Lack of Association of Apolipoprotein E (Apo E) ε2/ε3/ε4 Polymorphisms with Primary Open-Angle Glaucoma: A Meta-Analysis from 1916 Cases and 1756 Controls

Wei Wang*, Minwen Zhou*, Wenbin Huang, Shida Chen, Xiulan Zhang*
Zhongshan Ophthalmic Center, State Key Laboratory of Ophthalmology, Sun Yat-Sen University, Guangzhou, People’s Republic of China

Abstract

Background: A number of case-control studies were conducted to investigate the association of apolipoprotein E (Apo E) polymorphisms with primary open angle glaucoma (POAG). But the results remain controversial. This meta-analysis aims to comprehensively evaluate the relationship between a common ε2/ε3/ε4 polymorphism in Apo E gene on the risk of POAG.

Method: A comprehensive literature search for studies published up to April 2013 was performed. Summary odds ratios (ORs) and 95% confidence intervals (CI) were calculated employing random-effects models irrespective of between-study heterogeneity. Publication bias of literatures was evaluated using funnel plots and Egger’s test.

Results: A total of 12 studies including 1916 cases and 1756 controls meeting the predefined criteria were involved in this meta-analysis. Overall, the Apo E ε2 allele and ε4 allele were not associated with POAG, compared with those carrying ε3 allele, with ORs of 0.98 (95% CI, 0.79 to 1.23; P = 0.872) and 1.05 (95% CI, 0.78 to 1.41; P = 0.743), respectively. Genotypic analysis also found no significant association between the ε4 carriers (ε3/ε4+ε4/ε4), ε2 carriers (ε2/ε3+ε2/ε2) and POAG, compared with participants with Apo E ε3/3, with ORs of 0.91 (95% CI, 0.66 to 1.25; P = 0.543) and 1.08 (95% CI, 0.74 to 1.57; P = 0.694), respectively. In the subgroup analysis by ethnicity, source of controls, genotyping methods, Hardy-Weinberg equilibrium or not, or type of the POAG, still no obvious associations were found.

Conclusions: This meta-analysis suggests that Apo E ε2/ε3/ε4 polymorphisms may not be associated with the risk of POAG. However, well-designed studies with larger sample size and more ethnic groups are required to further validate the results.

Introduction

Glaucoma is a leading cause of irreversible blindness, estimated to affect 79.6 million people by 2020, of which over 8 million will suffer from bilateral blindness [1]. Primary open-angle glaucoma (POAG), clinically classified into high tension glaucoma (HTG) and normal tension glaucoma (NTG), is the most prevalent form of glaucoma in most populations, and affects 70 million individuals worldwide [2].

POAG is considered to be caused by multiple genetic and environmental factors, and interactions among these factors [2,3]. Three causative genes, namely optineurin (OPTN), myocilin (MYOC), and WDR36, have been identified thus far, but these account for fewer than 10% of patients with sporadic, adult-onset POAG [2]. Quite a number of POAG susceptibility genes have been identified. One of the good potential candidate susceptibility gene had been studied is apolipoprotein E (Apo E) [4].

Apo E is the principal apolipoprotein within the central nervous system and polymorphic variants of Apo E have been associated with a number of neurodegenerative diseases, including Alzheimer’s disease [5,6]. It is a ligand for the low density lipoprotein family receptors and plays a pivotal role in cholesterol metabolism. Apo E has three common isoforms, ε2, ε3, and ε4, respectively, at a single locus in chromosomal region 19q13.2. These alleles define six Apo E phenotypes: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4. Apo E ε3/ε3 is the most predominant genotype and ε3 is the most common allele in majority of populations. Individuals with one ε4 allele gene are three- to four-times more likely to develop AD than those without an ε4 allele gene [6]. The neuronal injuries associated with Alzheimer disease have several similarities with the optic nerve changes often seen with POAG [7]. Thus, the Apo E gene appears to be a potential genetic marker for POAG.

To date, many case–control studies have been carried out to investigate the role of the Apo E gene polymorphism in the
development of POAG, but these have produced conflicting or inconclusive results. Some of the studies showed an association between certain types of Apo E alleles and POAG, whereas others found no association. In a study by Junemann et al [8], they found a significant association between the level of IOP and the Apo E \(e2/e3\) allele, however, Nabuchi et al [9] and Yuan et al [10] found a reduction in POAG risk in people with \(e2\) allele in a Japanese population and Chinese population, respectively. Vickers et al [11], Al-Dabbagh et al [12] and Yuan et al [10] reported that the Apo E \(e4\) gene is associated with elevated risk of POAG or NTG. On the contrary, Lam and his colleagues [13] reported that the Apo E \(e4\) gene confers a protective effect against NTG. Their findings, however, could not be replicated in other researchers [14,15,16,17,18,19,20]. As a result, the role of Apo E in POAG remains to be established.

To date, no meta-analysis has been conducted to evaluate the association of the polymorphisms of Apo E with POAG. Hence, we performed a meta-analysis of all eligible studies to derive a more precise estimation of the association, to help us better understand its possible influence on POAG.

Methods

This meta-analysis was performed according to a predetermined protocol described in the following paragraph. MOOSE guidelines were followed at all stages of the process [21].

Literature Search

The PubMed, Embase, ISI Web of Knowledge, Cochrane Library and Chinese databases such as the China National Knowledge Infrastructure (CNKI) and Wanfang were searched (up to April 1, 2013). The Medical Subject Terms (MeSH), keywords and free text words used for this research were apolipoprotein E or Apo E, polymorphism (s) or allele (s) variation or genotype (s) and glaucoma or intraocular hypertension. Hand-searching of the references of included articles identified was also performed to identify other relevant studies. If the overlapping patient population was included in several studies, the latest study was included. If more than one geographical or ethnic population were included in one article, each population was considered separately. Two investigators (Wang W and Zhou MW) independently screened the information including the titles, abstracts and full texts to determine inclusion carefully. If the two reviewers disagreed with each other, a third reviewer (Zhang XL) may be sought.

Quality assessment

The qualities of included studies were assessed independently by the same two investigators using the Newcastle-Ottawa Scale (NOS) [22]. The NOS uses a ‘star’ rating system to judge quality based on 3 aspects of the study: selection, comparability, and exposure (case–control studies) or outcome (cohort studies). Scores were ranged from 0 star (worst) to 9 stars (best). Studies with a score of 7 stars or greater were considered to be of adequate quality. Disagreement was settled as described above.

Inclusion/Exclusion Criteria

The inclusion criteria were as follows: (1) studies on the relationship between Apo E \(e2/e3/e4\) gene polymorphism and POAG; (2) case-control study using either a hospital-based or a population-based design (Studies were classified as population-based if the controls were selected from the same source population as the case-patients, including the community and the general population. Studies using hospital- or clinic-based patients with other illnesses as controls and studies that used an unidentified healthy control group were considered hospital-based); (3) studies with full text articles; (4) sufficient data for estimating an odds ratio (OR) with 95% confidence interval (CI); and (5) not republished data. Studies were excluded if they were family studies; or published abstracts from meeting.

Data extraction

The same two reviewers independently extracted data, cross-checked, discussed all conflict, and reached consensus on all items. Following data were extracted from each study: first author’s last name, publication date, population ethnicity, study design, study location, age, sex, methods of genotyping, number of cases and controls, and available allele and genotype frequencies information.

Statistical Analysis

Hardy-Weinberg equilibrium (HW-E, \(p<0.05\) was considered significant) was assessed using the chi-squared test. The \(I^2\) statistic was used to quantify the inconsistency between study estimates, and the Q statistic were used to formally test for heterogeneity (\(p<0.10\) was considered representative of significant statistical heterogeneity). In this meta-analysis, a random-effects model was applied irrespective of between-study heterogeneity (DerSimonian & Laird). The association between Apo E polymorphism and POAG was estimated by calculating pooled odd ratios (ORs) and 95% CIs. The significance of the pooled OR was determined by Z
test (P<0.05 was considered statistically significant). For allelic analysis, we examined the risk of POAG associated with e2 and e4 allele using e3 as the reference group. For genotypic analysis, we defined e3/e3 genotype as the reference group. The e2 carriers were defined as patients with the e2/e2 or e2/e3 genotype. The e4 carriers included patients with the e3/e4 and e4/e4 genotype. The e2 and e4 carriers were separately compared with the e3 genotype as the reference group. For genotypic analysis, we
e3 as the reference group. For genotypic analysis, we defined e3/e3 genotype as the reference group. The e2 carriers were defined as patients with the e2/e2 or e2/e3 genotype. The e4 carriers included patients with the e3/e4 and e4/e4 genotype. The e2 and e4 carriers were separately compared with the e3/e3 group. (The e2/e4 genotype was excluded in genotypic analysis). All statistical analyses were carried out by using the Stata 12.0 (Stata Corporation, College Station, TX, USA).

Sensitivity analysis
Subgroup analysis was used to investigate which factors (ethnicities, sources of controls, genotyping methods, HW-E or not, types of POAG) might contribute to the heterogeneity. One-way sensitivity analyses were performed by iteratively removing one study at a time to assess the stability of the meta-analysis results. Cumulative meta-analysis was performed to evaluate the accumulation of evidence on the association between Apo E polymorphisms and POAG.

Publication bias
Publication bias was assessed using Begg’s funnel plots and Egger’s test. An asymmetric plot suggests a possible publication bias and the P value of Egger’s test less than 0.05 was considered representative of statistically significant publication bias.

Results

Literature Search and Characteristics
The study selection process is detailed in Fig. 1. The initial search strategy identified 203 studies. 184 were excluded (23 were duplicate studies, 159 were unrelated topic, two were letters), leaving 19 studies for full publication review. Of these, one article which contained overlapping data from the same patient source, 2 articles which absence of sufficient data for estimating OR and 95%CI, two article which were not about Apo E e2/e3/e4 gene, two article which were not about POAG, were excluded. Thus, 12 studies were included in the final meta-analysis, including 1916 cases and 1756 controls.

All studies were case–control in design. Table 1 shows the studies identified and their main characteristics. Among these studies, two studies were not in HW-E, one study was unavailable for performing HW-E test. The NOS results showed that the average score was 7.42 (range 7 to 9), indicating that the methodological quality was generally good. There were 7 studies of Caucasian and 5 studies of Asian. Controls were mainly healthy populations and non-glaucoma participants. Five studies were population-based and 7 were hospital-based. The cases of 2 studies were patients with NTG, 3 studies were patients with HTG. Seven studies were mixed patients, among of them, 3 studies provided data concerning HTG and 2 studies had enough data for NTG, allowing subtype specific meta-analysis. Therefore, six studies were combined for HTG subtype and four for NTG subtype. MOOSE checklist was generated to provide detailed description of this meta-analysis (Table S1). Genotype and allele distributions for each case–control study are shown in Table S2.

Main results and subgroup analyses
Allelic analysis. Table 2 give the summary results for the association of the Apo E polymorphism with the risk of POAG based on allelic analysis. The overall random effects pooled OR of e2 versus e3 for POAG showed no statistical significance: OR = 0.98 (95% CI: 0.79–1.23, P(Z) = 0.0872) (shown in Figure S1). Modest heterogeneity was present among the 11 studies (I² = 30.6%, P(Q) = 0.155). For e4 versus e3, the results were also not statistically significant (shown in Figure S2). The summary OR was 1.05 (95% CI: 0.78–1.41; P(Z) = 0.743). The I² statistic indicated substantial between-study heterogeneity (I² = 70%, P(Q) < 0.001).

Considering the fact that ethnic differences, sources of the controls, fulfilling HW-E or not, genotyping method, or type of POAG might bias the overall association, we conducted separate analysis according to these factors. The pooled OR for e2 allele and e4 allele versus e3 allele were also not statistical significant in all subgroups.

Table 1. Characteristics of eligible studies included in the present meta-analysis.

| First author(year) | Country | Ethnicity | Design | Genotyping method | Patients | Control |
|--------------------|---------|-----------|--------|------------------|----------|---------|
| Vickers(2002)      | Australia | Caucasian | PB     | PCR              | 142      | 51      |
| Junemann(2004)     | Germany  | Caucasian | PB     | NA               | 41       | 32      |
| Lake(2004)         | UK       | Caucasian | PB     | PCR-RFLP         | 155      | 349     |
| Resnissiotis(2004) | UK       | Caucasian | PB     | Taqman assay     | 137      | 75      |
| Mabuchi(2005)      | Japan    | Asian     | HB     | PCR-RFLP         | 310      | 179     |
| Lam(2006)          | China    | Asian     | HB     | PCR-RFLP         | 400      | 300     |
| Yuan(2007)         | China    | Asian     | PB     | PCR-RFLP         | 36       | 57      |
| Zetterberg(2007)   | Sweden   | Caucasian | HB     | minisequencing technique | 242     | 187     |
| Hu(2007)           | China    | Asian     | HB     | PCR-RFLP         | 142      | 77      |
| Al-Dabbagh(2009)   | Saudi    | Caucasian | HB     | PCR-RFLP         | 60       | 130     |
| Jia(2009)          | China    | Asian     | HB     | PCR              | 176      | 200     |
| Saglar(2009)       | Turkey   | Caucasian | HB     | PCR-RFLP         | 75       | 119     |

| NOS score |
|-----------|
| 9         |
| 7         |
| 7         |
| 8         |
| 7         |
| 7         |
| 7         |
| 7         |
| 7         |
| 7         |
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PB: population based; HB: hospital based; NA indicates data not available; PCR: polymerase chain reaction; RFLP restriction fragment length polymorphisms, M/F: male/ female; NOS: Newcastle-Ottawa Scale.

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Table 2. Summary estimates for the OR of Apo E polymorphism in various allele/genotype contrasts: overall analysis and subgroup analyses.

| Studies | Test of association | Test of heterogeneity | overall test |
|---------|---------------------|-----------------------|--------------|
|         | OR                  | 95%CI                 | Q            | P(Q) | I²      | Z   | P(Z) |
| Allelic analysis: e<sub>2</sub> allele vs e<sub>3</sub> allele |
| Overall | 11                  | 0.98                  | 0.79         | 1.23 | 14.42   | 0.155 | 30.60% | 0.16 | 0.872 |
| Ethnicity | Caucasian | 6                  | 1.05                  | 0.75 | 1.45 | 7.93 | 0.160 | 36.90% | 0.27 | 0.784 |
|          | Asian               | 5                  | 0.90                  | 0.65 | 1.26 | 6.16 | 0.188 | 35.10% | 0.60 | 0.551 |
| Source of controls | PB | 5                  | 0.88                  | 0.60 | 1.28 | 5.55 | 0.236 | 27.90% | 0.68 | 0.497 |
|          | HB                 | 6                  | 1.04                  | 0.78 | 1.40 | 8.32 | 0.139 | 39.90% | 0.28 | 0.780 |
| Genotyping Method | PCR-RFLP | 6                  | 0.98                  | 0.79 | 1.23 | 9.24 | 0.100 | 45.90% | 0.07 | 0.942 |
|          | Others             | 5                  | 0.99                  | 0.69 | 1.41 | 5.14 | 0.274 | 22.10% | 0.19 | 0.846 |
| HW-E Yes | 8                  | 1.02                  | 0.80 | 1.29 | 10.33 | 0.171 | 32.20% | 0.15 | 0.884 |
|          | No                 | 3                  | 0.77                  | 0.39 | 1.54 | 3.60 | 0.166 | 44.40% | 0.74 | 0.458 |
| Type of POAG | HTG | 6                  | 0.81                  | 0.52 | 1.26 | 11.30 | 0.046 | 55.70% | 0.93 | 0.352 |
|          | NTG              | 4                  | 1.00                  | 0.72 | 1.37 | 1.52 | 0.677 | 0.00% | 0.03 | 0.975 |
| Allelic analysis: e<sub>4</sub> allele vs e<sub>3</sub> allele |
| Overall | 12                  | 1.05                  | 0.78         | 1.41 | 36.68 | <0.001 | 70.00% | 0.33 | 0.743 |
| Ethnicity | Caucasian | 7                  | 1.07                  | 0.76 | 1.51 | 13.88 | 0.031 | 56.80% | 0.37 | 0.709 |
|          | Asian               | 5                  | 1.06                  | 0.61 | 1.82 | 21.62 | <0.001 | 81.50% | 0.20 | 0.844 |
| Source of controls | PB | 5                  | 1.18                  | 0.71 | 1.94 | 13.29 | 0.010 | 69.90% | 0.64 | 0.524 |
|          | HB                 | 7                  | 0.97                  | 0.67 | 1.40 | 19.71 | 0.003 | 69.60% | 0.17 | 0.868 |
| Genotyping Method | PCR-RFLP | 7                  | 1.11                  | 0.71 | 1.75 | 28.20 | <0.001 | 78.70% | 0.46 | 0.643 |
|          | Others             | 5                  | 1.01                  | 0.70 | 1.48 | 8.25 | 0.083 | 51.50% | 0.07 | 0.943 |
| HW-E Yes | 9                  | 1.03                  | 0.76 | 1.39 | 23.09 | 0.003 | 65.40% | 0.19 | 0.851 |
|          | No                 | 3                  | 1.03                  | 0.36 | 2.96 | 12.71 | 0.002 | 84.30% | 0.05 | 0.961 |
| Type of POAG | HTG | 6                  | 1.09                  | 0.67 | 1.78 | 17.74 | 0.003 | 71.80% | 0.36 | 0.715 |
|          | NTG              | 4                  | 1.07                  | 0.59 | 1.93 | 10.24 | 0.017 | 70.70% | 0.21 | 0.832 |
| Genotypic analysis: e<sub>2</sub> carrier vs e<sub>3</sub>/e<sub>3</sub> |
| Overall | 10                  | 0.91                  | 0.66         | 1.25 | 16.35 | 0.060 | 45.00% | 0.61 | 0.543 |
| Ethnicity | Caucasian | 5                  | 1.04                  | 0.69 | 1.55 | 5.36 | 0.252 | 25.40% | 0.17 | 0.867 |
|          | Asian               | 5                  | 0.76                  | 0.44 | 1.32 | 10.67 | 0.031 | 62.50% | 0.97 | 0.331 |
| Source of controls | PB | 4                  | 0.77                  | 0.48 | 1.24 | 2.52 | 0.472 | 0.00% | 1.07 | 0.287 |
|          | HB                 | 6                  | 0.97                  | 0.64 | 1.46 | 12.75 | 0.026 | 60.80% | 0.15 | 0.883 |
| Genotyping Method | PCR-RFLP | 6                  | 0.87                  | 0.51 | 1.48 | 13.69 | 0.018 | 63.50% | 0.53 | 0.599 |
|          | Others             | 4                  | 0.97                  | 0.69 | 1.37 | 2.66 | 0.447 | 0.00% | 1.05 | 0.879 |
| HW-E Yes | 8                  | 0.91                  | 0.65 | 1.29 | 15.20 | 0.033 | 54.00% | 0.52 | 0.604 |
|          | No                 | 2                  | 0.72                  | 0.18 | 2.91 | 1.03 | 0.310 | 3.20% | 0.47 | 0.640 |
| Type of POAG | HTG | 6                  | 0.84                  | 0.49 | 1.42 | 9.72 | 0.083 | 48.60% | 0.65 | 0.513 |
|          | NTG              | 4                  | 0.89                  | 0.61 | 1.31 | 0.56 | 0.906 | 0.00% | 0.58 | 0.564 |
| Genotypic analysis: e<sub>4</sub> carrier vs e<sub>3</sub>/e<sub>3</sub> |
| Overall | 11                  | 1.08                  | 0.74         | 1.57 | 35.15 | <0.001 | 71.60% | 0.39 | 0.694 |
| Ethnicity | Caucasian | 6                  | 1.11                  | 0.73 | 1.69 | 9.65 | 0.086 | 48.20% | 0.50 | 0.620 |
|          | Asian               | 5                  | 1.08                  | 0.56 | 2.11 | 23.84 | <0.001 | 83.20% | 0.23 | 0.820 |
| Source of controls | PB | 4                  | 1.62                  | 0.62 | 4.28 | 15.11 | 0.002 | 80.10% | 0.98 | 0.327 |
|          | HB                 | 7                  | 0.90                  | 0.62 | 1.30 | 15.97 | 0.014 | 62.40% | 0.58 | 0.561 |
| Genotyping Method | PCR-RFLP | 7                  | 1.04                  | 0.63 | 1.74 | 26.08 | <0.001 | 77.00% | 0.16 | 0.872 |
|          | Others             | 4                  | 1.19                  | 0.70 | 2.02 | 6.44 | 0.092 | 53.40% | 0.65 | 0.518 |
| HW-E Yes | 9                  | 0.99                  | 0.70 | 1.39 | 22.88 | 0.004 | 64.10% | 0.07 | 0.946 |
|          | No                 | 2                  | 1.65                  | 0.14 | 19.81 | 10.03 | 0.002 | 90.00% | 0.40 | 0.692 |
substantial heterogeneity in both comparisons ($Z = 0.543$) and $1.08$ (95% CI: $0.74–1.57$; $Z = 0.694$), showed that the pooled OR remained centered on 1 with not changed substantially, suggesting a high stability of the meta-analysis. Even after the deletion of any single study, the random-effect estimates were not materially changed, indicating that Apo E genotype is unlikely risk variants for POAG (The data is not shown but is available on request.).

### Publication Bias.
Publication bias were qualitatively assessed by Begg’s funnel plot and quantitatively assessed by Egger’s test. Neither Begg’s funnel plot nor Egger’s test detected obvious evidence of publication bias n relation to allele or genotype. (shown in Figure S5).

### Discussion
The pathogenesis of POAG is complex and genetic factor play an important role in POAG susceptibility. An increasing number of articles on genetic association studies, genome-wide association studies (GWAS), and relate meta-analyses have been published to clarify the association between gene polymorphisms and POAG [23,24]. To the authors’ knowledge, this is the first meta-analysis investigating the association between Apo E $e2/e3/e4$ polymorphisms and POAG. However, no significant association was found between Apo E polymorphisms and POAG after merging the results from 12 case-control studies. In subgroup analysis, associations between Apo E polymorphisms and POAG were also negative, although the number of articles included in this meta-analysis was limited.

Apo E is the major apolipoprotein of the central nervous system, where it is synthesized by glia, macrophages, and neurons [25]. In the rat eye, Apo E has been demonstrated to be synthesized by Muller cells, and secreted into the vitreous, where lipoproteins are assembled [26]. Apo E is absorbed by the ganglion cells (RGC), transported down the optic nerve, and may have a role in axonal nutrition [27]. There is a considerable body of evidence to show that Apo E genotype affects vulnerability of neurons to ischemia, survival and recovery after head injury, as well as its role in Alzheimer’s disease [28]. Possession of the $e4$ allele was shown to be associated with a reduced outcome after traumatic head injury and increased risk of earlier development of Alzheimer’s disease [29]. Several case-control studies have investigated the association between Apo E polymorphism and risks of POAG. Some of them showed positive results, while others found no association. There are also some studies demonstrated that common polymorphisms in MYOC, OPTN, and Apo E might interactively contribute to POAG. Fan et al [18] observed that Apo E $e2/e3/e4$ interact with OPTN Arg545Gln and MYOC -83G/A, Jia et al [15] identified another interactions between MYOC -83G>A and Apo E $e2/e3/e4$. However, neither allele frequency nor genotype distribution was significantly associated with susceptibility to POAG in this meta-analysis.

Heterogeneity is a potential problem that may affect the interpretation of the results. In our meta-analysis, significant heterogeneity was detected in some comparisons. To eliminate heterogeneity, we carried out subgroup analysis and used a random-effects model to pool the results whenever significant heterogeneity was present. Although the publication bias was maximally avoided, presence of between-study heterogeneity could not be fully explained by our subgroup analysis. It is unclear what factors contribute to the conflicting results reported in these studies. We speculate that several factors account for heterogeneity. Firstly, the diversity in the population characteristics may account for it. Different populations have different genetic backgrounds, which contribute to genetic heterogeneity. Secondly, environmental exposures and diet might play role in these differences as well [30,31]. In addition, some unpublished, eligible publications were not available in the present meta-analysis, which might affect the results. Thus, the results should be considered with caution, and in the future, more studies should be performed to assess these results.

Genome-wide association studies (GWAS) are a powerful tool for the identification of genetic risk factors for complex disease. POAG genetics has been the subject of several large scale GWAS in the past several years, and none has implicated Apo E [32,33,34,35,36,37,38]. Consistent with these studies, despite being an attractive candidate gene, our meta-analysis results do not support Apo E $e2/e3/e4$ to have a major effect to POAG susceptibility.

However, caution should be made when interpreting the results due to some limitations of this study. Firstly, POAG is a multifactorial disease that results from complex interactions between various genetic and environmental factors. Our results were based on unadjusted estimates, data were not stratified by other factors such as gender status, major systemic illness and family history, because sufficient information could not be extracted from the...
original studies. Secondly, this meta-analysis was limited by the number of cases and controls as well as small sample size, especially in subgroup analysis. However, given the available sample sizes and data, the study had 79% power to show a statistically association. This suggests that increasing the sample size would not change current results. All the included studies were carried out in Asians and Caucasians. Thus, the results may be applicable to these ethnic populations only. Thirdly, controls were not uniformly defined. This study is a meta-analysis of case-control studies, only 5 were population-based. Thus, some inevitable selection bias might exist in the results, and they may not be representative of the general population. Fourthly, all included studies were case-control design, which precludes further comments on cause-effect relationship. The results of long-term prospective, designed for the investigation of gene–gene and gene-environment interactions, in different ethnicity subgroups might produce more conclusive claims about the association between Apo E and POAG.

Despite these limitations, this study also has some advantages. First, it provides pooled data on a substantial number of cases and controls for better understanding the association between Apo E polymorphism and POAG. In addition, the methodological issues for meta-analysis, such as, heterogeneity, publication bias, and stability of results were all well investigated. In conclusion, despite the limitation, this meta-analysis suggests that Apo E e2/e3/e4 polymorphisms may not be associated with the risk of POAG. However, to reach a definitive conclusion, well-designed studies with larger sample size and more ethnic groups should be considered to further clarify the association. Moreover, gene–gene and gene-environment interactions studies should also be considered in future studies.

**Supporting Information**

*Figure S1* Forest plot for alleles of apolipoprotein E (Apo E) polymorphism and POAG risk in the overall study (e2 allele vs e3 allele). (TIF)

*Figure S2* Forest plot for alleles of apolipoprotein E (Apo E) polymorphism and POAG risk in the overall study (e2 allele vs e3 allele). (TIF)

*Figure S3* Forest plot for genotypes of apolipoprotein E (Apo E) polymorphism and POAG risk (e2 carrier vs e3/e3). (TIF)

*Figure S4* Forest plot for genotypes of apolipoprotein E (Apo E) polymorphism and POAG risk (e4 carrier vs e3/e3). (TIF)

*Figure S5* Begg’s funnel plots of publication bias analyses. (DOC)

**Table S1** MOOSE checklist. (DOC)

**Table S2** The distribution of the ApoE genotypes and allele frequencies for cases and controls. (DOC)

**Author Contributions**

Conceived and designed the experiments: WW MZ XZ. Performed the experiments: WW MZ WH. Analyzed the data: WW MZ. Contributed reagents/materials/analysis tools: MZ WH SC. Wrote the paper: WW.

**References**

1. Qiujie HA, Broman AT (2006) The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 90: 262–267.
2. Gremmenucci M, Yang Y, Lotery AJ (2012) Current concepts on primary open-angle glaucoma genetics: a contribution to disease pathophysiology and future treatment. Eye (London) 26: 355–369.
3. Burdon KP (2012) Genome-wide association studies in the hunt for genes for primary open-angle glaucoma. J Glaucoma 15: 218–222.
4. Ward A, Crean S, Mercaldi CJ, Collins JM, Boyd D, et al. (2012) Prevalence of apolipoprotein E4 genotype and homozygotes (APOE e4/e4) among patients diagnosed with Alzheimer’s disease: a systematic review and meta-analysis. Neuroepidemiology 38: 1–17.
5. Sadigh-Ezghad S, Talebi M, Farhoudi M (2012) Association of apolipoprotein E epsilon 4 allele with sporadic late onset Alzheimer’s disease. A meta-analysis. Neurosciences (Riyadh) 17: 321–328.
6. Tsolaki F, Gogaki E, Tiganita S, Skaritaroudi C, Lopatztzidt C, et al. (2011) Alzheimer’s disease and primary open-angle glaucoma: is there a connection? Clin Ophthalmol 5: 987–990.
7. Junemann A, Bleich S, Reulbach U, Henkel K, Wakili N, et al. (2004) Prospective case control study on genetic association of apolipoprotein epsilon2 with intraocular pressure. Br J Ophthalmol 88: 581–582.
8. Mabschi F, Tang S, Ando D, Yamakita M, Wang J, et al. (2005) The apolipoprotein E gene polymorphism is associated with open angle glaucoma in the Japanese population. Mol Vis 11: 609–612.
9. Yuan HP, Xiao Z, Yang TC, et al. (2006) Association of apolipoprotein E polymorphisms with normal tension glaucoma in a Chinese population. J Glaucoma 15: 218–222.
10. Saglar E, Yucel D, Bozkurt B, Ozgul RK, Irkec M, et al. (2009) Association of polymorphisms in APOE, p53, and p21 with primary open-angle glaucoma in Turkish patients. Mol Vis 15: 1270–1276.
11. Jia IY, Tam PO, Chia SW, Ding N, Chen LJ, et al. (2009) Multiple gene polymorphisms analysis revealed a different profile of generic polymorphisms of primary open-angle glaucoma in northern Chinese. Mol Vis 15: 89–98.
12. Zetterberg M, Tasa G, Palmer MS, Juronen E, Teesalu P, et al. (2007) Apolipoprotein E polymorphisms in patients with primary open-angle glaucoma. Am J Ophthalmol 143: 1059–1060.
13. Hu Y (2007) The APOE gene and its interactions with SNPs of other genes in primary open angle glaucoma and age-related macular degeneration [Master degree]. Shantou, China: Joint Shantou International Eye Center of Shantou University and Chinese University of Hongkong.
14. Fan BJ, Wang DY, Fan DS, Tam PO, Lam DS, et al. (2005) SNPs and interaction analyses of myocilin, optineurin, and apolipoprotein E in primary open angle glaucoma patients. Mol Vis 11: 625–631.
15. Lake S, Laverani E, Desai M, Casson R, James B, et al. (2004) Normal tension glaucoma is not associated ith the common apolipoprotein E gene polymorphisms. Br J Ophthalmol 88: 491–493.
16. Resinotis T, Griffiths PG, Birch M, Keers S, Chimney PF (2004) The role of apolipoprotein E gene polymorphisms in primary open-angle glaucoma. Arch Ophthalmol 122: 258–261.
17. Stang A (2010) Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 25: 603–605.
18. Gremmenucci M, Yang Y, Lotery AJ (2012) Current concepts on primary open-angle glaucoma genetics: a contribution to disease pathophysiology and future treatment. Eye (London) 26: 355–369.

Supporting Information

*Figure S1* Forest plot for alleles of apolipoprotein E (Apo E) polymorphism and POAG risk in the overall study (e2 allele vs e3 allele). (TIF)

*Figure S2* Forest plot for alleles of apolipoprotein E (Apo E) polymorphism and POAG risk in the overall study (e2 allele vs e3 allele). (TIF)

*Figure S3* Forest plot for genotypes of apolipoprotein E (Apo E) polymorphism and POAG risk (e2 carrier vs e3/e3). (TIF)

*Figure S4* Forest plot for genotypes of apolipoprotein E (Apo E) polymorphism and POAG risk (e4 carrier vs e3/e3). (TIF)

*Figure S5* Begg’s funnel plots of publication bias analyses. (DOC)

**Table S1** MOOSE checklist. (DOC)

**Table S2** The distribution of the ApoE genotypes and allele frequencies for cases and controls. (DOC)
24. Burdon KP (2012) Genome-wide association studies in the hunt for genes causing primary open-angle glaucoma: a review. Clin Experiment Ophthalmol 40: 358–363.

25. Xuan C, Zhang BB, Li M, Deng KF, Yang T, et al. (2011) No association between APOE epsilon 4 allele and multiple sclerosis susceptibility: a meta-analysis from 5472 cases and 4727 controls. J Neurol Sci 308: 110–116.

26. Amaratunga A, Abraham CR, Edwards RB, Sandell JH, Schreiber BM, et al. (1996) Apolipoprotein E is synthesized in the retina by Muller glial cells, secreted into the vitreous, and rapidly transported into the optic nerve by retinal ganglion cells. J Biol Chem 271: 5628–5632.

27. Lorber B, Berry M, Douglas MR, Nakazawa T, Logan A (2009) Activated retinal glia promote neurite outgrowth of retinal ganglion cells via apolipoprotein E. J Neurosci Res 87: 2645–2652.

28. Mahley RW, Huang Y (2012) Apolipoprotein E sets the stage: response to injury triggers neuropathology. Neuron 76: 871–885.

29. Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. Nat Rev Neurol 9: 106–118.

30. Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, et al. (2008) Assessment of cumulative evidence on genetic associations: interim guidelines. Int J Epidemiol 37: 120–132.

31. Daly AK, Day CP (2001) Candidate gene case-control association studies: advantages and potential pitfalls. Br J Clin Pharmacol 52: 489–499.

32. Scheetz TE, Fingeret JH, Wang K, Kurki MH, Knudtson KL, et al. (2013) A genome-wide association study for primary open angle glaucoma and macular degeneration reveals novel loci. PLoS One 8: e58657.

33. Wiggs JL, Hsueh MA, Abdabou W, Allingham RR, Budenz DL, et al. (2012) The NEIGHBOR Consortium Primary Open-Angle Glaucoma Genome-wide Association Study: Rationale, Study Design, and Clinical Variables. J Glaucoma.

34. Gibson J, Griffiths H, De Salvo G, Cole M, Jacob A, et al. (2012) Genome-wide association study of primary open angle glaucoma risk and quantitative traits. Mol Vis 18: 1083–1092.

35. Osman W, Low SK, Takahashi A, Kubo M, Nakamura Y (2012) A genome-wide association study in the Japanese population confirms 9q21 and 14q23 as susceptibility loci for primary open angle glaucoma. Hum Mol Genet 21: 2836–2842.

36. Burdon KP, Macgregor S, Hewitt AW, Sharma S, Chadlow G, et al. (2011) Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. Nat Genet 43: 574–578.

37. Meguro A, Inoko H, Ota M, Mizuki N, Bahram S (2010) Genome-wide association study of normal tension glaucoma: common variants in SRBD1 and ELOVL5 contribute to disease susceptibility. Ophthalmology 117: 1331–1338.

38. Nakano M, Ikeda Y, Taniguchi T, Yagi T, Fuwa M, et al. (2009) Three susceptible loci associated with primary open-angle glaucoma identified by -wide association study in a Japanese population. Proc Natl Acad Sci U S A 106: 12038–12042.