Estrogen Receptors Alpha and Beta and the Risk of Open-angle Glaucoma

The Rotterdam Study

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Objective: To investigate whether polymorphisms in the estrogen receptor alpha (ESR1) and beta (ESR2) genes were a risk factor for open-angle glaucoma (OAG).

Methods: Participants 55 years and older from the population-based Rotterdam Study underwent, at baseline and at follow-up, the same ophthalmic examination, including visual field screening and stereo optic disc photography. A diagnosis of OAG was based on an algorithm using optic disc measures and visual field loss. Haplotypes of the ESR1 and ESR2 genes were determined.

Results: We diagnosed incident OAG in 87 of 3842 participants (2.3%) at risk after a mean follow-up of 6.5 years. We could not detect any association with ESR1 haplotypes. Haplotype 1 of ESR2 showed a 3.6-fold (95% confidence interval, 1.4-9.2) higher risk of incident OAG in men. In women, no association was found between ESR2 and incident OAG.

Conclusion: Polymorphisms in the ESR1 gene are unrelated to OAG, but ESR2 polymorphisms seem to lead to increased risk of OAG in men.

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Primary open-angle glaucoma (OAG) may be described as a retinal ganglion cell disorder characterized by cupping of the optic disc as a result of loss of nerve fibers, so-called glaucomatous optic neuropathy (GON). In a later stage, glaucomatous visual field loss (GVFL) develops. Owing to aging populations, the burden of OAG on society is predicted to increase. Little is known about the pathogenesis and etiology of OAG, and several studies have shown that genetics has a role. Seven chromosomal loci for OAG and associations with other genes have been identified. Through formation of heterodimers, ESR2 is thought to be able to inhibit the transcription activation of ESR1. As a result, specific sets of estrogen-dependent genes could be activated in different tissues, depending on the presence or absence of one or both receptors. We tested the nuclear receptor hormone superfamily. It is possibly that a third, membrane-associated ER is involved. Estrogens diffuse into the cell nucleus and bind to the receptor to form an estrogen-ER complex. This estrogen-ER complex can subsequently bind to an estrogen response element on a gene to activate its transcription. Both nuclear receptors have been located in several tissues of the eye, including the retinal ganglion cell layer. Single-nucleotide polymorphisms (SNPs), subtle but common changes in the DNA sequence of the genes encoding the 2 ERs (ESR1 and ESR2), can lead to modified activity or structure of the ER protein, resulting in different responsiveness to circulating estrogens. The specific functions of the ESR1 and ESR2 genes are under study, and distinct, perhaps even opposing, effects have been described. Through formation of heterodimers, ESR2 is thought to be able to inhibit the transcription activation of ESR1. As a result, specific sets of estrogen-dependent genes could be activated in different tissues, depending on the presence or absence of one or both receptors. We tested

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the hypothesis that certain SNPs in the ESR1 and ESR2 genes led to higher risk of OAG in a general elderly population based on the presumption that changes in the structure or function of the ERs are likely to alter the response to estrogens.

METHODS

STUDY DESIGN

This study was performed within the Rotterdam Study, a prospective, population-based cohort study of residents 55 years or older living in a district of Rotterdam, the Netherlands. Home interviews and examinations at the research center were conducted after the medical ethics committee of Erasmus University had approved the study protocol, and all participants gave written informed consent according to the Declaration of Helsinki. After the baseline examination (January 1, 1990, to October 1, 1993) for prevalent OAG, a follow-up examination to study incident OAG was performed between March 1, 1997, and December 31, 1999.

ASSESSMENT OF OAG

The procedure for the assessment of OAG included supra-threshold visual field screening followed by ophthalmoscopy and stereoscopic fundus photography after pharmacologic pupillary dilation of each eye. Similar procedures were performed at baseline and at follow-up.

For GON evaluation, simultaneous stereo color transparencies were digitized and analyzed with a semiautomatic image analyzer (ImageNet; Topcon Optical Company, Tokyo, Japan). If the transparencies were missing or of bad quality, ophthalmoscopic estimates were used. The GON cutoff points were determined by the 97.5 and 99.5 percentiles in this population. Possible GON was defined as a vertical cup-disc ratio of 0.7 or higher, asymmetry of the vertical cup-disc ratio between the eyes of 0.2 or more, or minimum rim width less than 0.1, and probable GON as vertical cup-disc ratio of 0.8 or higher, asymmetry between the eyes of 0.3 or more, or minimum rim width less than 0.05. Visual fields were screened with automated suprathreshold perimetry, and defects on repeated screening were checked with Goldmann perimeter. Glaucomatous visual field loss was defined as visual field loss compatible with OAG (thus, excluding hemianopia, quadrantanopia, or isolated central defects) and not explained by other neuro-ophtalmic causes.

The diagnosis of OAG was based on an algorithm using GON and GVFL, independent of intraocular pressure, and could only be made in participants who had an open anterior chamber angle in 1 eye and no history or sign of angle closure or secondary glaucoma in that eye. Definite OAG was defined as the presence of possible or probable GON plus GVFL; probable OAG as probable GON without GVFL; or presence of GON without GVFL; and incident OAG as no OAG in either eye at baseline and probable or definite OAG in at least 1 eye at follow-up. We excluded from the incident OAG group participants with, as the only change, possible GON at baseline and probable GON at follow-up because a tiny increase in one of the GON criteria could lead to a change in this classification. This exclusion was made primarily because we wanted to be as confident as possible for the risk analyses that we analyzed only cases with true incident OAG. We prefer the term OAG rather than primary OAG because at baseline we did not specifically exclude pseudoxofoliation OAG in all participants. This, however, was never found at additional examinations at baseline or follow-up.

GENOTYPING

The ESR1 gene is located on chromosome 6q25, and the ESR2 gene on chromosome 14q22-24. Two well-known SNPs of the ESR1 gene are PvuII (rs2234693), in intron 1, located 397 base pairs upstream of exon 2, and XbaI (rs7940799), in intron 1, located 351 base pairs upstream of exon 2. Polymorphisms of the ESR2 gene have been studied less extensively. On the basis of their allele frequencies and linkage disequilibrium analysis, the most interesting SNPs seem to be rs1256031, in intron 2, located 10 550 base pairs upstream from the start of exon 3, and rs1060039, located 38 base pairs downstream from the 3' untranslated region. To our knowledge, specific studies of SNPs of ERs and OAG have not been published previously; thus, the selection of the SNPs was based on their availability within the Rotterdam Study and our experience with them in other research areas.

Genotypes of the ESR1 and ESR2 SNPs were determined using the TaqMan allelic discrimination assay (Applied Biosystems Inc [ABI], Nieuwkerk aan den IJssel, the Netherlands). Primer and probe sequences were optimized using the SNP assay-by-design service of ABI; details are available at http://store.appliedbiosystems.com. Reactions were performed with the TaqMan PRISM 7900HT Sequence Detection System (ABI), with 384 wells. We used the genotype data for each of the 2 SNPs of ESR1 and ESR2 to infer frequency of the haplotype alleles present in the population using the PHASE program. For ESR1, the alleles were defined as haplotypes such as “T-A,” representing a thymidine (T) nucleotide for the PvuII SNP and an adenosine (A) nucleotide for the XbaI SNP. We coded ESR2 haplotype alleles with numbers 1 through 4 in order of decreasing frequency in the population (1=T-A, 2=C-G, 3=C-A, and 4=T-G). For ESR2, the haplotypes were constructed for the combination of intron 2 SNP plus 3' untranslated region SNP. In order of decreasing frequency, the following haplotypes could be coded: 1=C-C, 2=T-T, 3=T-C, and 4=C-T.

DATA ANALYSIS

At baseline, 6780 participants (78% of those eligible) underwent an ophthalmologic examination. After excluding 221 persons with prevalent definite or probable OAG and 7 without data for both perimetry and optic disc measurements, 6552 participants formed the cohort at risk for incident OAG.

Data for haplotypes of ESR1 were available in 6008 persons, and for ESR2 in 5826. Analyses of ESR1 and ESR2 are only presented for their haplotype 1 because these were previously reported as risk haplotypes. Analyses of haplotypes 2, 3, and 4 were performed; the results cannot be seen as independent analyses because homozygous carriers of a certain haplotype are among the control subjects in the analyses of the other haplotypes. The genetic (Hardy-Weinberg) equilibrium was calculated using Pearson χ² analysis. We used univariate analyses of covariance to compare baseline characteristics of participants and nonparticipants at the follow-up examination, adjusted for age and sex, when applicable. Differences in the distribution of the ER haplotypes were evaluated with Kruskal-Wallis tests. Logistic regression analyses were used to calculate odds ratios with corresponding 95% confidence intervals, which can be interpreted as relative risk. We tested statistical significance for trends in increasing exposure by adding categorical determinants continuously in the model. Because estrogens and ERs are thought to have different effects in men and women, we stratified the analyses by sex, adjusted for age and follow-up time. Analyses were additionally adjusted for the following possible confounders: mean perfusion pressure (calculated as ⅓ times diastolic blood pressure plus ⅓ times systolic blood pressure minus intraocular...
At baseline, the frequencies of the 4 possible ESR1 haplotype alleles were as follows: 1, 53.3%; 2, 34.8%; 3, 11.9%; and 4, 0%. The frequencies of the 4 ESR2 haplotypes were as follows: 1, 45.1%; 2, 37.0%; 3, 17.2%; and 4, 0.7%. At follow-up, the frequency distributions were similar. The genotype distributions were in the genetic equilibrium. After a mean follow-up of 6.5 years (range, 5.0-9.4 years), 1244 participants had died and 1466 declined or were unable to participate in the follow-up examination, leaving 3842 persons (72% participation rate) at risk of incident OAG (Table 1). Most variables differed significantly between participants and those who declined the follow-up examination or died. This did not hold for the distribution of the ESR1 and ESR2 haplotypes and most of the OAG-related variables. We detected 87 incident OAG cases (2.3%) at follow-up.

Table 2 gives associations between ESR1 haplotype 1, ESR2 haplotype 1, and incident OAG. We did not find any association for ESR1 haplotype 1 and incident OAG. There was an allele dose-dependent increased risk in men carrying ESR2 haplotype 1 (P = .007), but not in women. Analyses of ESR2 haplotype 2 revealed a significant allele dose-dependent inverse relationship (P = .001) with incident OAG, again only in men (data not shown). Additional adjustment for other possible confounders had little influence on the relative risk estimates.

Table 3 is similar to Table 2 except that 25 persons with prevalent definite OAG newly diagnosed at baseline were added to the model. This led to a lower risk of OAG associated with ESR2 haplotype 1 in men. Both intraocular pressure-lowering treatment and perfusion pressure were added to the models. We also performed 2 analyses with only 1 of these confounders in the models, to prevent overadjustment. This resulted in only slight and nonsignificant differences.

To detect bias from dropout, we compared the prevalence of probable or definite OAG at baseline in persons who participated in the follow-up vs those who did not participate in the follow-up. Adjusted for age and sex, there was no difference between those who died (3.7%), declined the follow-up examination (3.4%), or participated in follow-up (3.1%).

In this ethnically homogeneous population, we found a higher risk for incident OAG with ESR2 haplotype 1 in men but not in women. The inverse association with ESR2 haplotype 2 and incident OAG supports the presence of an association between ESR2 SNPs and OAG in men. We could not demonstrate any differences in risk of OAG in relation to ESR1 genotype.

In theory, genotypes do not change over a lifetime; therefore, analyses between ER SNPs and prevalent OAG should yield the same results. We found at baseline no association for either ESR1 or ESR2. One explanation for this result is the presence of a prevalence-incidence bias. This bias means that one tends to underestimate the number of cases in cross-sectional studies when the determinant of the disease under study predisposes to shorter survival in patients with the disease. In the present study, this would imply that men with OAG carrying ESR2 haplotype 1 would have died earlier than similar men without this haplotype. After calculating the mortality risk in persons with prevalent OAG, we found that men with OAG carrying ESR2 haplotype 1 would have died earlier than similar men without this haplotype. Because calculating the mortality risk in persons with prevalent OAG, we found that men with OAG carrying ESR2 haplotype 1 (n = 8) were at 4.8-fold (95% confidence interval, 1.6-14.5, fully adjusted model) higher mortality risk compared with men with OAG who did not carry this haplotype (n = 14). When we combined persons with newly diagnosed prevalent OAG, assuming they had not changed their lifestyle yet, with those with newly detected incident OAG, the estimates decreased but still showed an increased risk of 2.2 (95% confidence interval, 1.0-4.9) in male carriers of ESR2 haplotype 1.

Different effects of ESR2 polymorphisms in men and women were also found in studies on high blood pressure, low bone mineral density, and Alzheimer disease. This strengthens the hypothesis that some effects of ESR2 are sex-specific.

### Table 1. Baseline Characteristics of the Study Population at Risk for Incident Open-Angle Glaucoma

<table>
<thead>
<tr>
<th>Status at Follow-Up</th>
<th>Participated (n=3842)</th>
<th>Declined or Unable to Participate (n=1466)</th>
<th>Died (n=1244)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.7±6.9</td>
<td>71.2±8.7a</td>
<td>77.4±9.1b</td>
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<tr>
<td>Female sex</td>
<td>57.8</td>
<td>68.6b</td>
<td>54.3c</td>
</tr>
<tr>
<td>ESR1 haplotype 1 carrier</td>
<td>53.1</td>
<td>53.1</td>
<td>54.0</td>
</tr>
<tr>
<td>ESR2 haplotype 1 carrier</td>
<td>44.3</td>
<td>46.1</td>
<td>46.8</td>
</tr>
<tr>
<td>Vertical cup-disc ratio</td>
<td>0.53±0.13</td>
<td>0.50±0.15b</td>
<td>0.48±0.17b</td>
</tr>
<tr>
<td>Intraocular pressure, mm Hg</td>
<td>15.1±3.1</td>
<td>15.2±3.3</td>
<td>14.8±3.4</td>
</tr>
<tr>
<td>Intracellular pressure-lowering treatment</td>
<td>1.6</td>
<td>2.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Body mass index</td>
<td>26.3±3.5</td>
<td>26.7±4.0b</td>
<td>25.8±3.9b</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6.9</td>
<td>10.6b</td>
<td>20.9b</td>
</tr>
<tr>
<td>Systemic hypertension</td>
<td>28.9</td>
<td>38.4b</td>
<td>45.5b</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>258.7±46.3</td>
<td>258.7±46.3</td>
<td>243.2±50.2b</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>54.0±15.4</td>
<td>54.0±15.4</td>
<td>50.19±15.4b</td>
</tr>
<tr>
<td>History of stroke</td>
<td>1.3</td>
<td>3.5b</td>
<td>7.2b</td>
</tr>
<tr>
<td>Dementia</td>
<td>0.2</td>
<td>3.4</td>
<td>15.2b</td>
</tr>
</tbody>
</table>

Abbreviations: ESR1 and ESR2, estrogen receptors alpha and beta, respectively; HDL, high-density lipoprotein. a SI conversion factor: To convert total and HDL cholesterol to millimoles per liter, multiply by 0.0259. b Data are given as mean±SD for continuous variables and percentages for dichotomous variables unless otherwise indicated. c Significant (P<.05) compared with participants, adjusted for age and sex if applicable. d Measured as maximum vertical cup-disc ratio for both eyes. e Only given for persons without intraocular pressure-lowering treatment. f Measured as maximum intraocular pressure in either eye. g Calculated as weight in kilograms divided by height in meters squared.

### RESULTS

In this ethnically homogeneous population, we found a higher risk for incident OAG with ESR2 haplotype 1 in men but not in women. The inverse association with ESR2 haplotype 2 and incident OAG supports the presence of an association between ESR2 SNPs and OAG in men. We could not demonstrate any differences in risk of OAG in relation to ESR1 genotype.

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One limitation of our study could be the relatively large group of persons who were unavailable for follow-up. The large number of deaths that occurred in this elderly cohort during follow-up can partially explain this. If persons who declined participation or died between baseline and follow-up developed OAG more often than those who participated, this would have biased the results toward the null value. At baseline, the determinants were similar in those who died, those who participated in follow-up, and those who declined follow-up (Table 1). Persons with OAG were not at increased risk of death, except for a possible subgroup of men with ESR2 haplotype 1, making bias from selective death at follow-up less likely. We still found an association despite a possible bias toward the null value. Although the differences in age probably resulted in fewer incident cases, leading to larger confidence intervals in this study, because the prevalence of OAG is higher at older ages, we are confident that loss to follow-up had a limited role.

In conclusion, we found an association with ESR2 polymorphisms and OAG in men. We could not detect any associations with ESR1 or ESR2 in women. The exact mechanism of why there is a sex difference in OAG remains to be elucidated.
Role of the Sponsor: The role of the funding organizations and companies was limited to approval of the study design. The funders had no role in the conduct of the study; collection, management, analysis, and interpretation of the data; or preparation, review, or approval of the manuscript.

Additional Contributions: Pascal Arp, BSc, performed genotyping of ESR1 and ESR2 SNPs.