G20210A Mutation in Prothrombin Gene and Risk of Myocardial Infarction, Stroke, and Venous Thrombosis in a Large Cohort of US Men

Paul M. Ridker, MD; Charles H. Hennekens, MD; Joseph P. Miletich, MD, PhD

**Background**—A single base pair mutation in the prothrombin gene has recently been identified that is associated with increased prothrombin levels. Whether this mutation increases the risks of arterial and venous thrombosis among healthy individuals is controversial.

**Methods and Results**—In a prospective cohort of 14,916 men, we determined the prevalence of the G20210A prothrombin gene variant in 833 men who subsequently developed myocardial infarction, stroke, or venous thrombosis (cases) and in 1,774 age- and smoking status–matched men who remained free of thrombosis during a 10-year follow-up (control subjects). Gene sequencing was used to confirm mutation status in a subgroup of participants. Overall, carrier rates for the G20210A mutation were similar among case and control subjects; the relative risk of developing any thrombotic event in association with the 20210A allele was 1.05 (95% CI, 0.7 to 1.6; \( P = 0.8 \)). We observed no evidence of association between mutation and myocardial infarction (RR = 0.8, \( P = 0.4 \)) or stroke (RR = 1.1, \( P = 0.8 \)). For venous thrombosis, a modest nonsignificant increase in risk was observed (RR = 1.7, \( P = 0.08 \)) that was smaller in magnitude than that associated with factor V Leiden (RR = 3.0, \( P < 0.001 \)). Nine individuals carried both the prothrombin mutation and factor V Leiden (5 controls and 4 cases). One individual, a control subject, was homozygous for the prothrombin mutation.

**Conclusions**—In a large cohort of US men, the G20210A prothrombin gene variant was not associated with increased risk of myocardial infarction or stroke. For venous thrombosis, risk estimates associated with the G20210A mutation were smaller in magnitude than risk estimates associated with factor V Leiden. (Circulation. 1999;99:999-1004.)

Key Words: myocardial infarction ■ stroke ■ thrombosis ■ genetics ■ risk factors

Over the past decade, it has been recognized that inherited abnormalities of coagulation are frequent among patients with a history of thrombophilia. In particular, with the description of factor V Leiden as an inherited risk factor for both first and recurrent venous thrombosis, clinical interest in genetic causes of thrombosis has greatly increased. Most recently, Poort and colleagues have described a single-point mutation in the 3′ untranslated region of the prothrombin gene (G-to-A transition at nucleotide position 20210) that appears to be associated with increased prothrombin levels. Moreover, these investigators and others have suggested that this mutation may be associated with increased risks of venous thrombosis, particularly among individuals with a family history of such events.

In the United States, the population frequency of the G20210A prothrombin mutation and its impact on the occurrence of venous thrombosis are uncertain. However, mutation in the prothrombin gene is hypothesized to confer a prothrombotic state, data relating this mutation to risks of myocardial infarction and stroke are sparse, and their interpretation has been controversial. We therefore evaluated in a large cohort of apparently healthy US men whether mutation in the prothrombin gene was associated with the future occurrence of thrombosis in the venous, arterial, and cerebral circulations.

**Methods**

We determined the presence of the G20210A mutation in the prothrombin gene in a nested case-control analysis within the Physicians’ Health Study, a prospective cohort of 22,071 apparently healthy US male physicians aged 40 to 84 years at study entry who were followed over a period of 10 years for the occurrence of myocardial infarction, stroke, and venous thromboembolism. As described elsewhere, Physicians’ Health Study participants had no history of myocardial infarction, stroke,
transient ischemic attack, or cancer at study entry and were randomly assigned to receive aspirin or beta-carotene as part of a randomized trial of these agents in the primary prevention of cardiovascular disease and cancer.20 Reported cardiovascular disease during follow-up were confirmed through a detailed review of hospital records, death certificates, and autopsy reports. The diagnosis of myocardial infarction was confirmed with the presence of symptoms plus either increased cardiac enzyme changes indicative of infarction, with diagnostic changes on ECG, or with autopsy reports. The diagnosis of stroke was confirmed in the presence of a new focal neurologic deficit with signs and symptoms persisting for ≥24 hours; computed tomography scans were available in >95% of cases. The diagnosis of venous thrombosis was confirmed with a positive venography or ultrasound report, whereas the diagnosis of pulmonary embolism was confirmed with a positive angiogram or a ventilation-perfusion scan that showed ≥2 segmental defects.

At study entry, 14,916 participants provided a baseline blood sample sufficient for DNA analysis. Case subjects (patients) were defined as study participants who provided a baseline blood sample and subsequently had a first myocardial infarction, stroke, or venous thrombosis. For each patient, 2 or 3 control subjects were randomly selected from study participants who provided a baseline blood sample and remained free of reported cardiovascular disease during study follow-up. Control subjects were matched to patients by age (±1 year) and smoking status (past, current, never); using these criteria, we were able to analyze the genotype distributions and allele frequencies for the G20210A mutation among 833 patients who had had myocardial infarction, stroke, or venous thrombosis and among 1774 control subjects.

Each study participant had DNA samples that were obtained at study entry evaluated for the G20210A prothrombin mutation using the strategy and primers described by Poort et al.10 The amplification protocol included an initial denaturation at 94°C for 1 minute; 30 cycles of 40 seconds at 92°C, 40 seconds at 57°C, and 90 seconds at 72°C; and a final extension at 72°C for 5 minutes. An additional polymerase chain reaction (PCR) product, corresponding to nucleotides 19979 to 20246, was amplified from the DNA of an individual homozygous for the G20210A mutation and from 10 individuals heterozygous for the mutation and from 10 homozygous normal subjects. This amplification protocol consisted of initial denaturation at 94°C for 1 minute; 35 cycles of 30 seconds at 92°C, 30 seconds at 54°C, and 1 minute at 72°C; and a final extension at 72°C for 5 minutes. The forward and reverse primers were 5’-AACAACGCCTGATCAAATGG-G and 5’-GAGCTGCCCATGATAGCACTG-3’, respectively. The sense primer was also used for Dye Terminator Cycle Sequencing as recommended by the manufacturer (PE Applied Biosystems).

Mean and proportion values for baseline characteristics were calculated for patients and control subjects, and differences were tested for significance using the Student’s t test or the χ2 statistic. Genotype distributions and allele frequencies for the G20210A mutation were compared with use of the χ2 analysis. Relative risks of thrombosis associated with the 20210A allele were computed with the use of logistic regression analysis; all risk estimates were adjusted for randomized treatment assignment to aspirin and beta-carotene. Prespecified analyses were performed for any thrombotic event and separately for myocardial infarction, stroke, and venous thrombosis. For study participants who had >1 end point, only the first event was counted. For venous thrombosis, we also computed relative risks for secondary events (those associated with cancer surgery or trauma) and for primary events. Subgroup analyses were further performed on the basis of age, smoking status, and the presence or absence of other cardiovascular risk factors. All probability values are 2-tailed and confidence intervals are computed at the 95% level.

Results

Table 1 displays baseline characteristics of the participants. As expected, the prevalence of diabetes, hypertension, obesity, and hyperlipidemia was higher among men who subsequently developed thrombotic events than among those who remained free of vascular disease during the 10-year follow-up period. Due to the matching, age and smoking status were virtually identical.

Among the 1774 men who remained free of vascular disease, 1705 (96.1%) were homozygous for the 20210G allele, and 68 (3.8%) were heterozygous for both the
2021G and 2021A alleles. One control subject (0.06%) was homozygous for the 2021A allele (Table 2). Thus, the observed allele frequency for the 2021G allele among control participants was 98.0% (95% CI, 97.5% to 98.5%), and that of the 2021A allele was 2.0% (95% CI, 1.5% to 2.5%).

As also shown in Table 2, the genotype distribution (95.9 GG, 4.1 GA, and 0.0 AA) and allele frequency (98.0 G and 2.0 A) of the prothrombin mutation among study participants who subsequently developed vascular events were virtually identical to those of the control group (P < 0.8). The relative risk of developing any thrombotic event associated with carriage of the 2021A allele was 1.05 (95% CI, 0.7 to 1.6; P < 0.8) (Table 3).

No evidence of association was observed between the prothrombin mutation and myocardial infarction (relative risk [RR] = 0.8; 95% CI, 0.4 to 1.4) or stroke (RR = 1.1; 95% CI, 0.6 to 2.1). The relative risk for any arterial event associated with mutation was 0.9 (95% CI, 0.6 to 1.5; P = 0.8). There was no significant evidence of any modification of this lack of effect in subgroup analyses stratified by age, smoking status, and other cardiovascular risk factors or in analyses limited to strokes considered to be thromboembolic (Table 4). To evaluate the possibility that randomized aspirin use (325 mg PO QD) might have modulated the effect of the prothrombin mutation on risks of myocardial infarction, we performed an additional stratified analysis for events that occurred before the unblinding of the aspirin component of the Physician’s Health Study. In these analyses, we found no evidence of association between the G20210A mutation and risks of myocardial infarction among those randomly assigned to receive aspirin (RR = 0.8, P = 0.6) or placebo (RR = 0.7, P = 0.4).

Among patients with venous thrombosis, the frequency of the 2021A allele was 3.2% compared with 2.0% among control subjects, such that the relative risk of any venous thromboembolic event associated with the prothrombin mutation was 1.7 (95% CI, 0.9 to 3.1; P = 0.08) (Table 3). There appeared to be no evidence of effect modification based on whether the venous thrombotic events were associated with cancer, surgery, or trauma. Specifically, the relative risk of primary venous thromboembolism (n = 99) associated with the presence of the prothrombin mutation was 1.9 (95% CI, 0.8 to 4.2; P = 0.1), whereas the relative risk of secondary venous thromboembolism

### Table 2. Distribution and Allele Frequency of G20210A Polymorphism Among 2607 Apparently Healthy Men Participating in the Physicians' Health Study, According to Development of Cardiovascular Disease During Follow-Up

<table>
<thead>
<tr>
<th>Cardiovascular Disease During Follow-Up</th>
<th>None (n=1774)</th>
<th>Any Event (n=833)</th>
<th>Myocardial Infarction (n=404)</th>
<th>Stroke (n=259)</th>
<th>Venous Thrombosis (n=218)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype distribution, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1705 (96.1)</td>
<td>799 (95.9)</td>
<td>392 (97.0)</td>
<td>248 (95.8)</td>
<td>204 (93.6)</td>
</tr>
<tr>
<td>GA</td>
<td>68 (3.8)</td>
<td>34 (4.1)</td>
<td>12 (3.0)</td>
<td>11 (4.2)</td>
<td>14 (6.4)</td>
</tr>
<tr>
<td>AA</td>
<td>1 (0.1)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Allele frequency, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>3478 (98.0)</td>
<td>1632 (98.0)</td>
<td>796 (98.5)</td>
<td>507 (97.9)</td>
<td>422 (96.8)</td>
</tr>
<tr>
<td>A</td>
<td>70 (2.0)</td>
<td>34 (2.0)</td>
<td>12 (1.5)</td>
<td>11 (2.1)</td>
<td>14 (3.2)</td>
</tr>
</tbody>
</table>

All P values for the differences between patients (or subgroups of cases) and control subjects in genotype distribution or allele frequencies were nonsignificant.

### Table 3. Crude and Adjusted RR of Myocardial Infarction, Stroke, or Venous Thrombosis Associated With Carriage of 20210A Allele

<table>
<thead>
<tr>
<th></th>
<th>Crude</th>
<th>Adjusted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Any event</td>
<td>1.05</td>
<td>0.7–1.6</td>
</tr>
<tr>
<td>Any arterial event</td>
<td>0.9</td>
<td>0.6–1.5</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>0.8</td>
<td>0.4–1.4</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.1</td>
<td>0.6–2.1</td>
</tr>
<tr>
<td>Any venous thrombosis</td>
<td>1.7</td>
<td>0.9–3.1</td>
</tr>
<tr>
<td>Primary venous thrombosis</td>
<td>1.9</td>
<td>0.8–4.2</td>
</tr>
<tr>
<td>Secondary venous thrombosis</td>
<td>1.6</td>
<td>0.7–3.6</td>
</tr>
</tbody>
</table>

All models matched on smoking and age and controlled for randomized treatment assignment. Adjusted models additionally controlled for body mass index, hypertension, hyperlipidemia, and diabetes.
was 1.6 (95% CI, 0.7 to 3.6; \(P=0.2\)). We found no evidence of effect modification by age, smoking status, or other risk factors (Table 4). By contrast, the relative risks of venous thrombosis in this cohort associated with factor V Leiden were 3.0 for any venous thrombosis (\(P=0.001\)) and 4.5 for primary venous thrombosis (\(P=0.001\)).

Of the 2607 individuals screened in the present analysis, 9 were identified who carried both the prothrombin mutation and factor V Leiden: 5 were control subjects and 4 were patients (1 myocardial infarction and 3 venous thromboses). One individual, a control subject, was found to be homozygous for the prothrombin mutation.

Gene sequencing was performed on 10 study participants determined with use of the PCR to be homozygous normal subjects, 10 determined to be heterozygous carriers, and 1 determined to be a homozygous carrier for the prothrombin mutation. In each case, gene sequencing confirmed PCR-based mutation status.

### Table 5

**Sample Size, Number of 20210A Carriers Identified and Carrier Frequencies for Prothrombin Mutation in Control Groups From Several Published Studies**

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Population, n</th>
<th>Carriers, n</th>
<th>%</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>United States</td>
<td>1774</td>
<td>69</td>
<td>3.9</td>
<td>3.0–4.9</td>
</tr>
<tr>
<td>Brown et al(^{15})</td>
<td>United Kingdom</td>
<td>508</td>
<td>13</td>
<td>2.6</td>
<td>1.4–4.3</td>
</tr>
<tr>
<td>Poort et al(^{10})</td>
<td>Netherlands</td>
<td>471</td>
<td>11</td>
<td>2.3</td>
<td>1.2–4.1</td>
</tr>
<tr>
<td>Hillarp et al(^{13})</td>
<td>Sweden</td>
<td>282</td>
<td>5</td>
<td>1.8</td>
<td>0.6–4.1</td>
</tr>
<tr>
<td>Rosendaal et al(^{16})</td>
<td>United States</td>
<td>381</td>
<td>6</td>
<td>1.6</td>
<td>0.6–3.4</td>
</tr>
<tr>
<td>Doggen et al(^{18})</td>
<td>Netherlands</td>
<td>646</td>
<td>8</td>
<td>1.2</td>
<td>0.5–2.4</td>
</tr>
<tr>
<td>Cumming et al(^{14})</td>
<td>United Kingdom</td>
<td>164</td>
<td>2</td>
<td>1.2</td>
<td>0.1–4.3</td>
</tr>
<tr>
<td>Franco et al(^{17})</td>
<td>Netherlands</td>
<td>400</td>
<td>4</td>
<td>1.0</td>
<td>0.3–2.5</td>
</tr>
<tr>
<td>Makris et al(^{11})</td>
<td>United Kingdom</td>
<td>150</td>
<td>1</td>
<td>0.7</td>
<td>0.1–3.6</td>
</tr>
<tr>
<td>Aruda et al(^{12})</td>
<td>Brazil</td>
<td>295</td>
<td>2</td>
<td>0.7</td>
<td>0.1–2.4</td>
</tr>
</tbody>
</table>

### Discussion

Prothrombin (factor II) is a precursor of thrombin and plays a critical role in fibrin formation; thus, the recent demonstration that a single-point mutation in the prothrombin gene is associated with increased prothrombin levels\(^{10}\) has generated considerable clinical interest, and it has been suggested that this polymorphism may lead to increased risks of both arterial and venous thrombosis.\(^{10–19}\)

We performed a large-scale, prospective, nested case-control study of the G20210A prothrombin mutation in an otherwise healthy population of US men who were followed over a 10-year period for the occurrence of myocardial infarction, stroke, or venous thrombosis. With regard to arterial thrombosis, we found no evidence of association between the 20210A allele and risks of either myocardial infarction or stroke, nor any evidence of effect modification by known cardiovascular risk factors or by...
The prevalence of prothrombin mutation among control subjects in our study was 3.9%, a rate higher than that reported in several prior studies (Table 5). As has been observed for factor V Leiden, it is possible that there are different rates of the prothrombin mutation in different populations. It is also possible, however, that previously reported mutation rates based on smaller samples may have underestimated the prevalence of the 20210A allele. For example, in 1 study from the United States that involved 381 control subjects, a mutation rate of 1.6% was reported, indicating that only 6 heterozygotes were identified.19 Similarly, investigators in the Netherlands have reported allele frequencies that vary by >2-fold despite sampling from similar population groups.20,21,22 In our study, we investigated the presence of the prothrombin mutation in a total of 1774 control subjects, a sample size substantially greater than that reported in any prior analysis; as a result, our estimate of the control prevalence of the G20210A mutation has quite narrow 95% CIs (Table 5).

It is interesting to compare and contrast our findings for the prothrombin mutation in this cohort to those for the factor V Leiden mutation, particularly with regard to venous thrombosis.2,3 Specifically, although the prevalence of factor V Leiden among control subjects in our study population (5.0%) is only modestly greater than that of the prothrombin mutation (3.9%), the clinical impact of factor V Leiden appears to be greater. As demonstrated in the Figure, compared with normal individuals or with those with the prothrombin mutation, those with factor V Leiden had substantially greater risks of developing any venous thrombosis as well as thrombotic events considered to be idiopathic. Moreover, in contrast to estimates for the prothrombin mutation, the risks of venous thrombosis associated with the factor V Leiden mutation were highly statistically significant (all \( P < 0.001 \)). Thus, at least for these data, any potential risk of venous thrombosis attributable to the prothrombin mutation appears to be small in comparison with that associated with factor V Leiden.23

In summary, these prospective data for a large cohort of US men show no evidence of association between the prothrombin mutation and risks of myocardial infarction or stroke. For venous thrombosis, we observed a modest increase in risk associated with the G20210A mutation (RR = 1.7, \( P = 0.08 \)) that was smaller in magnitude than that associated with factor V Leiden (RR = 3.0, \( P < 0.001 \)).

Acknowledgments

This work was supported by grants from the National Heart, Lung, and Blood Institute and by an Established Investigator Award from the American Heart Association (Dr Ridker).

References

G20210A Mutation in Prothrombin Gene and Risk of Myocardial Infarction, Stroke, and Venous Thrombosis in a Large Cohort of US Men
Paul M. Ridker, Charles H. Hennekens and Joseph P. Miletich

Circulation. 1999;99:999-1004
doi: 10.1161/01.CIR.99.8.999

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/99/8/999

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/