Primary open angle glaucoma (POAG; OMIM 137760) is a major cause of late onset visual failure of unknown etiology. Recent genetic association studies have implicated the apolipoprotein E (APOE) gene in the pathophysiology of primary open angle glaucoma, but there have been conflicting findings.

**Methods:** To resolve this issue we studied 140 cases and 73 controls that were carefully phenotyped, and used a logistic regression model to simultaneously analyze the effect of apolipoprotein E genotype and functional polymorphisms in the apolipoprotein E gene promoter while controlling for potentially confounding variables.

**Results:** We found no evidence of an association between the apolipoprotein E promoter region polymorphisms and primary open angle glaucoma.

**Conclusions:** Apolipoprotein E promoter polymorphisms are unlikely to have a major impact on the pathophysiology of primary open angle glaucoma.

Primary open angle glaucoma is a major cause of blindness affecting 70 million individuals worldwide [1]. The etiology of POAG is poorly understood, with both genetic and environmental factors contributing to the pathophysiology [2,3]. A small proportion of families with autosomal dominant POAG have mutations in MYOC (OMIM 601652) which codes for the trabecular meshwork inducible glucocorticoid response protein TIGR (also called myocilin) [4], but MYOC mutations are rare in patients with sporadic POAG [3]. Apolipoprotein E (APOE) is the principal apolipoprotein within the central nervous system and polymorphic variants of APOE have been associated with a number of neurodegenerative diseases, including Alzheimer’s disease (OMIM 104300) [5]. There is clinical evidence of an association between POAG and Alzheimer’s disease [6], raising the possibility that genetic variants in the APOE gene might predispose to focal neurodegeneration of the retinal ganglion cells and the optic nerve in POAG. Recent studies provide support for this hypothesis. The APOE ε4 allele was associated with normal tension glaucoma in a Tasmanian cohort [7], but not in a British cohort [8] or in a French cohort [9] of patients with POAG. In the French cohort, an association was reported between the APOE (-219G) promoter polymorphism and the degree of optic nerve damage in POAG; the APOE (-491T) promoter polymorphism appeared to interact with a single nucleotide polymorphism (SNP) in MYOC to cause raised intraocular pressure (IOP) and reduced efficacy of IOP lowering drugs in patients with POAG [9]. However, this area is fraught with controversy, particularly in the definition of normal tension glaucoma (which is based on an arbitrary cut off of 21 mm Hg) and the variables used to define the extent of optic nerve damage (cup:disc ratio [CDR] and the extent of visual field loss). As a result, the role of APOE in POAG remains to be established.

**METHODS**

**Case selection:** To address these issues, we studied an independent cohort of 140 unrelated POAG cases and 73 unrelated healthy controls from the northeast of England. The controls were the partners of the cases with POAG; they were only included if they did not have glaucoma and were older than the corresponding proband (see Table 1 for descriptive statistics). Using this approach, the mean age of the controls was greater that the mean age of the cases (Table 1). This is desirable for a genetic association study of a late onset disorder. This approach reduces the likelihood of a pre-clinical POAG patient being included in the control group, reducing the potential for misclassification bias. POAG was defined by characteristic cupping of the optic disc, open iridocorneal angle, and typical glaucomatous visual field defects. CDR was determined clinically by an ophthalmologist with specialty training in glaucoma. Intraocular pressure was measured using Goldmann applanation tonometry; no attempt was made to grade the severity of the visual field defect. Patients with secondary glaucoma (pseudoexfoliative, pigment dispersion syndrome, trauma or steroid induced) were excluded. Controls had normal visual acuity, a normal intraocular pressure, visual fields, and optic discs.

**Molecular genetic analysis:** The APOE genotype was determined as described previously [10,11]. The APOE promoter region was amplified using primers 5′-CAA GGT CAC ACA GCT GGC AAC-3′ (forward) and 5′-TCC AAT CGA CCG CTA GCT ACC-3′ (reverse) with an annealing tempera-
tecture of 54 °C and standard cycling conditions. The two APOE promoter polymorphisms previously associated with POAG were genotyped by nested PCR. The second round PCR incorporated fluorescent primers. The final product was digested with Dral to discriminate the -491A/T SNP and TaqI to discriminate the -219T/G SNP. Samples were analysed by electrophoresis on a Beckman-Coulter CEQ 8000 capillary fluorescence analyzer (Foster City, CA).

Statistical analysis: We used a logistic regression model to simultaneously analyze the effect of the different genotypes and their interactions (APOE genotype, -219 and -491 APOE promoter allele genotype), while controlling for age and gender by including these variables in the model using Minitab version 13.1 software (State College, PA).

RESULTS & DISCUSSION

Logistic regression revealed no evidence of an association between POAG and APOE genotype, the APOE (-219G) or APOE (-491T) promoter polymorphisms, or their interactions (Table 2). These findings contrast with a study by Copin et al. [9]. They studied APOE genotype and the two APOE promoter polymorphisms (-219 and -491) and concluded that the -219G allele increased an individual’s susceptibility to optic nerve damage as measured by the CDR and the severity of the associated visual field defect [9]. However, the difference in mean CDR and visual field score between -219G and -219T carriers was subtle. Although the results reached statistical significance, other authors have questioned their clinical significance [12]. It is notable that Copin et al. did not detect an association between APOE genotype and the risk or severity of developing glaucoma, nor the -427 promoter polymorphism and glaucoma [9]. Given the well-established linkage disequilibrium between the -219 and -491 promoter polymorphisms and both the -427 promoter polymorphism and APOE genotype [13,14], one would expect that a cohort showing an association between APOE genotype and POAG would also show evidence of an association between the -219 and -491 APOE promoter polymorphisms and POAG. This inconsistency is not apparent in our data set as we saw no evidence of an association between POAG and both promoter and coding region [8] polymorphisms of APOE.

Power calculations indicate that we had 80% power to detect an odds ratio of 2.25 for the -219 APOE promoter polymorphism at the 0.05 level. Since the power to detect an association was not as strong for the less frequent alleles, we cannot exclude the possibility that genetic variation at the APOE locus makes some contribution to the risk of developing glaucoma or its severity. However, this study shows that neither the APOE genotype nor the -219 and -491 APOE promoter polymorphisms are likely to have a major influence on the pathophysiology of POAG.

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REFERENCES


