Increased prevalence of the G20210A prothrombin gene variant in acute coronary syndromes without metabolic or acquired risk factors or with limited extent of disease

F. Burzotta¹, K. Paciaroni², V. De Stefano², P. Chiusolo², A. Manzoli¹, I. Casorelli², A. M. Leone¹, E. Rossi², G. Leone², A. Maseri¹ and F. Andreotti¹

¹Departments of Cardiology and ²Haematology, Catholic University, Rome, Italy

Aims To investigate the prevalence of the G20210A prothrombin and G1691A factor V gene variants in patients with acute coronary syndrome stratified according to risk factor profile and to extent of coronary disease, in comparison with matched healthy controls.

Methods and Results The 20210 prothrombin and the 1691 factor V loci were genotyped in 247 patients ≤65 years of age (190 myocardial infarction and 57 unstable angina as first presentation of disease) and in 247 healthy age- and sex-matched controls. The prevalence of the 1691A factor V allele was similar in cases and controls. The frequency of heterozygotes for the 20210A prothrombin allele was 6·5% among patients and 2·8% among controls (OR 2·4, 95% CI 1·0–5·9), increasing to 8·7% in patients with a family history of myocardial infarction (OR 3·3, 95% CI 1·2–9·1), to 9·9% in patients (n=81) with ≤1 vessel disease (OR 3·8, 95% CI 1·3–10·8), and to 13·0% in patients who were normocholesterolaemic, non-diabetic, normotensive and non-smokers (OR 5·1, 95% CI 1·2–21·4).

Conclusions These findings suggest that the 20210A prothrombin allele represents an inherited risk factor for acute coronary syndrome among patients who have limited extent of coronary disease at angiography or who lack major metabolic and acquired risk factors.


Key Words: Genetic polymorphism, prothrombin, acute coronary syndrome, risk factors.

Introduction

In 1996 a single base (G/A) substitution was identified at position 20210 within the 3'-untranslated region of the prothrombin gene on chromosome 11[1]. The 20210A, compared with the G allele, was associated with higher plasma levels of prothrombin[1–3], thrombin antithrombin complexes [3], and prothrombin fragment 1+2[3], suggesting a hypercoagulable state. Further studies convincingly showed that this polymorphism was a moderate risk factor for venous thrombosis[4]. Moreover, a significant association was observed between the 20210A allele and myocardial infarction in young women[5]. Subsequent studies, however, have provided conflicting results on the role of this gene variant in ischaemic heart disease[6–17]. Since a putative prothrombotic risk factor, if relevant to ischaemic heart disease, is likely to be so in clinical conditions where thrombosis is indeed critical (e.g. unstable coronary syndromes) but not in stable ones where atheroma is the predominant pathogenetic factor, it is possible that the divergent findings in the literature reflect the broad clinical heterogeneity of patients with ischaemic heart disease enrolled in published reports[6–17].

The aim of the present study was to investigate the prevalence of the G20210A prothrombin gene variant in a selected series of patients aged less than 65 years with an acute coronary syndrome as first manifestation of ischaemic heart disease. The prevalence of the factor V
(G1691A) Leiden, a strong risk factor for venous thrombosis\cite{16,19}, was also investigated.

**Methods**

**Study population**

The study design was a 1:1 case-control study. Enrolled subjects were Italian Caucasians living in the central or southern regions of the country. Cases were patients $\leq$ 65 years of age, admitted to our Cardiology Division with a confirmed diagnosis of acute myocardial infarction or unstable angina, in whom clinical history revealed the event to be the first manifestation of disease. Myocardial infarction was diagnosed according to World Health Organization criteria\cite{20}. Patients with unstable angina fell into class III B of Braunwald’s classification\cite{61}. A detailed history was taken to assess cardiovascular risk factors. Hypercholesterolaemia, diabetes mellitus and systemic hypertension were considered present if drugs for these conditions had been prescribed by a physician before admission or if these conditions were diagnosed during the hospital stay. A family history of myocardial infarction was defined as at least one first-degree relative with a fatal or non-fatal infarction before the age of 60. Of the 247 patients, 156 underwent coronary angiography for clinical reasons; 81 underwent angiography for clinical indications. In 45 patients, a $>50\%$ lumen diameter stenosis was considered significant disease.

For each patient, a healthy individual of the same sex and of similar age ($\pm$ 3 years) was enrolled from the Hospital’s blood donors. Clinical history and physical examination were performed to rule out hypertension, diabetes or dyslipidaemia. All cases and controls gave their informed consent to take part in the study.

**Genetic polymorphisms**

Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures. The factor V (G1691A) Leiden (FVL) gene was detected according to Poort et al.\cite{19}. The G20210A nucleotide substitution within the 3’-untranslated region of the prothrombin gene was detected according to Bertina et al.\cite{18}.

**Statistical analyses**

Continuous variables were compared by Student’s unpaired t-test. Frequencies were compared by Fisher’s exact test. Odds ratios (OR) in comparison with controls were calculated by simple cross-tabulation, with 95% confidence intervals (95% CI).

**Results**

The clinical characteristics of the 247 cases and 247 controls are shown in Table 1. Smoking, hypercholesterolaemia, hypertension, familial myocardial infarction and diabetes were present in 64%, 50%, 44%, 42% and 16% of patients, respectively.

For both mutations, no homozygotes were found. The prevalence of individuals carrying the 20210A prothrombin allele was 6.5% in cases and 2.8% in controls, with an associated OR of 2.4 (95% CI 1.0–5.9) (Table 2). In carriers of factor V Leiden, the overall OR for an acute coronary syndrome was 1.1 (95% CI 0.4–3.2). Only one subject, who developed a myocardial infarction at 32 years of age, carried both variants (Table 2). Among the 190 patients with myocardial infarction, the prevalence of the 20210A prothrombin allele was 6.8% (13/190). Among those with unstable angina it was 5.3% (3/57, OR=1.0 vs myocardial infarction). The prevalence of factor V Leiden among patients with myocardial infarction and unstable angina was 3.2% (6/190) and 3.5% (2/57), respectively (P=1.0). The prevalence of these variants did not differ significantly between patients above or below the median age of 51 years (20210A-carriers: 7/123 and 9/124, respectively, OR=0.8; FVL-carriers: 5/123 and 3/124, P=0.5). No significant difference in the carrier rate of the 20210A prothrombin allele was found between male (6.5%) and female (6.4%) patients (P=1.0).

Among the 103 patients with a family history of myocardial infarction (42%), the prevalence of the 20210A prothrombin allele (8.7%) was significantly higher compared with controls (P=0.02), with an associated odds ratio of 3.3 (95% CI 1.2–9.1) (Fig. 1). On the basis of coronary angiography (performed in 156 cases), patients were divided into those with three- (n=31) and

<table>
<thead>
<tr>
<th>Sex:</th>
<th>Cases (n=247)</th>
<th>Controls (n=247)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>216 (87.4%)</td>
<td>216 (87.4%)</td>
</tr>
<tr>
<td>Females</td>
<td>31 (12.6%)</td>
<td>31 (12.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years):</th>
<th>Mean ± SD</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>50 ± 10</td>
<td>51</td>
</tr>
<tr>
<td>Females</td>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Myocardial infarction</th>
<th>Cases (n=247)</th>
<th>Controls (n=247)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior</td>
<td>190 (76.9%)</td>
<td>190 (76.9%)</td>
</tr>
<tr>
<td>Inferior</td>
<td>75 (39.5%)</td>
<td>75 (39.5%)</td>
</tr>
<tr>
<td>Non-Q</td>
<td>30 (15.8%)</td>
<td>30 (15.8%)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>57 (23.1%)</td>
<td>57 (23.1%)</td>
</tr>
</tbody>
</table>

**Table 1 Clinical characteristics of cases and controls**

**Table 2 Prevalence of the prothrombin (FII) 20210A and FV Leiden (FVL) carriage in cases and controls**

**Eur Heart J, Vol. 23, issue 1, January 2002**
two-vessel disease (n=44), or single (n=64) and non-significant vessel disease (n=17). The 81 patients with single or non-significant disease had a higher prevalence of the prothrombin variant allele (9.9%) compared with controls (P=0.01), with an OR of 3.8 (95% CI 1.3–10.8) (Fig. 1). Among patients without a family history of myocardial infarction (OR 1.7, 95% CI 0.6–5.1) or with ≥2-vessel disease (OR 0.9, 95% CI 0.2–4.7), the prevalence of the 20210A prothrombin allele was not significantly increased compared with controls (P=0.4 and P=1.0, respectively) (Fig. 1).

When the cases were divided according to the presence or absence of smoking, hypercholesterolaemia, hypertension or diabetes, the odds ratio for acute coronary syndromes associated with the 20210A prothrombin allele increased substantially (five-fold) in patients with none of these risk factors, compared with controls (OR 5.1, 95% CI 1.2–21.4) (Fig. 1). In this group, a family history of myocardial infarction was present in 39% (nine of 23, two of which carried the 20210A prothrombin allele). Patient stratification according to the presence or absence of each of the afore mentioned risk factors consistently revealed increased odds ratios compared with controls among the normocholesterolaemic, normotensive, non-smoking or non-diabetic patients (Fig. 2). Conversely, in our patient population, the prevalence of the 20210A allele did not significantly increase the risk associated with smoking or metabolic cardiovascular risk factors, considered either in combination (OR 2.1, 95% CI 0.8–5.4) (Fig. 1) or individually (Fig. 2).

The prevalence of factor V Leiden in the subgroups of patients stratified according to family history, extent of coronary artery disease, and metabolic or acquired risk factors was not significantly different from that of controls (data not shown).

Discussion

The available literature concerning the role of the G20210A prothrombin gene variant in the pathogenesis of ischaemic heart disease provides conflicting results, with most authors reporting no significant association between this variant and disease [3,5–17]. In the present study, we found in the overall patient cohort a 2.4-fold increased risk of acute coronary syndromes associated with the 20210A prothrombin allele, which achieved only borderline significance compared with controls. Moreover, in the presence of multivessel coronary disease or of major metabolic or acquired coronary risk factors, we found that the variant allele was not significantly associated with the risk of acute coronary syndromes, in agreement with previous studies [6,7,11–17]. However, among patients with a family history of myocardial infarction, without other major cardiovascular risk factors, or with limited coronary artery disease at angiography, the prevalence of the 20210A prothrombin allele conferred a significantly increased risk of disease in comparison with controls.

The role of this genetic variant in the inheritance of acute coronary syndromes is supported by its significantly higher prevalence in patients with a family history of myocardial infarction (3.3-fold increased risk). Moreover, patients without other major risk factors who carried the variant allele had a five-fold increase in the
risk of disease compared with controls. This finding fits with previous data showing a significant association between the G20210A prothrombin gene variant and juvenile stroke[22] or coronary, cerebral or peripheral arterial thrombosis[23] in patients without classic risk factors. Conversely, the studies that did not find an increased risk associated with the 20210A allele were not aimed to specifically analyse patients without any major metabolic or acquired risk factors. Some reports, but not the present one, have found a substantially increased risk of myocardial infarction when the G20210A prothrombin gene variant coexisted with smoking or dysmetabolic diseases[5,10]. Therefore, while this polymorphism may increase the probability of thrombosis additively or synergistically with other risk factors, our data suggest that it may also explain the occurrence of thrombotic events in individuals without major known cardiovascular risk factors, other than heritability.

We found that patients with less extensive disease at angiography showed a substantially higher prevalence of the 20210A prothrombin allele compared with controls (3.8-fold increased risk), whereas the prevalence among patients with double- or triple-vessel disease did not differ from that of controls. This finding is consistent with the high carrier-rate of the 20210A allele in the subgroup of patients without major metabolic or acquired risk factors.

A possible drawback of our study is the lack of coronary angiography in 91 cases (37%), since angiography was performed purely on clinical grounds. This could have led to the selection of patients with more unstable disease, in whom the prevalence of a prothrombotic risk factor might have been overestimated. Such hypothesis however was discarded, given the similar prevalence of the 20210A prothrombin allele in patients who did and did not undergo angiography (6.4% vs 6.5%, \(P=0.8\)).

Only three previous studies have investigated the association between the G20210A prothrombin gene variant and angiographically-proven coronary artery disease. Two of these either did not consider the extent of disease[13] or excluded patients who had non-significant coronary stenoses[7], and consequently cannot be compared with our investigation. In a third, large study of approximately 2000 patients with or without previous myocardial infarction, the prevalence of the 20210A prothrombin allele did not differ significantly among patients with triple-, double-, single-, or non-significant vessel disease, when each group was compared to one another separately[14]. However, in a post hoc reanalysis of the overall cohort of this latter study, the comparison of patients with single- or non-significant vessel disease to those with double- or triple-vessel disease did yield a significantly higher 20210A carrier-rate among patients with \(\leq 1\)-vessel disease compared to those with multiple stenoses[23].

The overall lack of association between factor V Leiden and acute coronary syndromes in our study is in line with other published data[19].

The present results should be evaluated with some caution in view of two study limitations. First, the case–control design may have introduced a selection bias.
risk factor may play a significant role in individuals with acute coronary syndromes who have limited atherosclerotic involvement, who presumably have been less exposed to major metabolic or acquired cardiovascular risk factors, and in whom the prevailing mechanism of disease may be hypercoagulability.

References


