Research Article

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Association between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head: An update meta-analysis

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Abstract: **Objective** To investigate the correlation between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head. **Methods** Open published studies relevant to *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head were electronic systematic searched in the databases of cochrane central register of controlled trials, EMBSE and CNKI. The correlation between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head was calculated by odds ratio (OR) and corresponding 95% confidence interval (95%CI). The publication bias for the included studies were assessed by Begg's funnel plot and Egger's line regression text. **Results** After systematic searching the electronic databases, 11 original studies were finally included the present work. The I^2 test indicated significant statistical heterogeneity ($I^2=53.5\%$, $P=0.018$) across the included 11 publications. The pooled results indicated that subjects of Caucasians with CC genotype had decreased risk of developing non-traumatic osteonecrosis of the femoral head (OR=0.65, 95%CI: 0.44-0.96, $P=0.031$). However, there was no correlations between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head in American Jewish and East Asian races ($p>0.05$). Sensitivity analysis indicated the pooled ORs were not sensitive to any included single study. The Begg's funnel plot was generally left and right symmetrical which indicated no

obviously publications. The Egger's line regression test also demonstrated no statistical publication bias ($t=1.57$, $P=0.15$). **Conclusion** According to the present evidence, *MTHFR* C677T polymorphism was correlated with non-traumatic osteonecrosis of the femoral head especially for Caucasians race. Subjects of Caucasians race with CC genotype had decreased risk of developing non-traumatic osteonecrosis of the femoral head.

Keywords: *MTHFR* gene; non-traumatic osteonecrosis of the femoral head; polymorphism; meta-analysis.

Introduction

Non-traumatic osteonecrosis of the femoral head is a disease characterized by acquired ischemic lesions of the femoral head. Although globally epidemiological data are still lacking, some studies have shown that the highest incidence of the disease is in males aged 20 to 50 years [1-3]. As hip dysplasia and rapid progressive hip joint destruction, if the femoral head necrosis area reaches or exceeds 30% and remains untreated, non-traumatic osteonecrosis of the femoral head will develop into end-stage hip arthritis, which will lead to serious hip joint destruction, thus requiring surgical intervention. The etiology of non-traumatic osteonecrosis of the femoral head is not yet fully understood. Most studies indicated that the main pathophysiological mechanisms were excessive use of hormones and excessive intake of ethanol [3, 4]. Recently, several studies have evaluated correlations between some *MTHFR* gene polymorphism and susceptibility for non-traumatic osteonecrosis of the femoral head [5, 6]. A meta-analysis included 8 trials published 7 years ago also indicated the correlation between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head [7]. However, we found that this meta-analysis only included 8 studies and one of the included studies was about children, which may lead to significant clinical heterogeneity. Since 7 years had

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past, there were new studies published in the databases and may provide more evidence for the correlation between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head. Therefore, we performed this up-date meta-analysis in order to provide more accurate information for the etiology of non-traumatic osteonecrosis of the femoral head

Methods

Studies electronic searching strategy

Open published studies relevant to *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head were electronic systematically searched in the databases of Cochrane central register of controlled trials, EMBSE and CNKI. The case-control or cohort clinical trials published in English or Chinese were systematic searched by the text words of: “*MTHFR*”, “osteonecrosis of the femoral head”, “Methylene tetrahydrofolate reductase”, “polymorphism”. The references of the included publications were also screened in order to further identify additional potential suitable publications that were not indexed in the electronic databases.

Publication inclusion criteria

Eligible publications should meet the below criteria: (1) Case-control or cohort designed clinical trials relevant to *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head. (2) Paper was published in English or Chinese; (3) Genotype frequency of both case and control groups can be extracted or calculated from the original studies; (4) The genotyping methods were correct; (5) There was no deviation of Hardy–Weinberg equilibrium (HWE) in the controls and case groups.

Data extraction and evaluation

Two reviewers (Yunmiao Ma & Xiufeng Wang) independently review the included publications and extracted the data according to the Cochrane Handbook. If there was a disagreement of the data extraction, Two reviewers (Yunmiao Ma & Xiufeng Wang) discuss the disagreement and consulted the third reviewer (Zhiyang Gao) to solve the problem. The general information of authors, year of the paper published, journals, ethnicity

and genotyping methods of each included publication were extracted. The genotype frequency was carefully extracted or calculated by two reviewers from each included individual study independently and made cross-checking.

Statistical analysis

Stata/SE 11.0 (StataCorp LP, <http://www.stata.com>) statistical software was applied for data managing. The correlation between *MTHFR* C677T polymorphism and non-traumatic osteonecrosis of the femoral head was calculated by OR. Statistical heterogeneity among the included 11 studies was assessed through I^2 test. Begg’s funnel plot and Egger’s line regression test were used for publication bias evaluation.

Results

General character of the included 11 original publications

After systematic searching, the electronic databases of Medline, the Cochrane central register of controlled trials, EMBSE and CNKI databases, 11 original studies [5, 6, 8-16] were finally included in the present work, **Figure 1**. Of the included 11 publications, 5 studies included the subjects of East Asian, 4 studies were about Caucasians and the other 2 were American Jewish. The general characteristics of the included 11 publications were shown in **Table 1**.

Meta-analysis

Before pooling the data, we first test the statistical heterogeneity across the included 11 studies by I^2 test. The I^2 test indicated significant statistical heterogeneity among the 11 publications ($I^2=53.5\%$, $P=0.018$). Therefore, the data were pooled by random effect model. The pooled results indicated that subjects with CC genotype had decreased risk of developing non-traumatic osteonecrosis of the femoral head (OR=0.72, 95%CI: 0.54-0.96, $P=0.023$), **Figure 2**.

Table 1: General information for the included 11 studies.

First author	Year	Country	Ethnicity	Case			Control			Methods
				CC	CT+TT	Total	CC	CT+ TT	Total	
Glueck	1999	U.S	American Jewish	29	30	59	20	20	40	PCR
Glueck	2002	U.S	American Jewish	10	26	36	120	115	235	PCR
Kutlar	2001	U.S	Caucasians	29	16	45	54	8	62	PCR
Zalavras	2002	U.S	Caucasians	22	44	66	117	183	300	PCR
Asano	2004	Japan	East Asian	14	17	31	39	67	67	PCR
Liu	2009	China	East Asian	13	76	89	18	59	77	PCR
Kim	2010	Korea	East Asian	160	278	438	85	184	269	PCR
Chang	2008	Korea	East Asian	18	53	71	80	120	200	PCR
Gagala	2013	Poland	Caucasians	29	39	68	51	49	100	PCR
Li	2015	China	East Asian	20	73	93	24	59	83	PCR
French	2008	U.S	Caucasians	21	22	43	118	95	213	PCR

Figure 1: The publication searching flow chat of MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head.

Figure 2: The forest plot of OR for MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head(CC vs CT+TT).

Figure 3. Subgroup analysis (American Jewish, Caucasians and East Asian) of the forest plot of OR for MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head(CC vs CT+TT)

Table 2: Subgroup analysis of MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head(CC vs CT+TT).

Race	OR	95%CI	p-value	Weight
American Jewish	0.59	0.23-1.53	0.279	15.34%
Caucasians	0.65	0.44-0.96	0.031	35.84%
East Asian	0.83	0.53-1.28	0.399	48.83%

Table 3: The sensitivity analysis of MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head(CC vs CT+TT).

Study omitted	Year	Estimate	95% Confidence interval		p-value
			Lower	Upper	
Glueck	1999	0.70	0.51	0.95	0.02
Kutlar	2001	0.77	0.59	1.00	0.05
Glueck	2001	0.77	0.58	1.00	0.05
Zalavras	2002	0.70	0.51	0.97	0.03
Asano	2004	0.68	0.51	0.91	0.01
Chang	2008	0.75	0.55	1.00	0.05
French	2008	0.71	0.52	0.97	0.04
Liu	2009	0.73	0.54	0.99	0.05
Kim	2010	0.66	0.51	0.84	0.01
Gagala	2013	0.71	0.52	0.98	0.04
Li	2015	0.72	0.52	0.98	0.04

Subgroup analysis

A subgroup analysis according to different races (American Jewish, Caucasians and East Asian) was made **Figure 3**. The pooled results indicated that subjects of Caucasians with CC genotype had decreased risk of developing non-traumatic osteonecrosis of the femoral head (OR=0.65,95%CI: 0.44-0.96, P=0.031). However, there were no correlations between MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head in American Jewish and East Asian races ($p>0.05$), **Table 2**.

Sensitivity analysis

The sensitivity analysis of pooled results was performed by omitting each of the included studies, **Figure 4**. The results indicated the pooled ORs were not sensitive to any included single study, **Table 3**.

Publication bias evaluation

The publication bias in evaluation MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head were evaluated by Begg's funnel plot and Egger's line regression test. The Begg's funnel plot was generally left and right symmetrical which indicated no obviously publications, **Figure 5**. The Egger's line regression test also demonstrated no statistical publication bias($t=1.57$, $P=0.15$).

Discussion

Evidence has shown that intravascular coagulation and genetic factors are one of the pathogenesis of necrosis of the femoral head [1, 17]. Homocysteine, the product of methylenetetrahydrofolate reductase gene, can promote the formation of thromboembolic lesions by damaging vascular endothelial cells and enhancing the coagulation activity of vascular [18]. The hyperhomocysteinemia caused by homocysteinemia is considered to be an independent risk factor for thrombosis. The polymorphism of methylenetetrahydrofolate reductase gene C677T locus has been confirmed to be associated with many ischemic diseases [7] and lung cancer [19]. In recent years, the relationship between the polymorphism of methylenetetrahydrofolate reductase gene C677T locus and the occurrence of femoral head necrosis has been widely discussed [5, 9].

The C>T transition of methylenetetrahydrofolate reductase gene at nucleotide 677 may result in valine replacing highly conserved alanine in mature proteins, reducing enzyme activity and causing hyperhomocysteinemia. Homocysteine causes thromboembolic lesions by damaging vascular endothelial cells, proliferating vascular smooth muscle cells and enhancing the coagulation activity of vascular [20]. Therefore, this C>T SNP changes may increase the risk of developing necrosis of the femoral head.

A large number of studies have proved that the level of nitric oxide in atherosclerosis patients with methylenetetrahydrofolate reductase C677TT genotype is significantly reduced, while the high concentration of homocysteine can inactivate nitric oxide, resulting in vascular endothelial dysfunction [21-23]. Chang et al. [11] reported that 71 cases of femoral head necrosis were associated with methylenetetrahydrofolate reductase C677T polymorphism, of which 51 cases (71.8%) were alcoholic femoral head necrosis patients. However, Asano et al.

Figure 4: Sensitivity analysis of pooled results was performed by omitting each of the included study.

Figure 5: The Begg's funnel plot s indicated no significant publication bias in evaluation of MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head(CC vs CT+TT).

[6] reported that 31 patients with femoral head necrosis secondary to renal transplantation in Japan were not associated with methylenetetrahydrofolate reductase C677T polymorphism. A meta-analysis included 8 trials published 7 years ago also indicated the correlation between MTHFR C677T polymorphism and non-traumatic osteonecrosis of

the femoral head. However, we found that this meta-analysis only included 8 studies and one of the included study was about children, which may lead to significant clinical heterogeneity. Therefore, the methylenetetrahydrofolate reductase C677T polymorphism is still controversial for the occurrence of femoral head necrosis.

In our present work, we up-date the recently published case control studies relevant MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head and pooled the results. We found that MTHFR C677T polymorphism was correlated with non-traumatic osteonecrosis of the femoral head especially of the Caucasian's race. Subjects of the Caucasian's race with CC genotype had decreased risk of developing non-traumatic osteonecrosis of the femoral head. Subjects of Caucasians race with CC genotype had 0.65 risk of developing non-traumatic osteonecrosis of the femoral head compared to subjects of CT+TT genotype. And publication evaluation indicated no significant publication bias.

However, there were still some limitations. Firstly, in the present study, patients with femoral head necrosis were caused by different etiologies. This may a key potential clinical heterogeneity which may affect its general quality. Second, because folic acid and vitamin B12 both participate in the metabolism of homocysteine [24], folic acid and vitamin B12 concentrations can impact on homocysteine levels. Reduction of folic acid and vitamin B12 in the diet may cause hyperhomocysteinemia. So, the different folic acid and vitamin B12 intake from dietary may be another potential clinical bias. Third, significant statistical heterogeneity was also found among the included 11 studies. Therefore, better designed and large-scale clinical studies are still needed to further evaluate the correlation between MTHFR C677T polymorphism and non-traumatic osteonecrosis of the femoral head. The interaction between genes and environmental factors should be further explored to provide a more comprehensive and reliable basis for the research and prevention of necrosis of the femoral head in order to alleviate the disease.

Conflict of interest: Authors state no conflict of interest.

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