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A Meta-analysis of β-fibrinogen Gene-455G/A Polymorphism and Plasma Fibrinogen Level in Chinese Cerebral Infarction Patients[1]

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Objective To evaluate the correlation between the β-fibrinogen gene-455G/A polymorphism and cerebral infarction in Chinese population by means of meta-analysis. **Methods** Genetic association studies on evaluating the β-fibrinogen gene -455G/A polymorphism and cerebral infarction involving Chinese population published before December 2005 were collected from database of PubMed, EMBASE, and CNKI. All the data in literature were abstracted based on the defined selection criteria by two independent investigators. Publication bias was tested by funnel plot and the odd ratios of all studies were combined dependent on the result of heterogeneity test among the individual studies. The software Review Manager (Version 4.2) was used for meta-analysis. **Results** Eleven studies including 1405 patients and 1600 controls met the selection criteria. There was no publication bias in 11 reviewed studies. Heterogeneity test of reviewed studies showed statistically significant differences (χ^2=24.58, P=0.006) among the ORs of individual studies. The combined OR of 11 studies of susceptibility to cerebral infarction in –455A allele carriers compared with the -455G/G wild homozygotes was 1.33 (95%CI 1.04-1.71, P=0.02). In the patients with cerebral infarction in 6 studies, the summarized average plasma fibrinogen level of allele A carrier was 0.29 g/L (95%CI 0.14-0.44, P=0.0002) higher than that of -455G/G homozygous ones. **Conclusions** β-fibrinogen gene -455G/A polymorphism might contribute to susceptibility of cerebral infarction in Chinese population; allele A increases the individual susceptibility to the disease.

Key words: β-fibrinogen; Gene polymorphism; Cerebral infarction; Meta-analysis

INTRODUCTION

Elevated plasma levels of fibrinogen are associated with an increased risk for vascular events including coronary heart disease, cerebral infarction, and venous thromboembolism[1-2]. The synthesis of its β-polypeptide chain is the rate-limiting step in fibrinogen formation. In turn the variations at β-fibrinogen gene promoter region loci as well as some environmental factors have been proved to affect blood fibrinogen level[3-4]. The G/A variability in the -455 locus of the β-fibrinogen promoter region, which was documented to be strong or complete linkage disequilibrium with the C-T mutation at nucleotide position -148，the closest polymorphism locus to the responsive elements of IL-6, is associated with increased plasma fibrinogen levels[5-7]. Recently, researches have focused on identifying the association between the polymorphism and susceptibility of vascular disease including coronary heart disease and cerebral infarction. Up to now, several investigations[8-18] on the relationship between the gene polymorphism and cerebral infarction in Chinese population have been completed. However, the results remained controversial. Some studies[8-10,14,17] have indicated that the -455A allele may be associated with an increased risk of cerebral infarction, but other investigations[11-13,15-16,18] have been apparently inconclusive. Single studies may have been underpowered to detect relationships or even overall effects. Therefore, we sought to investigate whether the presence of the -455A allele was associated with

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an increased susceptibility risk of cerebral infarction among 11 individual studies performed in Chinese population by means of meta-analysis.

METHODS

Data Source

Genetic association studies on evaluating the β-fibrinogen gene -455G/A polymorphism and cerebral infarction involving Chinese population published before December 2005 were collected by computer-based searches, scanning of the reference lists for all case-control studies. Computer searches of CNKI (http://www.cnki.net), PubMed and EMBASE were conducted using both MeSH terms and text words "fibrinogen", "cerebral infarction", "ischemic stroke" in combination with "genetic", "polymorphism(s)", "mutation", "genotype" or "gene(s)" without language restriction.

Selection Criteria

For inclusion, studies should be case-control in design, involving unrelated subjects and examination of the relationship between the β-fibrinogen gene -455Gg/A polymorphism and cerebral infarction. The criteria for diagnosis of cerebral infarction are internationally recognized. Studies were excluded if subjects were ⩽18 years or ⩾80 years old. Those performed on minority subjects or reported only as abstracts were also not included. Articles investigating the same or subset population of reported articles, or not having an appropriate case-control design were also excluded. In all of the studies, the genotype frequencies should be consistent with Hardy-Weinberg equilibrium. When the genotyping information of the polymorphism was not reported in detail, authors were contacted to obtain the relevant information.

Data Abstraction

Two investigators independently extracted data and reached a consensus on all of the items. The following information was collected from each study according to a fixed protocol: author, journal, and year of publication, geographical location, numbers of cases and controls, genotyping information, and frequencies of alleles. If the plasma fibrinogen levels were available in the studies, the data were also abstracted for analysis.

Meta-analysis

In the majority of selected studies, the authors only presented the number of genotypes and allele frequencies and the results of Chi-square tests for them. For further analysis, we calculated the odds ratios (ORs) and respective 95% confidence intervals comparing the carriers of rare allele with wild homozygotes, as (G/A + A/A) *versus* G/G. Chi-square test was used for Hardy-Weinberg equilibrium of genotypes in control group of each reviewed study and heterogeneity of two allele frequencies in control groups among all studies. The Peto Mantel-Haenszel fixed-effect model or DerSimonian Laird random-effect model was selected to summarize the ORs dependent on the results of heterogeneity test among individual studies. The differences of plasma fibrinogen level between A allele carriers and G/G homozygotes in cerebral infarction patients were also combined dependent on the heterogeneity of the studies. Inverted funnel plot was used to provide evaluation of publication bias. All analyses were done using Review Manage (v.4.2; Oxford, England) software. All the P values were two-sided. The difference was considered statistically significant when $P<0.05$.

RESULTS

Study Selection and Subject Characteristics

The primary search generated 19 potentially relevant articles, of which 11 met the selection criteria[8-18], including 1405 patients and 1600 controls. All of the cerebral infarction were diagnosed based on clinical characteristics and documentation of CT scan or MR image. Most of the controls were individuals receiving routine health examination. The gene polymorphism were detected by means of PCR-RFLP using Hae Ⅲ to digest the PCR product in all of reviewed studies. There was no significant heterogeneity for two alleles frequencies in control groups of all reviewed studies (χ^2= 9.41, P=0.49). The detailed information of each study is described in Table 1. The genotype distribution, allele information, and odds ratio of individual studies are described in Table 2. The results of Hardy-Weinberg equilibrium test for all of control groups are shown in Table 3, and the plasma fibrinogen level of patients with cerebral infarction were presented in Table 4.

Meta-analysis of Relationship Between Gene Polymorphism and Disease

Figure 1 shows the distribution of the ORs from individual studies in relation to their respective SD being symmetric in funnel plot, which suggested that there was no significant publication bias in 11 reviewed

TABLE 1

Characteristics of Published Studies of Association between β-fibrinogen -455G/A Gene Polymorphism and Cerebral Infarction Included in Meta-analysis

Author	Journal and Year of Publication	Diagnose of Disease	Geographical Location	Sample Size	
				Cases	Controls
QIAN JJ, *et al*.[8]	J Apoplexy Nerv Dis, 2004	Cerebral Infarction	Jiangsu	90	102
SUN H, *et al*.[9]	Chin J Geriatr. 2004	Cerebral Infarction	Shandong	101	108
LIU Y, *et al*.[10]	Natl Med J China, 2000	Cerebral Infarction	Zhejiang	91	74
LIU R, *et al*.[11]	Chin J Hematol, 2002	Cerebral Infarction	Tianjin	96	273
BI S, *et al*.[12]	Chin J Neuromed. 2003	Cerebral Infarction	Helongjiang	134	166
ZHAO WJ, *et al*.[13]	Chin J Clin Rehab.2005	Cerebral Infarction	Guangxi	111	123
ZHOU SN, *et al*.[14]	J Cerebral Neruol. 2002	Cerebral Infarction	Shandong	102	53
ZHENG H, *et al*.[15]	Chin J Hematol, 2001	Cerebral Infarction	Guangdong	52	47
MA LY, *et al*.[16]	Chin J Geriatr Cariovas, 2001	Cerebral Infarction	Beijing	294	279
CAI YM, *et al*.[17]	Chin J Hematol, 2003	Cerebral Infarction	Guangdong	202	204
FU Y, *et al*.[18]	Chin J Intern Med 2005	Cerebral Infarction	Shanghai	132	171

TABLE 2

Genotype Information and Odds Ratio of Case-control Studies on Correlation of β-Fibrinogen -455G/A Polymorphism and Cerebral Infarction

Study (*n*)		Phenotype			G/A Frequency (%)	(G/A+A/A) *vs* G/G OR	95% CI	Weight (%)
		G/G	G/A	A/A				
1	DIS (90)	46	36	8	71.1/28.9	2.092	1.162-3.767	8.46
	Ctrl (102)	70	26	6	81.4/18.6			
2	DIS (101)	55	38	8	73.3/26.7	1.901	1.079-3.350	8.76
	Ctrl (108)	75	30	3	83.3/16.7			
3	DIS (91)	58	32	1	81.3/18.7	2.238	1.100-4.551	6.96
	Ctrl (74)	59	14	1	89.2/10.8			
4	DIS (96)	72	14	10	82.3/17.7	0.667	0.394-1.128	9.36
	Ctrl (273)	182	81	10	81.5/18.5			
5	DIS (134)	93	37	4	83.2/16.8	0.914	0.560-1.493	9.89
	Ctrl (166)	112	46	8	81.3/18.7			
6	DIS (111)	55	43	13	68.9/31.1	1.646	0.979-2.770	9.44
	Ctrl (123)	76	42	5	78.9/21.1			
7	DIS (102)	52	44	6	72.5/27.5	2.036	1.016-4.081	7.11
	Ctrl (53)	36	16	1	83.0/17.0			
8	DIS (52)	29	21	2	76.0/24.0	1.692	0.744-3.849	5.83
	Ctrl (47)	32	14	1	83.0/17.0			
9	DIS (294)	185	96	13	79.3/20.7	0.991	0.706-1.391	12.37
	Ctrl (279)	175	92	12	79.2/20.8			
10	DIS (202)	98	92	12	71.0/29.0	1.579	1.066-2.339	11.45
	Ctrl (204)	122	73	9	78.0/22.0			
11	DIS (132)	79	50	3	78.8/21.2	0.819	0.517-1.298	10.36
	Ctrl (171)	94	68	9	74.9/25.1			

Note. DIS: patient with cerebral infarction; Ctrl: control group.

TABLE 3

Results of Hardy-Weinberg Test for Genotype Distribution of β-fibrinogen -455G/A Polymorphism in Control Groups of Reviewed Studies

Case of Control	G/G		G/A		A/A		χ^2	P
	CIF	CIT	CIF	CIT	CIF	CIT		
102	70	67.58	26	30.89	6	3.53	2.59	>0.05
108	75	74.94	30	30.04	3	3.01	0.001	>0.05
74	59	58.88	14	14.26	1	0.86	0.26	>0.05
273	182	181.33	81	82.32	10	9.34	0.05	>0.05
166	112	109.72	46	50.47	8	5.80	1.28	>0.05
123	76	76.57	42	40.95	5	5.48	0.08	>0.05
53	36	36.51	16	14.96	1	1.53	0.26	>0.05
47	32	32.38	14	13.26	1	1.36	0.14	>0.05
279	175	175.01	92	91.92	12	12.07	0.001	>0.05
204	122	124.11	73	70.01	9	9.87	0.20	>0.05
171	94	95.93	68	64.30	9	10.77	0.61	>0.05

Note. CIF: cases in fact; CIT: cases in theory.

TABLE 4

Plasma Fibrinogen Level of Cerebral Infarction Patients with Different Genotypes (g/L)

Study	Fibrinogen Level of GG Homogenous (*n*)	Fibrinogen Level of Allele A Carriers (GA+AA) (*n*)
QIAN J J, *et al*.[8]	3.31±0.97 (46)	3.80±0.64 (44)
LIU Y, *et al*.[10]	3.71±1.19 (58)	3.99±0.85 (32)
LIU R, *et al*.[11]	3.56±1.29 (72)	3.28±0.83 (24)
ZHAO W J, *et al*.[13]	4.24±1.01 (55)	4.52±1.29 (56)
ZHENG H, *et al*.[15]	3.08±0.54 (29)	3.59±1.05 (23)
FU Y, *et al*.[18]	3.1±0.6 (79)	3.4±0.8 (53)

studies. Furthermore, there was significant heterogeneity among individual estimates of the ORs (χ^2=24.58, P=0.006), so the original data were combined by means of random-effect model. As shown in Fig. 2, there was a statistically significant increase (33%) of risk to cerebral infarction in-55A allele carriers, compared with the wild-455G/G homozygotes (OR=1.33, 95%CI 1.04-1.71, P=0.02).

Meta-analysis of Plasma Fibrinogen Differencebetween Allele A Carriers and G/G Homozygous Patients

The plasma fibrinogen levels were not available in detail in 2 studies[16-17]. In the remaining 9 ones, 3 studies[9,12,14] presented the fibrinogen level of G/A and A/A genotypes separately and in 6 studies combined as A carriers. Figures 3 and 4 show the result

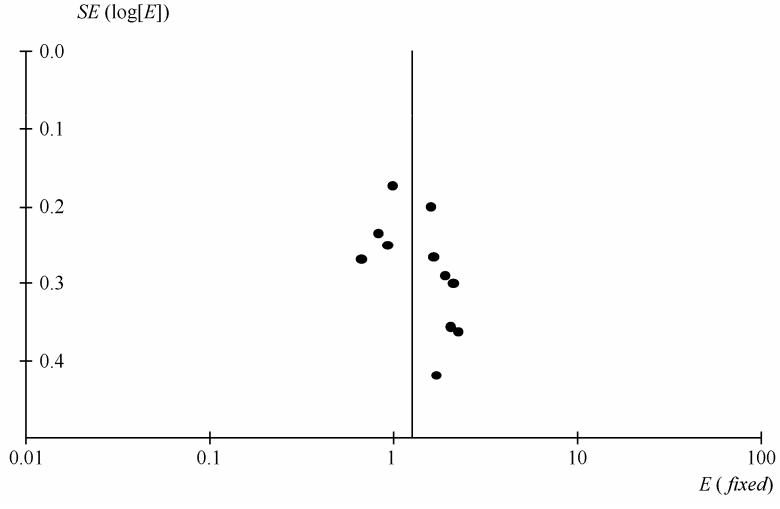

FIG. 1. Funnel plot of 11 studies on -455G/A polymorphism and cerebral infarction.

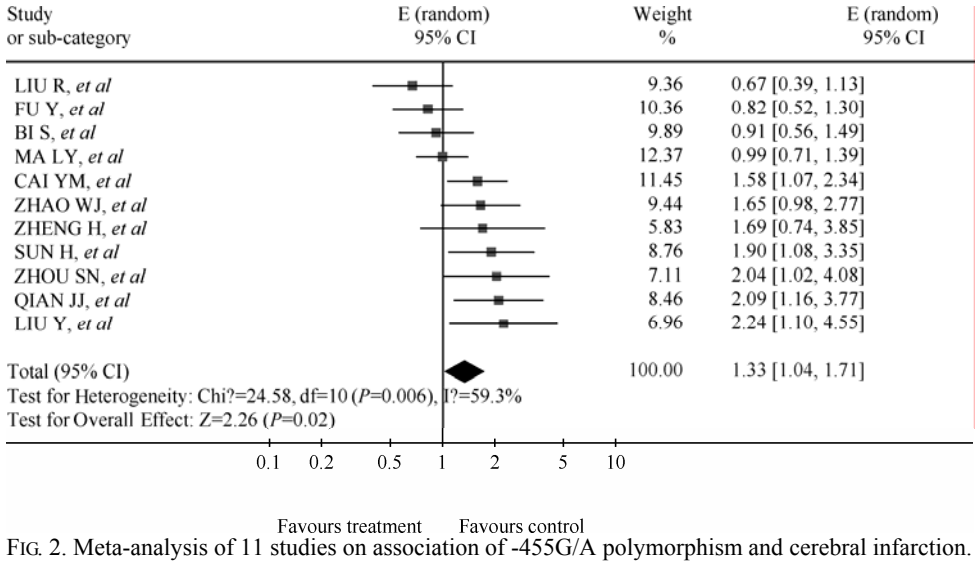

FIG. 2. Meta-analysis of 11 studies on association of -455G/A polymorphism and cerebral infarction.

of meta-analysis of the difference of plasma fibrinogen levels in A carriers and G/G homozygous patients. There were no significant publication bias and heterogeneity among the 6 studies (χ^2=8.46, *P*=0.13). The plasma fibrinogen levels of allele A carriers (G/A+A/A) patients were 0.29 g/L (95%CI 0.14-0.44, *P*=0.0002), higher than that of G/G homozygous patients.

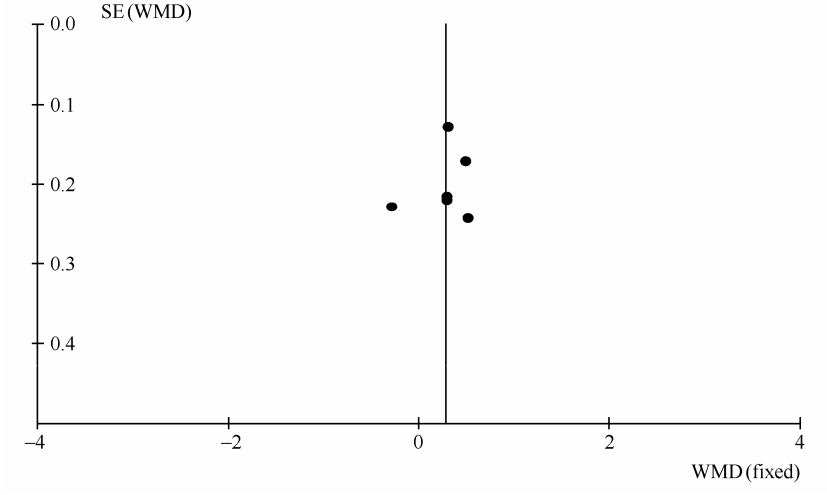

FIG. 3. Funnel plot of 6 studies comparing plasma fibrinogen levels among different genotypes in cerebral infarction patients.

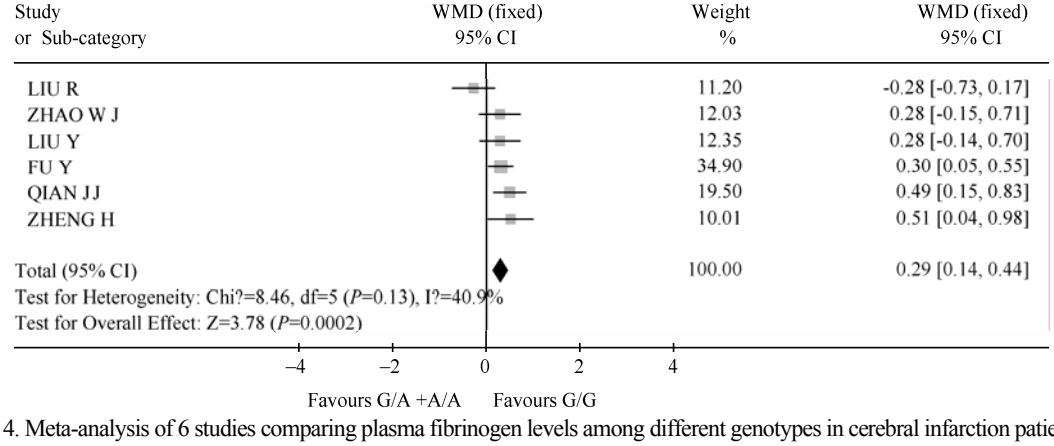

FIG. 4. Meta-analysis of 6 studies comparing plasma fibrinogen levels among different genotypes in cerebral infarction patients.

DISCUSSION

Five common polymorphisms on the proximal promoter region of β-fibrinogen have been detected, including -148C/T, -249C/T, -455G/A, -854G/A, and -993C/T, of which the –148 locus is the closest one to the responsive elements of IL-6, the regulating element of the activating repression of fibrinogen gene transcription[7]. It has been reported that the binding of nuclear proteins to the -148C and -148T alleles is different[19]. Several reports[5-6] have confirmed that the -455G/A polymorphism is in strong or complete linkage disequilibrium with the -148C/T polymorphism, and it was also documented by Cai *et al*. in Chinese population[17]. In the present study, we summarized the results of 6 studies and found that the average plasma fibrinogen level in patients with allele A was higher than that of allele G/G homozygous patients. Furthermore, in the other three reviewed studies[9,12,14], it was reported that the plasma fibrinogen level of A/A homozygotes was higher than both G/A heterozygotes and G/G homozygotes. On the other hand, the meta-analysis of relationship between gene polymorphism and disease in 11 reviewed studies showed that there was 33% increased risk of cerebral infarction for the variant genotypes (G/A + A/A), compared with the wild G/G homozygotes. It was suggested that the allele A might be a genetic risk factor in increasing susceptibility to cerebral infarction in Chinese population.

The aim of meta-analysis is to combine same studies to increase the sample size and statistic power, so as to draw a more authentic result; however, it also inevitably intermixes various factors. A common source of bias in meta-analysis is publication bias because the likelihood of publishing a study could be related to the results of that study. However, in our meta-analysis, there have been several studies published without statistically significant findings between the gene polymorphism and disease[11-13,15-16,18], and the funnel plot for all reviewed papers was symmetric, indicating that there was no significant publication bias affecting the final results. Generally, heterogeneity of studies could be caused by a wide range of reasons, such as study design and methodological difference. In our meta-analysis, some limitations obviously existed in individual studies. Firstly, although there was no significant heterogeneity among the two alleles frequencies of control groups among all selected studies, the primary design of most reviewed studies had no strict restriction on the patients, nationality, and the patients were dispersed among different geographical locations, both of which might lead to difference of genetic background. Secondly, the main method of genotyping in all the reviewed studies was PCR-RFLP without confirmation with DNA sequencing, so that the quality deficiencies might sometimes affect the magnitude of the observed association. Furthermore, the difference in disease type and severity could be one of the most important sources of heterogeneity. Wang *et al*.[20] proved that the elevated plasma fibrinogen plays a different role in different type of cerebral infarction, including cerebral arterial main trunk infarction (MCI) and cerebral penetrating arterial infarction (PCI), implying that the role of fibrinogen is various even in the same kind of disease with different types. Therefore, the different composition of patients could result in different conclusion. In our meta-analysis, most of the selected studies did not provide the type and severity of cerebral infarction, so the detailed information about composition of patients was absent.

The mechanisms by which fibrinogen gene polymorphism or elevated plasma fibrinogen level may promote atherosclerosis and thrombosis were complicated. Hemorheologic consequences of hyperfibrinogenemia might act at various levels: by reducing flow, by predisposing to thrombosis, and by enhancing atherogenesis[1,19]. Some reviews[10,14] presented more detailed information about association of plasma fibrinogen level, gene polymorphism and other factors, such as smoking, age, and gender, with the disease. Rather than direct effect on the blood levels of fibrinogen, gene polymorphisms always regulate the final phenotype by amplifying the effect of environment or other intermediate conditions[3-4]. In a study on the relationship between fibrinogen gene polymorphism and cerebral infarction, Li *et al*.[21] found that there was no relationship between -148C/T polymorphism and cerebral infarction in their total sample, but in subgroup of smoking population the risk of cerebral infarction in allele T carriers to C/C homozygotes was almost 5 times higher, suggesting that the interaction of genetic determination and other cardiovascular risk factors, including smoking, play more important role than gene polymorphism itself in the pathogenesis of disease.

In conclusion, our present study supports that β-fibrinogen gene-455G/A polymorphism may be associated with cerebral infarction in Chinese population, and allele A increases the individual susceptibility to the disease. To further evaluate the influence of gene polymorphism or plasma fibrinogen on the disease, larger studies with big sample size in good study design would provide more authentic information.

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