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Associations of polymorphisms of *LOXL1* gene with primary open-angle glaucoma: a meta-analysis based on 5,293 subjects

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Objective: Previous studies indicated that the relationship between lysyl oxidase-like 1 (*LOXL1)* gene polymorphisms and primary open-angle glaucoma (POAG) remains inconsistent. In the present study, we aimed to perform a meta-analysis to investigate the association of *LOXL1* polymorphisms with POAG risk.
Methods: Literatures were electronically searched in the PubMed, EMBASE, CNKI, Wanfang, and VIP databases. The published literatures, which are case-control or cohort studies on the relationship between the polymorphisms (rs1048661, rs3825942, rs2165241) of the *LOXL1* gene and POAG, were documented.
Results: We included 13 literatures including 5,293 subjects for the present study. A meta-analysis showed that the risk of POAG in individuals carrying the C allele of rs2165241 was 1.26 times higher compared with those carrying the T allele (odds ratio (OR) = 1.26, 95% confidence interval (CI): 1.09 ~1.46) in the total population. In the Caucasian population, we also found that individuals carrying the C allele of rs2165241 have an increased risk for POAG compared to those subjects carrying the T allele (OR = 1.42, 95% CI: 1.19 ~1.69, p = 0.0001). In addition, we found that the rs1048661 polymorphism was associated with POAG in the Asian population (OR = 1.17, 95% CI: 1.02 ~1.35, p = 0.03), and rs3825942 was associated with POAG in the Caucasian population (OR = 2.69, 95% CI: 1.61 ~4.47, p<0.001).
Conclusions: The polymorphisms of the *LOXL1* gene were associated with the susceptibility of POAG.

Glaucoma is a common eye disease, and approximately 50% of glaucoma cases are primary open-angle glaucoma (POAG) [1-3]. In clinical practices, patients with POAG can experience glaucomatous optic neuropathy and visual field defects in the corresponding area for no obvious reasons. POAG can result in blindness if left untreated. The main clinical manifestations of POAG are optic neuropathy, including size increases of the optic disc, and the irregular loss of optic disc tissues. It is considered the second-most frequent cause of irreversible blindness globally, and it affects primarily the older population, estimated to affect about 80 million people worldwide by the year 2020 [1]. However, the etiology of glaucoma remains unclear. Epidemiological studies suggested that POAG is a complex multifactorial disease resulting from the interaction between genetic background and traditional risk factors, including diabetes, myopia, cigarette smoking, and a positive family history [4-6]. Recently, many genes were found to be associated with POAG, including the lysyl oxidase-like 1 (*LOXL1*; Gene ID: 4016) gene, which is a member of the lysyl oxidase family, which catalyzes the oxidative deamination of lysine residues of tropoelastin and is thought to be essential for elastogenesis [7,8]. Dysregulated expressions of *LOXL1* and elastic proteins were associated with pronounced structural alterations to the elastic fiber network in the laminar beams of pseudoexfoliation syndrome eyes [7]. Theoretically, there was a relationship between *LOXL1* gene polymorphisms and POAG. Recent studies suggested that there was an association between *LOXL1* gene polymorphisms, such as rs2165241, rs1048661, and rs3825942, and POAG susceptibility [9-14]. As well, a previous study [15] suggested that these three single nucleotide polymorphisms (SNPs) demonstrated a strong linkage disequilibrium (rs1048661-rs3825942: D' = 1; rs1048661- rs2165241: D' = 0.8; rs3825942- rs2165241: D' = 0.8). Furthermore, rs1048661 and rs3825942 have been identified to be associated with POAG, and this association was later independently replicated in other patient cohorts [16,17]. Although an association between rs2165241 and increased POAG risk was found in the Icelandic population, the association was not found in other populations [16,18]. Liu et al. [19] reported that subjects carrying the C allele had a significantly lower risk of suffering from POAG. However, the findings by Fuse et al. [20] were contrary. Therefore, to clarify the relationship between *LOXL1* gene polymorphisms and POAG further, we have systemically examined the association of these SNPs with POAG in the present study.

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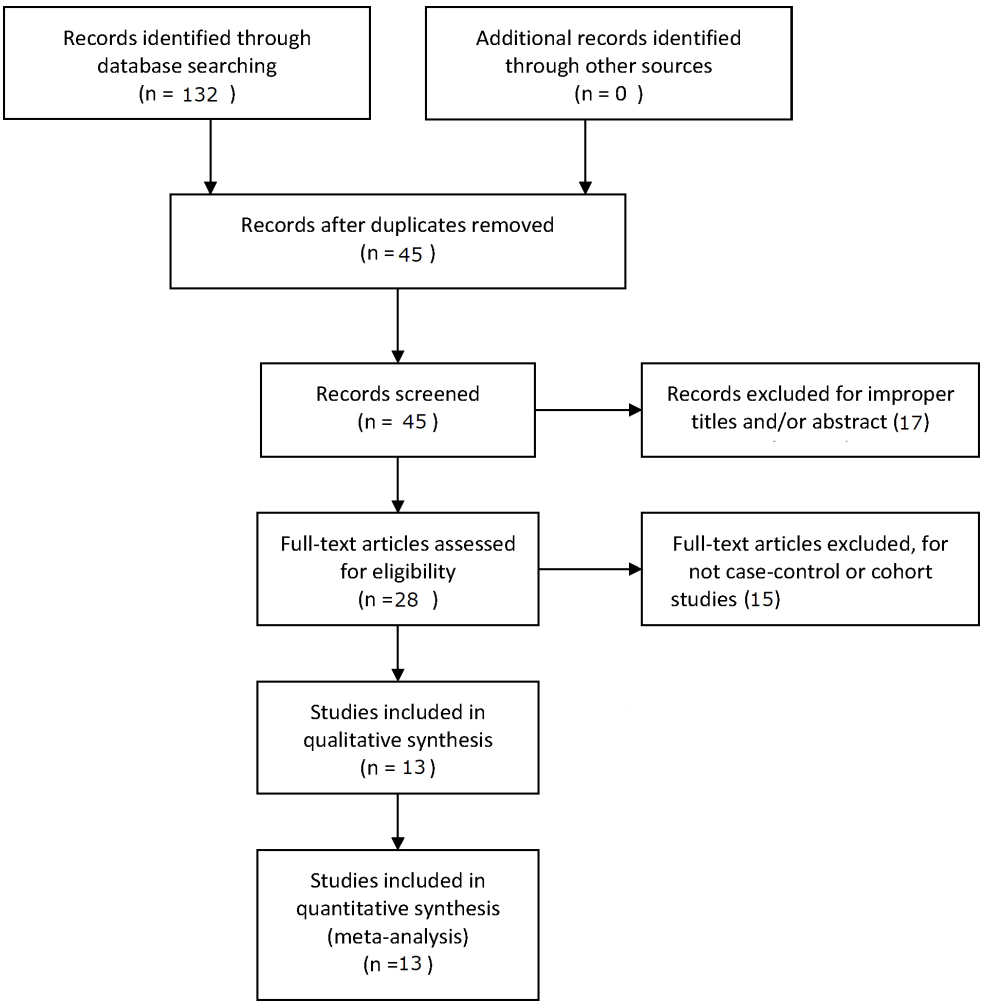

Figure 1. Flowchart of study inclusion.

METHODS

Literature inclusion criteria and exclusion criteria: All the included studies must meet the following criteria: (1) Type of study: case-control or cohort studies; (2) content of study: *LOXL1* gene polymorphisms and POAG susceptibility; (3) data: studies providing genotype and allele frequencies. We excluded studies that 1) provided incomplete data and that cannot be used to extract genotype and allele frequencies; 2) presented unreliable genotyping methods; 3) published repeated data from the same study.

Identification and eligibility of relevant studies: To identify all articles that examined the association of *LOXL1* polymorphisms with POAG, we conducted a literature search of the PubMed, EMBASE, CNKI, Wanfang, and VIP databases until February 2014 using the following MeSH terms and keywords: "*LOXL1*" of "lysyl oxidase-like 1"; and "polymorphism" or "SNP" or "mutation"; and "primary open angle glaucoma" or "POAG." Additional studies were identified by a manual search of references from original studies or review articles on this topic.

Statistical methods: Revman 5.2 statistical software was used to perform the meta-analysis. The odds ratios (OR) of the genetic *LOXL1* polymorphisms were combined and calculated, the 95% CIs were calculated, and the forest plots of the OR value distributions were drawn. Statistical heterogeneity was performed using an I^2 test analysis. If I^2 <50%, all the included studies had no significant statistical heterogeneity regarding OR quantity. The fixed effects model was adopted,

Table 1. The characteristics of included studies.

Authors	Publication year	Country	Ages (years)		rs1048661 (n)		H-W for	rs3825942 (n)		H-W for	rs2165241 (n)		H-W for
			Patients	Control	Patients	Control	Control	Patients	Control	Control	Patients	Control	Control
Lemmela et al.	2009	Finland	NA	NA	71	404	0.113	71	404	0.664	71	404	0.221
Fan et al.	2008	United States	75	72	331	88	0.227	331	88	0.112	331	88	0.327
Liu et al.	2008	United States	55.4±13.8	>55	-	-	0.365	642	462	0.435	642	462	0.164
Chakrabarti et al.	2008	India	NA	NA	112	105	0.443	112	105	0.665	112	105	0.143
Fuse et al.	2008	Japan	NA	68.0±7.0	62	138	0.556	62	138	0.221	62	138	0.779
Tanito et al.	2008	Japan	75.4±5.3	77.2±5.0	40	157	0.212	40	157	0.476	40	157	0.088
Cong et al.	2008	China	39.1±16.5	69.4±6.0	462	447	0.098	462	447	0.127	462	447	0.123
Thorleifsson et al.	2007	Europe	NA	NA	200	198	0.119	200	198	0.659	200	198	0.943
Abu-Amero et al.	2012	Saudi Arabia	63.7±14.7	69.3±12.4	96	101	0.876	96	101	0.212	96	101	0.558
Zanon-Moreno et al.	2014	Spain	NA	NA	-	-	-	-	-	-	232	241	0.332
Mabuchi et al.	2008	Japan	>40	>40	213	191	0.211	213	191	0.665	-	-	-
Williams et al.	2010	South Africa	NA	NA	50	50	0.332	50	50	0.443	-	-	-
Kasım et al.	2013	Turkish	67.7±9.3	66±5.7	100	100	0.126	100	100	0.119	-	-	-

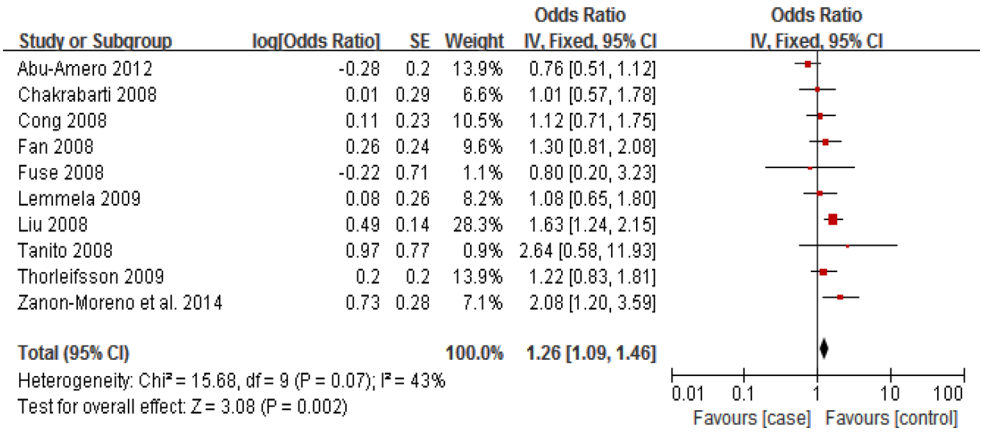

Figure 2. Forest plot of association of POAG with rs2165241 in the total population; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95% CI. In this analysis, a fixed-effects model was used.

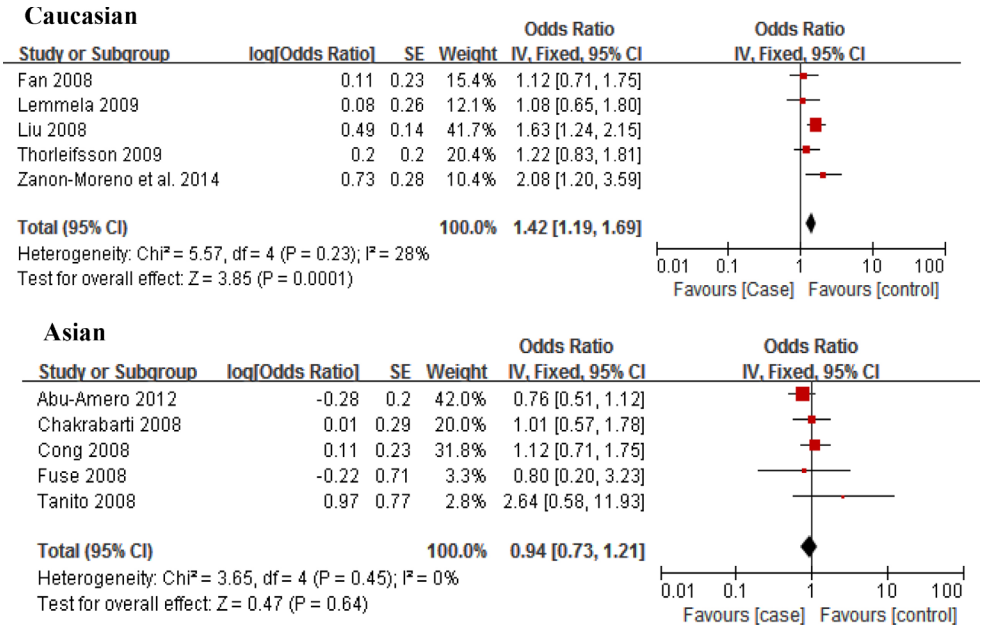

Figure 3. Forest plot of the association of POAG with rs2165241 in the Caucasian and Asian populations; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95% CI. In this analysis, a fixed-effects model was used.

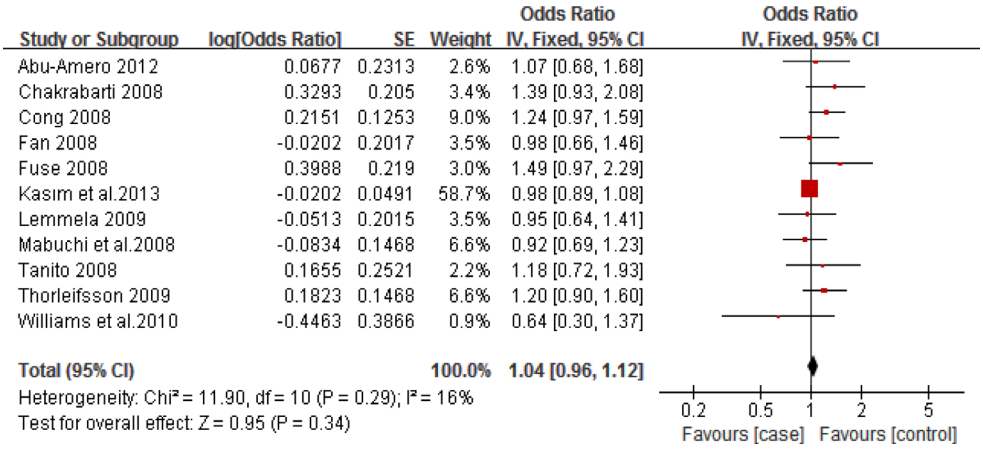

Figure 4. Forest plot of the association of POAG with rs1048661 in the total population; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95%CI. In this analysis, a fixed-effects model was used.

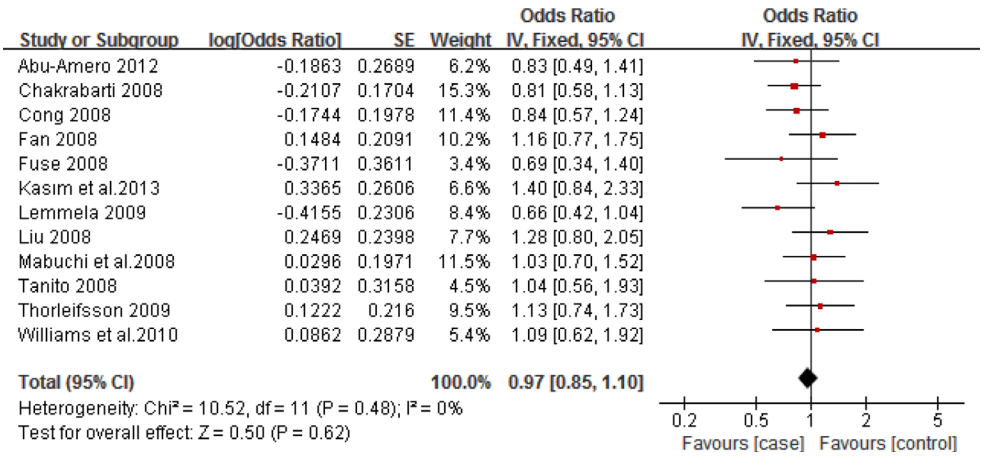

Figure 5. Forest plot of the association of POAG with rs3825942 in the total population; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95%CI. In this analysis, a fixed-effects model was used.

and the random effects model analysis was used if the status was conversed.

RESULTS

Literature inclusion: As shown in Figure 1, the relevant databases were reviewed and 132 literatures were found to meet the inclusion criteria for the meta-analysis. Of the 132, 119 literatures were excluded due to duplicated publications, non-clinical-based research, or non-availabilities of full texts. In total, 13 literatures [12,19-30,] were included, all of which were case-control studies totaling 5,293 subjects. The characteristics of the included studies were shown in Table 1.

Meta-analysis: All the publications including these three SNPs showed no significant heterogeneity (rs2165241: I^2 = 43%, p = 0.07; rs1048661: I^2 = 16%, p = 0.29; rs3825942: I^2 = 0%, p = 0.48), Therefore, the data were combined using the fixed effects model. For rs2165241, the meta-analysis results showed that the risk of POAG in individuals carrying the C allele was 1.26 times compared to those carrying the T allele (OR = 1.26, 95% confident interval (CI): 1.09 ~1.46, p = 0.002) in the total population (Figure 2). In the Caucasian population, we found that individuals carrying the C allele of rs2165241 have an increased risk for POAG compared to those subjects carrying the T allele (OR = 1.42, 95% CI: 1.19 ~1.69,

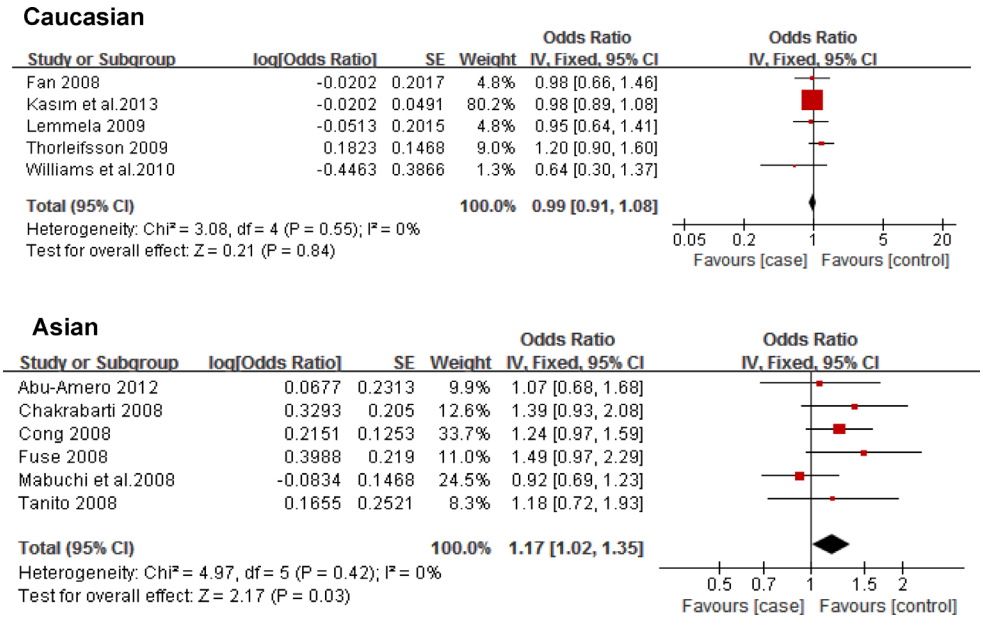

Figure 6. Forest plot of the association of POAG with rs1048661 in the Caucasian and Asian populations; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95%CI. In this analysis, a fixed-effects model was used.

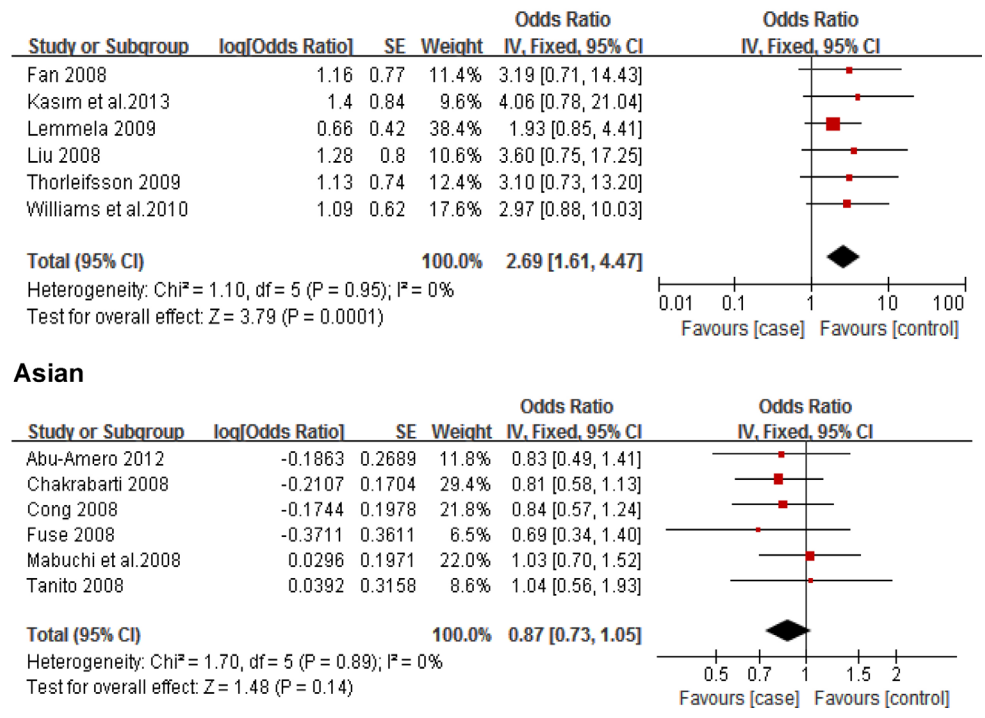

Figure 7. Forest plot of the association of POAG with rs3825942 in the Caucasian and Asian populations; the horizontal lines correspond to the study-specific OR and 95% CI, respectively. The area of the squares reflects the study-specific weight. The diamond represents the pooled results of the OR and 95%CI. In this analysis, a fixed-effects model was used.

p = 0.0001). However, we found no association in the Asian population (p = 0.64, Figure 3).

For rs1048661 and rs3825942, we found no associations between the genotype or allele and POAG in the total population (Figure 4 and Figure 5). However, we found that the rs1048661 polymorphism was associated with POAG in the Asian population (OR = 1.17, 95% CI: 1.02 ~1.35, p = 0.03, Figure 6), and rs3825942 was associated with POAG in the Caucasian population (OR = 2.69, 95% CI: 1.61 ~4.47, p<0.001, Figure 7).

Publication bias analysis: We analyzed the publication bias using Revman 5.2 software; the funnel plot shows the points as evenly distributed and symmetric, and most of the points are within the 95% CI. This indicates no publication bias, and the result of the study is credible (Figure 8).

DISCUSSION

In the present study, 13 literatures were included in a meta-analysis to investigate the relationship between the *LOXL1* gene polymorphisms and POAG. The results showed that the genetic polymorphisms of *LOXL1* were associated with a risk of POAG.

LOXL1 is located on human chromosome 15q22, and it is a member of the lysyl oxidase family [31], members of which can encode a kind of copper-dependent amino oxidase. This enzyme acted on the cell, and it catalyzes the first step of the

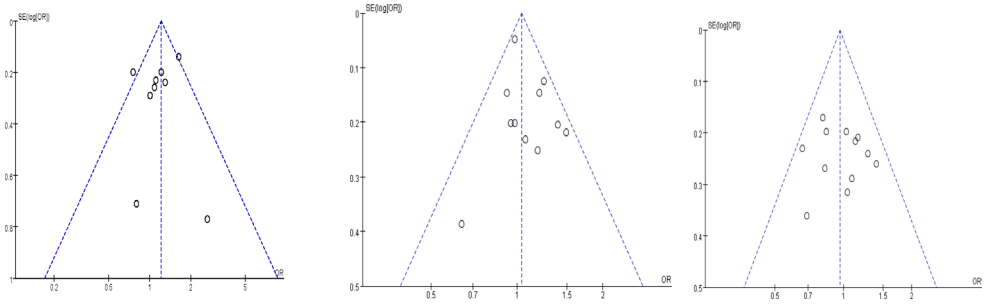

Figure 8. Begg's funnel plot to test for a publication bias. Each circle denotes an independent study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line stands for mean effect size. A: rs2165241; B: rs1048661; C: rs3825942.

cross-linking reaction between collagen and elastin [32]. The gene-encoded protein is a secreted protein; after that, it is synthesized in the form of a precursor, and it is glycosylated in the Golgi complex and secreted out of the cells in the plasminogen state. Under the action of the proteolytic enzymes, *LOXL1* can convert to an active form and act on the elastic and collagen fibers in the extracellular matrix. *LOXL1* expression upregulation and abnormally high levels of enzyme activity can cause excessive collagen accumulation and result in the occurrence and development of related diseases, such as POAG. The rs2165241 of the *LOXL1* gene is located in the coding region of the gene. Thus, the polymorphism may be associated with the expressed products. The *LOXL1* protein encoded by the C allele is different from the protein encoded by the T allele in the primary and spatial structures [12], which will result in changes to the biologic function of the protein, and it will eventually result in different incidences of POAG in individuals carrying different alleles. The other two SNPs (rs1048661 and rs3825942) are non-synonymous variants, which may affect protein function or expression. In the present study, we used meta-analysis methods to investigate the relationships between these three SNPs in the *LOXL1* gene and POAG, which has certain advantages. We found that in the Caucasian population, individuals carrying the C allele of rs2165241 have an increased 1.42-fold risk for POAG compared to those subjects carrying the T allele. In addition, we found that the rs1048661 polymorphism was associated with POAG in the Asian population and rs3825942 was associated with POAG in the Caucasian population. We found neither heterogeneity nor a publication bias among the studies. In addition, the sensitivity analysis showed that the results were stable and reliable. In conclusion, the present study indicated that *LOXL1* genetic polymorphisms are associated with susceptibility to POAG.

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