Variation in the Estrogen Receptor α Gene and Risk of Stroke
The Rotterdam Study

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Background and Purpose—Variations in the −397T>C (rs2234693) and −351A>G (rs9340799) single nucleotide polymorphisms of the estrogen α receptor (ESR1) gene were found to be strongly associated with risk of ischemic heart disease, although not all studies could replicate this finding. One study also reported an association with stroke. We assessed whether variations in the ESR1 gene are associated with the risk of stroke in the general population.

Methods—This prospective population-based study was based on 6229 Rotterdam Study participants who at baseline (1990–1993) were aged 55 years or older, free from stroke, and had assessment of the ESR1 rs2234693 and rs9340799 single nucleotide polymorphisms. Follow-up for incident stroke was complete until January 1, 2005. Data were analyzed with Cox proportional hazards models for men and women separately with adjustment for age.

Results—During an average follow-up time of 10.1 years, 659 strokes occurred, of which 386 were ischemic. Three common haplotypes were identified: −397T/−351A (carried by 78% of all participants), −397C/−351G (carried by 57%), and −397C/−351A (carried by 22%). Although we had at least 89% power to detect a relative risk of 1.5 (α=0.05) in all subgroups, we did not find any association between ESR1 haplotype carriership and risk of stroke and ischemic stroke.

Conclusions—We have not been able to replicate the previously reported association between variations in the ESR1 gene and risk of stroke. (Stroke. 2008;39:000-000.)

Key Words: cerebrovascular disease ■ risk factors ■ stroke

The relatively low incidence rate of ischemic heart disease in premenopausal women has drawn attention to the role of estrogen in cardiovascular disease. Estrogen exerts its effect through estrogen receptors α and β. Previous research has demonstrated that the estrogen receptor α is involved in atherosclerosis, and several epidemiological studies found associations between variations in the −397T>C (rs2234693) and −351A>G (rs9340799) single nucleotide polymorphisms of the estrogen α receptor (ESR1) gene and risk of ischemic heart disease. Studies on the association between variations in the −397T>C single nucleotide polymorphism and risk of stroke are also contradictory. We assessed whether variations in the ESR1 gene are associated with the risk of stroke in the general population.

Materials and Methods
The present study was part of the Rotterdam Study, a prospective study on chronic and disabling diseases in 7983 white Dutch (98.5%) community-dwelling participants aged 55 years and older (participation rate 78%). The Medical Ethics Committee of Erasmus University Rotterdam approved of the study. Written informed consent to retrieve information from treating physicians was obtained from all participants.

All Rotterdam Study participants who at baseline (1990–1993) were free from stroke and had successful assessment of both ESR1 single nucleotide polymorphisms were included in the present study (N=6229, 2511 men). After enrollment into the Rotterdam Study, participants were continuously monitored for strokes through automated linkage of the study database with files from general practitioners and the municipality. Strokes were subclassified as ischemic strokes, hemorrhagic strokes, and unspecified strokes, as described previously. Follow-up was complete until January 1, 2005, for 97.1% of potential person-years.

All participants were genotyped for the −397T>C (rs2234693) and −351A>G (rs9340799) polymorphisms as described previously. We used the genotype data for each of the 2 polymorphisms to infer the haplotype alleles present in the population by using the program PHASE, which implements a Bayesian statistical method for reconstructing haplotypes. Haplotype alleles were numbered in order of decreasing prevalence.

We calculated HRs and 95% CIs for the associations between ESR1 haplotypes (dominant model) and risk of stroke and ischemic disease.
stroke using Cox proportional hazards models with SPSS for Windows (Rel. 11.0.1).

Results
During an average follow-up time of 10.1 years, 659 strokes occurred, of which 386 were ischemic, 62 were hemorrhagic, and 211 could not be subclassified. Baseline characteristics are presented in Table 1. Neither in women nor in men were variations in individual single nucleotide polymorphisms associated with risk of stroke and ischemic stroke (P>0.37). Of all participants, 78% carried haplotype 1 (−397T/−351A; 29% homozygous), 57% carried haplotype 2 (−397C/−351G; 12% homozygous), 22% carried haplotype 3 (−397C/−351A; 1.3% homozygous), and 1 participant carried haplotype 4 (−397C/−351A). For women there was no association between ESR1 haplotypes and risk of stroke and ischemic stroke (Table 2). For men, risk of stroke and ischemic stroke might be slightly increased in haplotype 1 carriers compared to noncarriers (HR, 1.21; 95% CI, 0.89 to 1.64 for stroke; and HR, 1.40; 95% CI, 0.94 to 2.09 for ischemic stroke), which was not statistically significant (α=0.05). Further adjustment did not change the associations.

Discussion
ESR1 haplotypes were not associated with risk of stroke in our population-based cohort.
For men, our power (α=0.05) to detect RR of 1.3 in haplotype carriers compared to noncarriers ranged from 50% for haplotype 1 (H1) to 80% for haplotype 2 (H2), the power to detect RR of 1.4 ranged from 74% (H1) to 96% (H2); and the power to detect RR of 1.5 ranged from 89% (H1) to 100% (H2). For women, the power to detect RR of 1.3 ranged from 66% for H1 to 83% for H2, the power to detect RR of 1.4 ranged from 88% (H1) to 97% (H2), and the power to detect RR of 1.5 ranged from 98% (H3) to 100% (H2). Therefore, we cannot completely rule out the possibility that the risk of stroke might be slightly increased (with HR<1.5) in male haplotype 1 carriers compared to noncarriers; similarly, the risk of ischemic stroke might be slightly increased in male haplotype 3 carriers compared to noncarriers.

Strengths of our study are the large study population (N=6229), the intense stroke case finding, and the nearly complete follow-up (loss of potential person-years 2.9%). Our stringent stroke monitoring procedures enabled the ascertainment of stroke cases that were not referred to a hospital. Because in these cases neuroimaging was often lacking, 32% of the total number of strokes could not be subclassified into ischemic or hemorrhagic.

Four large studies with a total of 7134 participants found an association between variation in the ESR1 gene and the risk of ischemic heart disease, whereas 1 study with 4868 participants could not replicate this finding. According to a study in 2709 men, those with the −397CC genotype were at higher risk for stroke than those with −397CT or TT (adjusted HR, 1.92; 95% CI, 1.06 to 3.48). A study in 5063 participants found no relation between this polymorphism and risk of stroke. We found no associations between variations in the ESR1 gene and risk of stroke, neither in 3718 women nor in 2511 men.

Table 1. Baseline Characteristics of the Study Population (n=6229)*

<table>
<thead>
<tr>
<th></th>
<th>Women (N=3718)</th>
<th>Men (N=2511)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>69.1 (62.2–76.7)</td>
<td>66.7 (61.6–73.0)</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>138 (123–154)</td>
<td>136 (123–152)</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Ever smoking, %</td>
<td>46</td>
<td>92</td>
</tr>
<tr>
<td>Antihypertensive drug use, %</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Previous myocardial infarction, %</td>
<td>9</td>
<td>18</td>
</tr>
</tbody>
</table>

|                        | Values are median (25th and 75th percentile) if appropriate. |

Table 2. ESR1 Haplotypes and Risk of Stroke and Ischemic Stroke (Dominant Model)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>N</th>
<th>Strokes (n=388)</th>
<th>Ischemic Strokes (n=211)</th>
<th>N</th>
<th>Strokes (n=271)</th>
<th>Ischemic Strokes (n=175)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplotype 1 (−397T/−351A)</td>
<td>No</td>
<td>811</td>
<td>1 (ref)</td>
<td>547</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Yes (model 1)*</td>
<td>2907</td>
<td>0.98 (0.77–1.25)</td>
<td>0.95 (0.69–1.31)</td>
<td>1964</td>
<td>1.22 (0.90–1.66)</td>
<td>1.10 (0.94–2.09)</td>
</tr>
<tr>
<td>Haplotype 2 (−397C/−351G)</td>
<td>No</td>
<td>1598</td>
<td>1 (ref)</td>
<td>1055</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Yes (model 1)*</td>
<td>2120</td>
<td>0.94 (0.77–1.15)</td>
<td>0.89 (0.68–1.16)</td>
<td>1456</td>
<td>0.95 (0.75–1.21)</td>
<td>0.89 (0.66–1.21)</td>
</tr>
<tr>
<td>Yes (model 2)*</td>
<td>2120</td>
<td>0.95 (0.78–1.16)</td>
<td>0.90 (0.69–1.19)</td>
<td>1456</td>
<td>0.95 (0.75–1.21)</td>
<td>0.89 (0.66–1.21)</td>
</tr>
<tr>
<td>Haplotype 3 (−397C/−351A)</td>
<td>No</td>
<td>2903</td>
<td>1 (ref)</td>
<td>1932</td>
<td>1 (ref)</td>
<td>1 (ref)</td>
</tr>
<tr>
<td>Yes (model 1)*</td>
<td>815</td>
<td>1.03 (0.81–1.31)</td>
<td>0.89 (0.63–1.24)</td>
<td>124</td>
<td>1.04 (0.94–1.62)</td>
<td>0.93 (0.65–1.34)</td>
</tr>
<tr>
<td>Yes (model 2)*</td>
<td>815</td>
<td>1.05 (0.83–1.33)</td>
<td>0.90 (0.64–1.26)</td>
<td>579</td>
<td>1.22 (0.93–1.60)</td>
<td>0.94 (0.66–1.35)</td>
</tr>
</tbody>
</table>

*Model 1, adjusted for age; model 2, adjusted for age, systolic blood pressure, antihypertensive drug use, current smoking, former smoking, diabetes mellitus, serum C-reactive protein, previous myocardial infarction, atrial fibrillation, and waist-to-hip ratio. ref indicates reference category.
Conclusion
In conclusion, we have not been able to replicate the previously reported association between variations in the \textit{ESR} gene and risk of stroke.

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Disclosures
None.

References
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