G20210A prothrombin gene variant and clinical outcome in patients with a first acute coronary syndrome

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A B S T R A C T

Background and Objectives. The prognostic value of the G20210A prothrombin gene polymorphism in patients with a first acute coronary syndrome has not been previously assessed. We conducted a prospective study to investigate this issue.

Design and Methods. Genotyping at the 20210 prothrombin gene locus was performed in 162 patients with a first episode of myocardial infarction (MI) or unstable angina (UA) occurring before 65 years of age. Patients were stratified according to cardiovascular risk factors and to treatment strategy. The subsequent two-year relative risk (RR) of adverse events (death, MI and UA) was adjusted for possible confounders and analyzed according to genotype, risk factor category, and treatment allocation.

Results. In the entire study population, the prothrombin variant did not significantly increase the two-year risk of events: the adjusted RR for GA vs GG carriers was 1.82 (95% CI 0.68-4.89). However, in the absence of traditional cardiovascular risk factors the risk of events was consistently higher: among the 46 patients without hypertension, diabetes and hypercholesterolemia, GA vs GG carriership was associated with an adjusted RR at two years of 5.64 (95% CI 1.07-29.84). The gene variant also enhanced the risk of events among the 98 patients who did not undergo myocardial revascularization procedures (RR for GA vs GG: 2.89, 95% CI 1.04-8.00), but not among those who did.

Interpretation and Conclusions. The present prospective study suggests that heterozygosity for the G20210A prothrombin polymorphism adversely affects prognosis after a first acute coronary syndrome in the subgroup of patients without metabolic risk factors and in those not treated by revascularization procedures.

Key words: prothrombin, gene polymorphism, acute coronary syndromes, cardiovascular risk factors, outcome, myocardial revascularization.

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The single-base G to A substitution at the 20210 locus of the prothrombin gene has been known since 1996 to be associated with a hypercoagulable state that favors venous thrombosis.[1] Approximately 20 clinical studies have assessed the possible role of this gene variant in the pathogenesis of coronary atherothrombosis, providing conflicting data.[2-4] The heterogeneous results of these various trials may be attributed, at least in part, to important gene-environment interactions, causing an increased risk of disease for carriers of the variant allele in some environments but not in others. Several cross-sectional observations have suggested, indeed, that the risk of coronary artery thrombosis associated with the 20210A allele may be significantly modulated by age,[2-5] extent of atherosclerosis,[6-8] and pattern of major cardiovascular risk factors.[7] To date, there are no prospective data regarding the possible prognostic role of this polymorphism among patients who have had a first acute coronary event. We therefore investigated the relation between the prothrombin genotype at the 20210 locus and the occurrence of clinical events during a two-year follow-up in a cohort of subjects who experienced a first acute coronary syndrome before the age of 65 years. We prospectively sought for possible relations among the 20210A variant, clinical outcome, and traditional cardiovascular risk factors. In a secondary retrospective analysis, we also sought for possible associations among this gene variant, outcome, and treatment strategies.

Design and Methods

Study population

The study was designed as a prospective cohort investigation. Italian Caucasians <65 years of age were asked to participate if they were discharged from our Coronary Care Unit with a confirmed diagnosis of acute myocardial infarction (MI) or unstable angina (UA) and if routine clinical investigations revealed the event to be the first manifestation of disease. Out of 246 eligible patients, 162 accepted a follow-up assessment and gave their informed consent to take part in the study.

MI was diagnosed according to WHO criteria on the basis of symptoms, raised levels of cardiac enzyme, and electrocardiographic changes.[9] UA was defined as class IIIB of Braunwald's classification.[10] Systemic hypertension, diabetes mellitus, and hypercholesterolemia were considered present if drugs for these conditions had been prescribed before admission or if these conditions were diagnosed during the hospital stay; the respective criteria for diagnosis were: blood pressure values >140/90 mmHg on more than one occasion, fasting glycemia >126 mg/dL on at least two occasions, and a total serum cholesterol concentration >240 mg/dL. Smokers were defined as smokers of >1 cigarette/day at the time of admission. A family history of MI was defined as at least one first degree relative with a fatal or non-fatal MI before the age of 60.

Patients with ST-elevation acute MI who were eligible for thrombolysis received intravenous recombinant tissue-type plasminogen activator (rt-PA) over 90 minutes, in addition to aspirin and heparin; those with UA or non-ST-elevation MI were started on antiplatelet therapy (aspirin as first choice; ticlopidine if aspirin was contraindicated) and heparin (unfractionated or low-molecular weight). After a percutaneous coronary intervention, a combination of aspirin and ticlopidine was prescribed for 4 weeks.

At the time of recruitment (1997-1998), our institutional practice was to follow an initially conservative diagnostic and treatment strategy, limiting invasive procedures to patients with spontaneous recurrent ischemia or evidence of inducible ischemia during stress tests. Coronary angiography was performed on clinical grounds in 113 patients (60.8%); a >50% lumen diameter stenosis was considered to indicate significant obstructive disease. Left ventricular ejection fraction was measured by echocardiography using the area-length method.

Genetic analyses

Genomic DNA was extracted from peripheral blood leukocytes using standard procedures. The G20210A nucleotide substitution within the 3'-untranslated region of the prothrombin gene was detected according to Poort *et al.*[1] The factor V Leiden (FVL) G1691A polymorphism was identified using standardized techniques.[7]

Follow-up assessment and clinical end-points

Follow-up was performed through regular out-patient visits at 6 months, 1 year and 2 years. Clinical evaluations were blind to the results of genotyping. Patients were treated by the usual standard therapy prescribed by their attending physicians. The clinical end-points considered in our analysis were all cause death, non-fatal MI and UA. Patients were censored at the time of their first event. MI and UA during follow-up were defined in the same way as the initial index events.

Data analysis

Statistical analyses were performed using StatSoft Software (Tulsa, OK, USA). Discrete and continuous variables were analyzed by estimating the relative risk (RR) and by Mann Whitney U test. Kaplan Meier event-free survival curves were compared by the log-rank test. A Cox proportional hazard analysis was used to adjust for the possible confounding effects on outcome of age, sex, cardiovascular risk factors, type of acute coronary syndrome, reduced (<40%) left ventricular ejection fraction, number of significantly diseased vessels at angiography, and coronary revascularization. The RR of adverse events was also calculated at two years after adjustment for the above variables. The concomitant prognostic values of the serum concentrations of cardiac troponins and C-reactive protein were not assessed, as their measurement was not well established when this study began.

Results

Baseline characteristics

One hundred and nine patients with a confirmed diagnosis of a first acute MI and 53 patients with a first episode of Braunwald class IIIB UA found our study population. The clinical and angiographic characteristics of these patients, shown in Table 1, did not differ significantly from those of the group who declined to take part in the follow-up assessment (*data not shown*).

On genetic testing, 5 patients were heterozygotes for the FVL variant (3.1%) and 11 patients were heterozygotes for the prothrombin 20210A allele (6.8%). One of these patients was heterozygous for both polymorphisms, while no homozygotes were found.

Patients with the GG prothrombin genotype did not differ significantly from those with the GA genotype with respect to clinical and angiographic characteristics, except for a family history of MI and for the absence of metabolic risk factors (hypertension, diabetes mellitus, and hypercholesterolemia), which were significantly

more frequent among GA patients. Rates of treatment with angiotensin converting enzyme inhibitors, β-adrenergic blockers, and *statins* were also similar in the two genotypic groups (Table 1).

During hospitalization for the index event, 64 patients (39.5%) underwent coronary revascularization procedures: 12 had bypass surgery and 52 a percutaneous intervention. The rate of revascularization did not differ significantly between the two prothrombin genotypic groups (RR=0.35, 95% CI 0.08-1.57, for GA vs GG).

Two-year outcome according to FVL and to G20210A genotype

All 162 patients were assessed at 6 months and 1 year. Thereafter, 142 (88%) patients completed follow-up at 2 years. After the index event, a subsequent adverse event (death, MI, or UA) occurred in 20 patients (12.3%) within 6 months (11 UA, 9 MI, 0 deaths), in 31 patients (19.1%) within the 1st year (17 UA, 12 MI, 2 deaths), and in 45 patients (33.1%) within the 2nd year (24 UA, 19 MI, 2 deaths). No adverse event was observed during follow-up in the 5 patients carrying the FVL variant.

In the overall population, the two-year event-free Kaplan Meier survival curves did not differ significantly between prothrombin genotypic groups, with an adjusted RR of events at two years for GA vs GG of 1.82 (95% CI 0.68-4.89). Event-free survival curves by single end-point (death, MI, or UA) also revealed no significant differences between GA vs GG genotypes at two years (RR=1.82, 95% CI 0.69-4.85 for death, RR=2.39, 95% CI 0.46-12.14 for MI, and RR=1.34, 95% CI 0.28-6.29 for UA).

However, when patients were stratified according to the absence of metabolic risk factors, a consistent relation emerged between carriership of the A allele and risk of adverse events. The adjusted two-year RR of death, MI or UA for GA, compared with GG, carriers was 2.65 (95% CI 0.90-20.82) among those without hypertension, 2.93 (95% CI 1.36-26.51) among those without diabetes mellitus, and 2.11 (95% CI 0.70-11.78) among those without hypercholesterolemia. By pooling together the 46 patients without any metabolic risk factor (hypertension, diabetes, or hypercholesterolemia), the two-year Kaplan Meier survival curves differed significantly according to genotype, with carriers of the A allele showing a worse outcome compared to GG homozygotes (p=0.016, Figure 1). In patients without metabolic risk factors the adjusted two-year RR for the composite end-point was 5.64 (95% CI 1.07-29.84) for GA compared to GG carriers. Among non-smokers, GA carriers tended to have a worse two-year outcome compared to GG homozygotes (adjusted RR 2.27, 95%CI 0.73-14.26). In contrast, no significant risk modulation by genotype was observed among the 114 patients who had at least one metabolic risk factor (RR=0.95, 95%CI 0.13-6.65 for GA vs GG) or who smoked (RR 0.97, 95%CI 0.13-6.80 for GA vs GG). Table 2 shows the estimated adjusted RR of death, MI or UA associated with carriership of the A allele in the presence or absence of each risk factor. Among the 113 patients who underwent

Table 1. Baseline clinical characteristics of the entire population and of the two genotypic groups (GG vs GA) at the 20210 prothrombin locus.

	Overall n (%) 162 (100)	*GG n (%) 151 (100)*	*GA n (%) 11 (100)*	*Crude RR* (GG vs GA)*
Males	133 (82)	124 (82)	9 (82)	1.00 (0.75-1.34)
Acute myocardial infarction	109 (67)	100 (62)	9 (82)	0.81 (0.60-1.09)
Cardiovascular risk factors				
Hypercholesterolemia	73 (45)	70 (46)	3 (27)	1.70 (0.64-4.53)
Diabetes mellitus	22 (14)	20 (13)	2 (18)	0.73 (0.19-2.72)
Hypertension	76 (47)	73 (48)	3 (27)	1.77 (0.67-4.72)
No metabolic RF	46 (28)	39 (26)	7 (64)	0.41 (0.24-0.68)
Smoking	102 (63)	98 (65)	4 (36)	1.78 (0.88-3.93)
Family history of MI	56 (35)	49 (32)	7 (64)	0.51 (0.31-0.84)
Coronary angiography	113 (70)	105 (69)	8 (73)	0.96 (0.66-1.39)
No significant disease	12 (10)	11 (10)	1 (12)	0.84 (0.12-5.70)
1-vessel disease	51 (45)	46 (44)	5 (62)	0.70 (0.39-1.25)
2-vessel disease	35 (31)	34 (32)	1 (12)	2.59 (0.40-16.55)
3-vessel disease	15 (14)	14 (14)	1 (14)	1.07 (0.16-7.11)
Medical therapy				
Beta-blockers	80 (49)	74 (49)	6 (54)	0.90 (0.51-1.60)
ACE-inhibitors	76 (47)	71 (47)	5 (45)	1.03 (0.53-2.02)
Statins	49 (30)	47 (31)	2 (18)	1.71 (0.48-6.13)
LV ejection fraction (mean±SD)	51±9%	51±9%	51±10%	*p*=0.88
Age: years (mean±SD)	52±10	52±10	52±2	*p*=1.01

LV: left ventricular; MI: myocardial infarction; RF: risk factor; SD: standard deviation.

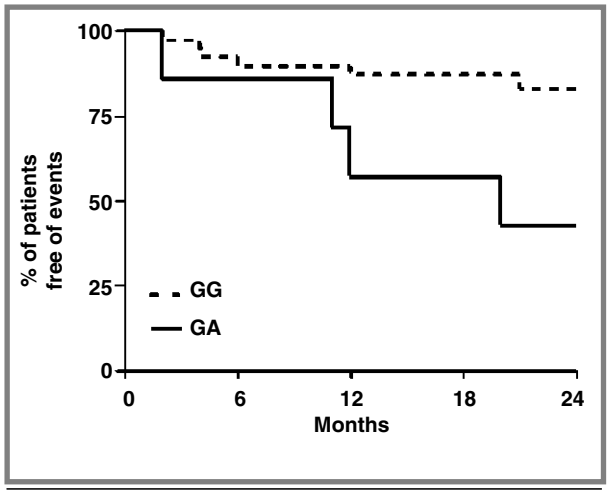

Figure 1. Comparison of the Kaplan Meier survival curves free of death, myocardial infarction and unstable angina in the 46 patients without metabolic risk factors (hypertension, diabetes, hypercholesterolemia) according to their prothrombin genotype (p=0.016). At two years, the relative risk of events in patients with the GA compared to the GG genotype (adjusted for age, sex, cardiovascular risk factors, type of acute coronary syndrome, left ventricular ejection fraction, number of diseased vessels at angiography, and coronary revascularization) was 5.64 (95%CI 1.07-29.84, p=0.04).

Table 2. Estimated adjusted RR of death, MI or UA associated with carriership of the prothrombin A allele in the absence or presence of each traditional risk factor.

Risk factor	*Number of patients*	*RR (95%CI)*
Smoking		
No	60	2.27 (0.73-14.26)
Yes	102	0.97 (0.13-6.80)
Diabetes mellitus		
No	140	2.93 (1.36-26.51)
Yes	22	0.40 (0.01-8.70)
Hypertension		
No	86	2.65 (0.90-20.82)
Yes	76	1.30 (0.10-13.83)
Hypercholesterolemia		
No	89	2.11(0.70-11.78)
Yes	73	1.50 (0.13-19.62)

coronary angiography during the index hospitalization, the extent of coronary artery disease did not seem to alter the overall effect of the prothrombin genotype on the clinical outcome at two years [adjusted RR for GA vs GG: 1.57 (95% CI 0.29-10.49) in those with single or no significant vessel disease, and 1.47 (95% CI 0.14-18.30) in those with multivessel disease].

Because myocardial revascularization may be expected to change the clinical course of patients with acute coronary syndromes by reducing the rate of subsequent adverse events,[11-13] we analyzed the effect of the prothrombin genotype on the clinical outcome of patients who did undergo an initial revascularization procedure (either surgical or percutaneous) or who did not. Among the 98 patients who did not have revascularization procedures, carriership of the GA genotype was associated with a significantly worse two-year prognosis, with an adjusted RR of 2.89 (95% CI 1.04-8.00). Conversely, among patients who did undergo a revascularization procedure, no significant difference in outcome was observed according to genotype.

Discussion

Subjects who develop an acute coronary syndrome in the absence of traditional cardiovascular risk factors represent a unique group in whom to search for possible new predisposing conditions. On the one hand, the absence of potent major risk factors may unmask the more modest effects of putative new pathogenetic factors. On the other hand, the presence of new predisposing conditions may contribute to explain the occurrence of ischemic events in patients otherwise considered at low risk on the basis of current knowledge.

The application of secondary prevention strategies to patients who present with an acute coronary syndrome in the absence of modifiable traditional risk factors currently constitutes a clinical challenge which may benefit from the identification of new prognostic markers.

The results of the present study indicate that, among patients without hypertension, diabetes, or hypercholesterolemia, the G20210A gene variant may be associated with a significant (approximately 5-fold) increase in the risk of death or recurrent acute coronary syndrome during the two years that follow the index event. This finding confirms and extends previous observations showing a significantly raised risk of a first coronary event associated with the G20210A gene variant among subjects with an otherwise low baseline cardiovascular risk profile[7,14] or with limited extent of coronary artery disease.[6-8] Conversely, among patients in whom the traditional, more potent, and more frequent cardiovascular risk factors are present, or within unselected series of patients, the effect of the prothrombin genotype may become undetectable, being probably masked or diluted by the various, stronger, and more prevalent pathogenetic mechanisms operating in the entire population.[2]

Myocardial revascularization, either surgical or percutaneous, is known to modify the clinical course of patients with acute coronary syndromes by causing an overall reduction of subsequent major adverse events,[11-13] although it does introduce other important variables, such as vascular restenosis or peri-procedural myocardial necrosis. In contrast, patients who do not undergo revascularization exhibit a more *natural* course of their disease, and in these the prognostic role of a potential genetic risk factor may be easier to identify. In the present study, patients who did not undergo an initial revascularization procedure, but not those who did, showed a significantly different event-free survival curve according to the prothrombin G20210A polymorphism, GA patients faring worse than GG homozygotes. Thus, when revascularization is not performed, the natural history of the disease may be less altered and the risk associated with the prothrombin polymorphism may become detectable. Alternatively, the prothrombin polymorphism may prevalently influence the outcome of subjects with a lower risk profile for recurrent events, who are more often managed conservatively. Both of these interpretations, however, should be regarded with caution and, at most, as hypothesis generating, since the study was not designed to analyze the relation among genotype, revascularization procedures, and outcome.

The major limitation of the present study is the relatively small sample size, with its limited statistical power: indeed, in the overall population, for the estimated 1.82 two-year relative risk of events associated with the G20210A variant to achieve statistical significance, 664 patients would have been necessary. On balance, the small clinical impact of such a potential modest increase in risk does not encourage further larger prospective investigations. On the other hand, the considerable increase in risk observed in our prospective analysis of patients without metabolic risk factors is in agreement with previous cross-sectional studies, and is likely to be a true finding. The significantly higher prevalence of a family history of MI among carriers of the A allele compared to GG homozygotes is a further element in support of a true role for the G20210A polymorphism in the pathogenesis of acute coronary syndromes. Since gene-gene interactions may occur and concur to modulate the phenotypic expression of disease,[5] another limitation of the present study is the lack of a concomitant assessment of other genetic variants, in addition to the FVL polymorphism.

In conclusion, the single-base G to A variant at the 20210 locus of the prothrombin gene, shown in humans to be associated with a hypercoagulable state,[1] may represent a significant new risk factor for an adverse long-term outcome of patients who develop a first acute coronary syndrome in the absence of major metabolic cardiovascular risk factors.

FB, AML, KP, VDS and FA conceived and designed the study and drafted the article; all authors analyzed and interpreted the data, critically reviewed the manuscript, and gave final approval. The authors reported no potential conflicts of interest.

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