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The apolipoprotein E gene polymorphism is associated with open angle glaucoma in the Japanese population

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Purpose: To assess whether genetic polymorphisms of the apolipoprotein E (APOE) gene are associated with open angle glaucoma (OAG) in the Japanese population.

Methods: Genomic DNA was examined in a cohort of 310 Japanese patients with OAG and 179 control subjects. The average age was 63.5±14.4 years (mean±SD) for the OAG patients and 65.5±11.6 years for the control subjects. The presence or absence of OAG in patients and controls was based on clinical examination and/or ophthalmic records. The APOE allele frequency (ε2, ε3, and ε4 alleles) was studied by restriction fragment length polymorphism, and compared between OAG patients and control subjects. The association between the intraocular pressure (IOP) and the APOE alleles was also evaluated.

Results: There was a significant difference in the APOE genotype frequencies between these groups (p=0.0006 χ^2 test). The frequencies of the ε2 and ε4 alleles were significantly lower in the OAG patients (ε2: 2.6%; ε4: 6.0%) compared to the control subjects (ε2: 5.0%, p=0.048; ε4: 10.6%, p=0.012; Fisher's exact test). The frequency of the ε3 allele was significantly higher in the OAG patients (91.4%) compared to the control subjects (84.4%, p=0.0010; Fisher's exact test). Adjusted for age, gender, and IOP, an appropriate three fold reduction in OAG risk (odds ratio [OR] 0.29, 95% confidence interval [CI] 0.10 to 0.80; p=0.018) was found with the ε2 allele and a two fold increased risk of OAG (OR 1.97, 95% CI 1.06 to 3.67; p=0.033) was found with the ε3 allele. The maximum IOP (18.3±6.0 mm Hg) in patients with the ε4 allele was significantly lower than that (21.3±9.1 mm Hg) in patients without the ε4 allele (p=0.006, Student's t-test).

Conclusions: The APOE gene polymorphism is associated with OAG in the Japanese population. Further studies in the other ethnic populations should be performed to elucidate the relationship between APOE and OAG.

Open angle glaucoma (OAG) is one of the most common eye diseases and can potentially result in bilateral blindness. It has been estimated that nearly 66.8 million people worldwide are affected by OAG [1]. The disease is characterized by optic nerve atrophy with an excavated optic nerve head and progressive visual field loss. Although elevation of intraocular pressure (IOP) is recognized as a major risk factor for optic nerve damage in OAG [2], multiple factors other than IOP, including genetic factors [3], are likely to have a role in the pathogenesis of glaucomatous optic neuropathy.

Apolipoprotein E (APOE) is the major apolipoprotein of the central nervous system. The gene encoding APOE on chromosome 19q has three polymorphic variants in humans designated as ε2, ε3, and ε4. Inheritance of the ε4 allele has been associated with central nervous diseases such as Alzheimer's disease [4], Parkinson disease [5], and amyotrophic lateral sclerosis [6]. It is therefore possible that the APOE allele is a common risk factor for neurodegeneration, and may therefore be associated with glaucoma. However the association between APOE allele and OAG is controversial with previous reports showing positive association [7,8] while others showed negative ones [9,10]. Additionally, the previous studies on the APOE allele and OAG have been performed in predominantly white populations, and there have been no studies in other populations. Further studies in the different ethnic population are desirable to understand more fully the relationship between APOE and glaucoma, because there is a ethnic difference in the APOE allele frequencies [11,12]. In this study, we therefore investigated whether the APOE allele and genotype were associated with OAG in the Japanese population.

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METHODS

Subjects: Unrelated Japanese patients with OAG were recruited from ophthalmology practices in the University of Yamanashi Hospital, Enzan City Hospital, Uenohara Town Hospital, and Oizumi Clinic in Yamanashi or Nagano prefectures, Japan. Diagnosis of OAG required open angles on gonioscopic examination, typical glaucomatous cupping of the optic disc, and visual field defects characteristic of glaucoma by automated static perimetry (Humphrey Visual Field Analyzer 30-2, Humphrey Instruments, San Leandro, CA). Patients had no other ocular diseases that would cause an increase in IOP. Additionally, patients with IOP less than 21 mm

Hg had no signs of intracranial disease that would cause optic nerve atrophy in x-ray computerized tomography or magnetic resonance imaging.

As control subjects, unrelated Japanese patients with eye diseases unrelated with OAG or optic nerve diseases were recruited from the participant institutions. Control subjects were over 40 years of age, had IOP below 20 mm Hg, had no glaucomatous cupping of the optic disc (cup to disc ratio less than 0.4), and had no family or personal history of glaucoma. All participants received comprehensive ophthalmologic examinations, and then 2 ml of peripheral blood was collected from each one. The study protocol was approved by the Ethics Committee of University of Yamanashi, and informed consent was obtained from all study participants. The study was conducted in accordance with the Declaration of Helsinki.

Genomic DNA Genotyping: Genomic DNA was purified using the EZNA Blood DNA Kit II(Omega Bio-tek, Doraville, GA), and screened for the APOE genotype using restriction fragment length polymorphism (RFLP) analysis. The primers, as reported by Wenham et al. [13] amplified a region of DNA that spans both APOE polymorphic sites. The PCRs were carried out in a total volume of 20 µl containing 100 ng genomic DNA, 4 pmol of each primer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, and 0.5 U of Taq polymerase (TaKaRa TaqTM; Takara Bio Inc., Otsu, Shiga, Japan) in a thermocycler (model AB-1820; ATTO Corporation, Tokyo, Japan). Amplification was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. A final extension at 72 °C for 10 min completed the reactions. Amplification product (15 µl) was then digested with 6 units *Hha*I (New England Biolabs, Beverly, MA) for at least 5 h at 37 °C, loaded on polyacrylamide gels (GeneGel Excel 12.5/24 Kit; stacking gel: T=6%, C=3%; separating gel: T=12.5%, C=2%; Amersham Biosciences, Amersham Place, UK) and electrophoresed at 20 W for 1 h, using an electrophoresis unit (GenePhor; Amersham Biosciences, Amersham Place, UK). Following electrophoresis, gels were stained with silver nitrate on an automated gel stainer (Hoefer; Amersham Biosciences, Amersham Place, UK), and the genotypes were obtained.

Statistical analysis: Data were analyzed using SAS statistical software (version 8.2; SAS Institute Inc., Cary, NC). χ^2 analysis of the Hardy-Weinberg equilibrium for APOE genotypes were performed for patients and controls. Genotypic frequency difference was estimated by a χ^2 test. The Fisher's exact test was used to calculate the probabilities for the tests of whether there was any unadjusted association between OAG and alleles. The association between the maximum IOP and alleles was evaluated by Student's t-test. We also used a logistic regression model to simultaneously study the effect of multiple variables when comparing OAG patients with controls, especially enabling a direct analysis of the effects of APOE genotype and allele irrespective of the IOP. The predictor variables were age, gender, maximum IOP, and APOE genotype or allele. The odds ratio of IOP is per mm Hg. The ε4 allele was excluded in this model because the ε4 allele was not an independent variable. A $p<0.05$ was considered to be statistically significant.

Table 1. Frequency of APOE genotypes and alleles

Sample	OAG patients (n=310)	Control subjects (n=179)	p value
APOE genotypes			
ε2ε2	0 (0)	0 (0)	
ε2ε3	14 (4.5)	18 (10.1)	0.022
ε2ε4	2 (0.7)	0 (0)	0.53
ε3ε3	259 (83.5)	123 (68.7)	0.029
ε3ε4	35 (11.3)	38 (21.2)	0.0037
ε4ε4	0 (0)	0 (0)	
APOE alleles			
ε2	16 (2.6)	18 (5.0)	0.048
ε3	567 (91.4)	302 (84.4)	0.001
ε4	37 (6.0)	38 (10.6)	0.012

There were significant differences in the apolipoprotein E (APOE) genotype and allele frequencies between the open angle glaucoma (OAG) and control groups (p=0.0006 and p=0.0030, respectively; χ^2 test). Each genotype is being compared to all other genotypes collectively. Data are presented as counts (percentages are in parentheses) and are calculated from the alleles of 310 patients and 179 controls (2 in each individual). Statistical tests reported in this table used Fisher's exact test.

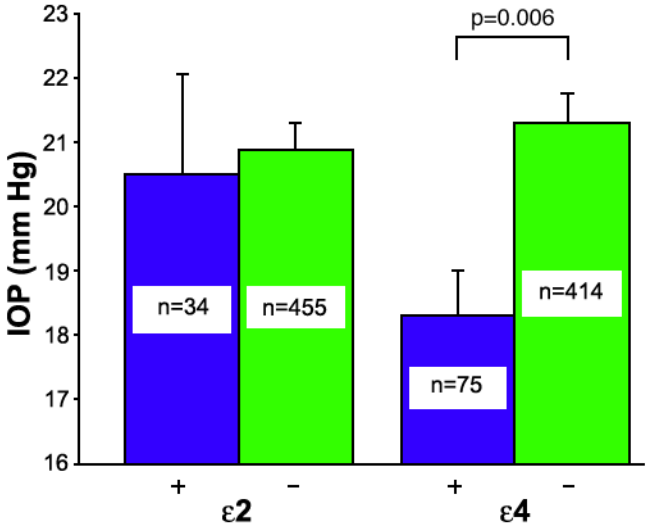

Figure 1. Association between the maximum IOP and the apolipoprotein E alleles. The maximum IOP in patients with the ε4 allele was significantly lower than that in patients without the ε4 allele (Student's t-test). The ε3 allele could not be analyzed because there were only 2 patients without the ε3 allele. IOP indicates intraocular pressure.

RESULTS

Three hundred and ten OAG patients, and 179 control subjects were enrolled in this study. The average age was 63.5±14.4 years (mean±SD) for the OAG patients and 65.5±11.6 years for the control subjects. The OAG patients and the control subjects included 50.3% and 34.6% of males, respectively. The average and standard deviation of maximum IOP was 24.3±9.2 mm Hg for the OAG patients and 15.0±2.7 mm Hg for the control subjects.

The frequency of APOE genotypes and alleles in the OAG patients and the control subjects is shown in Table 1. APOE genotypic and allelic frequencies were in Hardy-Weinberg equilibrium in both OAG patients and control subjects. There was a significant difference in the APOE genotype frequencies between these groups (p=0.0006, χ^2 test). The frequencies of the ε2 and ε4 alleles were significantly lower in the OAG patients (ε2: 2.6%; ε4: 6.0%) compared to the control subjects (ε2: 5.0%, p=0.048; ε4: 10.6%, p=0.012; Fisher's exact test). The frequency of the ε3 allele was significantly higher in the OAG patients (91.4%) compared to the control subjects (84.4%, p=0.0010; Fisher's exact test).

The association between the maximum IOP and the APOE alleles is shown in Figure 1. The mean of maximum IOP (18.3±6.0 mm Hg) in patients with the ε4 allele was significantly lower than that (21.3±9.1 mm Hg) in patients without the ε4 allele (p=0.006, Student's t-test).

Adjusted for age, gender, and IOP, a four fold reduction in OAG risk (odds ratio [OR] 0.26, 95% confidence interval [CI] 0.088 to 0.74; p=0.012) was found with the ε2/ε3 genotype compared to the ε3/ε3 genotype (Table 2). With the same adjustment, a three fold reduction in OAG risk (OR 0.29, 95% CI 0.10 to 0.80; p=0.018) was found with the ε2 allele and a two fold increased risk of OAG (OR 1.97, 95% CI 1.06 to 3.67; p=0.033) was found with the ε3 allele (Table 2).

Table 2. Results of logistic regression analysis

Variable	p value	Odds ratio (95% confidence interval)
Age	0.6	1.01 (0.98 to 1.03)
Gender	0.68	1.13 (0.63 to 2.03)
IOP	<0.0001	1.98 (1.73 to 2.25)
APOE genotypes		
ε2ε3	0.012	0.26 (0.088 to 0.74)
APOE alleles		
ε2	0.018	0.29 (0.10 to 0.80)
ε3	0.033	1.97 (1.06 to 3.67)

Logistic regression analysis was used to model the risk of developing open angle glaucoma (OAG) based on apolipoprotein E (APOE) genotype and allele with an adjustment for age, gender, and intraocular pressure (IOP). A four fold reduction in open angle glaucoma risk was found with the ε2ε3 genotype compared to the ε3ε3 genotype. With the same adjustments, a three fold reduction in OAG risk was found with the ε2 allele, and a two fold increased risk of OAG was found with the ε3 allele. The ε4 allele was excluded from this model because the ε4 allele was not an independent variable.

DISCUSSION

APOE is a plasma protein that serves as a ligand for low density lipoprotein receptors and, through its interaction with these receptors, participates in the transport of cholesterol and other lipids among various cells of the body [14]. APOE also appears to be involved in the repair response to tissue injury. For example, markedly increased amounts of APOE are found at sites of nerve injury and regeneration [14]. In the retina, APOE has been demonstrated to be synthesized by Müller cells, and secreted into the vitreous. APOE is absorbed by retinal ganglion cells, transported down the optic nerve, and may have a role in axonal nutrition [15]. This possible role in retinal ganglion cell metabolism, together with its documented effect on neuronal survival [14], has led to the hypothesis that APOE could be related to neuronal damage in glaucoma patients. In fact, the APOE promoter gene polymorphism has been shown to affect optic nerve damage and visual field loss [16]. Additionally, the APOE promoter and myocilin gene polymorphisms interact to affect optic nerve cupping and visual field loss in primary open angle glaucoma (POAG) [16], suggesting that APOE expression may have a role in glaucoma pathogenesis.

We noted a significantly lower frequency of the APOE ε2 and ε4 alleles in Japanese patients with OAG compared with control subjects. This differs from the previous studies, in which no significant association between the APOE ε2 and ε4 alleles and OAG was detected [9,10,16]. The APOE ε4 allele has also been associated with elevated risk of NTG in the Tasmanian population [7]. This suggests a reduction in disease risk with the ε2 and ε4 alleles. The prevalence of normal tension glaucoma (NTG) in the Japanese population is higher than that reported in the other races [17], this may reflect the low prevalence of APOE ε2 and ε4 alleles in the Japanese population [11]. Inheritance of ε3 allele was also associated with an elevated risk of OAG in this study. This finding has not been documented before. However, it may not be appropriate to consider the APOE ε3 allele as risk factor because it is a common allele. The frequency of the ε3 allele may be relatively high because the frequency of the ε2 and ε4 allele is low in patients with OAG.

As for the association between the IOP and the APOE alleles, the maximum IOP in patients with the ε4 allele was significantly lower than that in patients without the ε4 allele. This results from high frequency of the APOE ε4 allele in the control subjects or, alternatively, direct effect of the APOE ε4 allele to the IOP reduction. Junemann et al. [8] reported that IOP was significantly higher in subjects with the APOE ε2 allele. However, their sample sizes were smaller and it was only significant in the normal control subjects. Additionally, the frequency of the ε2 allele in the OAG patients was low rather than high compared to that in the control subjects, although it should be higher.

Our findings were in contrast with the findings in the previous studies. There are several possible explanations for these discrepancies. APOE might have a more obvious effect in populations exposed to different environmental factors or with a different genetic background. In fact, the association between

the APOE allele and Alzheimer's disease is not the case for all ethnic populations [18,19]. Ethnic differences in the APOE allele frequencies have been reported [11,12]. The reason for dissimilar findings may reflect a regional difference in the APOE allele frequencies. These indicate that the association between APOE and OAG may vary among different ethnic groups. An alternative explanations are linkage disequilibrium with an effect of a nearby polymorphism and different case definition of the patients in each study.

In conclusion, the APOE ε2 and ε4 alleles appear to be associated with reduced risk of OAG, and ε3 allele with elevated risk of OAG in the Japanese population. Further studies in other ethnic populations should be performed to elucidate the relationship between APOE and OAG.

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