Original Article
Correlation between CYP4F2 gene rs2108622 polymorphism and susceptibility to ischemic stroke

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Abstract: Objective: To conduct a meta-analysis for the correlation between cytochrome P450 4F2 (CYP4F2) rs2108622 (V433M) gene polymorphism and ischemic stroke. Methods: We retrieved the case-control studies on the correlation between CYP4F2 V433M polymorphism and ischemic stroke included in domestic and international databases before January 2015 and selected the best genetic model, using RevMan 5.2 software for meta-analysis. According to the heterogeneity test results of selected literature, the effect model of consolidated data was selected, and the combined OR and 95% CI were calculated. Results: A total of six documents were included. Recessive model (VM + MM vs. VV) was selected as the best genetic model. The combined results showed that: compared with wild-type VV, there are significant association between ischemic stroke and CYP4F2 polymorphism (OR merge = 1.37, 95% CI: 1.21~1.54, P < 0.001). Conclusion: CYP4F2 V433M may be the susceptibility gene for ischemic stroke.

Keywords: Ischemic stroke, cytochrome P450 4F2, gene polymorphism, susceptibility, meta-analysis

Introduction

Cytochrome P450 is a widespread heme-sulfur protease in the living body, playing an important role in the metabolism of exogenous and endogenous compounds [1]. CYP4F2 is expressed in the liver, heart, lungs, kidneys and leukocytes, which plays an important role in regulating the muscle-derived contraction of kidney, brain, skeletal muscle and mesenteric arterial smooth muscle through its participation in leukotriene B4 (LTB4) and 20-HETE metabolism. Recent studies have reported that, 20-HETE was involved in the development and progression of ischemic stroke [2, 3]. Therefore, research on the correlation between CYP4F2 gene polymorphism and ischemic stroke gradually increased, especially V433M loci polymorphism; but the conclusions in different races or genders remain controversial [4, 5]. In this study, the method of meta-analysis was used to comprehensively and systematically evaluate the correlation intensity between CYPV433M polymorphisms and ischemic stroke susceptibility, in order provide

appropriate references to the genetic etiology of ischemic stroke.

Materials and methods

Search strategy

With the recommendation of the Cochrane Collaboration search strategy, we collected the relevant researches about CYP4F2 V433M polymorphisms and the susceptibility of ischemic stroke. Retrieving relevant documents based on English words such as "ischemic stroke", "cerebral infarction", "CYP4F2", "Susceptibility", "polymorphism" from PubMed, EMbase, Web of Science, Chinese Journal Full-text database (CNKI), Chinese Biomedical literature Database (CBM), Wanfang and VIP databases. The language of the published articles were not limited. Searching time ended in January 2015.

Literature inclusion criteria

The included literatures for meta-analysis must meet the following criteria: (1) the included

Table 1. The characteristics of included studies

Authors	Publication Year	Country	Sample size	Genotyping methods	Genotype (Case)			Genotype (Control)		
					VV	VM	MM	VV	VM	MM
Deng et al.	2010	China	302/350	PCR-RFLP	161	117	34	171	144	35
Ding et al.	2010	China	694/551	TaqMan	-	-	-	-	-	-
Fava et al.	2008	Sweden	199/5592	TaqMan	-	-	-	-	-	-
Fu et al.	2008	Japan	175/246	TaqMan	97	60	18	133	80	33
Munshi et al.	2012	Saudi Arabia	507/487	PCR-RFLP	114	257	136	138	246	103
Yan et al.	2015	China	310/330	PCR-RFLP	167	188	25	152	142	36

studies must be case-control studies about CYP4F2 V433M polymorphisms and susceptibility of ischemic stroke. If the result of the research reported relationships of CYP4F2 V433M sites in different sex separately in the same article, the results of each associated study were combined as a study for analysis. (2) The distribution of genotype frequency, or evaluable indicators, such as odds ratios (odd ratios, OR) and 95% confidence intervals (confidential interval, CI) must be specific or can be calculated. (3) the frequency of population genotype must meet the Hardy-Weinberg equilibrium in the control group. (4) If there are duplicated publication or data, we selected the articles with the largest sample size or the latest published literatures.

Exclusion criteria

(1) Review articles. (2) The reports repeated the same population. (3) The frequency distribution of gene loci cannot be obtained. (4) Diagnostic criteria were different from other studies. (5) Genotype distribution in the control group did not meet the Hardy-Weinberg (HW) equilibrium.

Data extraction

Literature screening and data extraction were performed by two researchers independently for included documents. If there was disagreement, a third expert was referred or solve it through discussion. In each case-control study we extracted the contents of the following: first author, publication year, country, race, the average age of the sample size, genotypic methods, source control subjects.

Statistical analysis

Chi-square goodness of fit test was used for determining whether the genotype frequency distribution in the control group for the included studies met Hardy-Weinberg equilibrium law. If it did not meet the genotype frequency distribution of Hardy-Weinberg equilibrium law, the population in the control group maybe incorrect and the general characteristics were not presented. This type of literature needs to be excluded. OR value was the correlation strength indicator for the studies of CYP4F2 V433M polymorphisms and ischemic stroke. According to Thakkinstian et al. [6] promoted the best genetic model selection methods, we determined the best genetic models (VM + MM vs. VV) for evaluating CYP4F2 V433M polymorphism and susceptibility of stroke.

Q statistic test methods and I^2 quantitative assessment were used for evaluating the heterogeneity size between the studies. The significance level was defined 0.05, and the appropriate combined mode was selected according to the heterogeneity of the test results. If there is no significant heterogeneity among the findings (P > 0.05, using the Mantel-Haenszel fixed-effects model method to combined OR value; if there was significant difference in heterogeneity between studies (P < 0.05), we chose DerSimonian and Laird random effects model law to merge). The sensitivity analysis was used to assess the reliability and stability of results. Egger's and Begg's test were used for quantitative assessment of publication bias. Statistical analysis combination was completed by the RevMan 5.2 software.

Results

Results of literature searching

33 related articles were retrieved according to the formulated searching strategy. According to the title of the literature, 21 articles were ruled
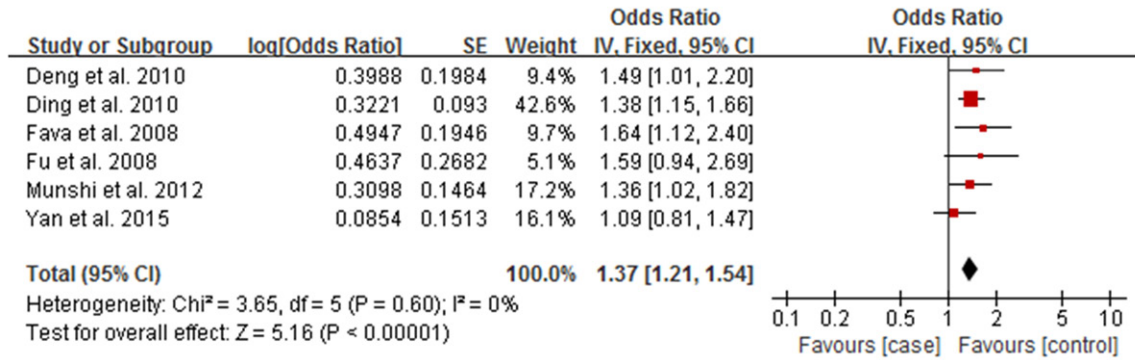
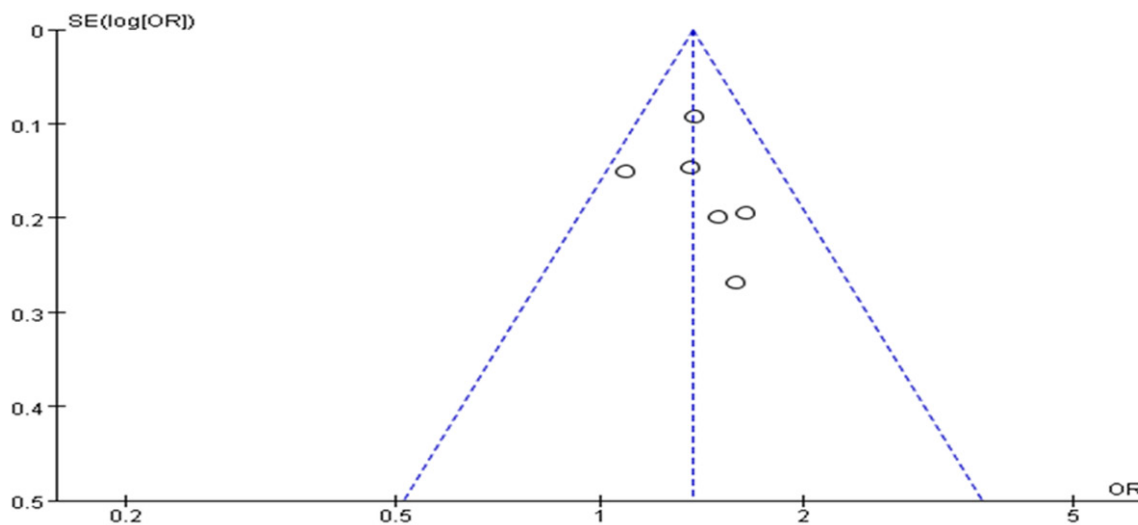Figure 1. Forest plot of stroke and rs2108622 polymorphism.

Figure 2. Funnel plot for publication bias tests.

out after initial assessment. Through reading the abstract and full text, two documents did not meet the inclusion criteria, two documents with duplicated data and two article lack of specific data. Ultimately six documents were included, the literatures were in the references [7-12]. All the six articles were wrote in English. The study involved a total of 2187 cases of ischemic stroke patients and 7556 cases of individuals in the control group which all met Hardy-Weinberg equilibrium. The main message of each study included first author, publication year, country, the sample size, genotypic methods, and genotype distribution, shown in **Table 1**.

Results of meta-analysis

We used the revessive genetic model (VM + MM vs. VV) to merge the OR value. Test for

heterogeneity showed that P = 0.60, I^2 = 0%, indicating the no heterogeneity existed between each studies. Therefore, random effect model was used. The results showed that, compared with wild VV genotype, ischemic stroke risk in carrying mutant heterozygous VM and mutant homozygous MM individuals showed significant difference (OR = 1.37; 95% CI: 1.21~1.54), shown in **Figure 1**.

Sensitivity analysis

Sensitivity analysis is mainly used for evaluating the impact of single study on the entire meta-analysis results. By gradually removing each study, the meta-analysis for rest research was carried out to evaluate the stability and reliability of results. Sensitivity analysis of this study showed that none of included studies could cause significant changes in the correla-

tion degree between CYP4F2 V433M polymorphisms and ischemic stroke, suggesting that the results of this meta-analysis were stable and reliable.

Assessment of publication bias

The character of the plot was basically inverted funnel-shaped, bilateral symmetry, which indicated that there was no publication bias and the conclusions were reliable (**Figure 2**).

Discussion

Ischemic stroke is the result of cooperation of genetic and environmental factors; many genetic factors play an important role in its pathogenesis. The incidence of ischemic stroke accounts for about 80 percent of all strokes, and it increases with age increasing. CYP4F2 as a member of CYP450 superfamily, plays a very important role in the metabolism of exogenous and endogenous compounds. It is located on human chromosome 19p13.11, about 20,000 bp, composed by 13 exons and 12 introns, encoding 520 amino acids [13]. 1347th nucleotide G → A polymorphism in the coding region of the 11th Exon causes changes in codon 433 (methionine M → V), leading to reduction in 20-HETE generation, thus affecting the susceptibility to ischemic stroke. CYP4F2 gene polymorphisms have obvious racial differences, and 1347G > A (V433M) distributes in all races; the distribution frequency of 1347A allele is higher in Sweden population, which is 46.5% [14]; in Chinese population, it is 27.50% [7].

At present, the sample size of the studies on the correlation between CYP4F2 V433M polymorphisms and ischemic stroke is generally small; OR and 95% CI values distributed dispersedly, therefore, statistical tests were difficult to be effective. In addition, the results of different studies on genetic polymorphism correlations were easily affected by disease heterogeneity, trial design, selection of control population and genetic models among ethnic groups. Advantages of the meta-analysis method used in this study were in that through quantitatively integrating the case-control studies on the correlation between the same gene polymorphism and susceptibility to ischemic stroke, it can increase the sample size and improve the performance of statistical analysis on one hand; on the other hand it can use subgroup

analysis to group the factors that may affect heterogeneity or use heterogeneity test to explore heterogeneity.

This study included six studies by meta-analysis, including a total of 2187 cases and 7556 controls, and the correlation between CYP4F2 V433M polymorphisms and susceptibility to ischemic stroke was evaluated truly by Recessive model. The combined results showed that, there was significant correlation between CYP4F2 V433M polymorphisms and ischemic stroke susceptibility. In the individuals carrying mutant heterozygous VM and homozygous MM were more susceptible to ischemic stroke compared with individuals carrying wild-type VV.

In this meta-analysis, there are also some limitations: on the one hand, due to the inability to obtain the OR value of each study after adjustment of age and sex, the study can only merge the crude OR values to evaluate the correlation. This may result in a deviation between the test association intensity and the real situation. On the other hand, we did not analysis the association in the subgroup by race due to the small number of included literature.

In conclusion, this study by meta-analysis found that in recessive model, those subjects who carried CYP4F2 V433M polymorphisms had a significantly higher risk of ischemic stroke. However, the incidence of ischemic stroke is a complex process mediated by the interactions between multiple genes and environmental factors, to fully reveal the impact of CYP4F2 gene on the susceptibility to ischemic stroke, more attention should be paid to the interactions between CYP4F2 gene and environmental factors in the future.

Disclosure of conflict of interest

None.

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