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Association between ACE gene polymorphism and carotid stenosisand and construction of related gene regulatory networks

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ABSTRACT

Objective: To investigate the association between DD, ID and II genotypes of ACE gene and carotid stenosis, and to construct a network of ACE-related genes regulating carotid stenosis.
Approaches: Meta-analysis was used to study the association between three genotypes DD, ID and II of carotid stenosis susceptibility gene ACE; functional annotation of ACE gene was conducted by GO analysis; and a network of ACE-related genes that regulate the mechanisms of carotid stenosis was established.
Results: Meta-analysis showed that DD and II genotypes of ACE gene were associated with carotid stenosis. GO analysis showed that the main biological processes involved in ACE include: the process of transforming angiotensinogen into mature angiotensin; angiotensin's mediation of the brain's response to alcohol consumption and thirst control; any chemical reaction involving the regulation of angiotensin; and the process of catalyzing the release of a C-terminal dipeptide from a polypeptide chain. A network of ACE gene regulation of carotid stenosis was constructed in combination with KEGG analysis.
Conclusion: The ACE gene is a susceptibility gene for carotid stenosis. Through the functional annotation and pathway analysis of ACE gene, an ACE gene-involved carotid stenosis regulatory mechanisms network was constructed.

1. Introduction

The main cause of carotid stenosis is atherosclerosis at the common carotid artery bifurcations or the base of intracranial artery (Buratti et al., 2014), that is, the carotid artery wall forms plaque and when these plaques enlarge or rupture, carotid stenosisor embolismis formed, causing a decrease in distal perfusion pressure and resulting in hypoperfusion cerebral infarction. Carotid stenosis caused by atherosclerosis is a common symptom among middle aged and elderly people, frequently accompanied with various cardiovascular risks. Carotid endarterectomy is currently recognized as the main approach for the treatment of carotid stenosis, and the United States now has nearly 100,000 cases of surgery each year. This operation can effectively improve brain blood supply and prevent stroke, and careful and meticulous observation and care before and after surgery are also very important, which can help reduce postoperative complications, cut down the morbidity rate, and improve the curative effect (Peng, 2015). It has been reported that angiotensin-converting enzyme ACE gene polymorphism is an adventurous factor for carotid atherosclerosis and stenosis in hemodialysis patients and ischemic stroke patients. ACE gene is located on human chromosome 17q23. The presence of insertion and deletion polymorphism in the 16 intron depended on the presence or absence of a 287 bp DNA fragment does not affect the ACE structure, but may be related to the functional imbalance (Lin et al., 2016). PCR detection revealed a 490 bp fragment (I allele) and a 190 bp fragment (D allele), showing three DNA genotypes – DD, ID and II (Sticchi et al., 2011). The ACE gene polymorphism is associated with a variety of cardiovascular diseases, but it is rarely reported whether it is related to carotid stenosis. In this study, the clinical literature of ACE gene mutation was used as the research object. The meta-analysis of the included literature was to systematically analyze the relationship between ACE gene polymorphism and carotid stenosis to explore the sensitivity of ACE gene mutation to carotid stenosis and to provide a basis for

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further research on carotid stenosis. On this basis, the GO database was used to functionally annotate the gene function, and KEGG analysis was combined to construct a network of ACE-related genes regulating carotid stenosis, providing a theoretical basis for studying the mechanism of carotid stenosis at the molecular level.

2. Materials and methods

2.1. Meta-analysis of ACE gene

The search databases include CNKI, WanFang Data, CQVIP, Google Scholar, PubMed database and other Chinese and English databases. Search terms include carotid artery, carotid stenosis, gene polymorphism, mutations. The literature inclusion and rejection criteria are as follows: Inclusion criteria: a. The study contents are the Chinese and English literature related to the carotid stenosis susceptibility gene ACE; b. The research type is retrospective study; c. All the included literatures include experimental group and control group, patients with carotid stenosis as the experimental group, and healthy people as the control group, and all subjects are not limited to ethnicity, age and gender; d. Research methods are correct, process standard; e. In case of multiple research reports from the same author or the same research unit, the latest or most complete report is adopted. Exclusion criteria: 1. Non-case-control study; 2. Review literature, comments or lectures; 3. Data analysis methods are incorrect or not provided; 4. Documents that cannot extract valid data; 5. Repeated publications.

2.2. GO analysis of ACE gene

In GO analysis, first enter the Ami GO homepage (http://geneontology.org), set "biological process", "Homo sapiens", "gene" as the screening conditions, and make a preliminary analysis of GO function annotation of ACE gene.

2.3. KEGG analysis of ACE gene

In KEGG analysis of ACE gene, first enter the homepage of the KEGG signaling pathway database (https://www.genome.jp/kegg/pathway.html), set the screening condition organism as "hsa", keyword as "ACE", and search the regulatory network of ACE gene in affecting carotid stenosis.

3. Results

3.1. Meta-analysis of ACE gene

A total of 301 related articles were retrieved. After reading the topics, abstracts or full texts, the non-randomized controlled clinical trials that do not match the purpose of this study or repeated publications were excluded. 19 articles were finally included, including 4434 cases of carotid stenosis and 4243 cases of the control group, and the basic information of the included literature is shown in Table 1.

3.1.1. Association between type II genotype and carotid stenosis

In the II genotype test, $P = 0.13 \geqslant 0.10$, which indicates that there is homogeneity between the studies. Therefore, the M-H fixed effect model Fixed is used to combine the effect size. In the I^2 test, $I^2 = 27\%$, which means that there is no heterogeneity between the selected documents. In the Z test, $Z = 2.95 > 1.96$, $P < 0.003 < 0.05$, and 95% confidence interval for the combined effect size is 0.87 [0.79, 0.95], which represents that the combined effect size of the test was statistically significant. It is suggested that the II genotype of angiotensin-converting enzyme gene ACE has a positive effect on the occurrence of carotid stenosis, refer to Fig. 1.

The bias analysis of the II genotype was carried out, and the sensitivity analysis was conducted by excluding each research method one by one, after which the combined OR value showed no significant change, indicating the meta-analysis results were reliable and stable. From the funnel plot analysis, the II genotype funnel plot is more symmetrical, indicating that its meta-analysis has less publication bias. That is, there is no significant publication bias in the study results, and the selected studies have good representativeness, refer to Fig. 2.

3.1.2. Association between ID genotype and carotid stenosis

In the ID genotype test, the Q test had $P = 0.72 \geqslant 0.10$, the I^2 test had $I^2 = 0\%$, showing no heterogeneity between the studies, so we used the Fixed of M-H model to combine the effect size. In the Z test, $Z = 0.27 < 1.96$, $P = 0.79 > 0.05$, and 95% confidence interval for the combined effect size is 0.99[0.90, 1.08], which represents that the combined effect size of the test was not statistically significant. It is suggested that the ID genotype of

Table 1
Baseline characteristics of the ACE gene inclusion study.

Name	Year	Genotype determination method	Research design	Sample size (carotid stenosis/control group)	Sample source	Hardy balance test
Cao Haitao	2011	PCR-RFLP	Case – control	49/443	Hospital	Yes
Chen Hongyi	2011	PCR-RFLP	Case – control	176/176	Hospital	Yes
Kong Xiangdong	2003	PCR-RFLP	Case – control	312/189	General public	Yes
Lai Yanxian	2015	PCR-RFLP	Case – control	54/50	Hospital	Yes
Lin Huizhong	2013	PCR-RFLP	Case – control	1380/888	General public	Yes
QiXiaohua	2009	PCR-RFLP	Case – control	100/100	Hospital	Yes
Ren Ming	2016	PCR-RFLP	Case – control	105/112	Hospital	Yes
Wang Yanhua	2005	PCR-RFLP	Case – control	432/583	Hospital	Yes
Wu Ken	2008	PCR-RFLP	Case – control	106/146	Hospital	Yes
Wu Lingning	2015	PCR-RFLP	Case – control	121/129	General public	Yes
Wu Xiaoying	2008	PCR-RFLP	Case – control	324/305	Hospital	Yes
XueTan	2015	PCR-RFLP	Case – control	110/43	Hospital	Yes
Yang Jianmin	2007	PCR-RFLP	Case – control	170/196	Hospital	Yes
Zhang Chunyu	2010	PCR-RFLP	Case – control	164/109	General public	Yes
Zhang Dongmei	2013	PCR-RFLP	Case – control	228/119	Hospital	Yes
Zhang Liping	2012	PCR-RFLP	Case – control	244/410	Hospital	Yes
Zhang Yuling	2007	PCR-RFLP	Case – control	115/96	Hospital	Yes
Zhan Yiyang	2006	PCR-RFLP	Case – control	190/94	Hospital	Yes
Zhou Biao	2010	PCR-RFLP	Case – control	54/55	General public	Yes

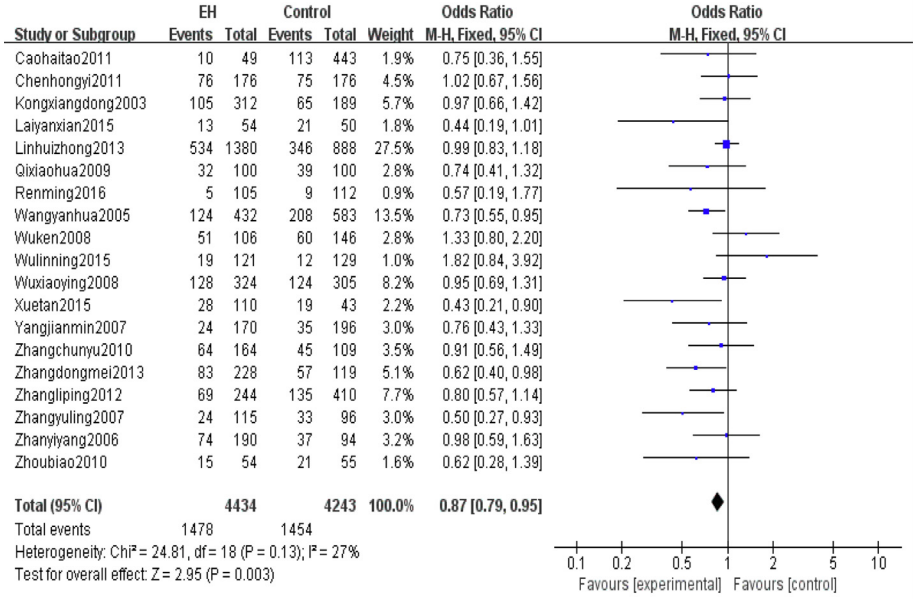

Fig. 1. ACE II genotype forest map.

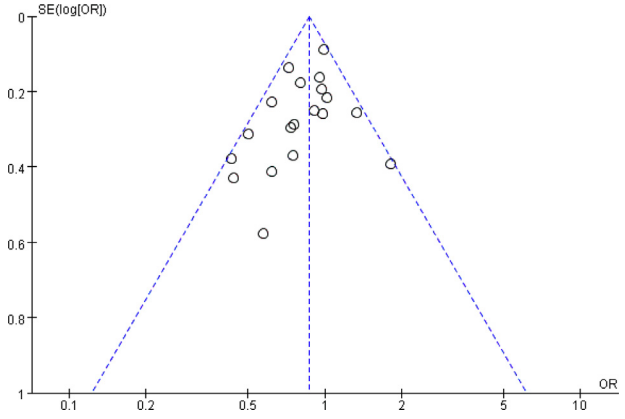

Fig. 2. ACE II genotype funnel plot.

angiotensin-converting enzyme gene ACE has no correlation with the occurrence of carotid stenosis, refer to Fig. 3.

The bias analysis of the ID genotype was carried out, and the sensitivity analysis was conducted by excluding each research method one by one, after which the combined OR value showed no significant change, indicating the meta-analysis results were reliable and stable. From the funnel plot analysis, the ID genotype funnel plot is more symmetrical, indicating that its meta-analysis has less publication bias. That is, there is no significant publication bias in the study results, and the selected studies have good representativeness, refer to Fig. 4.

3.1.3. Relationship between DD genotype and carotid stenosis

In the DD genotype test, the Q test had $P = 0.02 < 0.10$, the I^2 test had $I^2 = 45\%$, showing a slight heterogeneity between the studies, so we used the random effect model Random to combine

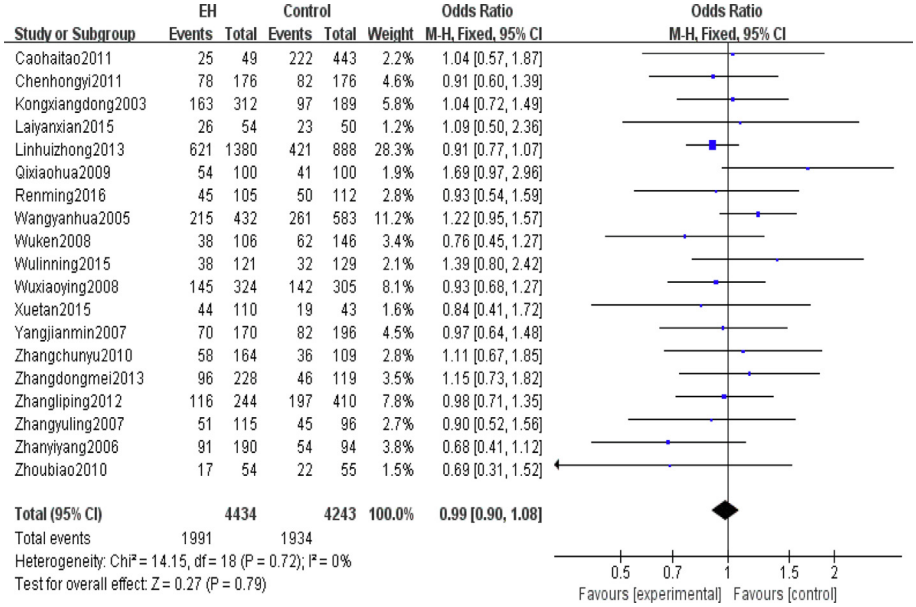

Fig. 3. ACE ID genotype forest map.

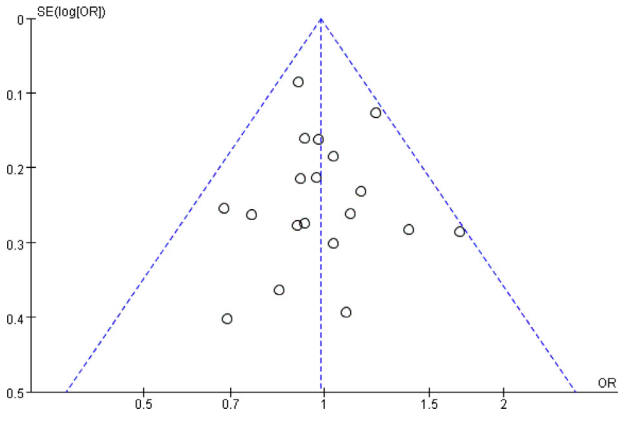

Fig. 4. ACEID genotype funnel plot.

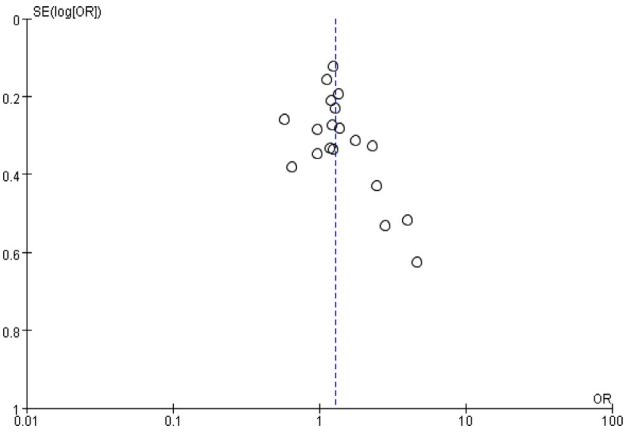

Fig. 6. ACEDD genotype funnel plot.

the effect size. In the Z test, $Z = 2.97 > 1.96$, $P = 0.003 < 0.05$, and 95% confidence interval for the combined effect size is 1.29[1.09, 1.53], which represents that the combined effect size of the test was statistically significant. It is suggested that the DD genotype of gene ACE has a positive effect on the occurrence of carotid stenosis, refer to Fig. 5.

The bias analysis of the DD genotype was carried out, seen from the funnel plot analysis, the points of the inverted funnel plot of the DD genotype were asymmetrically distributed, indicating the presence of publication bias. No matter the sensitivity was analyzed by fixed or random effect model, the results were consistent, indicating that the meta-analysis results were stable, refer to Fig. 6.

3.2. GO analysis results of ACE gene

The biological process functional annotation of carotid stenosis susceptibility gene ACE was performed by GO analysis, and the results are shown in Table 2. It can be seen that the ACE gene has two main functions – biological process and molecular function. The biological process mainly involves the process of transforming angiotensinogen into mature angiotensin. Angiotensin mediates the brain's response to alcohol consumption and thirst control, and any chemical reaction involved in the regulation of angiotensin; molecular function is primarily involved in the process of catalyzing the release of C-terminal dipeptides from a polypeptide chain.

3.3. Network construction

Based on the GO functional analysis, the KEGG database was used to analyze the carotid stenosis susceptibility gene ACE involved in this study. The KEGG signal pathway database was used to obtain a network of genes involved in the regulation of carotid stenosis, and the results are shown in Fig. 7.

4. Discussion

In the middle age, the arterial wall will generally show thickening of the intima, decreasing of vascular elasticity, and increasing of intimal stiffness (Xin et al., 2019). In patients with carotid stenosis, blood flow increases blood pressure, and dyslipidemia aggravates endothelial damage. Hemodynamic changes increase

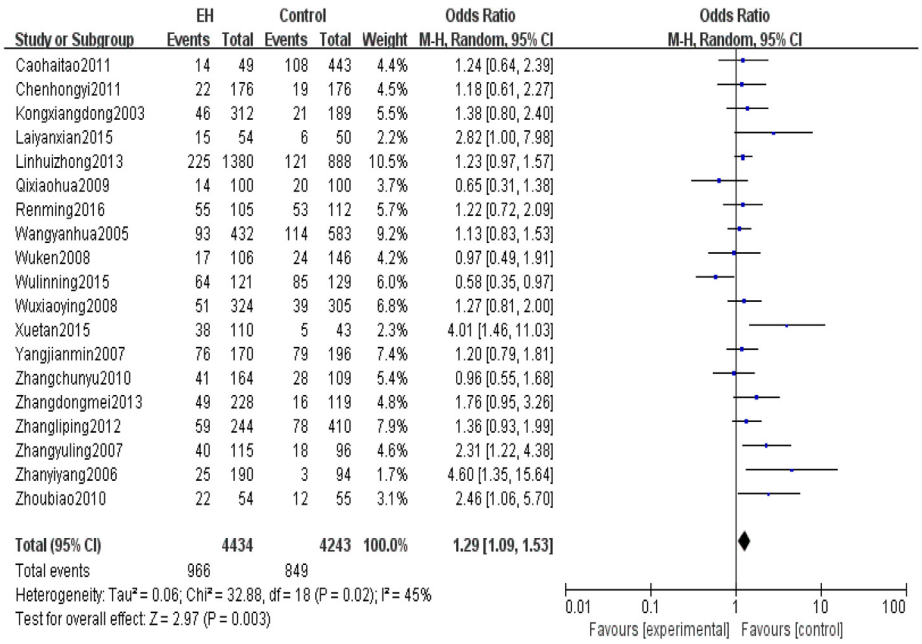

Fig. 5. ACE DD genotype forest map.

Table 2
GO analysis of ACE gene.

Accession	Name	Ontology	Definition
GO:0008241	peptidyl-dipeptidase activity	molecular_function	Catalysis of the release of C-terminal dipeptides from a polypeptide chain
GO:0002003 GO:0002005	angiotensin maturation	biological_process	The process leading to the attainment of the full functional capacity of angiotensin by conversion of angiotensinogen into mature angiotensin in the blood
GO:0003051	angiotensin-mediated drinking behavior	biological_process	The drinking behavior that is mediated by the action of angiotensin in the brain. Angiotensin stimulates the brain centers that control thirst
GO:0060177	regulation of angiotensin metabolic process	biological_process	Any process that modulates the frequency, rate or extent of the chemical reactions and pathways involving angiotensin

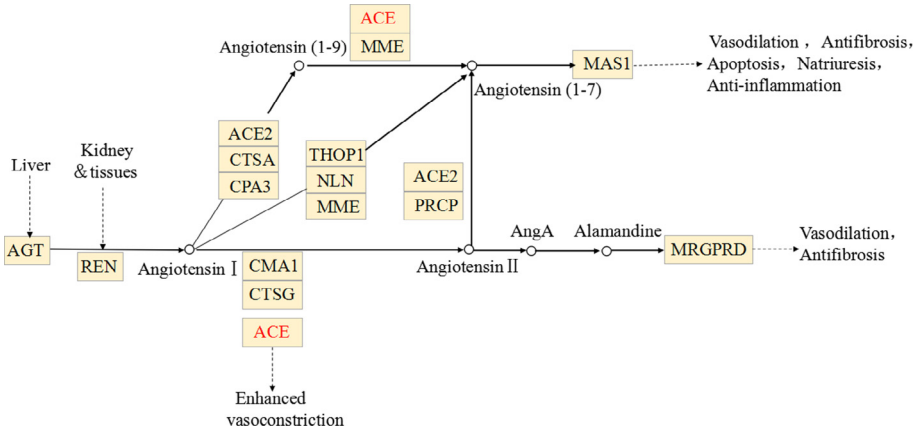

Fig. 7. Network of ACE gene regulation of carotid stenosis.

carotid turbulence and shear stress, vascular endothelium is easily damaged and dysfunctional, high concentration mucopolysaccharide and lipoprotein diffuse into the endothelium, promoting lipid deposition and hyperplasia of the intima; smooth muscle cell migration and proliferation, plus elastic fiber formation together promote localized intima thickening and accelerate the progression of carotid stenosis (Zhai et al., 2004). It has been reported that the ACE gene polymorphism is an adventurous factor for triggering carotid atherosclerosis and stenosis in patients with hemodialysis and ischemic stroke (Ihle-Hansen et al., 2019). This study used CNKI, CQVIP, WanFang Data, Pub Med and EMBASE database as the main source of searching the clinical research literature of carotid stenosis susceptibility gene ACE, and used meta-analysis to comprehensively and quantitatively analyze related literature. The results showed that ACE is a susceptibility gene for carotid stenosis, and DD and II genotypes play a role in the development of carotid stenosis.

The carotid stenosis susceptibility gene ACE is located on human chromosome 17q23. The presence of insertion and deletion polymorphism in the 16 intron due to the presence or absence of a 287 bp DNA fragment does not affect the ACE structure, but may be related to the functional imbalance (Lin et al., 2017). PCR detection revealed a 490 bp fragment (I allele) and a 190 bp fragment (D allele), showing three DNA genotypes – DD, ID and II (Sticchi et al., 2011). In this study, the clinical literature of ACE gene mutation was used as the research object. The meta-analysis of the included literature was to systematically analyze the relationship between ACE gene polymorphism and carotid stenosis to explore the sensitivity of ACE gene mutation to carotid stenosis and to provide a basis for further research on carotid stenosis. The results indicate that: the II genotype of gene ACE has a positive effect on the occurrence of carotid stenosis; the ID genotype of gene ACE has no correlation with the occurrence of carotid stenosis; the DD genotype of gene ACE has a positive effect on the occurrence of carotid stenosis. In the study of Li (2011), direct B-mode imaging was used to confirm the correlation between ACE gene I/D genotype and carotid IMT in 1–2 grade hypertension patients in northern China, suggesting that the DD genotype may be an independent risk factor for early atherosclerosis and may interact with hypertension to accelerate carotid thickening and plaque formation in hypertensive individuals. The mechanism by which the ACE gene affects carotid wall thickening is still unclear. Therefore, this study used the GO database to functionally annotate the ACE gene, and used the KEGG database to search the ACE gene-related network, and finally constructed a network of ACE-related genes that regulate the mechanisms of carotid stenosis.

Since the ACE gene I/D polymorphism is independent of plasma angiotensin level II, the local renin-angiotensin system may play be of vital significance in ACE gene (Cui et al., 2018). By using the living ACE gene transfection technique, it was found that the ACE gene transfected blood vessels developed a hypertrophic response (Xu et al., 2009). Therefore, a reasonable explanation is that the ACE genotype leads to thickening of the carotid artery by altering the genetic background of ACE activity in the blood vessels. DD genotype is associated with increased ACE activity in endothelial cells, and ACE activity may lead to neointimal proliferation, extracellular matrix degradation and vascular hypertrophy by increasing local angiotensin II (Ang II) production and increasing bradykinin degradation, thus causing thickening of the carotid wall (Zheng et al., 2009). However, the ACE gene I/D polymorphism may be merely a marker of unknown functional variation (ACE S/s) located in or near the ACE gene. Another hypothesis is that the DD genotype in the ACE gene is not involved directly, but rather acts as an indicator associated with other adjacent genes that have a direct effect on vascular growth regulation (Marrocco-Trischitta et al., 2006).

Meta-analysis uses a series of control measures to rigorously control the literature search methods, the criteria for screening

the included studies, the extracted data standards, and the software analysis methods. Therefore, the bias in the analysis process is significantly less than the traditional review (Kostulas et al., 2015; Kalyani, 2005), but a range of reasons makes the results of the meta-analysis inevitably biased, such as the most common publication bias. Since this research analysis only searches the documents published in the database, the data is less; in addition, because the language of the search literature is limited to Chinese and English, other languages are not included, which affects the comprehensiveness of the data, besides, the experimental study in the literature must have a control group. The inconsistency of the scoring standards for the screened documents and the incompleteness of the literature inclusion will affect the accuracy of the results of the meta-analysis. Therefore, as much literature and clinical research samples as possible are needed in the meta-analysis and standard testing methods are required.

In summary, this study provides a systematic and comprehensive qualitative and quantitative retrospective review of the clinical literature on ACE gene published at home and abroad. The results suggest that the II genotype and DD genotype of ACE gene play an important role in the occurrence and development of carotid stenosis. On this basis, the biological function annotation of ACE gene was analyzed by GO analysis, and KEGG analysis was used to construct a network of ACE gene regulation of carotid stenosis, laying a foundation for the study of carotid stenosis.

Declaration of Competing Interest

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