

Review Article

Plasma homocysteine levels may be associated with the subtypes of ischemic stroke: a meta-analysis

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Abstract: Objective: The Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification is an established etiological classification in routine practice, and is particularly important for the diagnosis and treatment of ischemic stroke. The aim of this meta-analysis is to investigate the potential association between the plasma homocysteine levels and the TOAST subtypes of ischemic stroke. Methods: The databases PubMed, Web of Science and Embase prior to December 31, 2017, were on-line retrieved. Case-control studies between plasma homocysteine levels and TOAST subtypes of ischemic stroke patients were statistically analyzed by Stata 12.0 software. The pooled effect size was evaluated by Z test. Results: A total of nine case-control studies were collected into this study, including 2,241 ischemic stroke patients and 1,378 normal controls. The plasma homocysteine levels in ischemic stroke patients were significantly higher than those of healthy controls. Furthermore, plasma homocysteine levels in all TOAST subtypes, i.e., the subtype of LASS, LAC, CEI, ODE and UDE, were higher than that of healthy volunteers. However, the serum contents of homocysteine can not differentiate the subtypes of this disease. Conclusion: Elevated plasma homocysteine levels may be a promising biomarker and associated with the pathogenesis of ischemic stroke.

Keywords: The Trial of Org 10172 in acute stroke treatment (TOAST), meta-analysis, ischemic stroke, homocysteine (Hcy)

Introduction

Stroke is a multifactorial dysfunction with complex interaction among intrinsic and extrinsic factors [1]. It is characterized by high morbidity, disability and mortality worldwide. Ischemic stroke is the main type of stroke caused by a blocked arterial blood vessel [2], accounting for approximately 80% to 88% of stroke patients. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification is an established etiological classification in routine practice [3]. According to TOAST criteria [4], the ischemic stroke can be divided into large artery atherosclerosis (LAAS), lacunar infarct (LAC), cardioembolic infarct (CEI), stroke of other determined etiology (ODE), and stroke of undetermined etiology (UDE). Both acquired and hereditary factors are associated with the development of ischemic stroke, such as the susceptible genotypes, diabetes, smoking, hypertension, and obesity [5]. Considering the rather low

diagnostic efficiency, identification of novel biomarkers is of particularly importance for ischemic stroke.

Homocysteine (Hcy) is a sulfur-containing amino acid derived from the demethylation of methionine. As one of the important intermediate products of methionine metabolism, it can maintain the normal plasma level of methionine, yet a reactive vascular-damaging amino acid [6]. Previous studies showed that an elevated levels of plasma Hcy might cause injury to vascular endothelium, promote vascular smooth muscle proliferation and atherosclerosis, and increase the risk of thrombogenesis [7]. There is growing evidence that excessive Hcy has been identified as a risk factor for ischemic stroke patients [8, 9], while other studies showed that there was no increase in risk with high plasma Hcy [10, 11]. For instance, Tan et al. [12] conducted a study in 109 young Asian adults with ischemic stroke and showed that

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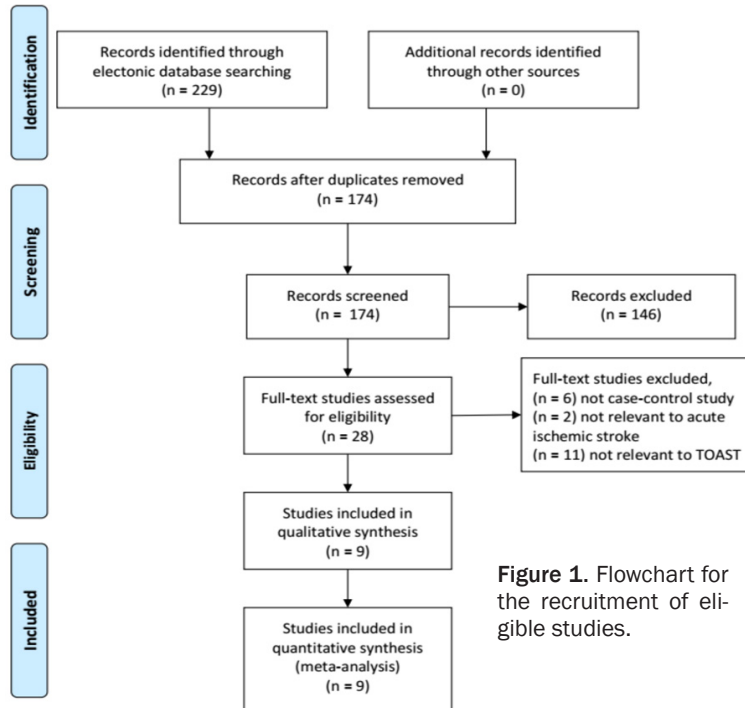


Figure 1. Flowchart for the recruitment of eligible studies.

LASS type of stroke was allied to elevated Hcy levels. However, Khan and colleagues [13] found that the Hcy levels were not elevated in LASS and ODE patients. These disparities may be partly explained by the difference of sample-collecting time, TOAST subtypes of ischemic stroke studied, or detection methods [14, 15].

Two meta-analysis [16, 17] demonstrated the closely interactions between plasma Hcy levels and ischemic stroke. However, most of their recruited studies referred TOAST subtypes of ischemic stroke were conducted in a single center, or with small samples [18-20]. On the other hand, the etiological distinction is clinically important, because different treatment programs are designed for different TOAST subtypes. Hence, we performed a meta-analysis to explore the potential association between plasma Hcy levels and TOAST subtypes of ischemic stroke, aiming to providing evidence for the promotion of clinical practice.

Materials and methods

Literature retrieve

This study was performed in accordance with the guidelines of Preferred Reporting Item for Systematic Reviews and Meta-Analysis

(PRISMA) [21]. Three authoritative databases, PubMed, Web of Science and Embase, were searched for eligible studies published prior to December 31, 2017. The retrieve terms were shown as follows: “ischemic stroke”, “stroke”, “cerebral infraction”, “brain ischemia”, “TOAST” or “subtype”, and “homocysteine” or “Hcy”. No language or other restrictions were applied. Furthermore, reference lists of all selected studies and relevant review articles were manually examined to identify more eligible studies.

Selection criteria

Publications recruited in the meta-analysis should meet the following criteria: (1) The study design was a case-control study; (2) First-ever acute ischemic stroke patients were classified using the TOAST criteria; (3) The plasma Hcy levels in the case and control group were reported; (4) The plasma Hcy levels of ischemic stroke patients were compared with those of the healthy volunteers; (5) The sample-collecting time was in the acute phase of stroke [22]. On the contrary, the following criteria were applied to exclude the candidate publications: (1) Ischemic stroke do not meet the TOAST criteria; (2) Studies published only as abstracts, letters, reviews, or case reports; (3) For duplicated studies with overlapping data, only the largest data was selected.

Data abstraction and quality assessment

Two reviewers, Y. J. Xu and Z. Zhang, independently checked the literature and collected information from eligible studies as follow items: study ID, year of publication, country or region, method used for qualification of the plasma Hcy, ethnicity criteria, mean age, gender and plasma Hcy levels derived from both ischemic stroke patients and control group. Any inconsistency was further affirmed by a third reviewer, Y. L. Wang. We performed study quality using the Newcastle-Ottawa Scale (NOS) in three aspects: (1) Selection of the participants;

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Table 1. Basic characteristics of the included studies

Study ID	Year	Country	Detecting methods	Number		Gender (M/F)		Age (years)		Quality
				Case	Control	Case	Control	Case	Control	
Zhang W [19]	2015	China	ECA	136	136	74/62	NR	32.3 ± 4.7	NR	6
Parnetti L [25]	2004	Italy	HPLC	161	152	76/85	69/73	72.0 ± 13.1	69.5 ± 13.0	8
Lehmann MF [20]	2015	Brazil	CMIA	121	96	68/53	23/73	NR	62.5-75.2	7
Khan U [13]	2008	UK	EIA	457	179	256/201	111/68	65.4 ± 12.2	65.4 ± 7.4	8
Perini F [26]	2005	Italy	RP-HPLC	775	421	399/376	201/220	71.6 ± 12.1	56.6 ± 14.8	8
Ashjazadeh N [14]	2013	Iran	EIA	171	86	74/97	35/51	67.9 ± 13.3	68.7 ± 8.4	7
Tan NC [12]	2002	Singapore	RP-HPLC	109	88	78/31	63/25	43.8 ± 5.87	43.1 ± 6.60	8
Üstündağ M [27]	2010	Turkey	CLIA	92	15	41/51	NR	65.6 ± 11.0	NR	6
Eikelboom JW [18]	2000	Australia	HPLC	219	205	140/79	131/74	67.0 ± 11.8	66.1 ± 12.4	7

M: male; F: female; NR: not report; ECA: enzymatic cycling assay; HPLC: high-performance liquid chromatography; CMIA: chemiluminescence microparticle immunoassay; EIA: enzyme immunoassay; RP-HPLC: reversed-phase high-performance liquid chromatography; CLIA: chemiluminescent immunoassay.

(2) Comparability of groups; (3) Exposure assessment for case-control studies [23]. According to the NOS scale, ranging from 0 through 9 points, only studies graded with 6 or more points were included.

Statistical analysis

Data analysis was performed with Stata 12.0 (www.stata.com). Prior to meta-analysis, standard deviation of plasma Hcy levels in some studies was calculated from the reported 95% confidence intervals (CI). Discrepancies in average levels of plasma Hcy between the case and control group were assessed using standard mean difference (SMD) and 95% CI. The pooled effect size was evaluated by using the Z test. The heterogeneity among studies was assessed using the Cochran's Q-test with I^2 statistic. Four scoring intervals, $I^2 < 25\%$, $25\% \leq I^2 < 50\%$, $50\% \leq I^2 < 75\%$, or $I^2 \geq 75\%$ were respectively considered to insignificant, low, moderate or high heterogeneity [24]. The fixed-effect model was applied as $I^2 < 50\%$; otherwise, the random-effect model was used. Furthermore, sensitivity analysis was applied to assess whether an individual study had the weight to impact on the overall risk estimate.

Results

Publications included in this study

All 229 potentially eligible studies were identified based on automatic on-line retrieve of databases and manual check. 28 publications were recruited for meta-analysis. 19 studies were further excluded as they were not case-

control (n = 6), not relevant to acute ischemic stroke (n = 2), or not relevant to TOAST subtypes (n = 11). Nine studies including 2,241 ischemic stroke patients and 1,378 healthy controls were finally enrolled into the meta-analysis (Figure 1). The baseline characteristics and NOS of the included studies are displayed in Table 1.

Hcy levels and ischemic stroke

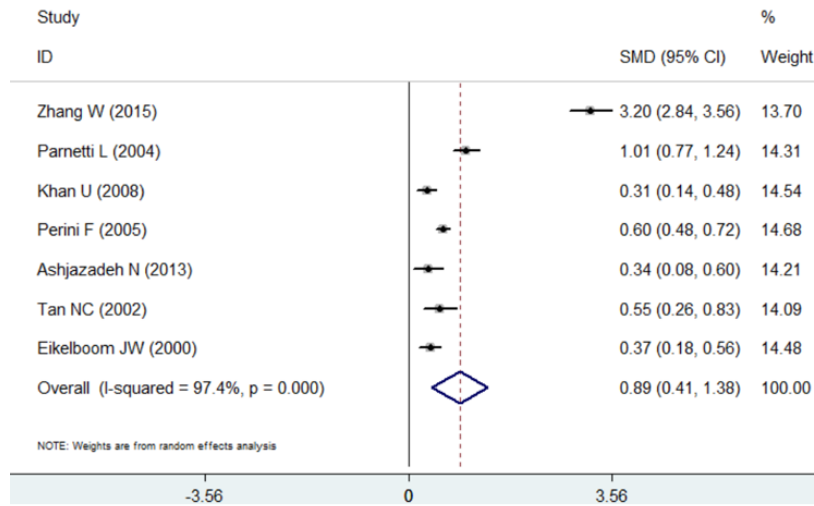
Nine studies were pooled to meta-analysis the potential association between plasma Hcy levels and ischemic stroke. A random-effect model was used to summarize the pooled prevalence in order to deal with the high heterogeneity ($I^2 = 97.4$, $P < 0.001$). As shown in Figure 2A, plasma Hcy levels in ischemic stroke patients were notably higher than that of healthy controls (SMD = 0.894, 95% CI = 0.408-1.379, $P < 0.001$).

Hcy levels and TOAST subtypes of ischemic stroke

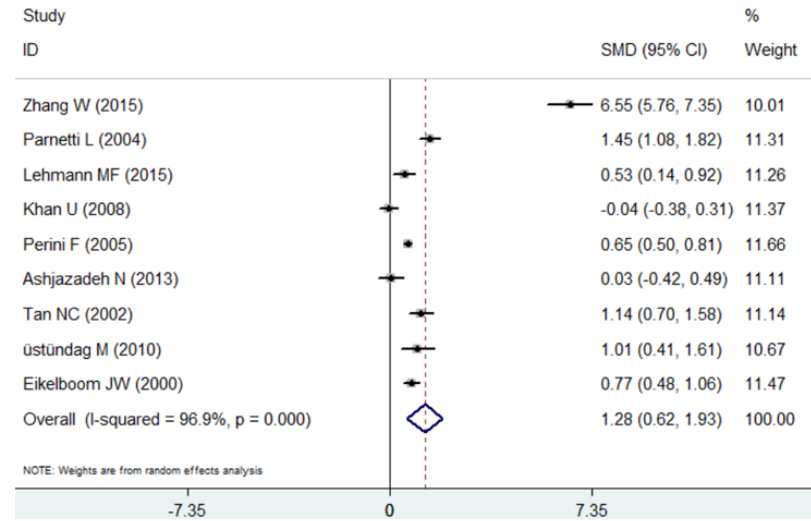
Results of meta-analysis demonstrate significant association between LASS, LAC, CEI type and plasma Hcy levels. A random-effect model was used to summarize the pooled prevalence for the high heterogeneity ($I^2 = 96.9\%$, $P < 0.001$; $I^2 = 84.3\%$, $P < 0.001$; $I^2 = 92.3\%$, $P < 0.001$). As shown in Figure 2B-D, plasma Hcy levels in ischemic stroke patients were notably higher than that of healthy controls (SMD = 1.279, 95% CI = 0.624-1.935, $P < 0.001$; SMD = 0.699, 95% CI = 0.431-0.967, $P < 0.001$; SMD = 1.059, 95% CI = 0.624-1.494, $P < 0.001$).

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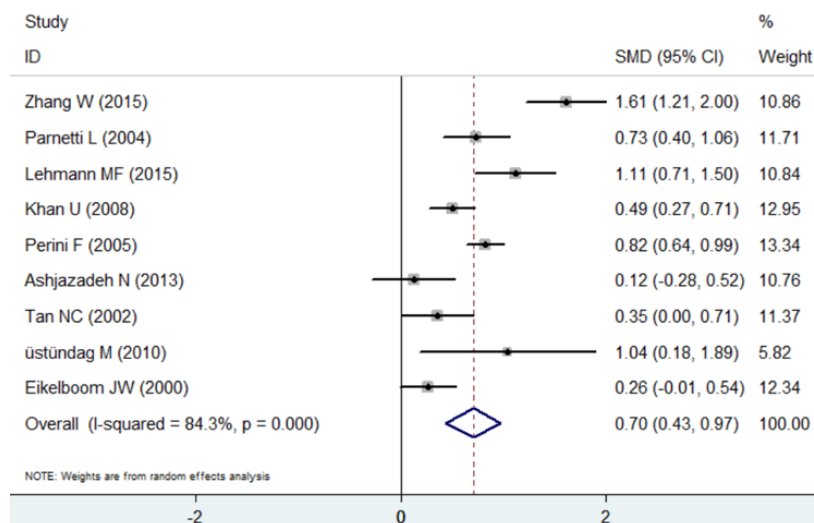
A



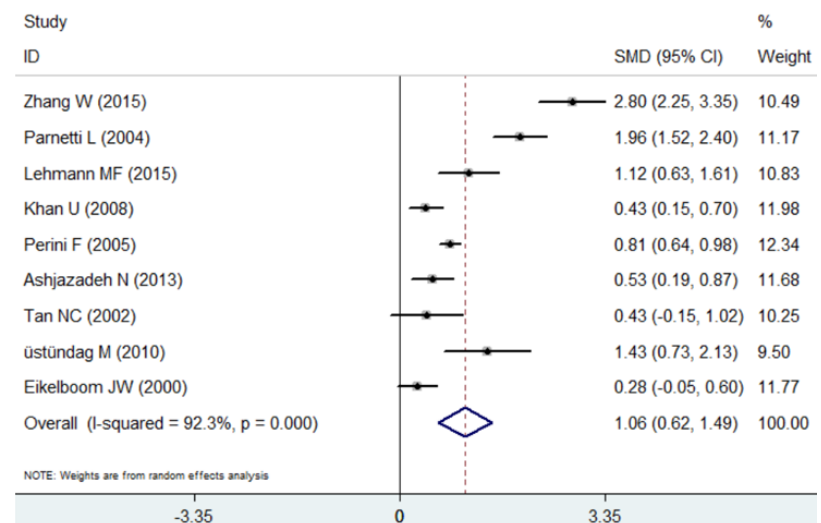
B



C



D



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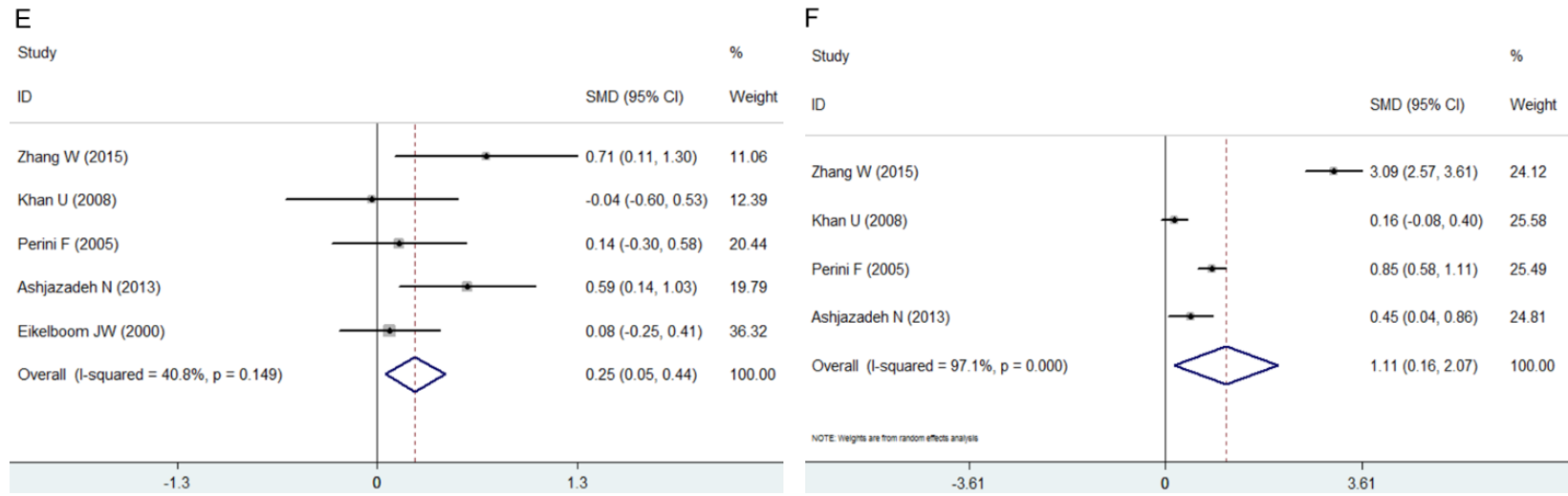


Figure 2. Forest plot for the relationship between plasma Hcy levels and ischemic stroke (A), LASS type (B), LAC type (C), CEI type (D), ODE type (E), UDE type (F).

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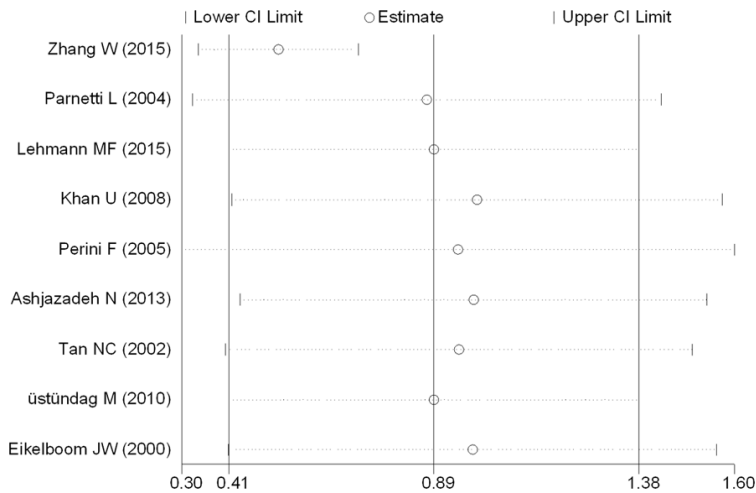


Figure 3. Sensitivity analysis on the differences of plasma Hcy levels between ischemic stroke patients and healthy controls.

Data were pooled from five studies reporting Hcy levels and ODE type of ischemic stroke. As showed in **Figure 2E**, plasma Hcy levels in ODE type patients were higher than that of healthy controls (SMD = 0.247, 95% CI = 0.049-0.445, $P = 0.015$) in a fixed-effect model with low heterogeneity ($I^2 = 40.8\%$, $P = 0.149$).

Four sets of UDE data were pooled together (**Figure 2F**). Results displayed that the plasma Hcy levels in UDE patients were higher than that of healthy controls (SMD = 1.115, 95% CI = 0.156-0.445, $P = 2.073$) in a random-effect model with high heterogeneity ($I^2 = 97.1\%$, $P < 0.001$).

Sensitivity analysis

Results of sensitivity analysis showed that each study in this meta-analysis had no obvious influence on the pooled SMDs for the relationship between plasma Hcy levels and TOAST subtypes of ischemic stroke, except Zhang's study [19] (**Figure 3**).

Discussion

Stroke is a heterogeneous illness which can be subtyped in view of pathophysiology or etiology. Metabolism of Hcy is a complex process tuned by several genetic components and environmental factors. Major processes of ischemic stroke, such as proatherogenic and prothrombotic state, can profoundly regulate Hcy metabolism [28]. Some studies have indicated strong

association between elevated plasma Hcy concentration and atherosclerotic vascular disease [29], mainly in LACS subtype [18]. The deleterious effect of elevated Hcy levels is mediated primarily via a proatherogenic effect, through damaging the vascular matrix, increasing oxidative injury to arterial endothelium and enhancing proliferation of vascular smooth muscle. LACS type of stroke is also associated with higher levels of Hcy, which is toxic to endothelium. Moreover, studies showed that Hcy levels was significantly relevant to the degree of leukoaraiosis, and most LACS patients had

lacunar infarct with leukoaraiosis [12]. Elevated Hcy level has been found as a potential risk factor for cardiovascular disease, which is one of the pathophysiological mechanisms of CEI type of ischemic stroke [14, 30, 31].

Complex pathological mechanisms of serum Hcy support our findings that abnormal high levels of Hcy were the potential biomarker for all subtypes of ischemic stroke. The overall results of this meta-analysis revealed that plasma Hcy levels were notably higher in ischemic stroke patients than that of healthy volunteers. To the best of our knowledge, this is the first meta-analysis to evaluate secure evidence on whether plasma Hcy levels are associated with TOAST subtypes of ischemic stroke. Our findings indicate that all etiological subtypes of ischemic stroke patients, i.e., LAAS, LACS, CEI, UDE and ODE, demonstrate prominent higher plasma Hcy levels, compared to the healthy controls.

Our meta-analysis need to be viewed with several limitations in mind. First, relatively few of publications available may increase the risk of random error. Consequently, our study did not conduct subgroup analysis due to limited sample size. Second, the age is another element need to be evaluated. A hospital-based prospective study demonstrated that etiological subtypes, risk factors were significant different between young and old people [32]. Then, TOAST criteria is not completely reliable [33] for the case that may have reduced the accuracy

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of our findings. Next, there were different sources of control in different studies, as hospital- or population-based controls might have increased selection bias. Finally, different analysis methods may influence the sensitivity and reliability of Hcy levels. For example, the Hcy level detected by enzymatic cycling assay showed notably higher results than those of other methods [19]. Additional well-designed studies and much larger sample sizes with subgroup analysis need to validate our results.

In conclusion, elevated plasma Hcy levels may contribute to the pathogenesis of ischemic stroke, despite fail to differentiate their subtypes.

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Disclosure of conflict of interest

None.

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