Synergistic Effects of Prothrombotic Polymorphisms and Atherogenic Factors on the Risk of Myocardial Infarction in Young Males

By Aida Inbal, Dov Freimark, Baruch Modan, Angela Chetrit, Shlomi Matetzky, Nurit Rosenberg, Rima Dardik, Zvia Baron, and Uri Seligsohn

Several recent studies evaluated a possible effect of the prothrombotic polymorphisms such as 5,10-methylenetetrahydrofolate reductase (MTHFR) nt 677C → T, factor V (F V) nt 1691G → A (F V Leiden), and factor II (F II) nt 20210 G → A on the risk of myocardial infarction. In the present study, we analyzed the effect of these prothrombotic polymorphisms, as well as apolipoprotein (Apo) E4, smoking, hypertension, diabetes mellitus, and hypercholesterolemia, on the risk of myocardial infarction in young males. We conducted a case-control study of 112 young males with first acute myocardial infarction (AMI) before the age of 52 and 187 healthy controls of similar age. The prevalences of heterozygotes for F V G1691A and F II G20210A were not significantly different between cases and controls (6.3% v 6.4% and 5.9% v 3.4% among cases and controls, respectively). In contrast, the prevalence of MTHFR 677T homozygosity and the allele frequency of Apo E4 were significantly higher among patients (24.1% v 10.7% and 9.4% v 5.3% among cases and controls, respectively). Concomitant presence of hypertension, hypercholesterolemia, or diabetes and one or more of the four examined polymorphisms increased the risk by almost ninefold (odds ratio [OR] = 8.66; 95% confidence interval [CI], 3.49 to 21.5) and concomitant smoking by almost 18-fold (OR = 17.6; 95% CI, 6.30 to 48.9). When all atherogenic risk factors were analyzed simultaneously by a logistic model, the combination of prothrombotic and Apo E4 polymorphisms with current smoking increased the risk 25-fold (OR = 24.7; 95% CI, 7.17 to 84.9). The presented data suggest a synergistic effect between atherogenic and thrombogenic risk factors in the pathogenesis of AMI, as was recently found in a similar cohort of women.

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CLINICAL OBSERVATIONS, INTERVENTIONS, AND THERAPEUTIC TRIALS

ACUTE MYOCARDIAL INFARCTION (AMI) frequently results from a rupture of an atherosclerotic plaque that is followed by thrombus formation. Major risk factors for atherosclerosis include current smoking, hypertension, diabetes mellitus, and dyslipidemia, including the apolipoprotein (Apo) E4 isoform.

The contribution of thrombogenic risk factors to the development of AMI has been less well characterized. The investigation of such factors is of particular interest in young patients with AMI whose coronary arteries are angiographically intact. Potential thrombogenic risk factors that have been studied include abnormalities of blood flow, platelet hyperreactivity, reduced fibrinolysis, and increased plasma levels of fibrinogen, factor VII, von Willebrand factor, tissue plasminogen activator, and tissue plasminogen activator inhibitor.

Polymorphism in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene that involves a nt 677C → T transition is associated in the homozygous state with an increased level of homocysteine, particularly when plasma folic acid level is reduced. The association between homozygous MTHFR 677T and myocardial infarction was intensely studied during the last 2 years. However, the results of these studies range from no effect to a mild to moderate effect. Resistance to activated protein C due to a substitution of arginine by glutamine at amino acid residue 506 in coagulation factor V (F V) nt G1691A is commonly observed in patients with venous thromboembolic disorders. No association of this polymorphism with myocardial infarction was established in the American Physician Health Study and in smaller scale studies. However, other studies showed that mutant F V G1691A was more frequent in patients with myocardial infarction. Recently, Rosendaal et al demonstrated that F V G1691A did exert a profound risk of myocardial infarction in young women who were smokers or who had other atherogenic metabolic risk factors. Similar observations were made in the same study group regarding another newly described prothrombotic polymorphism in factor II (F II). This polymorphism involves a G → A transition at position 20210 of the untranslated region of the F II gene. This substitution is associated with increased plasma levels of F II and was found to confer an excessive risk of venous thrombosis. Few recent reports suggest that F II G20210A is not associated with increased risk for myocardial infarction.

The studies reported by Rosendaal et al are the only ones that established a synergistic effect of atherogenic and thrombogenic risk factors. Similar studies in young men, in whom myocardial infarction is much more frequent, have not been published. In the present case-control study of young males with AMI, we evaluated the combined risks exerted by atherogenic risk factors, namely, smoking, hypertension, diabetes mellitus, hypercholesterolemia, and Apo E4, and by thrombogenic risk factors, ie, F V G1691A, F II G20210A, and homozygous MTHFR 677T.

MATERIALS AND METHODS

Cases and controls. The study group consisted of male patients aged less than 52 years who were consecutively admitted to the Coronary Care Unit from March 1994 to March 1998 with an
established diagnosis of first AMI as defined by the Cardiovascular Health Study. Of 169 eligible patients, five were deceased at the time of the study and 112 were willing to participate in this retrospective study. The control group consisted of 187 healthy male subjects of similar age who were enrolled during routine annual examinations at the clinic of the Israeli Defense Forces. None of the controls had a history or evidence of coronary artery disease (CAD) as determined by medical history, resting ECG, and ergometry. The study was approved by the Human Subject Ethics Committee of the hospital and written consent was obtained from all subjects.

Demographic characteristics. Demographic data were obtained for each subject from the medical records of the Medical Center and Israeli Defense Forces. The information included current age (for controls), age at the time of the AMI, ethnic background, smoking history, blood pressure, total serum cholesterol, diabetes status, and history of coronary events. Seventy-one of 112 patients underwent coronary angiography and the severity of CAD was determined by visual estimation. Stenosis of above 70% was defined as severe.

Determination of prothrombotic polymorphisms and Apo E4. Genomic DNA was isolated from 5 mL whole blood by a standard method. The G1691A in F V was detected by polymerase chain reaction (PCR) amplification of a 267-bp fragment and MnlI digestion as previously described. The C677T mutation in MTHFR gene was identified using HinfI cleavage of an 198-bp PCR-amplified product as described by Frosst et al. For detection of Apo E isoforms, a 242-bp fragment of the Apo E gene was amplified by PCR and digested simultaneously with ApoI and HaeII as described by Zivelin et al. For identification of the G20210A substitution in the F II gene, a slightly modified method of Poort et al was used. The A20210 and G20210 alleles were discernible by this procedure, since the A20210 allele bears a restriction site only for MspI. The G1691A was analyzed, the difference between cases and controls is estimated. Stenosis of above 70% was defined as severe.

Statistical analyses. Differences in baseline characteristics between patients and healthy controls were assessed by the chi-square test for categorical variables and t-test for continuous parameters. Univariate odds ratio (OR) and 95% confidence intervals (CIs) were estimated for each prothrombotic or Apo E4 polymorphism. Further analyses were undertaken to assess the effect of combinations of the polymorphisms on the risk of myocardial infarction. To estimate the effect of various risk factors on the occurrence of myocardial infarction, an unconditional logistic regression model was designed that included age, ethnic origin, smoking status, and presence of metabolic risk factors such as hypertension, hypercholesterolemia, or diabetes as controlling variables. It also included prothrombotic and Apo E4 polymorphisms. Combinations of the polymorphisms with smoking and metabolic factors were also included in the logistic regression model, and the adjusted OR with 95% CIs was estimated.

RESULTS

Characteristics of the study population. The demographic information and the prevalence of selected risk factors for CAD among cases and controls are shown in Table 1. Two thirds of AMI patients were of European-American origin, compared with 44% of the controls. As expected, major risk factors for coronary disease were more common in the patients than in the controls (Table 1).

Prevalence of prothrombotic and Apo E4 polymorphisms. Table 2 summarizes the prevalence of heterozygotes for F V G1691A and F II G20210A, homozygotes for MTHFR (677T), and of the Apo E4 allele frequency among cases and controls. None of the patients or controls was found to be homozygous for the F V or F II polymorphisms. The prevalences of heterozygotes for F V G1691A and F II G20210A were not significantly different between cases and controls. In contrast, the prevalence of MTHFR 677T homozygosity and the allele frequency of Apo E4 were higher among patients (24.1% vs 10.7% and 9.4% vs 5.3% among cases and controls, respectively). When the prevalence of any combination of the four studied polymorphisms was analyzed, the difference between cases and controls reached statistical significance (Table 3). It is notable that 45% of cases bore at least one of the four polymorphisms, whereas among controls only 23% carried one of these markers ($P = .0003$).

Among patients with AMI, 55% were current smokers and 57% had at least one atherogenic metabolic risk factor (hypertension, diabetes, or hypercholesterolemia). The corresponding figures among controls were 24.1% for current smoking and 24.6% for atherogenic risk factors.

Table 1. Demographic and Clinical Information on Patients With AMI and Controls

<table>
<thead>
<tr>
<th></th>
<th>AMI Patients</th>
<th>Controls</th>
<th>( P (\chi^2) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>Mean ± SD</td>
<td>42.5 ± 4.2</td>
<td>39.7 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>29-52</td>
<td>26-52</td>
</tr>
<tr>
<td>Origin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asia-Africa</td>
<td>36</td>
<td>32.1</td>
<td>104 55.6</td>
</tr>
<tr>
<td>Europe-America</td>
<td>72</td>
<td>64.3</td>
<td>82 43.9</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>5.3</td>
<td>1 0.6</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>28</td>
<td>25.0</td>
<td>100 53.5</td>
</tr>
<tr>
<td>Past</td>
<td>18</td>
<td>16.1</td>
<td>26 13.9</td>
</tr>
<tr>
<td>Present</td>
<td>62</td>
<td>55.4</td>
<td>45 24.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>4</td>
<td>3.6</td>
<td>16 8.5</td>
</tr>
<tr>
<td>Hypertension*</td>
<td>Yes</td>
<td>18</td>
<td>16.1 8.6</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>93</td>
<td>83.0 89.8</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>1</td>
<td>0.9 1.6</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes</td>
<td>17</td>
<td>15.2 0</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>92</td>
<td>82.1 100</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>3</td>
<td>2.7</td>
</tr>
<tr>
<td>Hypercholesterolemia†</td>
<td>Yes</td>
<td>53</td>
<td>47.3 25.1</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>53</td>
<td>47.3 69.5</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>6</td>
<td>5.4 10.3</td>
</tr>
</tbody>
</table>

* Systolic blood pressure \( \geq 140 \text{ mm Hg} \) on admission.
† Total serum cholesterol level > 200 mg/dL on admission.

Table 2. Prevalence of Prothrombotic and Apo E4 Polymorphisms Among Patients With AMI and Controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>AMI Patients (%)</th>
<th>Controls (%)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F V G1691A*</td>
<td>6.3</td>
<td>6.4</td>
<td>0.97</td>
<td>0.33-2.76</td>
</tr>
<tr>
<td>MTHFR 677T†</td>
<td>24.1</td>
<td>10.7</td>
<td>2.65</td>
<td>1.35-5.25</td>
</tr>
<tr>
<td>Apo E4 allele frequency</td>
<td>9.4</td>
<td>5.3</td>
<td>1.88</td>
<td>0.90-3.91</td>
</tr>
<tr>
<td>F II G20210A*</td>
<td>5.9</td>
<td>3.4</td>
<td>1.78</td>
<td>0.46-6.94</td>
</tr>
</tbody>
</table>

* Heterozygotes.
† Homozygotes.
The prevalence of normal coronary arteries is higher in young patients with AMI than in older patients. In the present study, among 71 patients with AMI who underwent coronary angiography, we were unable to demonstrate a difference in the prevalence of the four polymorphisms between patients who had normal or abnormal coronary arteries. If these findings will be confirmed in a larger cohort of young patients with AMI, it may suggest that prothrombotic polymorphisms confer a significant increase in risk of AMI. In young patients with AMI, the evidence for atherosclerotic CAD is not always discernible, which highlights a potential importance of prothrombotic risk factors. We therefore chose to evaluate the effect of several recently described prothrombotic polymorphisms and Apo E4 in relatively young patients with AMI and estimate their interaction with major atherogenic risk factors. Similarly to other investigators, we found that neither FV G1691A nor FII G20210A was associated with an increased risk of AMI. In contrast, Rosendaal et al did demonstrate an association between FII G20210A and FV G1691A polymorphisms and increased risk of AMI in young women. Conceivably, these differences may stem from variable prevalences of other risk factors in different populations.

Several studies have examined the association of homozygous 677T MTHFR and AMI, but the results have been inconsistent. Some studies, including our present study, demonstrated a higher prevalence of homozygous MTHFR 677T in patients with AMI, whereas others showed no difference in the prevalence of the mutation between coronary patients and controls. Conceivably, these inconsistencies stem from differences in the examined populations with regard to other genetic factors and intake of folic acid, which is known to ameliorate the effect of homozygous MTHFR 677T.

Our study supports the previous observations that indicated that Apo E4 polymorphism is associated with an increased risk for coronary heart disease, probably by alterations in lipid metabolism. When the presence of one or more of the three prothrombotic and Apo E4 polymorphisms was analyzed, only a mildly increased risk of AMI was observed. However, the relative risk increased significantly when major cardiovascular risk factors were also present. Thus, the estimated risk increased ninefold when the polymorphisms were associated with hypertension, hypercholesterolemia, or diabetes and almost 18-fold when associated with current smoking. Moreover, the estimated risk increased 25-fold when adjustment for atherogenic metabolic risk factors was undertaken. These results are in accordance with the studies of Rosendaal et al in which a similar synergism was established in a group of young women with myocardial infarction. Also consistent with our data is the finding of a synergistic effect that was recently reported in a cohort of older patients with myocardial infarction. Thus, it may well be that the presence of one or more of polymorphisms studied in combination with major cardiovascular risk factors plays a synergistic role in the etiology of AMI.

Table 3. Prevalence of Prothrombotic and Apo E4 Polymorphisms Among Patients With AMI and Controls

<table>
<thead>
<tr>
<th>Polymorphisms*</th>
<th>AMI Patients</th>
<th>Controls</th>
<th>Pt (χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>0</td>
<td>62</td>
<td>55.3</td>
<td>144</td>
</tr>
<tr>
<td>1</td>
<td>40</td>
<td>35.7</td>
<td>37</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>8.9</td>
<td>6</td>
</tr>
</tbody>
</table>

*Heterozygotes for F V G1691A or F II G20210A; homozygotes for MTHFR 677T; †Relates to the difference between 0 vs 1 and ≥ 2.

Further analyses were undertaken to identify possible interactions between the atherogenic and prothrombotic risk factors. To overcome the differences between the cases and controls with regard to ethnic origin and age, these variables, as well as metabolic variables, were included in a multivariate stepwise logistic regression model. The results presented in Table 4 were adjusted for age and ethnic origin and ORs were estimated for each combination of risk factors. The presence of atherogenic metabolic risk factors increased the risk of myocardial infarction fourfold (OR = 4.37; 95% CI, 2.20 to 8.70), whereas the combination of these factors with the prothrombotic polymorphisms and Apo E4 resulted in an almost ninefold increased risk (OR = 8.66; 95% CI, 3.49 to 21.5).

Current smoking with none of the polymorphisms present was associated with an almost fourfold increase in the risk for myocardial infarction (OR = 3.86; 95% CI, 1.94 to 7.68), whereas the combination of current smoking and the polymorphisms increased the risk almost 18-fold (OR = 17.6; 95% CI, 6.30 to 48.9).

When the atherogenic metabolic risk factors and current smoking were analyzed simultaneously by the logistic model, the combination of prothrombotic and Apo E4 polymorphisms with current smoking increased the risk 25-fold (OR = 24.7; 95% CI, 7.17 to 84.9). The prothrombotic and Apo E4 polymorphisms in the absence of smoking or atherogenic metabolic risk factors exerted only a threefold effect (OR = 3.0; 95% CI, 1.57 to 5.73).

The prevalence of the prothrombotic or Apo E4 polymorphisms in patients with angiographically defined CAD was similar to the prevalence in patients with angiographically normal coronary arteries. Thus, 21 of 57 patients (36.8%) with severe CAD bore one or more of the polymorphisms, compared with 6 of 14 patients (42.9%) who had normal coronary arteries (P = .6).

Table 4. ORs for Cardiovascular Risk Factors and Risk of AMI With and Without the Simultaneous Presence of Prothrombotic and Apo E4 Polymorphisms

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>3.86</td>
<td>1.94-7.68</td>
</tr>
<tr>
<td>Without prothrombotic or Apo E4 polymorphisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With prothrombotic or Apo E4 polymorphisms</td>
<td>17.6</td>
<td>6.30-48.9</td>
</tr>
<tr>
<td>Metabolic risk factors</td>
<td>4.37</td>
<td>2.20-8.70</td>
</tr>
<tr>
<td>Without prothrombotic or Apo E4 polymorphisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With prothrombotic or Apo E4 polymorphisms</td>
<td>8.66</td>
<td>3.49-21.5</td>
</tr>
</tbody>
</table>

Stepwise logistic analysis adjusted for age and origin.

DISCUSSION

In young patients with AMI, the evidence for atherosclerotic CAD is not always discernible, which highlights a potential importance of prothrombotic risk factors. We therefore chose to evaluate the effect of several recently described prothrombotic polymorphisms and Apo E4 in relatively young patients with AMI and estimate their interaction with major atherogenic risk factors. Similarly to other investigators, we found that neither FV G1691A nor FII G20210A was associated with an increased risk of AMI. In contrast, Rosendaal et al did demonstrate an association between FII G20210A and FV G1691A polymorphisms and increased risk of AMI in young women. Conceivably, these differences may stem from variable prevalences of other risk factors in different populations.

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cant risk of AMI, which is accentuated in young patients with angiographically normal coronary arteries. In accordance with this suggestion, two recent reports showed no difference in the prevalence of homozygous MTHFR 677T4 among subjects with normal or abnormal coronary arteries.

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