Factor V Arg506Gln Mutation Is Not Associated with Cardiovascular Mortality in Older Women

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Factor V Arg506Gln is the most common genetic risk factor for venous thrombosis and is associated with myocardial infarction in young women, especially among smokers. The authors studied the relation of factor V Arg506Gln to cardiovascular mortality in older women in a prospective cohort study of 12,239 women, living in the city of Utrecht, who were initially aged between 52 and 67 years. Women were followed on vital status between 1976 and 1995 (168,513 years). The factor V Arg506Gln mutation was determined in urine samples of 524 women who died of cardiovascular disease and in a reference group of 517 women who did not. Data were analyzed using a nested case-referent analysis. Factor V Arg506Gln heterozygosity was not associated with the risk of mortality by myocardial infarction, cerebrovascular disease, and other cardiovascular disease, with respective rate ratios and 95% confidence intervals being 1.1 (0.5–2.3), 1.2 (0.5–3.1), and 0.6 (0.2–1.7). No evidence of association was found in subgroups of smokers and age. Factor V Arg506Gln is not a risk factor for cardiovascular mortality in older women. Discrepancies with other studies may be explained by different study populations, as age and sex may modify both the frequency of cardiovascular disease and the effect of its risk factors. Am J Epidemiol 1999;149:665–70.

cardiovascular diseases; cerebrovascular disorders; cohort studies; factor V; mortality; myocardial infarction; postmenopause; women

Activated factor V (factor Va) is the receptor for activated factor X (factor Xa) at the platelet surface and is therefore an essential cofactor for the activation of prothrombin into thrombin. Factor Va is inactivated after cleavage by activated protein C at positions 506 and 306 (1–3).

An arginine into glutamine transition at position 506 (Arg506Gln) of the factor V protein is associated with reduced factor Va inactivation by activated protein C, leading to a tendency of venous thrombosis (4). Several case-control studies and one large cohort study reported an increased risk of venous thrombosis in factor V Arg506Gln heterozygotes (4–6). The factor V Arg506Gln mutation is found in 2–4 percent of the Dutch population (7), and the allele frequency appears to be highest in Western European countries (8). The relation of factor V Arg506Gln genotype to arterial thrombosis is not yet clear. Some have reported an increased frequency of factor V Arg506Gln heterozygotes in patients who suffer from arterial disease (9, 10), while several other studies did not disclose an association (11–14). In a large prospective cohort study among male physicians, no association was found between the factor V Arg506Gln genotype and arterial disease (6). Most studies on the factor V Arg506Gln genotype and arterial disease concerned men. Both age and sex modify the frequency of cardiovascular disease and the effects of its risk factors. Recently, two population-based case-control studies among younger women reported a statistically significant increased risk of myocardial infarction in factor V Arg506Gln heterozygotes, compared with women who had the normal factor V genotype (15, 16). The effect seemed to be largely confined to current smokers. The importance of factor V Arg506Gln genotype in arterial disease in older women at present is not known. We studied the relation of factor V Arg506Gln genotype to coronary mortality in a large cohort of 12,239 women (168,513 follow-up years) initially aged 52–67 years.
MATERIALS AND METHODS

Population

Between December 1974 and October 1980, 20,555 women, born between 1911 and 1925 and residing in the city of Utrecht, the Netherlands, were invited for an experimental program for breast cancer screening (Doorlopend Onderzoek Morbiditeit/Mortaliteit (DOM)) (17). The women were invited for repeated examinations at 1- to 6-year intervals. The baseline population of the present analysis consisted of 12,239 (60 percent) women who participated in the second examination (1976–1978) because the first screening examination did not include a questionnaire on smoking. These women were followed on vital status until December 1995.

Risk factors

At baseline, a questionnaire on cardiovascular risk factors, including medication, prescribed diets, the presence of cardiovascular disease, and smoking, was completed. In addition, blood pressure, height (m), and weight (kg) were measured. Women were classified as having diabetes mellitus if they reported use of insulin or oral blood glucose-lowering drugs or were on a diabetes diet. Women were defined as smokers if they reported being current smokers. Body mass index was calculated as the weight (kg)/height (m)². Obesity was defined as a body mass index of ≥30 (kg/m²). Hypertension was defined as a systolic blood pressure of >160 mmHg and/or a diastolic blood pressure of >90 mmHg and/or the use of antihypertensive medication.

Endpoints

Municipal registries informed the Department of Epidemiology (presently the Julius Center for Patient Oriented Research) about migration and mortality of the DOM cohort members. Causes of death were ascertained from the general practitioners. The 9,062 surviving women had a median follow-up time of 17 years, with a maximum of 18 years. A total of 1,447 women (12.3 percent) had moved outside the recruitment area and had a median follow-up of 10 years, with a maximum of 18 years. During follow-up (182,976 women-years), 1,714 women died: 608 of cardiovascular diseases (International Classification of Diseases, Ninth Revision (ICD-9), codes 390–459); 601 of neoplasms (ICD-9 codes 140–239); 299 of other causes; and 206 of unknown causes.

Design

We quantified the effect of factor V Arg506Gln heterozygosity on cardiovascular mortality, using a nested case-referent approach (18). The cases comprised 608 women who died of cardiovascular disease. The referents were a random sample of 605 women out of 11,425 women (12,239 – 608 cardiovascular mortality cases – 206 unknown mortality cases) who did not die of cardiovascular mortality (sampling fraction, 1:18.9) and were included for genotype analysis. Urine samples of 84 cardiovascular mortality cases and of 88 women of the reference group were not stored at baseline or were not suitable for DNA analyses, and these women were therefore excluded from the study. The final study group comprised 524 cardiovascular mortality cases and 517 noncases.

Genotyping

DNA was isolated from 50-ml urine samples using a DNA extraction kit (Puregene; Gentra Systems, Minneapolis, Minnesota). Factor V Arg506/Gln506 genotype was determined from each DNA fraction using polymerase chain reaction and hybridization with antigen-specific oligonucleotides (4, 19). The antigen-specific oligonucleotide for factor V Arg506 was 3'-TGGACAGGCAAGGAATAC-5' and, for factor V Gln506, 3'-GGACAGGCGAGGAATAC-5'. Dots were visualized on x-ray films (DuPont, Brussels, Belgium) after overnight radiation. Mutation analysis was performed with samples blinded for case or referent status.

Data analysis

Means and proportions of baseline cardiovascular risk factors were computed for women with factor V Arg506 genotypes (wildtypes), with both factor V Arg506 and Gln506 genotypes (heterozygotes), and with factor V Gln506 genotypes (homozygotes). The significance of difference in means was tested by unpaired t tests, and significance in proportions was tested by chi-square statistics. Allele frequencies were calculated by the Hardy-Weinberg law (20, 21). The chi-square goodness-of-fit test was used to determine whether the observed numbers of each genotype were in equilibrium.

A nested case-referent approach was used to estimate incidence rates and rate ratios. The reference group was a random sample of all women who did not die of cardiovascular disease (sampling fraction, 1:18.9). As referents are selected at random, all data of our reference group provide unbiased estimates of the entire cohort of women who did not die of cardiovascular mortality. After factor V 506 genotype was deter-
mined, the follow-up years were estimated for the entire cohort for each genotype by multiplying the follow-up years of the reference group by 18.9 (the inverse of the sampling fraction) and applying the follow-up years from the cardiovascular mortality cases. Incidence rates were calculated as the number of incidents per genotype divided by the estimated follow-up years for that genotype (18). The incidence rate ratio was calculated as the incidence rate for cardiovascular disease in heterozygotes divided by the incidence rate in wildtypes. Poisson regression was used to estimate incidence rates and risk ratios, while 95 percent confidence intervals were calculated with the method of Huber (22).

Similarly, crude relative risks, incidence rates, and rate ratios were estimated for women who died of myocardial infarction (ICD-9 codes 410–414), cerebrovascular disease (ICD-9 codes 430–438), and other cardiovascular disease (all remaining ICD-9 codes between 390 and 459), separately. The relation of factor V Arg506Gln genotype to cardiovascular mortality was also tested in subgroups of age (above or below the median) and smoking (yes/no). Women homozygous for factor V Arg506Gln were analyzed as a separate group.

RESULTS

The genotype distribution was similar for women who died of cardiovascular mortality and for women who did not die of cardiovascular mortality (table 1). Also, the genotype distribution appears not to be different among fatal myocardial infarction, cerebrovascular mortality, and other cardiovascular mortality. Of women homozygous for the factor V Arg506Gln polymorphism, one died of unspecified cardiac failure, another of unspecified heart disease, and a third one of stroke. One woman from the reference group was found to be homozygous for factor V Gln506. Remarkably, this woman reported a myocardial infarction in the baseline questionnaire. The allele frequency of the factor V Arg506Gln polymorphism in the reference group was 2.2 percent (95 percent confidence interval 1.4–3.2), and the population was in Hardy-Weinberg equilibrium.

The percentage of women with hypertension, the number of women with a history of symptomatic cardiovascular disease and diabetes mellitus, the number of smokers, and the number of obese women were not different among women who were wildtype or heterozygous for factor V genotypes (table 2).

The incidence rates for mortality by myocardial infarction, cerebrovascular events, and all other cardiovascular events were not higher in factor V Arg506Gln heterozygotes than in wildtypes (table 3). Rate ratios were 1.1 (95 percent confidence interval 0.5–2.3), 1.2 (95 percent confidence interval 0.5–3.1), and 0.6 (95 percent confidence interval 0.2–1.7). The rate ratio for all cardiovascular mortality events was 1.0 (95 percent confidence interval 0.5–1.8). Subgroup analysis on age and smoking had minor consequences for the effect of factor V Arg506Gln heterozygosity on cardiovascular mortality.

DISCUSSION

We studied the relation of factor V Arg506Gln genotype to cardiovascular mortality in women initially aged between 52 and 67 years. Factor V Arg506Gln heterozygosity had no effect on the risk of mortality from myocardial infarction, cerebrovascular disease, or all other cardiovascular disease. The risk of cardiovascular mortality associated with factor V Arg506Gln heterozygotes was not modified by age and smoking. Three factor V Arg506Gln homozygotes were found in the group of women who died of cardiovascular disease compared with one homozygote in the reference group. Remarkably, the factor V Arg506Gln homozygote in the reference group reported a history of myocardial infarction in the baseline questionnaire.

We found important evidence that factor V Arg506Gln genotype is not a major risk factor for cardiovascular mortality in older women. Our findings are in agreement with results from previously performed studies, which suggested that factor V Arg506Gln genotype is not associated with incidence of myocardial infarction in older men either (6, 13). On the other hand, factor V Arg506Gln appears to be associated with nonfatal myocardial infarction in younger women (15, 16). This discrepancy between

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Myocardial Infarction</th>
<th>Stroke</th>
<th>Other Cardiovascular Disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 517)</td>
<td>(n = 239)</td>
<td>(n = 119)</td>
<td>(n = 186)</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Wildtype (Arg506)</td>
<td>495</td>
<td>95.7</td>
<td>228</td>
<td>95.4</td>
</tr>
<tr>
<td>Heterozygote (Gln506)</td>
<td>21</td>
<td>4.1</td>
<td>11</td>
<td>4.6</td>
</tr>
<tr>
<td>Homozygote (Gln506)</td>
<td>1</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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young and old age might be explained by different mechanisms of symptomatic cardiovascular disease that dominate at different ages (23). Moreover, the absolute effect of genetic factors on chronic disease is commonly inversely associated with age, because exposure time to nongenetic factors increases with age (24).

The allele frequency of factor V Gln506 in our reference group was 2.2 percent (95 percent confidence interval 1.4–3.2). This is in agreement with findings from other large-scale studies on factor V Arg506Gln genotype in which an allele frequency of factor V 506Gln was between 1.5 and 3.0 percent (6, 25). Using the Hardy-Weinberg formula, the expected fraction of factor V Arg506Gln homozygotes in these populations is estimated to be between 0.02 and 0.09 percent. The fraction of homozygotes in our patients was 3 of 524, which is 0.6 percent. The number of homozygotes is too low to draw firm conclusions. Other large studies did not report an increased number of factor V Arg506Gln homozygotes in their cardiovascular patients (6, 13).

Data were analyzed using a nested case-referent approach, which is an alternative term for a nested case-control study. Nested analysis of prospectively collected material has the cost effectiveness of case-control studies and takes full advantage of cohort studies. The major advantage of nested case-referent analysis in favor of case-control studies is that incidence rates and relative risks can be directly estimated, while case-control studies can only approach relative risks with odds ratios. An additional advantage is that referents and cardiovascular patients are obtained from the baseline cohort and therefore have the same genetic background, while most case-control studies compare cases and controls from genetically nonidentical populations. Furthermore, questionnaire data, anthropometry, and urine samples are collected at baseline and are therefore not biased by events that occurred during follow-up. Fourth, the prospective approach enabled us to study cardiovascular mortality, while case-control studies rely on retrospective analysis and are therefore limited to morbidity of cardiovascular disease or to intermediate markers of disease.

Because our subjects were members of a normal population, vital status was therefore obtained from municipal registries, and the cause of death was ascertained from the general practitioner. Two hundred six women died of unknown cause. It is expected that a proportion of these women died of cardiovascular disease. As it was not possible to classify these women as either cardiovascular cases or noncardiovascular cases, we decided to leave them out of the analysis.

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<table>
<thead>
<tr>
<th>Cardiovascular mortality</th>
<th>Total follow-up time (person-years)</th>
<th>Incidence rates/1,000 years</th>
<th>Rate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myocardial infarction (n = 239)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>146,283</td>
<td>1.6 (1.3–1.9)</td>
<td>1.1 (0.5–2.3)*</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>3.2 (1.7–5.6)</td>
<td>1.1 (0.5–3.1)</td>
<td>0.6 (0.2–1.7)</td>
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<tr>
<td><strong>Cerebrovascular (n = 119)</strong></td>
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<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>242</td>
<td>0.8 (0.6–0.9)</td>
<td>0.9 (0.4–2.4)</td>
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<tr>
<td>Heterozygotes</td>
<td>1.1 (0.9–1.3)</td>
<td>0.6 (0.2–2.2)</td>
<td>0.8 (0.6–0.9)</td>
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<tr>
<td><strong>Other (n = 166)</strong></td>
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</tr>
<tr>
<td>Wildtype</td>
<td>3.4 (3.0–3.9)</td>
<td>1.0 (0.5–1.8)</td>
<td>1.1 (0.5–2.3)*</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>3.2 (1.7–5.6)</td>
<td>1.2 (0.5–3.1)</td>
<td>1.2 (0.8–3.5)</td>
</tr>
<tr>
<td><strong>Total (n = 524)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wildtype</td>
<td>6,651</td>
<td>3.2 (1.7–5.6)</td>
<td>1.2 (0.5–3.1)</td>
</tr>
<tr>
<td>Heterozygotes</td>
<td>1.0 (0.5–1.8)</td>
<td>1.1 (0.5–2.3)*</td>
<td>3.2 (1.7–5.6)</td>
</tr>
</tbody>
</table>

*Numbers in parentheses, 95% confidence interval.

In conclusion, we found no relation between factor V Arg506Gln heterozygosity and cardiovascular mortality in older women, suggesting that factor V Arg506Gln heterozygosity does not play an important role in end-stage atherothrombotic disease in older women.

**ACKNOWLEDGMENTS**

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**REFERENCES**


