Association of Glutathione S transferases Polymorphisms with Glaucoma: A Meta-Analysis

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Abstract

Background: Glutathione S transferase (GST) polymorphisms have been considered risk factors for the development of glaucoma, including primary open angle glaucoma (POAG) and other types of glaucoma. However, the results remain controversial. In this study, we have conducted a meta-analysis to assess the association between polymorphisms of GSTM1, GSTT1 and GSTP1 and glaucoma risk.

Methods: Published literature from PubMed and other databases were retrieved. All studies evaluating the association between GSTM1, GSTT1 and GSTP1 polymorphisms and glaucoma risk were included. Pooled odds ratio (OR) and 95% confidence interval (CI) were calculated using random- or fixed-effects model.

Results: Twelve studies on GSTM1 (1109 cases and 844 controls), ten studies on GSTT1 (709 cases and 664 controls) and four studies on GSTP1 (543 cases and 511 controls) were included. By pooling all the studies, either GSTM1 or GSTT1 null polymorphism was not associated with a POAG risk, and this negative association maintained in Caucasian. The GSTP1 Ile 105 Val polymorphism was significantly correlated with increased POAG risk among Caucasian in a recessive model (Val/Val vs. Ile/Ile+Ile/Val: OR, 1.62, 95%CI: 1.00–2.61). Interestingly, increased glaucoma risk was associated with the combined GSTM1 and GSTT1 null genotypes (OR, 2.20; 95% CI, 1.47–3.31), and with the combined GSTM1 null and GSTP1 Val genotypes (OR, 1.86; 95% CI, 1.15–3.01).

Conclusions: This meta-analysis suggests that combinations of GST polymorphisms are associated with glaucoma risk. Given the limited sample size, the associations between single GST polymorphism and glaucoma risk await further investigation.

Introduction

Glaucoma is a heterogeneous group of diseases characterized by the death of the retinal ganglion cells and progressive degeneration of the optic nerve. It is the second most frequent cause of irreversible blindness in the world and affects primarily the older population, estimated to affect about 80 million people worldwide by 2020 [1]. However, the etiology of glaucoma remains obscure. Risk factors for glaucoma include aging, elevated intraocular pressure, variable susceptibility of the optic nerve, vascular factors (ischemia), diabetes, myopia, cigarette smoking and positive family history [2]. Glaucoma can be inherited as a Mendelian autosomal-dominant or autosomal-recessive trait, or as a complex multifactorial trait [3]. Genetic approaches have defined the causative genes (e.g., MYOC, OPTN and WDR36) for juvenile-onset and late-onset primary open angle glaucoma (POAG) [4]. In addition to these genes, over 20 gene variants were found to be associated with glaucoma [5]. Recently, large-scale genome-wide association studies have been conducted to map the genes for glaucoma [6,7,8].

Growing evidence supports the involvement of oxidative stress as a common component of glaucomatous neurodegeneration in different subcellular compartments of retinal ganglion cells (RGCs), by acting as a second messenger and/or modulating protein function by redox modifications of downstream effectors through enzymatic oxidation of specific substrates [9]. There are many defensive mechanisms against this oxidative damage, including catalase, superoxide dismutase, glutathione peroxidase, and glutathione S transferase (GST) in the eye for protection. Among them, GST is a multigene family with different enzymes that play an important role in the anti-oxidation, detoxification and elimination of xenobiotics, including carcinogens, oxidants, toxins, and drugs [10]. Human GST enzymes mainly include members of eight classes, assigned on the basis of sequence similarity: Alpha (GSTA), Mu (GSTM), Pi (GSTP), Theta (GSTT), Sigma (GSTS), Zeta (GSTZ), Epsilon (GSTE) and Omega (GSTO) [11]. Among these classes, GSTA, GSTM, GSTP, and GSTT have attracted considerable attention in the study of glaucoma [12].

To further elucidate the genetic basis of glaucoma, many studies have focused on the role of glutathione S transferase (GST) polymorphisms. Glutathione is a tripeptide that consists of cysteine, glutamate, and glycine, and it is one of the most abundant intracellular thiols in the cell [13]. Glutathione is involved in many redox reactions, such as detoxification of xenobiotics, protection of cells from oxidative stress, and regulation of protein function [13]. GST is a central component of the antioxidant and detoxification system [14]. The enzymatic activity of GST is required for the normal physiological function of glutathione [15].

Different GSTs have been found to be expressed in certain tissues and organs, such as the eye [16], and the distribution of the GST genes is highly tissue-specific and organ-specific [17]. GSTs have been found to be expressed in the eye and play a role in protecting the eye from oxidative stress [16]. The enzymes of the GST family are critically important for maintaining intracellular glutathione homeostasis and thus are important for cellular survival and function [18]. The GST gene superfamily is a critical part of the antioxidant defense system, and the glutathione S transferase polymorphisms have been associated with a variety of diseases, including cancer, hypertension, infertility, and glaucoma [19].}

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GSTM1 conducted a meta-analysis to investigate the associations between effect of low penetrance genes; therefore, in this study we since individual studies are usually underpowered in detecting the conclusion, probably due to the relatively small size of subjects, (SNP) of GSTP1 by a deletion in each gene, and a single-nucleotide polymorphism (SNP) of GSTT1 or GSTM1, or GSTP1 Ile 105 Val polymorphism results in an absence of their enzyme activity [12,13,14], and these polymorphisms of GST have been associated with altered risk of a variety of pathologies including cancer [15], cardiovascular disease [16], respiratory disease [17], and ophthalmologic problems such as cataract [18,19]. The relationship between GST polymorphisms and risk of glaucoma has been studied for more than 10 years. Several studies have found GST polymorphisms to be protective or risk factors in POAG [20,21,22,23,24,25,26] or other types of glaucoma [27], but other studies show no association between GST polymorphisms and risk of glaucoma [28,29]. These studies revealed an inconsistent conclusion, probably due to the relatively small size of subjects, since individual studies are usually underpowered in detecting the effect of low penetrance genes; therefore, in this study we conducted a meta-analysis to investigate the associations between GSTM1, GSTT1, and GSTP1 polymorphisms and the risk for glaucoma.

Materials and Methods
Identification and Eligibility of Relevant Studies
To identify all articles that examined the association of GST polymorphism with glaucoma, we conducted a literature search in the PubMed databases up to August 2012 using the following MeSH terms and keywords: “glutathione S transferase”, “polymorphism” and “glaucoma”. Additionally studies were identified by a manual search from other sources (e.g., Web of Knowledge), references of original studies or review articles on this topic. Eligible studies included in this meta-analysis had to meet the following criteria: (a) evaluation of the association between GSTM1 or GSTT1 null genotypes, or GSTP1 Ile 105 Val polymorphism and glaucoma, (b) an unrelated case-control study, if studies had partly overlapped subjects, only the one with a larger sample size was selected, (c) available genotype frequency, (d) sufficient published data for estimating an odds ratio (OR) with 95% confidence interval (CI), and (e) papers published in English from 2000.

Data Extraction
Two investigators independently assessed the articles for inclusion/exclusion and extracted data, and reached a consensus on all of the items. For each study, the following information was extracted: name of the first author; publication year; ethnicity (country); sample size (numbers of cases and controls); gene polymorphisms investigated; types of glaucoma; sources of samples; genotyping methods.

Statistical Analysis
The association between GSTM1, GSTT1 or GSTP1 polymorphism and glaucoma 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). The risk of GSTM1 or GSTT1 null genotype on glaucoma was evaluated by comparing with their reference wild type homozygote. For the GSTP1 polymorphism, we first estimated the risks of the Ile/Val and Val/Val genotypes on glaucoma, compared with the reference Ile/Ile homozygote, and then evaluated the risks of (Ile/Val+Val/Val vs. Ile/Ile) and (Val/ Val vs. Ile/Val+Ile/Val) on glaucoma, assuming dominant and recessive effects of the variant Val/Val allele, respectively. The I²-based Q statistic test was performed to evaluate variations due to heterogeneity rather than chance. A random-effects (DerSimonian-Laird method) or fixed-effects (Mantel-Haenszel method) model was used to calculate pooled effect estimates in the presence (P≤0.10) or absence (P>0.10) of heterogeneity. Publication bias was detected by Egger’s test [30] and Beggs’s [31] test for the overall pooled analysis of GSTM1 and GSTT1 null genotypes, and recessive model of GSTP1. Additionally, Begg’s funnel plot was drawn. Asymmetry of the funnel plot means a potential publication bias. Stratified analyses were also performed by types of glaucoma and ethnicities of study populations. For the one-way sensitivity analysis, one single study was excluded each time, and the new pooled results could reflect the influence of that deleted study to the overall summary OR. All analyses were done with Stata software (version 11.0; Stata Corp LP, College Station, TX), using two-sided P values.

Results
Characteristics of Studies
Thirteen abstracts were retrieved through the search “glutathione S transferase”, “polymorphism” and “glaucoma”, and ten studies meeting the inclusion criteria were identified as eligible [20,21,22,23,24,25,26]. Out of the thirteen, one was commentary [34], and one was in vitro study to evaluate the sensitivity to oxidative stress of anterior chamber tissues [35]. One article was excluded due to the study on the relationship between GST polymorphisms and risk of age-related macular degeneration [36]. We also included two eligible studies with manual searching [21,25]. As a result, a total of twelve studies met the inclusion criteria and were identified as eligible articles (Figure 1).

Twelve studies were included in the meta-analysis of GSTM1 genotype (1908 cases, 1457 controls), ten studies for GSTT1 genotype (1414 cases, 1177 controls) and four studies for GSTP1 polymorphism (543 cases, 511 controls). For the ethnicities, eleven studies of Caucasians and one study of Asians were included on the GSTM1 genotype. As to GSTT1, night studies of Caucasians and one study of Asians were included. The four studies on GSTP1 were all based on the Caucasians. For the glaucoma type, this meta-analysis included ten, eight, and three studies on the relationship between GSTM1, GSTT1, and GSTP1 polymorphism and risk of POAG (the most common form of glaucoma), respectively. In addition, we included four and three studies on the association between the GSTM1 and GSTT1 polymorphism and risk of other types of glaucoma (including exfoliative glaucoma and primary closed angle glaucoma), respectively. In addition to the study by Juronen et al. [20], in which the GSTM1 and GSTT1 phenotypes were determined with monoclonal antibody based enzyme-linked immunosorbent assay (ELISA), the genotyping for GSTM1, GSTT1 or GSTP1 was performed using polymerase chain reaction (PCR) in all other studies. The detailed characteristics of each study included in the meta-analysis are presented in Table 1, and the GST polymorphism genotype distributions from each study are presented in Table S1 and S2.

Quantitative Synthesis
Table 2 shows the results of the meta-analysis on the association between GSTM1 or GSTT1 null polymorphism and risk of glaucoma. By pooling all the studies, either GSTM1 or GSTT1 null polymorphism was not associated with a glaucoma risk, and this negative association maintained in Caucasian (Table 2, and
When stratified by glaucoma types, no association was found between GSTM1 or GSTT1 null polymorphism and risk of POAG, or other types of glaucoma, in all populations or in Caucasians.

We also examined the association between GSTP1 Ile 105 Val polymorphism and glaucoma risk, and the overall result showed that GSTP1 polymorphism was not correlated with glaucoma risk in all four models by pooling all four studies (Table 3 and Figure S3). In subgroup analysis, we found that GSTP1 Ile 105 Val polymorphism was significantly correlated with increased POAG risk in a recessive model (Val/Val vs. Ile/Ile + Ile/Val: OR, 1.62; 95%CI, 1.00–2.61; \( P = 0.049 \)) but not in other three models. Interestingly, these three studies were all based on Caucasian populations, thus, GSTP1 Ile 105 Val polymorphism in recessive model was associated with increased POAG risk in Caucasians (Table 3).

To investigate if the profiles of GST genotypes were associated with the risk of glaucoma, we first examined the association between combinations of GSTM1 and GSTT1 null genotypes and the risk of glaucoma, in which the reference group consisted of individuals with both putative low-risk genotypes, i.e., the presence of GSTM1 and GSTT1 genotypes [22]. Table 4 displays the risk of glaucoma associated with combinations of GST null genotypes as well as the trend in risk associated with each putative high-risk null genotype. The data showed a significant association between increased glaucoma risk and the combined GSTM1 and GSTT1 null genotypes in all population (OR, 2.20; 95% CI, 1.47–3.31; \( P<0.001 \)). When stratified by the types of glaucoma, combination of GSTM1 and GSTT1 null genotypes was associated with increased risk of POAG (OR, 1.90; 95% CI, 1.15–3.13; \( P=0.013 \)) but not other types of glaucoma (OR, 3.04; 95% CI, 0.95–9.72; \( P=0.061 \)). We also examined if the risk of glaucoma was associated with combinations of GSTP1 and GSTM1, or GSTT1 genotypes, in which the homozygous Ile/Ile genotype for GSTP1 was used as reference and the individuals heterozygous and homozygous for the Ile 105 Val allele was combined [22]. The
results showed that that increased glaucoma risk was associated with the combined \textit{GSTM1} null and \textit{GSTP1} Val genotypes (OR, 1.86; 95\% CI, 1.15–3.01; \(P = 0.012\)), but the combined \textit{GSTT1} null and \textit{GSTP1} Val genotypes played a protective role in glaucoma risk which, which remained of borderline statistical significance (OR, 0.60; 95\% CI, 0.36–1.00; \(P = 0.051\)).

Potential Publication Bias and Sensitivity Analysis

Publication bias was firstly detected by Begg's test for the overall pooled analysis of \textit{GSTM1} and \textit{GSTT1} null genotypes, and the recessive model of \textit{GSTP1} polymorphism. The Begg's test showed that the \(P\) value for \textit{GSTM1}, \textit{GSTT1} and \textit{GSTP1} polymorphism was 1.00, 0.371 and 1.00 respectively, and the corresponding funnel plots showed symmetric distribution (Figure 2). The Egger's test also showed that All the \(P\) values were more than 0.05 (Data not shown). Thus, no evident publication bias was found in present study. Sensitivity analysis was conducted by deleting each study in turn from the pooled analysis to examine the influence of the removed data set to the overall ORs. As shown in Figure S4, S5, S6, exclusion of each study did not influence the result in specific genotype comparison for \textit{GST} polymorphism, suggesting that the results of synthetic analysis were robust.

Discussion

In the present study, we systemically reviewed all available published studies and performed a meta-analysis to examine the association between the \textit{GST} polymorphisms and susceptibility to glaucoma. Our meta-analysis showed that single \textit{GSTM1} or \textit{GSTT1} null polymorphism was not associated with glaucoma risk, and \textit{GSTP1} Ile 105 Val polymorphism in recessive model was positively correlated with increased glaucoma risk. The combination of \textit{GSTM1} null and \textit{GSTT1} null, or \textit{GSTM1} null and \textit{GSTP1} genotype was associated with increased risk of glaucoma. Although different types of glaucoma have their own clinical characteristics and pathogenesis, our meta-analysis indicates that \textit{GST} polymorphisms may contribute to increased risk of glaucoma.

Table 1. Characteristics of literatures included in the meta-analysis.

| Author/Year (Reference) | Origin  | Ethnicity | Case/control | GST family | Glaucoma Type* | Samples | Genotype |
|-------------------------|---------|-----------|--------------|------------|----------------|---------|----------|
| Juronen 2000 [20]       | Estonia | Caucasian | 250/202      | \textit{GSTM1}/\textit{GSTT1}/\textit{GSTP1} | POAG   | Blood    | ELISA    |
| Izzotti 2003 [21]       | Italy   | Caucasian | 45/46        | \textit{GSTM1}/\textit{GSTT1} | POAG   | Trabecular meshwork | PCR      |
| Jansson 2003 [33]       | Sweden  | Caucasian | 388/200      | \textit{GSTM1} | POAG/Others | Blood   | PCR      |
| Yilmaz 2005 [28]        | Turkey  | Caucasian | 53/65        | \textit{GSTM1}/\textit{GSTT1}/\textit{GSTP1} | Others | Blood    | PCR      |
| Yildirim 2005 [22]      | Turkey  | Caucasian | 153/159      | \textit{GSTM1}/\textit{GSTT1}/\textit{GSTP1} | POAG   | Blood    | PCR      |
| Unal 2007 [23]          | Turkey  | Caucasian | 144/121      | \textit{GSTM1}/\textit{GSTT1} | POAG   | Blood    | PCR      |
| Abu-Amero 2008 [24]     | Saudi Arabia | Caucasian | 107/120      | \textit{GSTM1}/\textit{GSTT1} | POAG/Others | Blood | PCR      |
| Rasool 2010 [25]        | Egypt   | Caucasian | 32/16        | \textit{GSTM1}/\textit{GSTT1} | POAG   | Trabeculectomy specimens | PCR      |
| Fan 2010 [29]           | China   | Asian     | 405/201      | \textit{GSTM1}/\textit{GSTT1} | POAG   | Blood    | PCR      |
| Khan 2010 [27]          | Pakistan | Caucasian | 165/162      | \textit{GSTM1}/\textit{GSTT1} | POAG   | Blood    | PCR      |
| Izzotti 2010 [32]       | Italy   | Caucasian | 100/100      | \textit{GSTM1} | POAG   | Trabecular meshwork | PCR      |
| Rocha 2011 [26]         | Brazil  | Caucasian | 87/85        | \textit{GSTM1}/\textit{GSTT1}/\textit{GSTP1} | POAG   | Blood    | PCR      |

*Others: including exfoliative and primary closed angle glaucoma.
Abbreviations: POAG, primary closed angle glaucoma; PCR, Polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay.

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Table 2. Subgroup Analysis of the Association between \textit{GSTM1} and \textit{GSTT1} Polymorphisms and the Risk for Glaucoma.

| Groups          | n\textsuperscript{1} | Statistical Method | OR (95\% CI) | \(P\) |
|-----------------|----------------------|--------------------|--------------|------|
| All Glaucoma    |                      |                    |              | 0.290|
| \textit{GSTM1} null |                    | 12 Random          | 1.25 (0.82- 1.90) | 0.290|
| Caucasian       | 11 Random            | 1.25 (0.77- 2.04)  | 0.361        |
| \textit{GSTT1} null |                    | 10 Random          | 1.37 (0.82- 2.28) | 0.229|
| Caucasian       | 9 Random             | 1.49 (0.83- 2.67)  | 0.183        |
| Asian           | 1                    |                    |              | 0.183|
| \textit{POAG}   |                      |                    |              |     |
| \textit{GSTM1} null |                    | 10 Random          | 1.23 (0.74- 2.03) | 0.426|
| Caucasian       | 9 Random             | 1.24 (0.68- 2.27)  | 0.474        |
| \textit{GSTP1} Ile 105 Val |                    | 8 Random          | 1.42 (0.80- 2.52) | 0.237|
| Caucasian       | 7 Random             | 1.61 (0.80- 3.24)  | 0.182        |
| \textit{Others}\textsuperscript{*} |                    | 4 Random          | 1.64 (0.82–3.28) | 0.164|
| \textit{GSTT1} null |                    | 3 Random          | 2.13 (0.59–7.72) | 0.248|

*Others: including exfoliative and primary closed angle glaucoma.
\textsuperscript{1}n: number of studies.

Abbreviations: POAG, primary closed angle glaucoma.
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null genotype is a risk factor for development of POAG [22,26], while another study showed otherwise [33]. By pooled 10 studies, we did not find an association between GSTP1 polymorphism and POAG, suggesting that previous controversial data may be due to small size of population. As to GSTP1, previous studies did not identify differences between POAG patients and control individuals in the frequencies of GSTP1 Ile 105 Val genotypes [20,22,26,28]; however, by pooled these studies, we found that GSTP1 polymorphism was significantly correlated with increased POAG risk in Caucasian in a recessive model. It should be noted that the statistical significance of the association between the GSTP1 polymorphism and POAG risk was at borderline level. The GSTP1 105-Val allele homozygote was correlated with increased POAG risk but the difference did not reach statistical significance (OR, 1.55; 95% CI, 0.94–2.55). Since the studies included were very limited, it is necessary to validate the association between GSTP1 Ile 105 Val polymorphism and glaucoma risk in future studies.

To the best of our knowledge, this is the first meta-analysis assessing the association between single GST polymorphism, or combination of GST polymorphisms and glaucoma. Previously, Yildirin et al. reported an increasing glaucoma risk with higher numbers of the combined of GSTM1 null and GSTP1 105-Val allele genotypes but this association was not significant [22]. Our meta-analysis results showed that the association between combined of GSTM1 and GSTT1 null genotypes, or GSTM1 null and GSTP1 Val genotypes, and the risk for glaucoma is statistically significant in Caucasians. The study by Yildirin et al. also found a trend of increasing glaucoma risk with higher numbers of the combined GSTM1 null, GSTT1 null and GSTP1 105-Val allele genotypes (OR, 2.3; 95% CI: 0.75–7.08) [22]. Due to the limited studies, we did not perform meta-analysis for association between the combined GSTM1 null, GSTT1 null and GSTP1 105-Val allele genotypes and glaucoma risk. The current available data support the multifactorial nature of glaucoma, and both genetic and environmental factors are involved in pathogenesis of glaucoma. However, most studies did not provide GST polymorphisms when stratified by environmental factors (e.g., smoking). The relationship between polymorphic GST with other genetic and environmental glaucoma risk factors may be highly complicated, and extensive research is required to ascertain how exactly the GST genotype affects the individual susceptibility to glaucoma.

Meta-analysis has advantages compared to individual studies, however, some potential limitations in our study should be considered. First, this meta-analysis was limited by the small number of studies. Number of studies,

Abbreviations: POAG, primary closed angle glaucoma.

**Table 3. Meta-analysis of the GSTP1 Ile105Val polymorphism on glaucoma risk.**

| Groups | n | Ile/Val vs. Ile/Ile | Val/Val vs. Ile/Ile | Ile/Val +Val/Val vs. Ile/Ile (dominant) | Ile/Val vs. Ile/Ile (recessive) |
|--------|---|------------------|------------------|------------------------------|-------------------------------|
| All glaucoma | | | | | |
| Pooled | 4 | 0.95(0.73–1.24) | 0.70 | 1.17(0.51–2.68) | 0.701 | 1.00(0.78–1.29) | 0.987 | 1.18(0.50–2.80) | 0.708 |
| Glaucoma type | | | | | |
| POAG | 3 | 0.92(0.70–1.23) | 0.587 | 1.55(0.94–2.55) | 0.087 | 1.03(0.79–1.34) | 0.836 | 1.62(1.00–2.61) | 0.049 |
| Others* | 1 | | | | |

*Others: including exfoliative glaucoma.

| OR (95% CI) | P |
|-------------|---|
| 1.00(0.78–1.29) | 0.987 |
| 1.18(0.50–2.80) | 0.708 |

Previously, the study by Juronen et al. suggests that the GSTM1 positive phenotype may be a genetic risk factor for development of POAG [20]. However, the following studies show that the GSTM1 Val allele and the GSTT1 null genotype is a risk factor for development of POAG [22,26], while another study showed otherwise [33]. By pooled 10 studies, we did not find an association between GSTM1 polymorphism and POAG, suggesting that previous controversial data may be due to small size of population. As to GSTP1, previous studies did not identify differences between POAG patients and control individuals in the frequencies of GSTP1 Ile 105 Val genotypes [20,22,26,28]; however, by pooled these studies, we found that GSTP1 polymorphism was significantly correlated with increased POAG risk in Caucasian in a recessive model. It should be noted that the statistical significance of the association between the GSTP1 polymorphism and POAG risk was at borderline level. The GSTP1 105-Val allele homozygote was correlated with increased POAG risk but the difference did not reach statistical significance (OR, 1.55; 95% CI, 0.94–2.55). Since the studies included were very limited, it is necessary to validate the association between GSTP1 Ile 105 Val polymorphism and glaucoma risk in future studies.

To the best of our knowledge, this is the first meta-analysis assessing the association between single GST polymorphism, or combination of GST polymorphisms and glaucoma. Previously, Yildirin et al. reported an increasing glaucoma risk with higher numbers of the combined of GSTM1 null and GSTP1 105-Val allele genotypes but this association was not significant [22]. Our meta-analysis results showed that the association between combined of GSTM1 and GSTT1 null genotypes, or GSTM1 null and GSTP1 Val genotypes, and the risk for glaucoma is statistically significant in Caucasians. The study by Yildirin et al. also found a trend of increasing glaucoma risk with higher numbers of the combined GSTM1 null, GSTT1 null and GSTP1 105-Val allele genotypes (OR, 2.3; 95% CI: 0.75–7.08) [22]. Due to the limited studies, we did not perform meta-analysis for association between the combined GSTM1 null, GSTT1 null and GSTP1 105-Val allele genotypes and glaucoma risk. The current available data support the multifactorial nature of glaucoma, and both genetic and environmental factors are involved in pathogenesis of glaucoma. However, most studies did not provide GST polymorphisms when stratified by environmental factors (e.g., smoking). The relationship between polymorphic GST with other genetic and environmental glaucoma risk factors may be highly complicated, and extensive research is required to ascertain how exactly the GST genotype affects the individual susceptibility to glaucoma.

Meta-analysis has advantages compared to individual studies, however, some potential limitations in our study should be considered. First, this meta-analysis was limited by the small

**Table 4. Subgroup Analysis of the Association between GSTM1, GSTT1 and GSTP1 Polymorphisms and the Risk for Glaucoma.**

| Groups | n | Statistical Method | OR (95% CI) | P |
|--------|---|--------------------|-------------|---|
| GSTM1 GSTT1 | | | | |
| Pooled | 7 | Random | 1.42 (0.66–3.04) | 0.373 |
| GSTM1 null | 7 | Random | 1.99 (0.64–6.19) | 0.236 |
| GSTT1 null | 7 | Random | 2.20 (1.47–3.31) | <0.001 |
| GSTM1 null+GSTT1 null | 7 | Fixed | 3.04 (0.95–9.72) | 0.061 |
| GSTM1 null | 5 | Random | 2.72 (1.05–7.00) | 0.039 |
| GSTT1 null | 5 | Random | 2.40 (0.61–9.53) | 0.212 |
| GSTM1 null+GSTT1 null | 3 | Random | 3.04 (0.95–9.72) | 0.061 |

*Others: including exfoliative, pseudoexfoliative and primary closed angle glaucoma.

**Abbreviations: POAG, primary closed angle glaucoma.**

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sample size, especially in subgroup analysis aforementioned (e.g., studies on GSTP1 polymorphism), which needs further investigations. Second, basic methodological differences among the studies might have affected the results. In addition to three studies [20,22,28], the controls recruited in other studies were hospital-based [21,23,24,25,26,27,29,32,33]. Most of studies used PCR methods for genotyping, but the study by Juronen et al. [20] used enzyme-linked immunosorbent assay. Although excluding this study did not affect the result of GSTM1 and GSTT1 genotypes, the association between GSTP1 polymorphism and POAG risk in Caucasian was not significant due to the limited studies (n = 2) (Data not shown). Third, the studies differed in their procedure for sampling. Three studies used trabeculectomy specimens [25,32,33] while other studies used blood. We found no association between each single GST polymorphism and glaucoma risk in each subgroup analysis when the samples were stratified as trabeculectomy specimens or blood, and excluding one study using trabeculectomy specimens as samples did not affect the results on the associations between the combinations of GST polymorphisms and glaucoma risk (Data not shown). Fourth, most of the studies included in this meta-analysis did not categorize the POAG patients as high- and normal-tension glaucoma. So, we did not analyze the association between GST polymorphism and risk of high- or normal-tension glaucoma, and future studies should address this point. Last, the Caucasian group might have been genetically heterogeneous, with differences in terms of lifestyle and environment (e.g., European vs Arabian). These factors may explain the heterogeneity in meta-analysis for Caucasian populations.

In summary, the present meta-analysis suggested that combination of GSTM1 and GSTT1 null genotypes, and GSTM1 null and GSTP1 105-Val allele genotypes are associated with increased risk for glaucoma in Caucasian populations. The association between single GST polymorphism and glaucoma is either negative or evidence limited. More epidemiologic studies are suggested to further ascertain the relationship between GST polymorphisms and genetic predisposition to glaucoma.

Supporting Information

Figure S1 Forest plots of the association between GSTM1 null polymorphism and glaucoma risk. (DOC)

Figure S2 Forest plots of the association between GSTT1 null polymorphism and glaucoma risk. (DOC)

Figure S3 Forest plots of the association between GSTP1 Ile 105 Val polymorphism and glaucoma risk. (DOC)

Figure S4 Sensitivity analysis for GSTM1 null polymorphism. (DOC)

Figure S5 Sensitivity analysis for GSTT1 null polymorphism. (DOC)

Figure S6 Sensitivity analysis for GSTP1 Ile 105 Val polymorphism. (DOC)

Table S1 GSTM1 and GSTT1 polymorphism genotype distribution of each study included in the meta-analysis. (DOC)

Table S2 GSTP1 Ile 105 Val polymorphism genotype distribution of each study included in the meta-analysis. (DOC)

Author Contributions
Conceived and designed the experiments: YY KY GC. Performed the experiments: YY YW JG. Analyzed the data: YY JG GC. Contributed reagents/materials/analysis tools: KY GC. Wrote the paper: YY YW KY GC.
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