The Role of Apolipoprotein E Gene Polymorphisms in Primary Open-angle Glaucoma

Thomas Ressiniotis, MRCOphth; Philip G. Griffiths, FRCOphth; Michael Birch, FRCOphth; Sharon Keers; Patrick F. Chinnery, PhD, MRCP

Objective: To test the hypothesis that genetic polymorphisms of the apolipoprotein E (APOE) gene are associated with primary open-angle glaucoma (POAG), based on the association between neurodegenerative diseases and the APOE genotype.

Methods: Genomic DNA was examined from an unrelated cohort of 137 POAG patients and 75 control subjects from the ophthalmology department of the Royal Victoria Infirmary. The APOE allele frequency (e2, e3, and e4 alleles) was studied by polymerase chain reaction amplification of the related locus (19q13.2), enzymatic digestion of the products, gel electrophoresis, and imaging under UV illumination. For statistical analysis, we used a logistic regression model that included intraocular pressure as a continuous variable to study the possible correlation between POAG and APOE allele frequency.

Results: Logistic regression analysis showed no statistically significant association between the frequency of the APOE allele and POAG for the population studied, irrespective of the IOP (e2 odds ratio, 0.82; 95% confidence interval, 0.12-5.79 [P = .84]; e3 odds ratio, 0.39; 95% confidence interval, 0.10-1.49 [P = .17]; and e4 odds ratio, 3.84; 95% confidence interval, 0.80-18.49 [P = .09]).

Conclusion: In our cohort, the APOE genotype does not constitute a risk factor for developing POAG, even in patients with normal-tension glaucoma.

Clinical Relevance: Apolipoprotein E polymorphisms do not appear to be contributory to POAG.

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Primary Open-angle Glaucoma (POAG) is one of the most common causes of blindness and affects approximately 70 million people worldwide. It is thought to be a neurodegenerative disease with a significant hereditary element. It is likely that POAG is a complex trait with multiple genes contributing to the phenotype. Defining the genetic factors behind glaucoma will advance our understanding of the pathogenesis of visual failure and may lead to the development of novel treatments.

Clinical studies have shown an association between glaucoma and Alzheimer disease (AD), which is also a complex trait. The most important identified genetic risk factor for the neurodegeneration in sporadic AD is the apolipoprotein E (APOE) genotype. Apolipoprotein E is the major apolipoprotein of the central nervous system, where it is synthesized by glia, macrophages, and neurons. Apolipoprotein E exists in 3 common isoforms, E2, E3, and E4, encoded by the corresponding APOE gene alleles, e2, e3, and e4. It is therefore possible that the APOE genotype is a common risk factor for neurodegeneration and explains the association between AD and glaucoma. This hypothesis was supported by the results of a single study reporting an association between normal-tension glaucoma and the APOE e4 allele. However, normal-tension glaucoma only accounts for approximately 30% of glaucoma cases, and the intraocular pressure (IOP) cutoff defining normal-tension glaucoma is in fact arbitrary. To clarify the role of APOE in glaucoma, we investigated the role of APOE polymorphisms as risk factors for POAG in a population in northeast England, using a statistical approach that controls for the effects of IOP without making a distinction between low and high pressure.

Methods: Having obtained ethics approval from our local research ethics committee, blood samples were analyzed from an unrelated cohort of 137 POAG patients and 75 control subjects, from the same population.

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The definition for POAG included characteristic cupping of the optic disc, open iridocorneal angle, and typical glaucomatous visual field defects. An experienced ophthalmologist (M.B.), specializing in glaucoma, clinically examined the patients and controls. The highest pretreatment IOP and the cup-disc ratio were recorded and analyzed as variables. We excluded patients with IOPs higher than 30 mm Hg and secondary types of glaucoma (pseudoxfoliative, pigment dispersion syndrome, and traumatocorticosteroid-induced). Patients with IOPs higher than 30 mm Hg were excluded to maximize the chance of detecting polymorphisms that exert their effect by increasing the vulnerability of the optic nerve to damage. All controls were older than 63 years and had normal IOPs and optic discs.

**GENOMIC DNA GENOTYPING**

The genomic DNA was amplified by polymerase chain reaction of the related locus (19q13.2). For each patient, 1 µL of DNA was mixed with 1 U of Taq DNA polymerase (Promega, Madison, Wis), 10× Promega buffer, 2-nmol deoxyribonucleoside triphosphate, 10% dimethyl sulfoxide, 0.25 mmol/L of each oligonucleotide (primer), and water to a total volume of 50 µL. The primers used were forward (5′-TCC AAG GAG CTG CAG GCG GCC GA-3′) and reverse (5′-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3′). Reactions were treated in a thermal cycle machine to incubation at 94°C for 2 minutes, followed by 40 cycles at 65°C for 1 minute, 72°C for 1 minute, 94°C for 30 seconds, and a final incubation at 72°C for 10 minutes.

The products were then digested separately per sample with 2 restriction enzymes, Hae II and Afl III (New England Biolabs Inc, Beverly, Mass). The Hae II digestion mixture contained 20 µL of polymerase chain reaction product, 5 U of enzyme, 0.3 µL of bovine serum albumin, and 3 µL of buffer (buffer 4, New England Biolabs Inc). The Afl III digestion mixture contained 20 µL of polymerase chain reaction product, 1 U of enzyme, 0.3 µL of bovine serum albumin, and 3 µL of buffer (buffer 3, New England Biolabs Inc). Water was added to create a total volume of 30 µL per sample. Both reactions were allowed to proceed for at least 12 hours at 37°C.

The resulting fragments were separated by electrophoresis on a 4% MicroSieve agarose gel (Flowgen; Ashby de la Zouch, Leicestershire, England) and visualized by ethidium bromide staining with a digital camera.

APOE ε3 alleles were digested by both enzymes (Afl III results in 50- and 177–base pair [bp] fragments, and Hae II results in 32- and 195-bp fragments) where ε4 alleles lack the restriction site for Afl III and ε2 alleles lack the restriction site for Hae II. Therefore, the genotype was obtained by combining the band patterns for the 2 enzymes per sample.11

**STATISTICAL ANALYSIS**

To minimize the chance of detecting a spurious statistical association, we used a logistic regression model to simultaneously study the effect of multiple variables and their interactions when comparing POAG patients with controls.12 The model assumes that the logarithm of the odds ratio (OR) is a linear function of the variables included in the model:

\[
\log\left( \frac{P}{1-P} \right) = B_0 + B_1X_1 + B_2X_2 + \ldots + B_nX_n,
\]

where \( P \) indicates the probability of being affected; \( X_1, X_2, \ldots, X_n \) the chosen predictor variables; \( B_0 \) the intercept for the regression equation; and \( B_1, B_2, \ldots, B_n \) the coefficients reflecting the nature of each predictor. For the analysis, the predictor variables were age, IOP, cup-disc ratio, and APOE genotype. All the variables were added to the model with stringent forward-selection criteria using Minitab version 13.1 statistical package (Minitab Inc, State College, Pa).

**RESULTS**

Our cohort consisted of 137 POAG patients and 75 controls. The mean±SD age was 73.0±8.0 years (range, 51-87 years) for the POAG patients and 78.0±4.4 years (range, 68-90 years) for the controls. The mean±SD IOP was 20.8±2.6 mm Hg for the POAG patients and 16.2±3.4 mm Hg for the controls (Figure 1). The median cup-disc ratios were 0.8 and 0.3 for POAG patients and controls, respectively (Figure 2).

The frequency of ε2, ε3, and ε4 alleles in affected and unaffacted individuals is shown in Table 1. Logistic re-

![Figure 1. Distribution of intraocular pressure in patients with primary open-angle glaucoma and control subjects.](http://archophthal.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/9919/ on 04/08/2017)

![Figure 2. Distribution of cup-disc ratio in patients with primary open-angle glaucoma and control subjects.](http://archophthal.jamanetwork.com/pdfaccess.ashx?url=/data/journals/ophth/9919/ on 04/08/2017)

**Table 1. Frequency of APOE Alleles in Patients and Control Subjects**

<table>
<thead>
<tr>
<th>APOE Allele</th>
<th>Patients (n = 137)</th>
<th>Controls (n = 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ε2</td>
<td>35 (25.4)</td>
<td>16 (21.3)</td>
</tr>
<tr>
<td>ε3</td>
<td>199 (72.6)</td>
<td>114 (76.0)</td>
</tr>
<tr>
<td>ε4</td>
<td>40 (14.8)</td>
<td>20 (13.3)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) and are calculated from alleles in 274 patients and 150 controls (2 in each individual).
regression analysis confirmed that our patient and control groups were matched for age and sex but showed no evidence of an association between $\varepsilon_2$, $\varepsilon_3$, and $\varepsilon_4$ allele frequency and POAG (Table 2), irrespective of IOP ($\varepsilon_2$ OR, 0.82; 95% confidence interval, 0.12-5.79 [P = .84]; $\varepsilon_3$ OR, 0.39; 95% confidence interval, 0.10-1.49 [P = .17]; and $\varepsilon_4$ OR, 3.84; 95% confidence interval, 0.80-18.49 [P = .09]).

### Table 2. Results of Logistic Regression Analysis Between Patients and Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>$P$ Value</th>
<th>Odds Ratio (95% Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracocular pressure</td>
<td>.002</td>
<td>1.72 (1.22 to 2.41)</td>
</tr>
<tr>
<td>Cup-disc ratio</td>
<td>&lt;.001</td>
<td>7.05 $\times$ 10$^3$ (3.29 $\times$ 10$^3$ to 1.51 $\times$ 10$^3$)</td>
</tr>
<tr>
<td>Age</td>
<td>.055</td>
<td>0.83 (0.68 to 1.00)</td>
</tr>
<tr>
<td>APOE allele</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\varepsilon_2$</td>
<td>.84</td>
<td>0.82 (0.12 to 5.79)</td>
</tr>
<tr>
<td>$\varepsilon_3$</td>
<td>.17</td>
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<td>$\varepsilon_4$</td>
<td>.09</td>
<td>3.84 (0.80 to 18.49)</td>
</tr>
</tbody>
</table>

### COMMENT

Apolipoprotein E, a 36-kDa glycoprotein, is the major apolipoprotein of the central nervous system, where it is synthesized by retinal Müller cells, glia, macrophages, and neurons.13,14 Apolipoprotein E plays an important role in neural function and is involved in neurite outgrowth and repair from injury.15 It is up-regulated in response to oxidative stress and appears to act as an antioxidant. Apolipoprotein E isoforms may affect the onset and severity of several neurodegenerative disorders, and the strongest association is with AD. The APOE $\varepsilon_4$ allele is associated with 40% to 50% of sporadic and familial AD, compared with a 30% frequency of the allele in the general population.8 APOE alleles have been reported to have an effect on recovery from head injury.8,15 There is also association between the $\varepsilon_4$ allele and severe progression of multiple sclerosis and reduced survival time in amyotrophic lateral sclerosis.7

Primary open-angle glaucoma can be considered a neurodegenerative disease16 with a significant hereditary element.2 The strongest risk factors for glaucomatous optic atrophy are raised IOP and age. However, it is clear that multiple risk factors operate, some of which are as yet undetermined.17 There is evidence from twin studies, case-control studies, and population studies of a heritable element to POAG.2 The risk of glaucoma in first-degree relatives is 2 to 4 times greater than that in the general population.18 Recently, several genes have been identified that may contribute to POAG,3,19 including myocilin,20 OPA1,21 and OPTN.22 It is likely that POAG is a complex trait with multiple genes contributing to the phenotype, along with environmental factors.

There is evidence that the prevalence of POAG is higher in AD patients. A retrospective study of medical records of patients with POAG found that those with AD had more rapid progression of their disc cupping than those without AD. Another study showed that AD and Parkinson disease occur more commonly in patients with POAG. Furthermore, at the cellular level, similar neurofilament triplet proteins susceptible to neurofibrillary tangle formation were identified in AD and POAG.23 This raises the interesting possibility that the APOE genotype is a common genetic risk factor for the neurodegeneration in POAG and other neurodegenerative diseases.

This study showed no association between APOE genotype and POAG. The frequency of the APOE $\varepsilon_4$ allele in our control population was 13%. Power calculations indicate that we had 87% power to detect an OR of 3.00 at the $P = .05$ significance level. Our findings are in agreement with a previous study carried out on a French cohort with POAG, but they contrast with those of a previous study that reported an increased frequency of the APOE $\varepsilon_4$ allele in a Tasmanian population with normal-tension glaucoma. There are several possible explanations for this discrepancy. It is possible that APOE might have a more obvious effect in populations exposed to different environmental factors or with a different genetic background. An alternative explanation is that our patients were defined differently compared with the previous study. The choice of a normotensive cohort, which has also been used by other authors, eliminates the strongest risk factor (IOP) and may make a study more sensitive to underlying neurodegenerative risk factors. However, the arbitrary division of POAG into normal- and high-tension types may be unhelpful and misleading because POAG represents a spectrum of phenotypes. Such a distinction based on a specific level of IOP, if wrongly chosen, could conceal the true risk factors that operate in POAG or could reveal spurious associations through artificial stratification.16 To resolve these issues, we used a logistic regression model that controls for any effect of IOP during the analysis, without drawing an arbitrary cutoff, enabling a direct analysis of the effects of APOE genotype irrespective of the IOP. This approach also minimizes the chance of detecting a false-positive result (type I error). This statistical approach may prove to be useful in further studies of POAG, as it allows for a more holistic view of the disease.

There is a strong rationale for pursuing further studies of apolipoprotein E as an etiological factor in glaucoma. Our study suggests that it is not the isoform itself that is important, but the effect may be more subtle, possibly involving different levels of APOE expression in the optic nerve. This is supported by the recent finding of an association between POAG and polymorphisms in the APOE promoter region.24 Further investigations are required to confirm these findings in other populations.

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REFERENCES


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