos (%).

Anexo 6c

Arritmias

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	13 (50%)	145 (58,5%)	0,413	0,71	0,31 - 1,59
Alelo Asp eNOS / Glu298Asp	19 (70,4%)	161 (64,4%)	0,672	1,31	0,55 - 3,12
Alelo T 5-HT / T102C	22 (81,5%)	168 (67,2%)	0,189	2,14	0,78 - 5,87
Alelo 5A 5A/6A	23 (85,2%)	181 (71,8%)	0,173	2,25	0,75 - 6,75
Alelo A fvW / -1185G	18 (75%)	170 (74,2%)	1,00	1,04	0,39 - 2,70
Alelo Q fVII / R353Q	3 (11,1%)	63 (25,2%)	0,151	0,37	0,10 - 1,27
Alelo I fVII / I/D	3 (11,1%)	82 (32,7%)	0,026	0,25	0,07 - 0,88
Alelo A β-fibrinogênio	11 (44%)	82 (34%)	0,379	1,52	0,66 - 3,50
Alelo A1 α-fibrinogênio	10 (37%)	104 (41,4%)	0,687	0,83	0,36 - 1,88
Alelo Q fV Leiden	-	3 (1,2%)	1,00	-	-
Alelo A G20210A	1 (3,7%)	4 (1,6%)	0,397	2,42	0,26 - 22,40
Alelo I t-PA / I/D	21 (77,8%)	196 (78,4%)	1,00	0,96	0,37 - 2,50
Alelo 4G PAI-1 / 4G/5G	22 (81,5%)	173 (68,9%)	0,267	1,98	0,72 - 5,43

Os valores estão apresentados como número de indivíduos (%).

Anexo 6d

Insuficiência ventricular esquerda

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	5 (50%)	153 (58%)	0,748	1,22	0,64 - 2,33
Alelo Asp eNOS / Glu298Asp	3 (30%)	177 (66,3%)	0,036	0,22	0,05 - 0,86
Alelo T 5-HT / T102C	5 (50%)	185 (69,3%)	0,295	0,44	0,12 - 1,57
Alelo 5A 5A/6A	8 (80%)	196 (72,9%)	1,00	1,49	0,30 - 7,18
Alelo A fvW / -1185G	6 (66,7%)	182 (74,6%)	0,698	0,68	0,16 - 2,80
Alelo Q fVII / R353Q	2 (20,%)	64 (24%)	1,00	0,10	0,16 - 3,83
Alelo I fVII / I/D	2 (20%)	83 (31%)	0,729	0,55	0,11 - 2,68
Alelo A β-fibrinogênio	4 (44,4%)	89 (34,6%)	0,724	1,51	0,39 - 5,76
Alelo A1 α-fibrinogênio	4 (40%)	110 (41%)	0,519	1,26	0,67 - 2,37
Alelo Q fV Leiden	-	3 (1,1%)	1,00	-	-
Alelo A G20210A	-	5 (1,8%)	1,00	-	-
Alelo I t-PA / I/D	10 (100%)	207 (77,5%)	0,125	-	-
Alelo 4G PAI-1 / 4G/5G	9 (90%)	186 (69,4%)	0,290	3,86	0,49 - 31,80

Os valores estão apresentados como número de indivíduos (%).

Anexo 7

Intercorrências pós-hospitalares

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	49 (58,3%)	109 (57,4%)	0,987	1,04	0,61 - 1,75
Alelo Asp eNOS / Glu298Asp	53 (62,4%)	127 (66,1%)	0,636	0,84	0,49 - 1,44
Alelo T 5-HT / T102C	59 (68,6%)	131 (68,6%)	1,00	1,00	0,57 - 1,53
Alelo 5A 5A/6A	58 (68,2%)	146 (75,3%)	0,284	0,70	0,40 - 1,23
Alelo A fvW / -1185G	59 (77,6%)	129 (72,9%)	0,525	1,29	0,68 - 2,43
Alelo Q fVII / R353Q	20 (23,5%)	46 (24%)	1,00	0,97	0,53 - 1,78
Alelo I fVII / I/D	23 (27,1%)	62 (32,1%)	0,482	0,78	0,44 - 1,38
Alelo A β-fibrinogênio	30 (36,6%)	63 (34,2%)	0,817	1,10	0,64 - 1,90
Alelo A1 α-fibrinogênio	39 (45,9%)	75 (38,9%)	0,335	1,33	0,79 - 2,23
Alelo Q fV Leiden	-	3 (1,5%)	0,231	-	-
Alelo A G20210A	5 (5,7%)	-	0,004	-	-
Alelo I t-PA / I/D	67 (78,8%)	150 (78,1%)	1,00	1,04	0,55 - 1,94
Alelo 4G PAI-1 / 4G/5G	68 (80%)	127 (65,8%)	0,025	2,07	1,13 - 3,82

Os valores estão apresentados como número de indivíduos (%).

Anexo 8a

Angina pós-IAM

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	29 (54,7%)	129 (58,4%)	0,645	0,86	0,47 - 1,57
Alelo Asp eNOS / Glu298Asp	36 (67,9%)	144 (64,3%)	0,749	1,17	0,62 - 2,22
Alelo T 5-HT / T102C	38 (70,4%)	152 (68,2%)	0,870	1,10	0,58 - 2,12
Alelo 5A 5A/6A	34 (64,2%)	170 (75,2%)	0,121	0,59	0,31 - 1,11
Alelo A fvW / -1185G	39 (79,6%)	149 (73%)	0,466	1,44	0,67 - 3,08
Alelo Q fVII / R353Q	9 (17%)	57 (25,4%)	0,214	0,59	0,27 - 1,30
Alelo I fVII / I/D	15 (28,3%)	70 (31,1%)	0,743	0,87	0,45 - 1,69
Alelo A β-fibrinogênio	23 (44,2%)	70 (32,7%)	0,144	1,63	0,88 - 3,02
Alelo A1 α-fibrinogênio	28 (52,8%)	86 (38,2%)	0,063	1,81	0,99 - 3,30
Alelo Q fV Leiden	-	3 (1,3%)	1,00	-	-
Alelo A G20210A	2 (3,6%)	3 (1,3%)	0,25	2,83	0,46 - 17,30
Alelo I t-PA / I/D	40 (75,5%)	177 (79%)	0,581	0,81	0,40 - 1,65
Alelo 4G PAI-1 / 4G/5G	39 (73,6%)	156 (69,3%)	0,619	1,23	0,62 - 2,41

Os valores estão apresentados como número de indivíduos (%).

Anexo 8b

Re-IAM

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	7 (77,8%)	151 (57%)	0,310	2,64	0,53 - 12,90
Alelo Asp eNOS / Glu298Asp	8 (88,9%)	172 (64.2%)	0,168	4,46	0,55 - 36,20
Alelo T 5-HT / T102C	8 (88,9%)	182 (67,9%)	0,281	3,78	0,46 - 30,70
Alelo 5A 5A/6A	6 (66,7%)	198 (73,3%)	0,706	0,72	0,17 - 2,98
Alelo A fvW / -1185G	5 (62,5%)	183 (74,7%)	0,427	0,56	0,13 - 2,43
Alelo Q fVII / R353Q	3 (33,3%)	63 (23,5%)	0,449	0,62	0,39 - 6,69
Alelo I fVII / I/D	3 (33,3%)	82 (30,5%)	1,00	1,14	0,27 - 4,60
Alelo A β-fibrinogênio	4 (44,4%)	89 (34,6%)	0,724	1,51	0,39 - 5,76
Alelo A1 α-fibrinogênio	5 (55,6%)	109 (40,5%)	0,494	1,84	0,48 - 6,98
Alelo Q fV Leiden	-	3 (1,1%)	1,00	-	-
Alelo A G20210A	1 (11,1%)	4 (1,5%)	0,15	8,43	0,84 - 84,20
Alelo I t-PA / I/D	9 (100%)	208 (77,6%)	0,213	-	-
Alelo 4G PAI-1 / 4G/5G	9 (100%)	186 (69,1%)	0,062	-	-

Os valores estão apresentados como número de indivíduos (%).

Anexo 8c

Insuficiência ventricular esquerda

	Com Intercorrência	Sem Intercorrência	P	odds ratio	I.C.
Alelo C eNOS / T-786C	13 (65%)	145 (57,1%)	0,640	1,39	0,53 - 3,61
Alelo Asp eNOS / Glu298Asp	9 (45%)	171 (66,5%)	0,086	0,41	0,16 - 1,03
Alelo T 5-HT / T102C	15 (75%)	175 (68,1%)	0,623	1,40	0,49 - 3,99
Alelo 5A 5A/6A	17 (85%)	187 (72,2%)	0,297	2,18	0,62 - 7,67
Alelo A fvW / -1185G	13 (72,2%)	175 (74,5%)	0,785	0,89	0,30 - 2,60
Alelo Q fVII / R353Q	6 (30%)	60 (23,3%)	0,585	1,40	0,51 - 3,82
Alelo I fVII / I/D	6 (30%)	79 (30,6%)	1,00	0,97	0,36 - 2,61
Alelo A β-fibrinogênio	3 (15,8%)	90 (36,4%)	0,082	0,32	0,09 - 1,15
Alelo A1 α-fibrinogênio	7 (35%)	107 (41,5%)	0,643	0,76	0,29 - 1,96
Alelo Q fV Leiden	-	3 (1,1%)	1,00	-	-
Alelo A G20210A	1 (5%)	4 (1,5%)	0,309	3,40	0,36 - 32,02
Alelo I t-PA / I/D	17 (85%)	200 (77,8%)	0,581	1,61	0,45 - 5,70
Alelo 4G PAI-1 / 4G/5G	15 (75%)	180 (69,8%)	0,801	1,30	0,45 - 3,70

Os valores estão apresentados como número de indivíduos (%).

Anexo 9

Presença de mais de 2 fatores de risco

	CASO	CONTROLE	P	odds ratio	I.C
Alelo C eNOS / T-786C	107 (55,7%)	26 (65%)	0,298	0,67	0,33 - 1,37
Alelo Asp eNOS / Glu298Asp	130 (67%)	16 (39%)	0,001	3,14	1,58 - 6,36
Alelo T 5-HT / T102C	130 (67,7%)	30 (71,4%)	0,716	0,83	0,40 - 1,74
Alelo 5A 5A/6A	143 (73,3%)	24 (57,1%)	0,042	2,06	1,03 - 4,10
Alelo A fvW / -1185G	129 (72,9%)	24 (57,1%)	0,061	2,01	1,10 - 4,03
Alelo Q fVII / R353Q	49 (25,3%)	11 (26,2%)	1,00	0,95	0,44 - 2,03
Alelo I fVII / I/D	61 (31,3%)	13 (31,7%)	1,00	0,98	0,45 - 2,02
Alelo A β-fibrinogênio	68 (36,6%)	9 (21,4%)	0,071	2,11	0,95 - 4,68
Alelo A1 α-fibrinogênio	81 (41,5%)	15 (36,6%)	0,603	1,23	0,61 - 2,47
Alelo Q fV Leiden	2 (1%)	-	1,00	-	-
Alelo A G20210A	3 (1,5%)	-	1,00	-	-
Alelo I t-PA / I/D	149 (76,4%)	30 (73,2%)	0,690	1,18	0,55 - 2,55
Alelo 4G PAI-1 / 4G/5G	137 (70,3%)	31 (75,6%)	0,572	0,76	0,35 - 1,65

Os valores estão apresentados como número de indivíduos (%).

Anexo 10

NÚMERO DE INFARTOS

	1 IAM	+ de 1 IAM	P	odds ratio	I.C.
Alelo C eNOS / T-786C	115 (56,7%)	43 (60,6%)	0,580	1,17	0,67 - 2,03
Alelo Asp eNOS / Glu298Asp	139 (67,5%)	41 (57,7%)	0,151	0,65	0,37 - 1,14
Alelo T 5-HT / T102C	141 (68,1%)	49 (70%)	0,882	1,09	0,60 - 1,96
Alelo 5A 5A/6A	153 (73,6%)	51 (71,8%)	0,759	0,91	0,50 - 1,67
Alelo A fvW / -1185G	138 (72,6%)	50 (79,4%)	0,322	1,44	0,72 - 2,88
Alelo Q fVII / R353Q	48 (23,3%)	18 (25,4%)	0,748	1,11	0,59 - 2,08
Alelo I fVII / I/D	62 (30%)	23 (32,4%)	0,766	1,12	0,62 - 2,00
Alelo A β-fibrinogênio	67 (34,2%)	26 (37,1%)	0,664	1,13	0,64 - 2,00
Alelo A1 α-fibrinogênio	85 (41,1%)	29 (40,8%)	1,00	0,99	0,57 - 1,71
Alelo Q fV Leiden	3 (1,4%)	-	0,575	-	-
Alelo A G20210A	1 (0,5%)	4 (5,6%)	0,015	12,59	1,38 - 114,6
Alelo I t-PA / I/D	161 (78,2%)	56 (78,9%)	1,00	1,04	0,54 - 2,01
Alelo 4G PAI-1 / 4G/5G	146 (70,5%)	49 (69%)	0,881	0,93	0,51 - 1,67

Os valores estão apresentados como número de indivíduos (%).

Anexo 11

COM E SEM ESTENOSE

	Com Estenose	Sem Estenose	P	odds ratio	I.C.
Alelo C eNOS / T-786C	144 (58,1%)	14 (53,8%)	0,680	1,18	0,52 - 2,67
Alelo Asp eNOS / Glu298Asp	167 (55,6%)	13 (50%)	0,120	1,98	0,88 - 4,47
Alelo T 5-HT / T102C	172 (68,3%)	18 (72%)	0,820	0,83	0,33 - 2,08
Alelo 5A 5A/6A	186 (73,5%)	18 (69,2%)	0,640	1,23	0,51 - 2,97
Alelo A fvW / -1185G	169 (73,8%)	19 (79,2%)	0,800	0,74	0,26 - 2,07
Alelo Q fVII / R353Q	63 (25,1%)	3 (11,5%)	0,150	2,59	0,74 - 8,84
Alelo I fVII / I/D	77 (30,6%)	8 (30,8%)	1,00	0,99	0,41 - 2,37
Alelo A β-fibrinogênio	83 (34,6%)	10 (38,5%)	0,670	0,84	0,36 - 1,94
Alelo A1 α-fibrinogênio	101 (40,1%)	13 (50%)	0,400	0,66	0,29 - 1,50
Alelo Q fV Leiden	2 (0,8%)	1 (3,8%)	0,250	0,19	0,01 - 2,27
Alelo A G20210A	5 (1,9%)	-	1,00	-	-
Alelo I t-PA / I/D	197 (78,5%)	20 (76,9%)	0,800	1,09	0,41 - 2,89
Alelo 4G PAI-1 / 4G/5G	177 (70,2%)	18 (69,2%)	1,00	1,04	0,43 - 2,51

Os valores estão apresentados como número de indivíduos (%).

Anexo 12

≥ 3 ESTENOSES E 1 OU 2 ESTENOSES

	≥ 3 Estenoses	1 ou 2 Estenoses	P	odds ratio	I.C
Alelo C eNOS / T-786C	25 (56,8%)	133 (57,8%)	1,00	0,96	0,50 - 1,84
Alelo Asp eNOS / Glu298Asp	26 (57,8%)	154 (66,4%)	0,300	0,69	0,36 - 1,32
Alelo T 5-HT / T102C	36 (82%)	154 (63,0%)	0,050	2,02	0,92 - 4,41
Alelo 5A 5A/6A	31 (68,9%)	173 (73,9%)	0,460	0,78	0,29 - 1,56
Alelo A fvW / -1185G	33 (80,5%)	155 (73,1%)	0,430	1,51	0,66 - 3,47
Alelo Q fVII / R353Q	10 (22,2%)	56 (24,1%)	0,850	0,89	0,41 - 1,92
Alelo I fVII / I/D	10 (22,5%)	75 (32,2%)	0,210	0,60	0,28 - 1,28
Alelo A β-fibrinogênio	16 (37,2%)	77 (34,5%)	0,730	1,12	0,57 - 2,21
Alelo A1 α-fibrinogênio	22 (48,9%)	92 (39,5%)	0,250	1,46	0,77 - 2,78
Alelo Q fV Leiden	-	3 (1,3%)	1,00	-	-
Alelo A G20210A	1 (2,1%)	4 (1,7%)	1,00	1,26	0,13 - 11,54
Alelo I t-PA / I/D	37 (82,2%)	180 (77,6%)	0,550	1,33	0,58 - 3,04
Alelo 4G PAI-1 / 4G/5G	28 (62,2%)	167 (71,1%)	0,210	0,65	0,33 - 1,26

Os valores estão apresentados como número de indivíduos (%).