Association of Common Variants in eNOS Gene with Primary Open Angle Glaucoma: A Meta-Analysis

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1. Introduction

Glaucoma is a common, complex, heterogenous disease and it constitutes the major cause of irreversible blindness worldwide [1]. In 2013, the number of people (aged 40–80 years) with glaucoma was estimated to be 64.3 million, increasing to 76.0 million in 2020 and 111.8 million in 2040, disproportionately affecting people residing in Asia and Africa [2]. Primary open angle glaucoma (POAG), the most common type of glaucoma in all populations, is characterized by progressive damage of retinal ganglion cells (RGCs) and their axons, leading to the pathognomonic remodeling of the optic nerve head and subsequent irrevocable vision loss [3]. The known risk factors for POAG include a higher age, African ancestry, refractive error, and a positive family history for glaucoma, apart from elevated intraocular pressure (IOP), an established risk contributor [4–6]. Furthermore, there is growing evidence that vascular [7, 8] and genetic [9–11] components may pose a potential risk to POAG patients, including both those with normal and elevated IOP.

Nitric oxide (NO) is an active biologic agent involved in diverse physiologic processes [12]. NO generated by endothelial nitric oxide synthase (eNOS) has been found to contribute to vasodilatation, increased local blood flow, and decreased vascular resistance in ocular circulation [13, 14]. Hence, changes in the activity of eNOS determined by genetic variations and environmental factors may play an important role in the pathogenesis of glaucoma. Several studies were conducted to evaluate the association of eNOS polymorphisms with risk of POAG but presented inconsistent results [15–20]. During seven functional single-nucleotide polymorphisms (SNPs) reported in relevant studies, the controversy was mainly centered on the two most important SNPs, T-786C (rs2070744) and Glu298Asp (rs1799983). Thus the current
meta-analysis aims to assess the strength of the evidence for an effect of these two polymorphisms on POAG risk by combining data from all relevant eligible studies.

2. Methods

2.1. Literature Search. A systematic literature search was conducted in the MEDLINE, EMBASE, and Web of Science databases (accessed on November 30, 2015) with the following free words and MeSH terms: “glaucoma”, “open angle”, “Endothelial nitric oxide synthase”, “eNOS”, “polymorphism(s)”, “single nucleotide polymorphism”, and “SNP”. We also supplemented our search by screening the reference lists of all the retrieved studies, as well as genome-wide association studies (GWAS) performed for glaucoma to which we have the access.

2.2. Inclusion and Exclusion Criteria. Eligible studies were included if they (1) evaluated the association between eNOS and POAG; (2) compared unrelated POAG cases and normal controls identified by complete ophthalmological examination in defined populations; (3) provided an odds ratio (OR) with 95% confidence interval (CI) in case and control groups, respectively, or other data which could be calculated to estimate an OR; and (4) were original research articles. All animal studies, case reports, abstracts from conferences, full-texts with incomplete data, and reviews were excluded. As for duplicate studies retrieving data from the same source, ones with available data and the largest sample size were brought into the analysis list. Although we did not define language in the review process, the articles in the final analysis were all in English.

2.3. Literature Review and Data Extraction. Two investigators (Yang Xiang and Yi Dong) extracted data from the retrieved records and confirmed the validity of the included articles independently. The following variables were extracted: author, year of publication, ethnicity of subjects, demographic information, the numbers of cases and controls, results of Hardy-Weinberg equilibrium (HWE) test when reported, and the allele and genotype counts or frequencies of each SNP. When the allele or genotype counts were not given specially in some articles, they were calculated from the frequencies and then rounded to the nearest integer. A final review was performed by other reviewers (Xuan Li and Xin Tang) while the discrepancy was resolved through discussion.

2.4. Meta-Analysis and Test for Potential Bias. The Chi-square test was utilized to check whether the genotype distribution in controls was consistent with HWE for studies that did not report relevant data. To assess the strength of association between the polymorphisms (rs2070744 and rs1799983) of eNOS gene and POAG susceptibility, we estimated crude OR with its 95% CI under allele model (T versus C, G versus A), homozygote model (TT versus CC, GG versus AA), heterozygote model (TC versus CC, GA versus AA), dominant model (TT + TC versus CC, GG + GA versus AA), and recessive model (TT versus TC + CC, GG versus GA + AA), respectively.

Interstudy heterogeneity was detected using the Chi-square-based Q statistic as well as the $I^2$ metric. If $P_Q \leq 0.10$ or $I^2 > 50\%$, which indicated significant heterogeneity in the comparison models among studies [21], the pooled ORs were calculated with a random-effects model (DerSimonian and Laird method) [22]. Otherwise, the fixed-effects model was considered more appropriate (Mantel-Haenszel method) [23]. We also conducted subgroup analysis based on ethnicity, as well as sex where applicable. Publication bias was investigated by Begg’s tests and Egger’s linear regression test [24, 25]. The statistical analysis was done with Stata 12.0 and the values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Literature Search and Characteristics. The workflow and results of the literature review are shown in Figure 1. A total of thirty-one records were initially identified for the meta-analysis. Of the thirty-one, twenty-six studies were excluded due to duplicated publications, unsuitable titles or abstracts, or incomplete data. In total, five eligible studies [15–19] were included and reviewed. Seven SNPs of eNOS gene, including rs2070744, rs1799983, rs743507, rs3793342, rs7830, rs1771443, and rs3918188, were evaluated for possible association with POAG while five SNPs, apart from rs2070744 and rs1799983, were reported in only one or two studies, the data of which were interpreted to be insufficient to perform a qualified meta-analysis. Consequently, the combined study population investigating rs2070744 (consisting of 1156 cases and 1879 controls) and rs1799983 (consisting of 1230 cases and 2035 controls) are involved in our meta-analysis, and the detailed characteristics of the included studies are listed in Tables 1 and 2.

3.2. Meta-Analysis Results. Tables 3 and 4 show the summary results for the association between eNOS rs2070744 and rs1799983 and risk for POAG. Low heterogeneity was present among all the publications involved for all the genetic models (Tables 3 and 4). Thus, the data were combined using the fixed-effects model. For rs2070744, the data was pooled from 4 sample collections without HWE deviation, and the overall results showed significant association between rs2070744 and POAG (OR = 0.736, 95% CI = 0.594–0.912 for T-allele versus C allele (Figure 2(a)); OR = 0.498, 95% CI = 0.296–0.838 for TT versus CC (Figure 2(b))); OR = 0.573, 95% CI = 0.348–0.943 for TT + CC versus CC (Figure 2(d)); OR = 0.746, 95% CI = 0.575–0.967 for TT versus TT + TC (Figure 2(e))). Statistically significant association was also observed between rs1799983 and POAG (OR = 0.753, 95% CI = 0.568–0.997 for GG versus AA (Figure 3(b))); OR = 0.745, 95% CI = 0.559–0.993 for GA versus AA (Figure 3(c))); OR = 0.752, 95% CI = 0.576–0.983 for GG + GA versus AA (Figure 3(d))).

To further explore the association, stratified analysis was performed based on ethnicity (Caucasians and Asians) and sex. For rs2070744, the results showed that the association between rs2070744 and POAG was significant in Caucasians.
Records identified through database searching (n = 31)

Records screened (n = 12)

Records after duplicates removed (n = 12)

Studies included in qualitative synthesis (n = 5)

Studies included in quantitative synthesis (meta-analysis) (n = 5)

Records excluded for unsuited titles or abstracts (n = 5)

Full-text articles excluded with incomplete data (n = 2)

Figure 1: PRISMA flow diagram of studies included in the meta-analysis.

Table 1: Principle characteristics of the studies included in the meta-analysis for association between eNOS rs2070744 and POAG.

| First author          | Cohorts | Year | Ethnicity   | Genotyping | Case Size | GG | GA | AA | MAF | Control Size | GG | GA | AA | MAF | HWE (p) |
|-----------------------|---------|------|-------------|------------|-----------|----|----|----|-----|-------------|----|----|----|-----|--------|
| Fan [16]              | All     | 2010 | Asians      | NA         | 397       | 319| 72 | 6  | 0.11| 201         | 157| 43 | 1  | 0.11| 0.44   |
| Kang [17]             | All     | 2011 | Caucasians  | Taqman     | 510       | 236| 203| 71 | 0.34| 1444        | 682| 598| 164| 0.32| 0.28   |
|                        | Male    | 2011 | Caucasians  | Taqman     | 147       | 72 | 60 | 15 | 0.31| 425         | 203| 170| 52 | 0.32| 0.06   |
|                        | Female  | 2011 | Caucasians  | Taqman     | 363       | 164| 143| 56 | 0.35| 1019        | 479| 428| 112| 0.32| 0.08   |
| Magalhães da Silva [18]| All     | 2012 | Caucasians  | Taqman     | 89        | 55 | 27 | 7  | 0.23| 124         | 72 | 46 | 6  | 0.23| 0.28   |
|                        | Male    | 2012 | Caucasians  | Taqman     | 28        | 20 | 7  | 1  | 0.16| 63          | 35 | 24 | 4  | 0.25| 0.70   |
|                        | Female  | 2012 | Caucasians  | Taqman     | 61        | 35 | 22 | 4  | 0.25| 61          | 37 | 23 | 1  | 0.20| 0.97   |
| Emam [19]             | All     | 2014 | Caucasians  | PCR-RFLP   | 160       | 81 | 59 | 20 | 0.31| 110         | 63 | 37 | 10 | 0.26| 0.22   |
|                        | Male    | 2014 | Caucasians  | PCR-RFLP   | 76        | 41 | 24 | 11 | 0.11| 56          | 32 | 19 | 5  | 0.11| 0.19   |
|                        | Female  | 2014 | Caucasians  | PCR-RFLP   | 84        | 40 | 35 | 9  | 0.34| 54          | 31 | 18 | 5  | 0.32| 0.39   |

NA: data not available; MAF: Minor Allele Frequency; HWE: Hardy-Weinberg equilibrium; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.
Table 2: Principle characteristics of the studies included in the meta-analysis for association between eNOS rs1799983 and POAG.

| First author | Cohorts | Year | Ethnicity | Genotyping | Case Size | TT | TC | CC | MAF | Control Size | TT | TC | CC | MAF | HWE (p) |
|--------------|---------|------|-----------|------------|-----------|-----|----|----|-----|--------------|-----|----|----|-----|--------|
| Lin [15]     | All     | 2005 | Asians    | Taqman     | 66        | 55  | 11 | 0  | 0.08| 100          | 84  | 16 | 0  | 0.08| 0.38   |
| Fan [16]     | All     | 2010 | Asians    | NA         | 397       | 310 | 10 | 2  | 0.08| 201          | 157 | 4  | 0.12| 0.38| 0.45   |
| Kang [17]    | All     | 2011 | Caucasians| Taqman     | 356       | 137 | 166| 62 | 0.40| 1044         | 414 | 458| 172 | 0.38| 0.02   |
|              | Male    | 2011 | Caucasians| Taqman     | 202       | 235 | 84 | 2  | 0.08| 1501         | 580 | 673| 248 | 0.39| 0.03   |
|              | Female  | 2011 | Caucasians| Taqman     | 283       | 493 | 85 | 2  | 0.08| 1501         | 580 | 673| 248 | 0.39| 0.03   |
| Magalhães da Silva [18] | All     | 2012 | Caucasians| Taqman     | 169       | 225 | 105| 55 | 0.08| 123          | 74  | 38 | 11  | 0.24| 0.07   |
|              | Male    | 2012 | Caucasians| Taqman     | 52        | 98  | 53 | 2  | 0.08| 61           | 35  | 22 | 4   | 0.25| 0.83   |
|              | Female  | 2012 | Caucasians| Taqman     | 117       | 195 | 57 | 8  | 0.08| 61           | 35  | 22 | 4   | 0.25| 0.83   |
| Emam [19]    | All     | 2014 | Caucasians| PCR-RFLP   | 160       | 63  | 59 | 38 | 0.08| 110          | 60  | 38 | 12  | 0.28| 0.12   |
|              | Male    | 2014 | Caucasians| PCR-RFLP   | 76        | 32  | 26 | 18 | 0.08| 56           | 30  | 19 | 7   | 0.08| 0.17   |
|              | Female  | 2014 | Caucasians| PCR-RFLP   | 84        | 31  | 33 | 20 | 0.08| 54           | 30  | 19 | 5   | 0.12| 0.44   |

NA: data not available; MAF: Minor Allele Frequency; HWE: Hardy-Weinberg equilibrium; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism.

Table 3: Summary risk estimates for association between eNOS rs2070744 and POAG.

| Comparisons | Studies (n) | Model | Pooled estimate OR (95% CI) | \( p_Z \) | Heterogeneity | \( I^2 \) (%) | \( p_Q \) | Egger’s test (p) | Begg’s test (p) |
|-------------|-------------|-------|-----------------------------|--------|---------------|----------------|--------|-----------------|-----------------|
| Overall     |             |       |                             |        |               |                |        |                 |                 |
| T versus C  | 4           | F     | 0.736 (0.594–0.912)        | 0.005  | 44.8          | 0.143          | 0.724  | 0.734           |
| TT versus CC| 4           | F     | 0.498 (0.296–0.838)        | 0.009  | 21.6          | 0.279          | 0.236  | 1.000           |
| TC versus CC| 4           | F     | 0.725 (0.425–1.239)        | 0.240  | 25.0          | 0.264          | 0.561  | 1.000           |
| TT + TC versus CC | 4 | F | 0.573 (0.348–0.943) | 0.029 | 25.1 | 0.263 | 0.379 | 1.000 |
| TT versus TC + CC | 4 | F | 0.746 (0.575–0.967) | 0.027 | 34.6 | 0.205 | 0.873 | 1.000 |
| Female      |             |       |                             |        |               |                |        |                 |                 |
| T versus C  | 2           | F     | 0.490 (0.333–0.721)        | 0.000  | 0.0           | 0.887          |        |                 |                 |
| TT versus CC| 2           | F     | 0.268 (0.112–0.642)        | 0.003  | 0.0           | 0.913          |        |                 |                 |
| TC versus CC| 2           | F     | 0.489 (0.200–1.196)        | 0.117  | 0.0           | 0.732          |        |                 |                 |
| TT + TC versus CC | 2 | F | 0.328 (0.144–0.749) | 0.008 | 0.0  | 0.764 |        |                 |
| TT versus TC + CC | 2 | F | 0.423 (0.260–0.689) | 0.001 | 0.0 | 0.896 |        |                 |
| Male        |             |       |                             |        |               |                |        |                 |                 |
| T versus C  | 2           | F     | 0.711 (0.467–1.081)        | 0.111  | 8.4           | 0.296          |        |                 |                 |
| TT versus CC| 2           | F     | 0.496 (0.211–1.175)        | 0.111  | 0.0           | 0.452          |        |                 |                 |
| TC versus CC| 2           | F     | 0.604 (0.244–1.496)        | 0.276  | 0.0           | 0.623          |        |                 |                 |
| TT + TC versus CC | 2 | F | 0.513 (0.230–1.144) | 0.103 | 0.0  | 0.476 |        |                 |
| TT versus TC + CC | 2 | F | 0.688 (0.406–1.165) | 0.164 | 0.0 | 0.328 |        |                 |
| Asians      |             |       |                             |        |               |                |        |                 |                 |
| T versus C  | 2           | F     | 0.971 (0.695–1.358)        | 0.864  | 0.0           | 0.967          |        |                 |                 |
| TT versus CC| 2           | F     | 0.790 (0.244–2.558)        | 0.694  | —             | —              |        |                 |                 |
| TC versus CC| 2           | F     | 0.770 (0.227–2.610)        | 0.675  | —             | —              |        |                 |                 |
| TT + TC versus CC | 2 | F | 0.786 (0.243–2.537) | 0.687 | —  | — |        |                 |
| TT versus TC + CC | 2 | F | 0.990 (0.684–1.431) | 0.955 | 0.0 | 0.921 |        |                 |
| Caucasians  |             |       |                             |        |               |                |        |                 |                 |
| T versus C  | 2           | F     | 0.607 (0.460–0.803)        | 0.001  | 3.2           | 0.310          |        |                 |                 |
| TT versus CC| 2           | F     | 0.444 (0.249–0.791)        | 0.006  | 46.1          | 0.173          |        |                 |                 |
| TC versus CC| 2           | R     | 0.715 (0.394–1.296)        | 0.269  | 62.4          | 0.103          |        |                 |                 |
| TT + TC versus CC | 2 | R | 0.534 (0.308–0.925) | 0.025 | 57.7 | 0.124 |        |                 |
| TT versus TC + CC | 2 | F | 0.563 (0.390–0.812) | 0.002 | 0.0  | 0.813 |        |                 |

OR: odds ratio; CI: confidence interval; \( p_Z \): \( p \) value for Z test; \( p_Q \): \( p \) value for Q-test; F: fixed-effects mode; R: random-effects model; —: data not available.
Table 4: Summary risk estimates for association between eNOS rs1799983 and POAG.

| Comparisons                  | Studies (n) | Model | Pooled estimate OR (95% CI) | 𝑃𝑍 | Heterogeneity 𝐼 2 (%) | 𝑃𝑄 | Egger's test (𝑝) | Begg's test (𝑝) |
|------------------------------|-------------|-------|----------------------------|-----|-----------------------|-----|-----------------|---------------|
| **Overall**                  |             |       |                            |     |                       |     |                 |               |
| G versus A                   | 4           | F     | 0.928 (0.817–1.053)        | 0.247 | 0.0                   | 0.694 | 0.851           | 0.734         |
| GG versus AA                 | 4           | F     | 0.753 (0.568–0.997)        | 0.048 | 0.0                   | 0.831 | 0.039           | 0.308         |
| GA versus AA                 | 4           | F     | 0.745 (0.559–0.993)        | 0.045 | 0.0                   | 0.720 | 0.149           | 0.089         |
| GG + GA versus AA            | 4           | F     | 0.752 (0.576–0.983)        | 0.037 | 0.0                   | 0.824 | 0.033           | 0.089         |
| GG versus GA + AA            | 4           | F     | 0.979 (0.832–1.153)        | 0.803 | 0.0                   | 0.584 | 0.832           | 1.000         |
| **Female**                   |             |       |                            |     |                       |     |                 |               |
| G versus A                   | 3           | F     | 0.852 (0.724–1.003)        | 0.054 | 0.0                   | 0.869 |                |               |
| GG versus AA                 | 3           | F     | 0.665 (0.471–0.938)        | 0.020 | 0.0                   | 0.651 |                |               |
| GA versus AA                 | 3           | F     | 0.674 (0.474–0.958)        | 0.028 | 0.0                   | 0.505 |                |               |
| GG + GA versus AA            | 3           | F     | 0.666 (0.481–0.923)        | 0.015 | 0.0                   | 0.626 |                |               |
| GG versus GA + AA            | 3           | F     | 0.894 (0.720–1.108)        | 0.306 | 0.0                   | 0.684 |                |               |
| **Male**                     |             |       |                            |     |                       |     |                 |               |
| G versus A                   | 3           | F     | 1.067 (0.839–1.358)        | 0.595 | 20.7                  | 0.283 |                |               |
| GG versus AA                 | 3           | F     | 1.085 (0.641–1.836)        | 0.124 | 0.0                   | 0.431 |                |               |
| GA versus AA                 | 3           | F     | 1.041 (0.604–1.793)        | 0.885 | 0.0                   | 0.557 |                |               |
| GG + GA versus AA            | 3           | F     | 1.066 (0.643–1.768)        | 0.804 | 0.0                   | 0.456 |                |               |
| GG versus GA + AA            | 3           | F     | 1.088 (0.798–1.485)        | 0.593 | 0.0                   | 0.378 |                |               |
| **Asians**                   |             |       |                            |     |                       |     |                 |               |
| G versus A                   | 1           |       | —                          | 1.065 | 0.726–1.564           | 0.746 |                |               |
| GG versus AA                 | 1           |       | —                          | 0.339 | 0.040–2.837           | 0.682 |                |               |
| GA versus AA                 | 1           |       | —                          | 0.279 | 0.032–2.397           | 0.245 |                |               |
| GG + GA versus AA            | 1           |       | —                          | 0.326 | 0.039–2.725           | 0.301 |                |               |
| GG versus GA + AA            | 1           |       | —                          | 1.146 | 0.756–1.737           | 0.520 |                |               |
| **Caucasians**               |             |       |                            |     |                       |     |                 |               |
| G versus A                   | 3           | F     | 0.912 (0.797–1.043)        | 0.180 | 0.0                   | 0.641 |                |               |
| GG versus AA                 | 3           | F     | 0.768 (0.578–1.021)        | 0.069 | 0.0                   | 0.855 |                |               |
| GA versus AA                 | 3           | F     | 0.765 (0.572–1.023)        | 0.071 | 0.0                   | 0.776 |                |               |
| GG + GA versus AA            | 3           | F     | 0.767 (0.585–1.006)        | 0.055 | 0.0                   | 0.866 |                |               |
| GG versus GA + AA            | 3           | F     | 0.952 (0.798–1.137)        | 0.803 | 0.0                   | 0.584 |                |               |

OR: odds ratio; CI: confidence interval; 𝑃𝑍: 𝑝 value for 𝑍 test; 𝑃𝑄: 𝑝 value for 𝑄-test; F: fixed-effects mode; —: data not available.

3.3. Publication Bias. Publication bias was quantitatively assessed by Begg's tests and Egger's tests. In the overall analysis, there was no evidence of publication bias detected for rs2070744 (Table 3). With regard to rs1799983, Egger's regression test suggested a weak indication of publication bias, whereas Begg's rank correlation test did not identify evidence of substantial publication bias (Table 4).

4. Discussion

We reviewed a broad selection of publications found in electronic databases and performed a meta-analysis, in an attempt to identify the effects of polymorphisms of the eNOS gene on the pathogenesis of POAG. Five eligible studies were involved and available data in this regard were conflicting [15–19]. After the results were pooled, the main finding of this study is that TT genotype and/or T-allele in rs2070744, as well as GG genotype and/or G-allele in rs1799983, could protect individuals from POAG risk. Stratified analysis based on ethnicity showed that the association of rs2070744 with POAG remained only in Caucasians, while no association between rs1799983 and POAG was found in either Caucasians or Asians. To further explore the association, we performed subgroup analysis by sex. The results indicated that TT genotype and/or T-allele in rs2070744 and GG genotype and/or G-allele in rs1799983 were favorable factors for POAG in female group, but not in male group.
Study ID rs2070744
T versus C

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Lin et al. (2005) | 0.96 (0.43, 2.13) | 6.19 |
| Fan et al. (2010) | 0.97 (0.67, 1.41) | 29.17 |
| Magalhães da Silva et al. (2012) | 0.72 (0.47, 1.11) | 24.51 |
| Emam et al. (2014) | 0.54 (0.37, 0.78) | 40.13 |
| Overall ($I^2 = 44.8\%, p = 0.143$) | 0.74 (0.59, 0.91) | 100.00 |

Overall ($I^2 = 21.6\%, p = 0.279$)

Study ID rs2070744
TT versus CC

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.79 (0.24, 2.56) | 15.67 |
| Magalhães da Silva et al. (2012) | 0.78 (0.29, 2.09) | 21.05 |
| Emam et al. (2014) | 0.33 (0.16, 0.69) | 63.28 |
| Lin et al. (2005) | (Excluded) | 0.00 |
| Overall ($I^2 = 21.6\%, p = 0.279$) | 0.50 (0.30, 0.84) | 100.00 |

Study ID rs2070744
TC versus CC

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.77 (0.23, 2.61) | 19.03 |
| Magalhães da Silva et al. (2012) | 1.41 (0.51, 3.89) | 19.74 |
| Emam et al. (2014) | 0.49 (0.23, 1.06) | 61.23 |
| Lin et al. (2005) | (Excluded) | 0.00 |
| Overall ($I^2 = 25.0\%, p = 0.264$) | 0.73 (0.42, 1.24) | 100.00 |

Study ID rs2070744
TT+TC versus CC

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.79 (0.24, 2.54) | 15.46 |
| Magalhães da Silva et al. (2012) | 0.99 (0.38, 2.58) | 19.83 |
| Emam et al. (2014) | 0.39 (0.19, 0.79) | 64.71 |
| Lin et al. (2005) | (Excluded) | 0.00 |
| Overall ($I^2 = 25.1\%, p = 0.263$) | 0.57 (0.35, 0.94) | 100.00 |

Figure 2: Continued.
Generated by eNOS via the conversion of L-arginine to L-citrulline, NO acts as a pivotal vasodilator mediator liberated from endothelial cells of ocular blood vessels. There is evidence that constant formation of NO by eNOS provides the maintenance of a basal vasodilator tone in the optic nerve head of humans and experimental animals [26–30], which is a precondition of sufficient blood supply to this tissue. Earlier studies suggested that vascular dysregulation played an important role in the etiology of glaucoma [31, 32]. In accordance with this, Polak et al. observed the perfusion of the optic nerve head during NOS inhibition and found differences in ocular blood flow response between patients with POAG and controls, indicating an abnormal NO system and NOS activity in POAG patients [33]. Further, it was reported that the increased presence of eNOS in vascular endothelium may be neuroprotective by causing vasodilatation and increased blood flow in the glaucomatous tissue [34]. Besides, the activity of NOS in trabecular meshwork was observed in patients with POAG [35]. Based on these evidences, it is reasonable to assume that the polymorphisms of eNOS are associated with the pathogenesis of POAG.

Rs2070744 and rs1799983 are the most important identified functional polymorphisms of the eNOS. The polymorphism of the promoter region of eNOS rs2070744 has been considered to be related to nonarteritic anterior ischemic optic neuropathy (NAION), coronary spasm, myocardial infarction, and coronary artery disease [36–39]. This polymorphism reduces the transcription rate of the eNOS gene and then lowers eNOS mRNA and serum nitrite/nitrate levels [40, 41]. As for eNOS rs1799983, the polymorphism has been associated with ischemic shock, coronary spasm, coronary artery disease, myocardial infarction, and NAION [38, 39, 42–44]. As this polymorphism is located in a coding region, it might be in relation to altered eNOS function and functional changes of the endothelium [45, 46]. Several investigations to date were conducted to explore the links between these two polymorphisms and POAG but achieved inconsistent conclusions. Therefore, the present meta-analysis was performed to determine whether or not these two polymorphisms could predict susceptibility to POAG.

In our study, we observed associations between eNOS gene variants and POAG, particularly among the women, revealing some sex-related facts in pathogenesis. Several lines of evidence suggest the sexually dimorphic effects of eNOS. In a series of animal studies, the expression levels of eNOS exhibited sex disparity [47] and displayed different degrees of inhibition under the sex-dependent miR-222 regulation [48]. In a human study of 373 glaucoma cases and 1082 controls, Kang et al. found that eNOS SNPs showed significant interactions with current postmenopausal hormone use in relation to high tension POAG [49]. These findings are in line with our results. Although the basis of molecular mechanisms is not clear, we believe that there are several factors that may influence this discrepancy. Concerning biological factors, circulating estrogen may act directly on eNOS through nongenomic effects, resulting in rapid dilatation of blood vessels [50, 51]. One recent study also indicates that estrogen induces NO production via NOS activation in endothelial cells [52]. Furthermore, women in most part of the world are more likely to adopt healthy lifestyle [53–56]. For example, cigarette smoking is proved to contribute to endothelial dysfunction through the uncoupling of the eNOS-mediated synthesis of NO [57, 58] and a series of studies indicate that women obtained lower tobacco consumption than their male fellows [59–61].

For our study, we have put considerable efforts and attempted to minimize every bias and gain stable and reliable results; however, there are still some limitations. Firstly, studies involved in the present meta-analysis were limited to published full-text articles in English. We failed to track the unpublished articles or ones published in other languages to obtain data for analysis, causing an influence on
**Figure 3: Continued.**

### Study ID rs1799983

#### G versus A

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 1.07 (0.73, 1.56) | 10.17 |
| Kang et al. (2011) | 0.92 (0.79, 1.07) | 70.28 |
| Magalhães da Silva et al. (2012) | 1.02 (0.65, 1.61) | 7.42 |
| Emam et al. (2014) | 0.78 (0.53, 1.15) | 12.13 |
| Overall ($I^2 = 0.0\%, p = 0.694$) | 0.93 (0.82, 1.05) | 100.00 |

### Study ID rs1799983

#### GG versus AA

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.34 (0.04, 2.84) | 3.56 |
| Kang et al. (2011) | 0.80 (0.58, 1.10) | 76.65 |
| Magalhães da Silva et al. (2012) | 0.65 (0.21, 2.06) | 6.57 |
| Emam et al. (2014) | 0.64 (0.28, 1.47) | 13.22 |
| Overall ($I^2 = 0.0\%, p = 0.831$) | 0.75 (0.57, 1.00) | 100.00 |

### Study ID rs1799983

#### GA versus AA

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.28 (0.03, 2.40) | 4.01 |
| Kang et al. (2011) | 0.78 (0.57, 1.08) | 77.74 |
| Magalhães da Silva et al. (2012) | 0.50 (0.15, 1.65) | 7.10 |
| Emam et al. (2014) | 0.80 (0.34, 1.89) | 11.14 |
| Overall ($I^2 = 0.0\%, p = 0.720$) | 0.75 (0.56, 0.99) | 100.00 |

### Study ID rs1799983

#### GG+GA versus AA

| Study ID | OR (95% CI) | % Weight |
|----------|-------------|----------|
| Fan et al. (2010) | 0.33 (0.04, 2.73) | 3.36 |
| Kang et al. (2011) | 0.79 (0.59, 1.07) | 77.77 |
| Magalhães da Silva et al. (2012) | 0.60 (0.19, 1.84) | 6.48 |
| Emam et al. (2014) | 0.70 (0.31, 1.56) | 12.39 |
| Overall ($I^2 = 0.0\%, p = 0.824$) | 0.75 (0.58, 0.98) | 100.00 |
the completeness of the data. Secondly, although we collected and reviewed all the relevant studies, only five eligible ones were included for analysis and the sample size of the individual studies was not sufficiently large, which could increase the likelihood of type I and type II errors. As for rs2070744, we excluded one study with significant HWE deviation, further decreasing the overall sample size of our study. Therefore, our results should be interpreted with caution until these findings can be replicated in other large datasets. Stratified analysis of ethnicity and sex also encountered the similar problem due to the lack of detailed data. Despite all of these limitations, we believe our study would be beneficial to a better understanding of the association between eNOS polymorphisms and POAG. Moreover, our analysis has also revealed the limitations in the current POAG genetic studies. Hence, large-scale and well-designed studies are warranted in the future. As stated, glaucoma was estimated to disproportionately affect people in Africa and thus more research needs to be conducted in the African population. Finally, since POAG is a multifactorial disease and the roles of several genes in the pathogenesis of POAG have been established, further investigations should be performed in this direction. It is possible that specific gene-gene and gene-environment interactions may alter those associations between gene polymorphisms and POAG. We expect that as more studies become available, a more accurate estimation of the relationship of eNOS with POAG will be obtained.

In summary, the current meta-analysis suggests that TT genotype and/or T-allele in rs2070744, as well as GG genotype and/or G-allele in rs1799983, was associated with decreased risk for POAG overall and in female group. To better understand the role of genetic factors in the physiopathology of this condition, further studies are needed in large, standardized, and ethnically diverse populations.

Competing Interests
The authors declare that they have no competing interests.

Authors’ Contributions
Yang Xiang and Yi Dong contributed equally to this study.

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