Estrogen Receptor α Gene Variation Is Associated With Risk of Myocardial Infarction in More Than Seven Thousand Men From Five Cohorts


Understanding the mechanisms by which estrogens affect cardiovascular disease risk, including the role of variation in the gene for estrogen receptor α (ESR1), may be key to new treatment strategies. We investigated whether the CC genotype at ESR1 c.454-397T>C is associated with increased risk among men. Study of more than 7000 whites in 5 cohorts from 4 countries provided evidence that genotype CC, present in roughly 20% of individuals, is a risk factor for nonfatal acute myocardial infarction (odds ratio = 1.44; \( P < 0.0001 \)), after adjustment for established cardiovascular risk factors. After exclusion of younger subjects from 2 cohorts, because of age interaction, the odds ratio increased (to 1.63).

In the Rotterdam Study, an estrogen receptor α (ESR1) haplotype, including the ESR1 c.454-397 T allele, also referred to in previous studies as the PvuII site in intron 1 (rs2234693), was associated with significantly increased risk of myocardial infarction (MI) among postmenopausal women (for homozygous carriers, the relative risk was 2.48; the 95% confidence interval [CI], 1.22 to 5.03 after adjusting for known cardiovascular risk factors). In men, however, no statistically significant association was found (relative risk, 0.82; 95% CI, 0.49 to 1.38). These results may be viewed as at odds with previous reports by us and others of a significantly higher risk of MI among men with the c.454-397CC genotype (PP of PvuII).2,3 We aimed to clarify whether the CC genotype at ESR1 c.454-397T>C is associated with increased odds of nonfatal MI among men.

Materials and Methods

We included only cohorts of white men not recruited on the basis of coronary heart disease risk factors (Table 1 in the online data supplement, available at http://circres.ahajournals.org). We studied men from the prospective population-based Second Northwick Park Heart Study (NPHSII) in the United Kingdom4 and 2 case-control studies of MI from Poland and the United States, selected from the Global Repository at Genomics Collaborative.5 Here we present a metaanalysis of the results of these studies and published results from the Framingham6 and Rotterdam Studies7: a total of more than 7000 men with detailed covariate information, including 731 men with acute, nonfatal MI. We also carried out analyses of total MI, including 47 fatal MIs in NPHSII and the Rotterdam Study, and ischemic heart disease (IHD) in the 3 prospective studies. Unless specified, odds ratios (ORs) are from a fixed effects model, adjusted for age, body mass index, serum total cholesterol level, hypertension, diabetes, and smoking status.

Results

The mean (SD) values for age (supplemental Table I), body mass index, and total cholesterol level, as well as the prevalence of current smoking, hypertension, and diabetes were not significantly different by genotype. Comparing men with the ESR1 c.454-397CC genotype with those with CT or TT genotypes, pooled OR=1.44 (95% CI, 1.17 to 1.76; \( P < 0.0001 \)) for MI (Figure). Power was low for detection of an association with fatal MI, with 20% power to detect an effect of the size seen in the nonfatal MIs. Of 47 fatal MIs, only 4 (8.5%) had an ESR1 c.454-397CC genotype. When fatal MIs were included in the Rotterdam and NPHSII analyses, the ORs for total MI were 1.11 (95% CI, 0.72 to 1.71) and 1.23 (95% CI, 0.83 to 1.82), respectively, with the pooled OR 1.31 (95% CI, 1.07 to 1.59; \( P = 0.008 \)). After exclusion of total MI, the combined IHD result from the 3 prospective studies was not significant, pooled OR=0.90

Original received November 15, 2004; first resubmission received January 3, 2005; revised first resubmission received February 7, 2005; second resubmission received November 15, 2005; revised second resubmission received December 15, 2005; accepted February 3, 2006.

From the Center for Cancer Research (A.M.S., D.E.H.), Massachusetts Institute of Technology, Cambridge, Mass; Center for Cardiovascular Genetics (J.A.C., P.J.K., S.E.H.), Department of Medicine, Royal Free and University College London Medical School, Rayne Institute, United Kingdom; Portex Anaesthesia, Intensive Therapy and Respiratory Medicine Unit (P.J.K.), Institute of Child Health, London, United Kingdom; Medical Research Council Cardiovascular Research Group (G.J.M.), Wolfson Institute of Preventive Medicine, London, United Kingdom; Genomics Collaborative (K.G.A., B.J., K.I., K.L.L.), Cambridge, Mass; Departments of Internal Medicine and Epidemiology & Biostatistics (S.C.E.S., A.G.U., H.A.P.P.), Erasmus Medical Center, Rotterdam, The Netherlands; Department of Biostatistics, Boston University School of Public Health (S.D., L.A.C.), Boston, Mass; Tufts–New England Medical Center (M.E.M.), Boston, Mass; and the Framingham Heart Study of the National Heart, Lung, and Blood Institute (D.L.), Framingham, Mass.

Sera Care Life Science employed K.G.A., K.I., B.J., and K.L.L. and donated the DNA samples and data from the case control studies free of charge. M.E.M. had minor speakers bureau appointments at Wyeth and Merck and honoraria for academic talks. D.E.H., D.L., M.E.M., and A.M.S. were named on a patent application filed through the Massachusetts Institute of Technology technology licensing office for medical uses related to estrogen receptor gene variation.

Correspondence to Dr Amanda M. Shearman, E17-536, Massachusetts Institute of Technology, Cambridge, MA 02139. E-mail shearman@mit.edu (Circ Res. 2006;98:590-592.)

© 2006 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/01.RES.0000210578.62102.a6
Sixty-eight percent of the weight of our model for nonfatal MI is derived from previously unstudied cohorts, making it unlikely that our results have been affected by publication bias. Our findings also show consistency across 5 white cohorts from 4 countries, providing additional evidence that the association is real and not attributable to population stratification or a context dependent factor. There was wide variability in participant age across the 5 studies, and we obtained evidence that the association we report may be affected by subject age. That a time window of maximum effect exists for this ESR1 genotype (which may itself vary with characteristics such as ethnicity, diet, estrogen levels, and established risk factors for MI) would be compatible with previous results and recent negative findings from an MI case-control study of men with mean age in the early sixties (SD 12 years), and should be tested in other populations including studies of hormone replacement therapy. Fatality for MI may potentially be affected by ESR1 variation and the 2 case-control studies recruited only survivors of MI. Therefore, only nonfatal cardiovascular events were initially included in this metaanalysis. Secondary analyses including 47 fatal MIs provided results that were significant; however, only 8.5% of subjects with a fatal MI had an ESR1 c.454-397T>C genotype. Although not statistically significant, there was a trend toward decreased risk of fatal MI in subjects with ESR1 c.454-397CC genotype, in both studies that included such variation and the older or younger subgroup of each cohort gave OR=1.58 (95% CI 1.19 to 2.10; P=0.002) and 1.22 (95% CI 0.92 to 1.62; P=0.17), respectively. Justified by these findings, metaanalysis excluding the younger case-control subgroups gave OR=1.63 (95% CI 1.29 to 2.07; P<0.0001).

Discussion

Sixty-eight percent of the weight of our model for nonfatal MI is derived from previously unstudied cohorts, making it unlikely that our results have been affected by publication bias. Our findings also show consistency across 5 white cohorts from 4 countries, providing additional evidence that the association is real and not attributable to population stratification or a context dependent factor. There was wide variability in participant age across the 5 studies, and we obtained evidence that the association we report may be affected by subject age. That a time window of maximum effect exists for this ESR1 genotype (which may itself vary with characteristics such as ethnicity, diet, estrogen levels, and established risk factors for MI) would be compatible with previous results and recent negative findings from an MI case-control study of men with mean age in the early sixties (SD 12 years), and should be tested in other populations including studies of hormone replacement therapy. Fatality for MI may potentially be affected by ESR1 variation and the 2 case-control studies recruited only survivors of MI. Therefore, only nonfatal cardiovascular events were initially included in this metaanalysis. Secondary analyses including 47 fatal MIs provided results that were significant; however, only 8.5% of subjects with a fatal MI had an ESR1 c.454-397T>C genotype. Although not statistically significant, there was a trend toward decreased risk of fatal MI in subjects with ESR1 c.454-397CC genotype, in both studies that included such events. Although this initially appears to be at odds with the findings for nonfatal MIs, results from women in the Rotterdam Study included higher mortality in the year after IHD among women without ESR1 c.454-397CC genotype. This is consistent with the highest OR being from the Framingham Study, where survival for blood sampling at the sixth examination cycle, almost 3 decades after the start of the study, was required for inclusion and might, in part, underlie the results we observe for nonfatal MIs. A genetic basis for increased risk of fatality among older subjects might be a reason for the observed interaction with age. Further studies
of fatal MI are required to resolve whether the impact of the c.454-397CC genotype on nonfatal events differs from that on fatal events.1–3

Estrogen receptors are required for normal vascular physiology in males,8 although the underlying mechanisms are not clear. Several studies suggest that c.454-397C is within a potential transcription factor binding site and results in a relatively high level of ESR1 transcription as compared with c.454-397T.1–10 A study of postmenopausal women reported that c.454-397CC genotype was associated with more severe atherosclerosis at baseline than in other genotypes but progressed more slowly in response to hormone-replacement therapy.11 The alleles of a polymorphic TA repeat, in the ESR1 promoter, in strong linkage disequilibrium with c.454-397C, have been associated with more severely narrowed coronary arteries, larger areas of complicated lesions, and lower adenosine-stimulated myocardial blood flow.12–14 A more comprehensive evaluation of ESR1 is required to determine the mechanism(s) underlying these observed associations.

Our results add substantially to recent evidence that a mechanism based on ESR1 variation contributes to a range of important estrogen-dependent characteristics, including responses of the lipid profile to hormone replacement therapy and risk of fracture.15,16

For nonfatal MI events in the populations included in this study, the population risk attributable to the ESR1 c.454-397CC genotype was 6.4%, which would correspond among US men17 to approximately 25 000 of such events each year and incur substantial hospital and medical expenses.

Acknowledgments
Support was from the National Heart, Lung, and Blood Institute (Tufts–New England Medical Center Specialized Center of Research in Ischemic Heart Disease, P30-HL63494; the Framingham Heart Study contract N01-HC-25195; RO1-HL65230; HL-33014; and HL-54502), the Netherlands Organization for Scientific Research under the Research Institute for Diseases in the Elderly (grant 014-90-001), the Dutch Research Organization NWO (RIDE grant 14-93-015), the European Commission (“GENOMOS”; QLK6-CT-2002-02629), the UK Medical Research Council, the British Heart Foundation (RG 2000/015), and Du Pont Pharma, Wilmington, Del. The National Heart, Lung, and Blood Institute reviewed the manuscript for scientific content and consistency of data interpretation with previous Framingham publications. We are indebted to all those who participated in the studies and thank the anonymous reviewers for helpful suggestions.

References

Key Words: genetics  myocardial infarction  estrogen receptor  risk factors
Estrogen Receptor α Gene Variation Is Associated With Risk of Myocardial Infarction in More Than Seven Thousand Men From Five Cohorts


Circ Res. 2006;98:590-592; originally published online February 16, 2006;
doi: 10.1161/01.RES.0000210578.62102.a6
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/98/5/590

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2006/02/16/01.RES.0000210578.62102.a6.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Research is online at:
http://circres.ahajournals.org/subscriptions/
Methods

We studied 2,709 men (with available DNA samples) from the prospective population-based Second Northwick Park Heart Study.

All subjects gave informed consent and the examination protocols were approved by the relevant Institutional Review Boards. These three previously unpublished studies used the Taqman 5' nuclease assay (Applied Biosystems) or other standard genotyping methods. Negative and positive controls, and random duplicate DNAs were included for quality control.

Fatal and silent events were excluded from our primary analysis because the two case-control studies included only survivors of clinical events. Our risk analyses exclude 441 prior MIs in the Rotterdam Study, ascertained at baseline ECG or by patient report. Meta-analysis was performed in Stata 8.2 (Stata Corporation, Texas) using the user-written command 'meta'. This uses inverse-variance weighting to calculate fixed and random-effects summary estimates.

We also carried out analysis of ischemic heart disease (IHD) in the three prospective studies, defined in NPHSII and Rotterdam using International Classification of Disease definitions: acute MI, IHD death, or revascularization procedures (percutaneous transluminal coronary angioplasty or coronary artery bypass surgery). In Framingham IHD was defined as recognized MI, coronary heart disease death, angina pectoris, or coronary insufficiency (revascularization procedure data were not available).
Results

The mean (SD) values for age (Table 1), BMI [26.4 (3.4) kg/m$^2$], and total cholesterol level [5.89 (1.08) mmol/L], as well as the prevalence of current smoking (2458/7512, 32.7%), hypertension (3063/7514, 40.8%), and diabetes (352/7517, 4.7%) were not significantly different by genotype.

In the three prospective studies, the combined total IHD result was a pooled OR of 1.26 (95% CI: 1.02-1.57), P=0.04 (Framingham OR=1.72 [95%CI:1.03-2.86]; Rotterdam OR=1.21 [95% CI:0.85-1.71]; Northwick Park OR=1.15 [95% CI:0.81-1.61]). After exclusion of total MI the combined IHD result was not significant, with a pooled OR of 0.90 (95% CI: 0.62-1.32), P=0.62 (Framingham OR= 0.60 [95%CI: 0.33-1.10]; Rotterdam OR=1.25 [95% CI:0.64-2.30]; Northwick Park OR=1.05 [95% CI: 0.54-2.18]). Limiting the exclusion to non-fatal MI gave similar results with a pooled OR of 0.96 (95% CI: 0.73-1.24), P=0.73 (Framingham OR= 0.60 [95%CI: 0.33-1.10]; Rotterdam OR=1.21 [95% CI:0.67-2.18]; Northwick Park OR=1.02 [95% CI: 0.68 -1.52]).

Using the fixed effects OR as the estimate of relative risk (RR), the attributable risk (AR) of the CC genotype for non-fatal MI was (RR-1)/RR=0.306. The CC genotype frequency from the three prospective studies was 1181/5756=0.21. Thus, population AR (AR × prevalence of exposure to risk in the population) was 0.306×0.21= 0.06 (6.4%).

References
## ONLINE TABLE 1. Characteristics, ESR1 c.454-397T>C genotype and allele frequencies in men, by study and disease status

<table>
<thead>
<tr>
<th>Study design</th>
<th>Study</th>
<th>Mean age (SD)</th>
<th>Genotype frequency, No. (%)</th>
<th>C Allele Freq, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>TT</td>
</tr>
<tr>
<td>Prospective</td>
<td>Framingham Heart Study, USA</td>
<td>Sample No.</td>
<td>(%) of total</td>
<td></td>
</tr>
<tr>
<td>cohort study</td>
<td>Total</td>
<td>875</td>
<td>36 (10) 59 (10)</td>
<td>273 (32)</td>
</tr>
<tr>
<td></td>
<td>IHD cases, Non-fatal</td>
<td>100 (11)</td>
<td>24 (24) 50 (50)</td>
<td>26 (26)</td>
</tr>
<tr>
<td></td>
<td>MI cases, Non-fatal</td>
<td>54 (6)</td>
<td>13 (24) 21 (39)</td>
<td>20 (37)</td>
</tr>
<tr>
<td>&quot;</td>
<td>Rotterdam Study, the Netherlands</td>
<td>Total</td>
<td>2172</td>
<td>68 (8) 75 (8)</td>
</tr>
<tr>
<td></td>
<td>IHD cases, Fatal</td>
<td>29 (1)</td>
<td>6 (21) 17 (59)</td>
<td>6 (21)</td>
</tr>
<tr>
<td></td>
<td>Non-fatal</td>
<td>153 (6)</td>
<td>49 (32) 68 (44)</td>
<td>36 (24)</td>
</tr>
<tr>
<td></td>
<td>MI cases, Fatal</td>
<td>11 (0.4)</td>
<td>1 (9) 10 (91)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td>Non-fatal</td>
<td>110 (4)</td>
<td>30 (27) 53 (48)</td>
<td>27 (25)</td>
</tr>
<tr>
<td>&quot;</td>
<td>Northwick Park Heart Study,* UK</td>
<td>Total</td>
<td>2709</td>
<td>56 (3) 67 (4)</td>
</tr>
<tr>
<td></td>
<td>IHD cases, Fatal</td>
<td>36 (1)</td>
<td>9 (25) 23 (64)</td>
<td>4 (11)</td>
</tr>
<tr>
<td></td>
<td>Non-fatal</td>
<td>182 (7)</td>
<td>42 (23) 96 (53)</td>
<td>44 (24)</td>
</tr>
<tr>
<td></td>
<td>MI cases, Fatal</td>
<td>36 (1)</td>
<td>9 (25) 23 (64)</td>
<td>4 (11)</td>
</tr>
<tr>
<td></td>
<td>Non-fatal</td>
<td>106 (4)</td>
<td>28 (26) 47 (44)</td>
<td>31 (29)</td>
</tr>
<tr>
<td>MI cases/</td>
<td>GCI-USA</td>
<td>Controls</td>
<td>414</td>
<td>64 (11)</td>
</tr>
<tr>
<td>matched healthy controls</td>
<td></td>
<td>MI cases, Non-fatal</td>
<td>226 (35)</td>
<td>125 (30)</td>
</tr>
<tr>
<td>&quot;</td>
<td>GCI-Poland</td>
<td>Controls</td>
<td>441</td>
<td>58 (10)</td>
</tr>
<tr>
<td></td>
<td>MI cases, Non-fatal</td>
<td>235 (35)</td>
<td>141 (32) 209 (47)</td>
<td>91 (21)</td>
</tr>
</tbody>
</table>

We observed no significant deviation from expectations of Hardy-Weinberg Equilibrium

*Exclusion criteria included previous cardiovascular disease, ASA or anticoagulants, and malignant disease
Online Table 1. In the prospective studies mean age (SD), years for Baseline to Follow-up examinations was 36 (10) to 59 (10) for Framingham, 68 (8) to 75 (8) for Rotterdam, 56 (3) to 67 (4) for the Northwick Park Studies; in the case control studies mean age (SD) were 64 (11) for GCI-USA, and 58 (10) for GCI-Poland.