RAPID COMMUNICATION

A Common Prothrombin Variant (20210 G to A) Increases the Risk of Myocardial Infarction in Young Women

By F.R. Rosendaal, D.S. Siscovick, S.M. Schwartz, B.M. Psaty, T.E. Raghunathan, and H.L. Vos

Using specimens from a population-based case control study among women ages 18 to 44 years in western Washington, we assessed the relationship between carriership of a genetic clotting factor II variant (20210 G→A) and myocardial infarction (MI). The factor II variant was previously shown to be present in 1% to 2% of the population, to increase the levels of factor II, and to be associated with venous thrombotic disease. Personal interviews and blood samples were obtained from 79 women with a first myocardial infarction and 381 control women identified through random-digit telephone dialing. Polymerase chain reaction (PCR) method was used to determine the factor II genotypes. The factor II 20210 G to A transition was present more often in women with MI (5.1%) than among control women (1.6%).

Prothrombin (factor II) is the precursor of thrombin, the final effector of the clotting cascade that leads to the formation of fibrin. Prothrombin is a key enzyme in the balance between procoagulation and anticoagulation because it potentiates coagulation by positive feedback loops and also promotes anticoagulation by the protein C pathway.

A recently described genetic variant of prothrombin is associated with an increased risk of venous thrombosis. The variant is located in the 3' untranslated region of the gene (on chromosome 11), at position 20210, where one nucleotide is changed (a G to A transition). Carriership of this variant is associated with elevated levels of prothrombin in plasma, which are related to an increased risk of thrombosis. Among 474 patients with a first deep-vein thrombosis we found the 20210 G→A variant in 6.2%, as opposed to 2.3% among 471 healthy control subjects. From these results we concluded that carriership of this variant increases the risk of deep-vein thrombosis 2.3-fold. Among patients with heritable thrombophilia, the variant was found in 18%. This high proportion is consistent with the hypothesis that the variant is a genetic cause of thrombosis. In a second sample of 646 unaffected individuals, we found the mutation in 1%, which led us to conclude that prevalence in the Netherlands is 1% to 2%.

Recently we have shown that another common genetic abnormality, a mutation in clotting factor V (the prothrombin 20210 G to A transition) and the protein C pathway, is a fairly common variant and thus of potential importance in the etiology of myocardial infarction.

The age-adjusted odds ratio for MI was 4.0 (95% confidence interval 1.1 to 15.1). The relative risk was high when another major cardiovascular risk factor was also present, such as smoking (odds ratio 43.3, 95% confidence interval 6.7 to 281), and the risk seemed limited to those with other risk factors. These results, in which the effect of major coronary risk factors is enhanced fourfold to sixfold by the prothrombin variant, are similar to those previously reported for another genetic clotting abnormality, factor V Leiden. We conclude that factor II 20210 G to A increases the risk of myocardial infarction in young women, especially in the women with other major risk factors for coronary heart disease

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MATERIALS AND METHODS

We conducted a population based case control study of myocardial infarction among women 18 to 44 years of age residing in three contiguous counties of western Washington. The methods of the study have been described extensively previously. Cases were women aged 18 to 44, without a prior history of coronary heart disease or cerebrovascular disease, who were diagnosed between July 1, 1991 and February 28, 1995 with a first acute MI. Criteria for myocardial infarction were defined by evidence of symptoms, elevated enzymes, and electrocardiographic changes. Of 165 eligible patients, 112 participated in an in person interview and 84 were willing to undergo venipuncture.

Controls were identified by random-digit telephone dialing. Eligible were women aged 18 to 44 years living in the same area during the time period of the study without a history of cardiovascular disease.

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disease. A household census was completed for 94.9% of the residences contacted. Of 684 randomly chosen eligible individuals, 525 agreed to a personal interview (response 78%) and of these 391 agreed to venipuncture.

Participating cases and controls were interviewed in person regarding histories of diabetes, hypertension, or hyperlipidemia (physician's diagnosis and drug-treatment), cigarette smoking, height and weight, reproductive and contraceptive histories, and demographic characteristics. All questions elicited information from a time period before each case's cardiovascular event, or an equivalent date for controls.

We obtained 30 mL venous blood from the antecubital vein in EDTA-treated vacutainers, from which the cells were separated by centrifugation at 2,000 g for 10 minutes, the buffy coat was resuspended in phosphate-buffered saline and frozen at -70°C. White blood cell aliquots were shipped to Leiden, The Netherlands, where DNA analysis was performed. DNA was extracted from these samples essentially as described by Miller et al. Analyzable DNA was available for 79 women with MI and 381 control subjects (5 women included in an earlier analysis were later classified as having unstable angina and were excluded from this analysis). The technician who performed DNA analyses was blinded as to whether a specimen was from a patient or a control subject.

The presence of the factor II variant (20210 G to A replacement) was first determined using an A-allele-specific polymerase chain reaction (PCR) according to the method of Glisic and Alavantic. The heterozygous AG status of putative positives was confirmed by the presence of a HindIII restriction site in the relevant PCR-fragment according to a previously described protocol.

Smokers were defined as women who reported smoking currently and regularly, while all others were classified as non-smokers. A woman was classified as diabetic, hypertensive, or hypercholesterolemic if she reported that she had ever been diagnosed by a physician as such. Additionally, we assessed which women were currently taking prescription drugs for these conditions. We considered obese any woman with a body mass index (BMI) equal to or exceeding 27.3 kg/m². In some analyses the variables for hypertension, hypercholesterolemia, diabetes mellitus, and obesity were grouped together as 'metabolic risk factors'.

The association of carnership of the factor II variant with MI was examined by unconditional logistic regression adjusted for the matching variable age, and expressed with the odds ratio (OR) as a measure for relative risk. Effect modification was assessed through stratified analyses. Confidence intervals (95%, CI95) were calculated by standard methodology.

RESULTS

The 79 women with an acute MI had a mean age of 39.7 (median 41), the control women of 37.7 (median 39). All major risk factors for coronary disease such as smoking, obesity, hypertension, hypercholesterolemia, and diabetes were reported more often among cases than among controls (Table 1). Smoking (75%) and obesity (58%) were particularly prevalent among the patients.

Five percent of women with an MI carried the factor II 20210A allele (4 of 79, 5.1%), compared to 1.6% of the control women (6 of 381) (Table 2). All carriers of the mutation were white women whereas none of the 51 non-white women in the study carried the mutation. The OR for MI (from the age-adjusted logistic model) was 4.0 (CI95 1.1 to 15.1, P = 0.038). These results did not materially change when the analyses were restricted to white women (n = 409, OR = 4.2), premenopausal women (n = 389, OR = 3.7), or users of oral contraceptives (n = 414, OR = 6.3). The complementary subgroups for these variables included too few women to allow risk estimation.

The risk associated with factor II 20210A was particularly high when other major risk factors were also present (Table 2). Among nonsmokers or women without metabolic risk factors, the risk was not increased when women carried the factor II variant, but among smokers who carried the mutation the risk was increased 43-fold (CI95 6.7-281) compared to nonsmoking women without the factor II variant, and, among women with one or more of four metabolic risk factors (obesity, hypertension, hypercholesterolemia, or diabe-

### Table 1 General Characteristics of Patients and Control Subjects

<table>
<thead>
<tr>
<th></th>
<th>Patients With MI (n = 79)</th>
<th>Control Women (n = 381)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>39.7</td>
<td>37.7</td>
</tr>
<tr>
<td>Median</td>
<td>41</td>
<td>39</td>
</tr>
<tr>
<td>Range</td>
<td>23-44</td>
<td>19-44</td>
</tr>
<tr>
<td>Current smokers</td>
<td>59 (74.7)</td>
<td>86 (22.6)</td>
</tr>
<tr>
<td>Ever diagnosed with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>27 (34.2)</td>
<td>37 (9.7)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>33 (41.8)</td>
<td>60 (16.7)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>12 (15.2)</td>
<td>11 (2.9)</td>
</tr>
<tr>
<td>Currently drug treated for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (16.6)</td>
<td>10 (2.6)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>2 (2.5)</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (6.3)</td>
<td>3 (0.8)</td>
</tr>
<tr>
<td>Obese</td>
<td>46 (58.2)</td>
<td>102 (27.0)</td>
</tr>
</tbody>
</table>

Data were not available for treated hypercholesterolemia in one control, and body mass index in three controls. Percentages are given for available data, in parentheses. All data, including diagnosis or treatment of the conditions mentioned above, refer to dates before the MI.

### Table 2 Factor II 20210A Among Patients With MI and Controls and ORs in Relation to Smoking and Metabolic Risk Factors

<table>
<thead>
<tr>
<th>Smoking</th>
<th>Other Risk Factor</th>
<th>Patients (n = 79)</th>
<th>Controls (n = 381)</th>
<th>OR</th>
<th>CI95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>No</td>
<td>20</td>
<td>621</td>
<td>1.0</td>
<td>0.51</td>
</tr>
<tr>
<td>AG</td>
<td>No</td>
<td>0</td>
<td>4</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>Yes</td>
<td>65</td>
<td>84</td>
<td>9.3</td>
<td>5.2 56.5</td>
</tr>
<tr>
<td>AG</td>
<td>Yes</td>
<td>4</td>
<td>2</td>
<td>43.3</td>
<td>6.7 281</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Metabolic risk factor</th>
<th>Other Risk Factor</th>
<th>Patients (n = 79)</th>
<th>Controls (n = 381)</th>
<th>OR</th>
<th>CI95</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>No</td>
<td>14</td>
<td>210</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>AG</td>
<td>No</td>
<td>0</td>
<td>4</td>
<td>0.245</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>Yes</td>
<td>61</td>
<td>162</td>
<td>5.3</td>
<td>2.999</td>
</tr>
<tr>
<td>AG</td>
<td>Yes</td>
<td>4</td>
<td>2</td>
<td>33.8</td>
<td>5.5 209</td>
</tr>
</tbody>
</table>

ORs are adjusted for age, and calculated separately for each stratum with a dummy variable model, age-adjusted and relative to the reference category (OR 1). Metabolic risk factors are either obesity or a physician's diagnosis of diabetes mellitus, hypertension, or hypercholesterolemia.

### RESULTS

The 79 women with an acute MI had a mean age of 39.7 (median 41), the control women of 37.7 (median 39). All major risk factors for coronary disease such as smoking, obesity, hypertension, hypercholesterolemia, and diabetes were reported more often among cases than among controls (Table 1). Smoking (75%) and obesity (58%) were particularly prevalent among the patients.

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Two fold increased risk of MI in young women

Factor II variant had a 5.3-fold increased risk (CI 95% 2.9 to 9.9), namely the already elevated risk among women with these risk factors was increased further by more than sixfold. Restriction of this analysis to drug-treated hypertension, hypercholesterolemia, or diabetes mellitus (instead of reported physician’s diagnosis of these conditions) led to similar results.

These results of a striking synergy with other major cardiovascular risk factors is similar to what we have previously described for the association of factor V Leiden (factor V 1691 G to A) and MI among these young women. Therefore, we combined these two mutations into one variable representing either clotting abnormality, which was present in 12 cases (15.2%) and 21 controls (5.5%).

The OR associated with any clotting abnormality was 3.1, with a 95% confidence interval of 1.5 to 6.7 (P = 0.003). This relative risk indicates a moderate strength risk factor that increases the risk threefold, however, when the clotting abnormality was present combined with other cardiovascular risk factors, the relative risks ranged from 21 (obesity) to 36 (smoking), compared to women without the other risk factor or any clotting abnormality. Table 3 shows the relative risks of several major determinants of coronary disease when present without the clotting abnormality, and when present in combination with the clotting abnormality. The presence of either prothrombotic mutation (factor V Leiden or factor II 20210A) increased further the already elevated risk of MI associated with smoking, obesity, and metabolic risk factors by fourfold to sixfold.

**DISCUSSION**

A common variant in the prothrombin gene, factor II 20210 G to A replacement, was associated with an overall threefold increased risk of MI in young women. The variant was found in 1.6% of healthy young women. The relative risk became particularly high when other major cardiovascular risk factors were also present.

MI is a rare event in young women. For MI to occur in a young individual, several risk factors need to be present simultaneously, as is the case for venous thrombosis. Therefore, young individuals form an excellent group to study the interaction of new risk factors as well as the interaction between risk factors. We have reported previously from this study population that over 95% of cases had at least one of the risk factors smoking, hypertension, hypercholesterolemia, diabetes mellitus, obesity, or factor V Leiden. Smoking and obesity both were extremely frequent and present in more than half of the women with MI. Because MI is uncommon in young women, the absolute individual risk of disease will remain low even when the relative risk is high. However, it should be kept in mind that all major diseases are rare in the young, and that therefore even small absolute risks contribute significantly to overall morbidity and mortality among the young.

The factor V Leiden mutation has a distinct racial and, to a lesser extent, geographical distribution. It is common in whites and very rare in Asians and Africans, among whites in Europe, there seems to be a North-South gradient, with prevalences ranging between 2% in Italy to 7% in Sweden. Among 51 nonwhite women in this study, we did not find any carriers of the factor II mutation. Because the overall prevalence of the factor II mutation was 1.6%, this is obviously well within the expected finding, nevertheless, our data suggest the possibility that this variant, like the factor V Leiden mutation, is also present mainly in whites.

The findings of an association of MI with factor II 20210 G to A only among young women with other risk factors are strikingly similar to our previous reports on factor V Leiden in young women. Both clotting abnormalities display a strong synergistic effect with other cardiovascular risk factors, in particular with smoking. These two recently discovered clotting abnormalities were present in over 5% of the population, and thus may affect a large number of people.

In young women, MI appears to be the result of combined atherogenic and thrombogenic factors. Without a concomitant atherogenic abnormality, there was little effect of the clotting abnormality with regard to the risk of MI. The reverse seemed true, too, as shown in Table 3 the various atherogenic risk factors, such as obesity, hypercholesterolemia, hypertension, and diabetes had a modest effect on the risk of MI in these women when present solely, and this risk was greatly enhanced when a clotting abnormality as factor V Leiden or factor II 20210A was simultaneously present.

Our results are based on a small number of mutant gene carriers and therefore the statistical uncertainty around the risk estimates remained considerable. The confidence intervals were particularly wide for the estimates in the interaction analysis. However, because the results for the overall effects of carriage of factor II 20210 A, as well as for the interaction with other major risk factors for coronary disease, are similar to those we reported previously for factor V Leiden, we feel confident about our conclusions of an increased risk and synergism with other risk factors. Although we found no increased risk for gene carriers who did not...
have another major risk factor for coronary heart disease, the confidence intervals for these estimates were wide and did not exclude the possibility of a mildly increased risk.

A further question is whether the association between the factor II mutation and the risk of MI is causal and indeed brought about by factor II. In our previous study in venous thrombosis we have shown a relation between the mutation and factor II levels, which appeared instrumental to the risk. Therefore, even though we could not measure clotting factor levels in the current study, it is likely that a prothrombotic effect of carriership of factor II 20210 A increases the risk, since we have shown before, among these same women with MI at a young age, a similar effect of another clotting abnormality (factor V Leiden).

Although generally there is no reason to assume that risk factors in the young qualitatively differ from those in middle-aged and older adults, they may differ in their strengths. Therefore, our findings cannot be generalized to older or male populations. It may well be that clotting factor abnormalities play a role in the etiology of MI in those populations, too, but it may be more difficult to discern because with advancing age both the presence and severity of atherosclerosis increases, which results in a higher background incidence of MI among those without a particular risk factor. Additionally, large groups need to be studied to examine specific interactions of atherogenic and prothrombotic risk factors in older populations. We believe that it will be worthwhile to look for specific interactions of risk factors in those populations as well.

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REFERENCES