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Original Article

GSTM1 and GSTT1 null genotype and diabetic retinopathy: a meta-analysis

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Abstract: Glutathione S-transferases (GSTs) have proved to be involved in the detoxifying several oxidants and may play an important role in diabetic retinopathy (DR). Previous studies on the association between glutathione S-transferase T1 (GSTT1) and GSTM1 polymorphism and DR risk reported inconclusive results. To clarify the possible association, we conducted a meta-analysis of eligible studies. We searched in the PubMed, Embase, and Wangfang Medicine databases for studies assessing the association between GSTM1 and GSTT1 null genotype and DR risk. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to assess the strength of association between GSTM1 and GSTT1 null genotype and DR risk. Five studies with 3563 subjects were included in this meta-analysis. The null genotypes of GSTT1 and GSTM1 were associated with a significantly increased risk of DR (OR = 1.69; 95% CI, 1.33-2.16; OR = 1.59; 95% CI, 1.22-2.06), respectively. When stratified by the type of DM, a significantly elevated DR risk was observed in T2DM patients. In conclusion, this meta-analysis suggested that an increased risk of DR was associated with the null polymorphism of GSTT1 and GSTT1, respectively.

Keywords: Diabetic retinopathy, GSTM1, GSTT1, meta-analysis, polymorphism

Introduction

Diabetic retinopathy (DR) is one of the most common microangiopathic complications of patients with diabetes mellitus (DM) [1]. DR is an ocular manifestation of DM, affecting up to 80% of all patients who have had DM for 10 years or more. Although glycemic control and diabetes duration are important predictors of retinopathy [2], genetic susceptibility also plays an important role in the pathogenesis of DR [3]. Identification and characterization of genetic factors that predispose individuals to DR could improve prevention and treatment measures for this debilitating condition.

Glutathione S-transferases (GSTs) belong to a family of ubiquitous and multifunctional enzymes that work as one of the endogenous antioxidants in the body [4]. GST enzymes are coded by at least eight distinct loci: α (GSTA), μ (GSTM), θ (GSTT), π (GSTP), σ (GSTS), κ (GSTK), ο (GSTO), and τ (GSTZ), each containing one or more homodimeric or heterodimeric isoforms. Two loci in particular, GSTM1 and GSTT1, have received most of the attention. The GSTM1 locus has been mapped on chromosome 1p13.3, while the GSTT1 locus exists on chromosome 22q11.2. Persons with homozygous deletions of either the GSTM1 or GSTT1 locus have no enzymatic functional activity of the respective enzyme [5].

Many investigators have investigated the association between the GSTM1 and SGTT1 null genotype and DR risk [6-10]. But the results were conflicted and inconclusive. As a single study may lack the power to provide reliable conclusion, we performed this meta-analysis.

Methods

Selection of published studies

We searched in the PubMed, Embase, and Wangfang Medicine databases for studies assessing the association between GSTM1 and GSTT1 null genotype and DR risk. The literature strategy used the following keywords: (“Glutathione S-transferase T1”, “GSTT1” or “GSTT” or “Glutathione S-transferase M1”, “GSTM1” or “GSTM”) and (“diabetic retinopa-

Table 1. Characteristics of the included studies

First author	Year	Ethnity	Gender	Age	No. of patients	DM	Gene
Doney	2005	Caucasian	Mixed	64.4	2015	Type 2	GSTT1
Hovnik	2009	Caucasian	Mixed	27.4	124	Type 1	GSTM1, GSTT1
Cilensek	2012	Caucasian	Mixed	65.6	704	Type 2	GSTM1, GSTT1
Dadbinpour	2013	Caucasian	Mixed	35-65	115	Type 2	GSTM1, GSTT1
Moasser	2014	Caucasian	Mixed	52.7	605	Type 2	GSTM1, GSTT1

DM, diabetes.

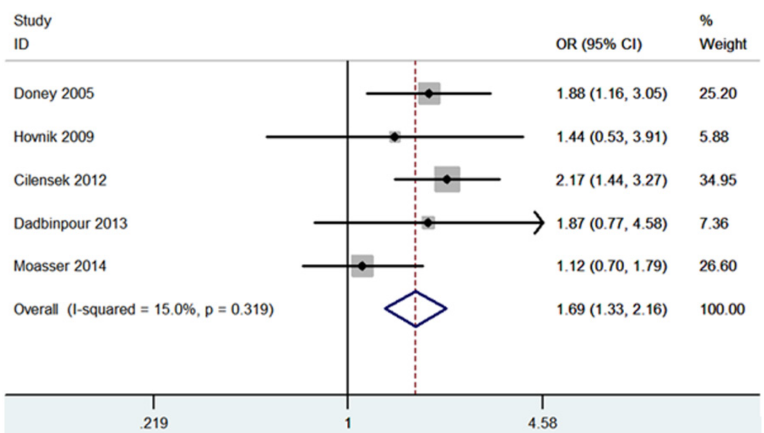

Figure 1. Forest plot of DR risk of GSTT1 polymorphism.

Table 2. Meta-analysis of the results

	OR (95% CI)	*P* Value	I^2 (%)	*P* Value
GSTT1				
All	1.69 (1.33-2.16)	<0.0001	15	0.32
Type 1	1.71 (1.33-2.20)	<0.0001	35	0.20
GSTM1				
All	1.59 (1.22-2.06)	0.0005	0	0.57
Type 1	1.51 (1.15-1.99)	0.003	0	0.71

thy" or "retinopathy"). The references of the retrieved articles were also hand searched at the same time to identify additional published articles. The references of eligible studies and relevant reviews were also checked for other literature not indexed into common databases. There was no language restriction applied in this meta-analysis. The inclusion criteria of eligible studies were as following: (1) Case-control or cohort study; (2) The cases were patients with diabetes; (3) The controls were DR-free individuals; (4) Reported the frequencies of GSTM1 and SGTT1 polymorphism in both cases and controls or the odds ratio (OR) and its 95% confidence interval (95% CI) of the association between GSTM1 and SGTT1 null genotype and DR risk. Family-based studies and studies containing overlapping data were all excluded.

Data extraction

Relevant data were extracted from all the eligible studies independently by two reviewers, and disagreements were settled by discussion and the consensus among all reviewers. The main data extracted from the eligible studies were as following: the first author, year of publication, ethnicity, characteristics of cases, total numbers of cases and controls, and ORs and corresponding 95% CIs.

Statistical analysis

For the GSTM1 and GSTT1 gene, we estimated the risk of the null genotype on DR compared with the non-null genotypes in the recessive model (null verses heterozygous + wild type). The strength of the association was measured by ORs with 95% CIs.

The ORs with corresponding 95% CIs from individual studies were pooled using fixed or random effects models according to the heterogeneity. When the *P* value for Cochran's Q statistic was less than 0.1, and a significant heterogeneity existed across the included studies, the random effects model (DerSimonian and Laird method) was used for meta-analysis, or else the fixed effects model (Mantel-Haenszel method) was used. Cumulative meta-analysis was performed. Sensitivity analysis was further performed by excluding single study in turn to assess the impact of individual study on the pooled estimate. Subgroup analyses were stratified by type of DM. Funnel plots and

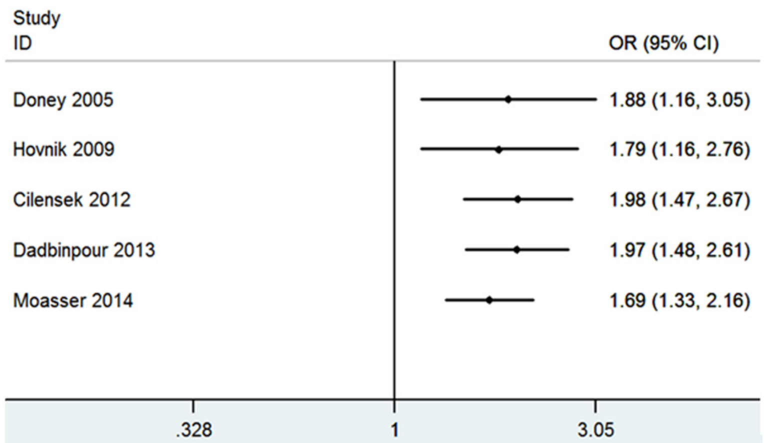

Figure 2. Cumulative meta-analysis of DR risk of GSTT1 polymorphism.

Egger's regression test were undertaken to assess the potential publication bias [11]. Data analysis was performed using STATA 12 (StataCorp LP, College Station, Texas, USA).

Results

Study characteristics

A total of 5 studies with 3563 subjects were retrieved based on the search criteria for DR susceptibility related to the GSTM1 and GSTT1 polymorphism [6-10]. All these studies were conducted in Caucasians. One study used Type 1 DM patients, while other studies used Type 2 DM patients. Five studies investigated the association between GSTT1 polymorphism and DR risk, while 4 studies investigated the association between GSTM1 polymorphism and DR risk. The main study characteristics are summarized in **Table 1**.

GSTT1 and DR risk

The null genotype of GSTT1 was associated with a significantly increased risk of DR when compared with present genotype (OR = 1.69; 95% CI, 1.33-2.16; **Figure 1**). When stratified by type of DM, a significantly elevated DR risk were observed in **Table 2** (OR = 1.71; 95% CI, 1.33-2.20).

As shown in **Figure 2**, significant associations were evident with each addition of more data over time. The results showed that the pooled ORs tended to be stable. A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (**Figure 3**).

Funnel plot and Egger's test were performed to assess the publication bias of literatures. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (**Figure 4**). Egger's test did not find the evidence of publication bias (*P* = 0.462).

GSTM1 and DR risk

The null genotype of GSTM1 was associated with a significantly increased risk of DR when compared with present genotype (OR = 1.59; 95% CI, 1.22-2.06; **Figure 5**). When stratified by type of DM, a significantly elevated DR risk were observed in **Table 2** (OR = 1.51; 95% CI, 1.15-1.99).

As shown in **Figure 6**, significant associations were evident with each addition of more data over time. The results showed that the pooled ORs tended to be stable. A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data set to the pooled ORs, and the corresponding pooled ORs were not materially altered (**Figure 7**).

Funnel plot and Egger's test were performed to assess the publication bias of literatures. The shape of the funnel plot did not reveal any evidence of obvious asymmetry (**Figure 8**). Egger's test did not find the evidence of publication bias (*P* = 0.308).

Discussion

Previous studies on the association between GSTT1 and GSTT1 polymorphism and DR risk reported inconclusive results. To clarify the possible association, we conducted a meta-analysis of a total of 5 studies with 3563 individuals. Overall, GSTT1 and GSTT1 null genotype was significantly associated with increased risk of DR, respectively. Significant association was also found in T2DM patients. Therefore, the meta-analysis provides strong evidence for the significant association between GSTT1 and GSTT1 null genotype and increased risk of DR. To our knowledge, this was the first meta-analysis which assessed the association between GSTT1 and GSTT1 null genotype and risk of DR.

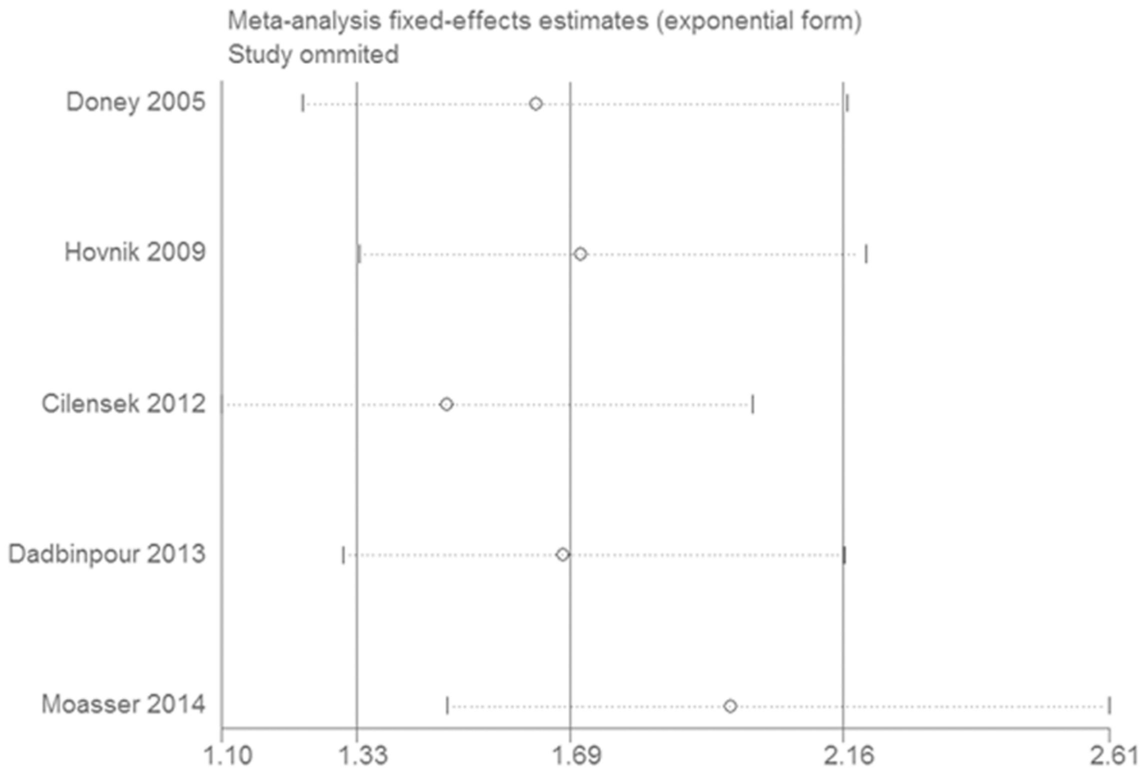

Figure 3. Sensitivity analysis of DR risk of GSTT1 polymorphism.

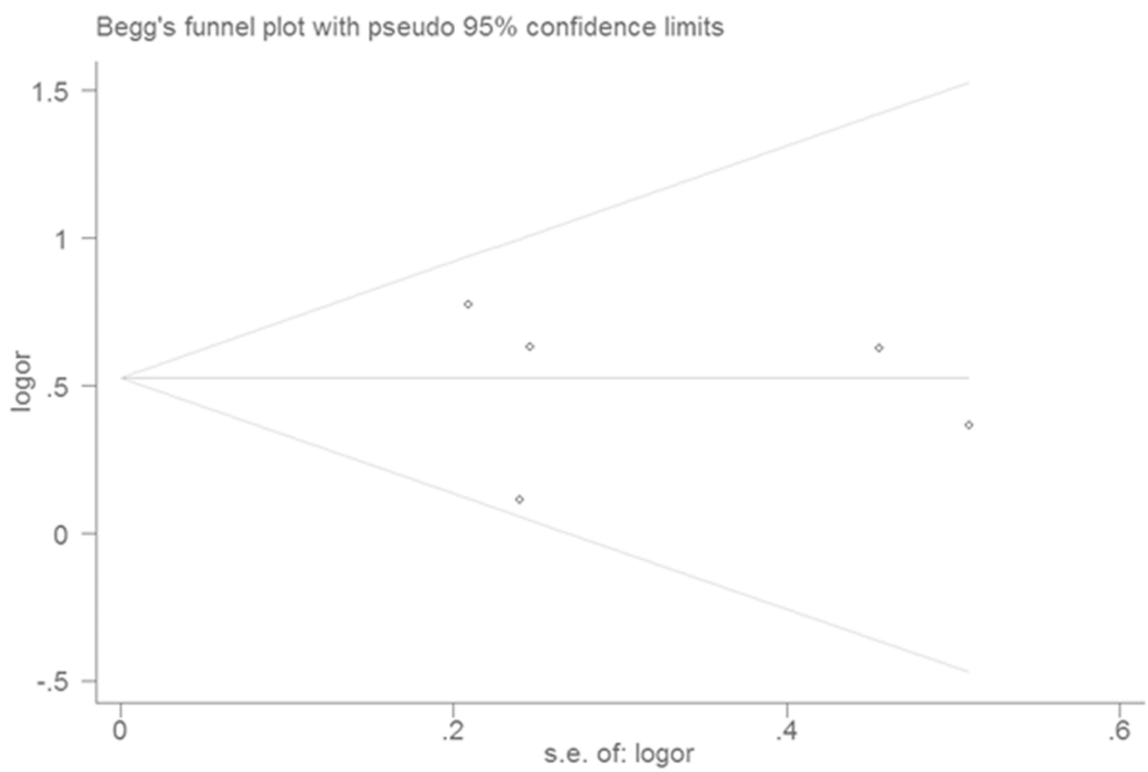

Figure 4. Funnel plot of association between DR risks of GSTT1 polymorphism.

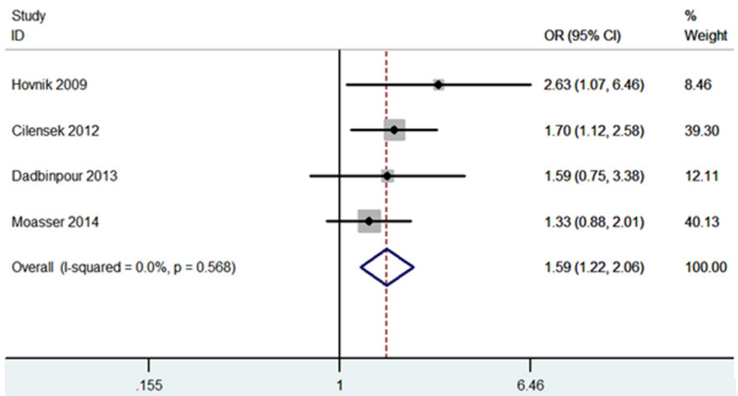

Figure 5. Forest plot of DR risk of GSTM1 polymorphism.

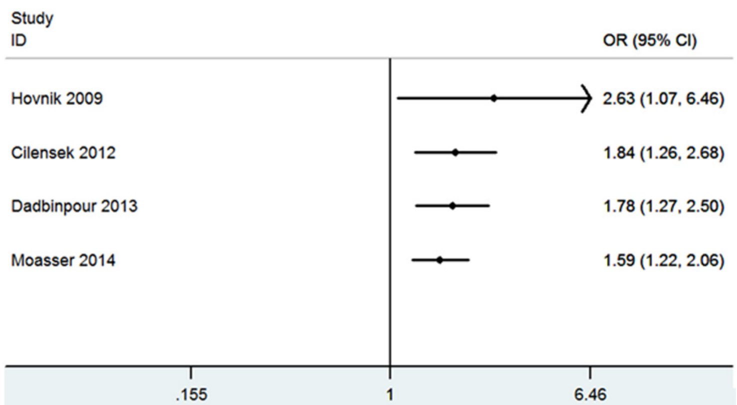

Figure 6. Cumulative meta-analysis of DR risk of GSTM1 polymorphism.

DR is characterized by gradual and progressive alterations in the retinal microvasculature. Damages to neurons and glia also occur during the course of DR. Individuals with diabetes, regardless of whether they are afflicted with type 1 or type 2, are all at risk of developing retinopathy. Increasing evidence emphasizes the critical involvement of elevated oxidative stress in the pathogenesis of diabetes and its complications. The retina is particularly susceptible to oxidative stress because of high energy demands and exposure to light [12]. A number of interconnecting biochemical mechanisms that contribute to the pathogenesis of DR have been identified, including inflammation, the polyol pathway, accumulation of advanced glycation end products (AGEs), the flux of hexosamine pathway, and protein kinase C (PKC) activation. All of these mechanisms appear to be associated with mitochondrial overproduction of reactive oxygen species (ROS) [13]. The GSTT1 and GSTT1 enzymes detoxifie products of oxidative stress and other reactive compounds such as the polycyclic aromatic hydrocarbons. Therefore, the polymorphisms of GSTT1 and GSTT1 might influence the risk of DR.

Heterogeneity is a potential problem that may affect the interpretation of the results. However, no significant heterogeneity was observed and thus heterogeneity did not influence the results. Results from one-way sensitivity analysis and cumulative meta-analysis suggested stability of these results. Additionally, funnel plots and Egger's tests did not find potential publication bias. All together, these results suggested that results of this meta-analysis were reliable.

Some limitations of this study should be acknowledged. Firstly, most studies in the meta-analysis were retrospective design which could suffer more risk of bias owing to the methodological deficiency of retrospective studies. Those there was no obvious risk of publication bias in the present meta-analysis, the risks of other potential bias were unable to be excluded. Some misclassification bias was possible because most studies could not exclude latent DR cases in the control group. Therefore, more studies with prospective design and low risk of other bias are needed to provide a more precise estimate of the association between GSTT1 and GSTT1 null genotype and DR risk. Secondly, we could not address gene-gene and gene-environmental interactions in the association between GSTT1 and GSTT1 null genotype and DR risk. The latter may be important for genes that code proteins with detoxifying function, but would require detailed information on exposures to various potential carcinogens and individual-level data and would be most meaningful only for common exposures that are found to be strong risk factors for the disease. Thus, more studies analyses on the gene-gene and gene-environmental interactions are needed.

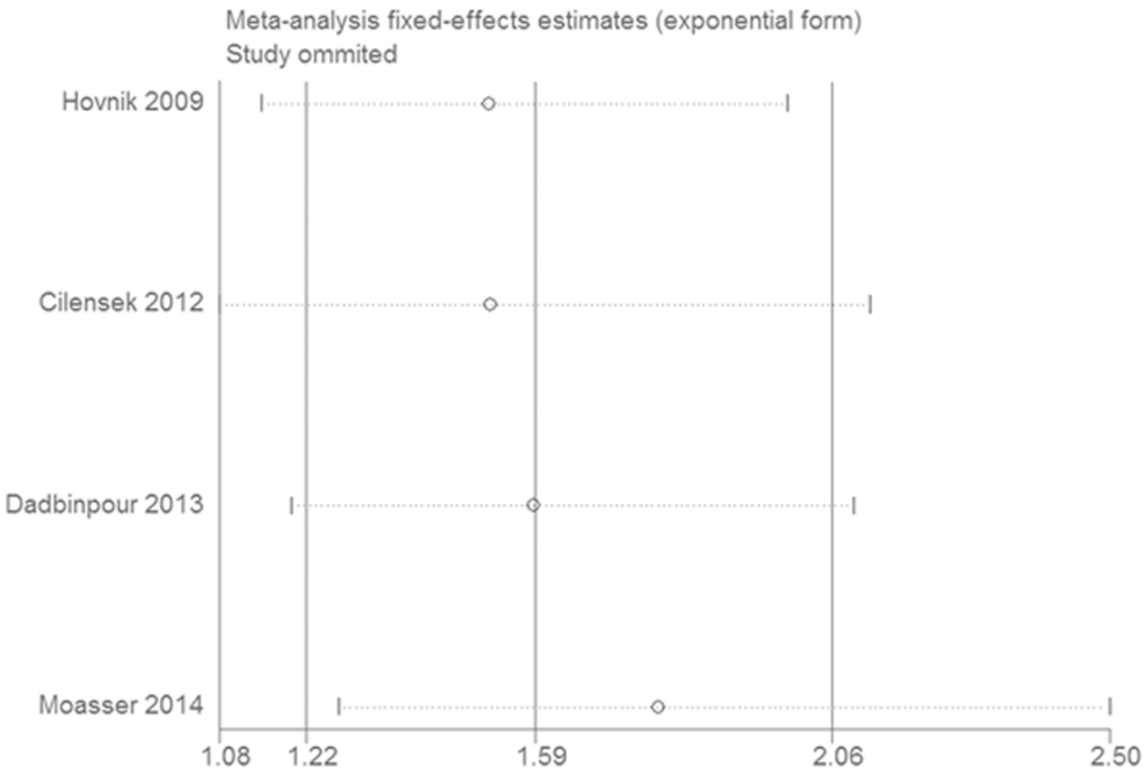

Figure 7. Sensitivity analysis of DR risk of GSTM1 polymorphism.

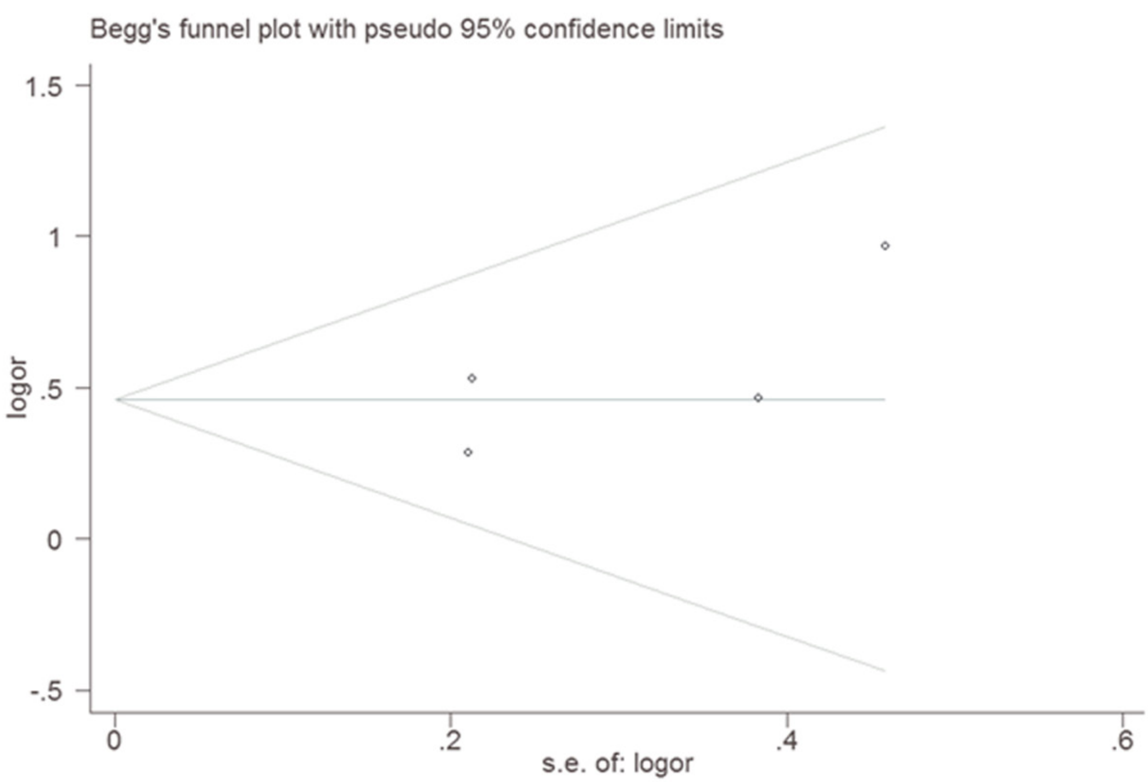

Figure 8. Funnel plot of association between DR risks of GSTM1 polymorphism.

In conclusion, this meta-analysis suggested that an increased risk of DR was associated with the null polymorphism of GSTT1 and GSTT1, respectively.

Disclosure of conflict of interest

None.

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