Associations of polymorphisms of \textit{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 (\textit{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 \textit{LOXL1} polymorphisms with POAG risk.

Methods: Literature 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 \textit{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 \textit{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 (\textit{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 \textit{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 \textit{LOXL1} gene polymorphisms and POAG. Recent studies suggested that there was an association between \textit{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 \textit{LOXL1} gene polymorphisms and POAG further, we have systemically examined the association of these SNPs with POAG in the present study.
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 I² test analysis. If I² <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) | rs3825942 (n) | rs2165241 (n) |
|--------------------|------------------|--------------|--------------|---------------|---------------|---------------|
| Lemmela et al.     | 2009             | Finland      | NA           | 71            | 0.113         | 71            | 0.664         | 71            | 0.221         |
| Fan et al.         | 2008             | United States| 75           | 72            | 331           | 0.227         | 331           | 0.112         | 331           | 0.327         |
| Liu et al.         | 2008             | United States| 55.4±13.8    | >55           | -             | 0.365         | 642           | 0.435         | 642           | 0.164         |
| Chakrabarti et al. | 2008             | India        | NA           | 112           | 0.443         | 112           | 0.665         | 112           | 0.143         |
| Fuse et al.        | 2008             | Japan        | NA           | 68.0±7.0      | 62            | 0.556         | 62            | 0.221         | 62            | 0.779         |
| Tanito et al.      | 2008             | Japan        | 75.4±5.3     | 77.2±5.0      | 40            | 0.212         | 40            | 0.476         | 40            | 0.088         |
| Cong et al.        | 2008             | China        | 39.1±16.5    | 69.4±6.0      | 462           | 0.098         | 462           | 0.127         | 462           | 0.123         |
| Thorleifsson et al.| 2007             | Europe       | NA           | 200           | 0.119         | 200           | 0.659         | 200           | 0.943         |
| Abu-Amero et al.   | 2012             | Saudi Arabia | 63.7±14.7    | 69.3±12.4     | 96            | 0.876         | 96            | 0.212         | 96            | 0.558         |
| Zanon-Moreno et al.| 2014             | Spain        | NA           | -             | -             | -             | -             | 232           | 241           | 0.332         |
| Mabuchi et al.     | 2008             | Japan        | >40          | >40           | 213           | 0.211         | 213           | 0.665         | -             | -             |
| Williams et al.    | 2010             | South Africa | NA           | 50            | 0.332         | 50            | 0.443         | -             | -             |
| Kasım et al.       | 2013             | Turkish      | 67.7±9.3     | 66±5.7        | 100           | 0.126         | 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.
and the random effects model analysis was used if the status was reversed.

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% 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,

Caucasian

| Study or Subgroup | log(Odds Ratio) | SE | Weight | Odds Ratio | CI | 95% CI |
|-------------------|----------------|----|--------|------------|----|--------|
| Fan 2008          | -0.0292        | 0.2017 | 4.8%  | 0.99 [0.86, 1.14] |
| Kasmr et al 2013  | -0.0232        | 0.0491 | 90.2% | 0.99 [0.99, 1.08] |
| Lemmela 2009      | -0.0513        | 0.2015 | 4.8%  | 0.95 [0.64, 1.41] |
| Thorleffson 2009  | 0.1823         | 0.1488 | 9.0%  | 1.20 [0.90, 1.60] |
| Williams et al 2010 | -0.4463     | 0.3886 | 1.3%  | 0.64 [0.30, 1.37] |

Total (95% CI)

| Heterogeneity: Chisq = 3.06, df = 4 (p = 0.55), I^2 = 0% |
| Test for overall effect: Z = 0.21 (p = 0.84) |

Asian

| Study or Subgroup | log(Odds Ratio) | SE | Weight | Odds Ratio | CI | 95% CI |
|-------------------|----------------|----|--------|------------|----|--------|
| Abu-Amero 2012    | 0.0677         | 0.2313 | 9.9%  | 1.07 [0.69, 1.68] |
| Chakrabarti 2008  | 0.3293         | 0.205  | 12.6% | 1.39 [0.93, 2.08] |
| Cong 2008         | 0.2151         | 0.1253 | 33.7% | 1.24 [0.97, 1.59] |
| Fuse 2008         | 0.3988         | 0.219  | 11.0% | 1.49 [0.97, 2.29] |
| Mabuchi et al 2008 | -0.0834    | 0.1488 | 24.5% | 0.92 [0.69, 1.23] |
| Tanilo 2008       | 0.1855         | 0.2521 | 9.3%  | 1.10 [0.72, 1.73] |

Total (95% CI)

| Heterogeneity: Chisq = 4.97, df = 5 (p = 0.42), I^2 = 0% |
| Test for overall effect: Z = 2.17 (p = 0.03) |

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.
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
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 plasma membrane state. Under the action of the proteolytic enzymes, LOXL1 can convert to an active form and play a role in 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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