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EDITED BY
Paraskevi Detopoulou,
General Hospital Korgialenio Benakio, Greece

REVIEWED BY
Lu Xiao,
First Teaching Hospital of Tianjin University of Traditional Chinese Medicine, China
Jing Gao,
Shanghai Jiao Tong University, China

*CORRESPONDENCE
Yaxi Zhang
✉ zhyx0723@163.com

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Folic acid for the primary prevention of stroke: a systematic review and meta-analysis

Jianjian Yang[1], Jia Wang[2], Bo Li[3] and Yaxi Zhang[4]*

[1]Department of Cardiology, Wenzhou Central Hospital, Wenzhou, Zhejiang, China, [2]Department of Public Health, Weihai Maternal and Child Health Hospital, The Affiliated Weihai Second Municipal Hospital of Qingdao University, Weihai, Shandong, China, [3]Zhangcun Town Health Center in Huancui District, Weihai, Shandong, China, [4]Department of Neurology, Wenzhou Central Hospital, Wenzhou, Zhejiang, China

Objectives: Results from studies were inconsistent with regard to the effect of folic acid on the primary prevention of stroke. The aim of this study was to analyze the association between folic acid and the primary prevention of stroke using the data from observational studies and randomized controlled trials (RCTs).

Methods: Eligible publications published until June 2024 were searched in the database of PubMed, Web of Science and Embase. This study included all observational studies and RCTs of folic acid with first stroke as the reporting endpoints. Relative risks (RRs) and 95% confidence intervals (CIs) were pooled in the random-effects model to assess the effect of folic acid on the primary prevention of stroke.

Results: Results from 12 observational publications with 16 research, including 312,320 participants, were combined to explore the association between dietary folic acid intake and the primary prevention of stroke. The results showed that high dietary folic acid intake was associated with a 17% reduction in stroke incidence (RR:0.83; 95% CI: 0.73–0.94), and the effect of dietary folic acid was greater in areas without grain fortification (RR:0.80; 95% CI: 0.67–0.95). The pooled results from 12 RCTs, totaling 75,042 participants, indicated that folic acid supplementation was not associated with the stroke primary prevention (RR:0.92; 95% CI: 0.80–1.05), but folic acid supplementation was effective in areas without grain fortification (RR:0.78; 95% CI: 0.68–0.89).

Conclusion: Our meta-analysis demonstrated that dietary folic acid is effective in stroke primary prevention, and folic acid supplementation is effective in stroke primary prevention only in areas without grain fortification.

Systematic review registration: https://www.crd.york.ac.uk/PROSPERO/#myprospero, identifier CRD42024516991.

KEYWORDS

folic acid, folate, supplementation, stroke, primary prevention, meta-analysis

1 Introduction

Stroke is the leading cause of death and disability globally (1). There has been a twofold increase in the number of new strokes over the last 30 years, with around 795,000 strokes reported per year. On average, one person dies from a stroke every 3 min and 30 s (2, 3). Even those who survive a stroke have high rates of disability, requiring long-term rehabilitation and chronic care (4, 5). Therefore, the primary prevention of stroke is very important.

Among the range of preventive tactics, homocysteine (Hcy)-lowering medications have garnered significant interest, since research has indicated that Hcy may have an impact on stroke (6–8). Folic acid is crucial regulator in the metabolism of Hcy, and a shortage in folate can cause an accumulation of Hcy (9, 10). The efficacy of folic acid for stroke prevention has been debated for almost 30 years (11). Resent studies were designed to explored the relationship of dietary folic acid or folic acid supplements with stroke risk, and they found that both dietary folic acid and folic acid supplementation reduced the risk of stroke, but the combined results included primary and secondary prevention of stroke (11, 12). There is growing acknowledgment that there are major differences in the efficacy of folic acid for primary vs secondary prevention of stroke (13). The efficacy of folic acid for stroke primary prevention remains uncertain.

Several observational studies (9, 14–24) and RCTs (25–36) have investigated the link between folic acid and stroke primary prevention, however the findings were inconsistent. Of these, seven research (9, 14, 15, 17, 20, 21, 23) discovered an inverse relationship between dietary folic acid and the incidence of stroke, and one RCT demonstrated the protective effects of folic acid supplementation for stroke primary prevention. Another nine studies (14, 16–20, 22) and eleven RCTs (25–35) showed that folic acid was not associated with stroke incidence. Thus, we conducted a systematic review and meta-analysis to combine and synthesize the findings from the existing research.

2 Materials and methods

All analyses in this study were based on the guidelines of Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) (37).

2.1 The literature search strategy

We conducted a literature search in PubMed, web of science and Embase for relevant work published before June 2024. The search phrases used were "folic acid" (or "folate" or "folvite" or "vitamin B9" or "vitamin M") and "Stroke" (or "cerebral infarction" or "intracranial hemorrhage" or "ischemic stroke" or "subarachnoid hemorrhage" or "cerebrovascular accident" or "brain ischemia" or "cerebral hemorrhage"). In addition, the references of the retrieved papers were manually examined for any relevant articles that may have been missed. The search approach was detailed in Figure 1 and Supplementary Tables 1, 2.

2.2 Inclusion criteria

The criteria for inclusion were as follows: (a) A published observational research or RCT in English. (b) The exposure interest in observational studies was the intake of dietary folic acid and the study intervention in RCTs included folic acid supplementation (with or without B vitamins and multivitamin supplementation). (c)The outcome interest was first stroke incidence. (d) Relative risks (RRs) and 95% confidence intervals (CIs) were shown. (e) The duration of the study intervention in the RCTs was at least 6 months. (f) For articles from the same population, the most comprehensive one would be selected.

Two investigators (Jianjian Yang and Jia Wang) meticulously searched and reviewed all studies separately. If two investigators disagreed on the eligibility of an article, a third reviewer will assess it to reach an agreement (Yaxi Zhang).

2.3 Data extraction

Two authors (Jianjian Yang and Bo Li) extracted the following information from each valuable article: first author's name, publication year, study period, country in which the study was carried out, age range or mean age at baseline, RRs (RR is used to consistently represent all results for simplicity) with 95% CIs, type of stroke, grain fortification status. We furtherly gathered study design, method of assessing folic acid consumption, number of cases and sample size, adjustment factors in observational studies, and number of cases and participants in treatment and control groups, intervention regimen, type of control, duration of intervention in RCTs.

2.4 Quality assessment

The Newcastle-Ottawa Scale was utilized to evaluate the quality of the cohort studies and case-control articles in this investigation (38). The scale consisted of three components (selection, comparability, and outcome), with a maximum rating of 9 stars. All RCTs were assessed for the quality of randomization, blinding, withdrawals, random numbers generation, and concealment of allocation, and studies that took all five aspects into account were given a score of 5 (39).

2.5 Grain fortification status

We reviewed published papers on folic acid fortification policies for staple foods to understand the specifics of implementing mandated or voluntary fortification policies by June 2024 (40, 41). Folic acid fortified staple foods mainly include maize flour, rice, and wheat flour.

2.6 Statistical analyses

Most observational studies presented RR of stroke incidence for the highest vs. the lowest level of folic acid intake, so for

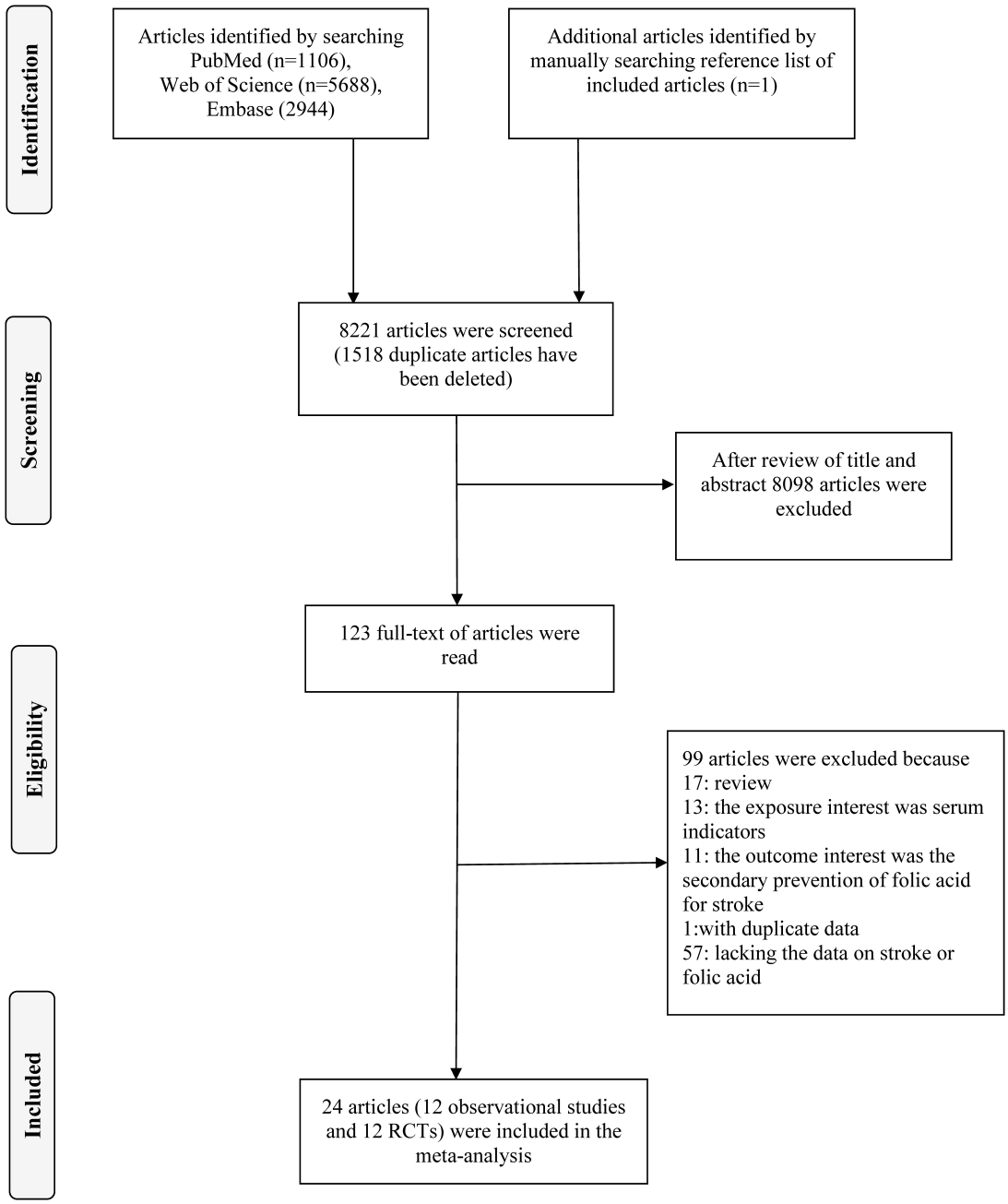

FIGURE 1
Flow chart of the selection of studies included in the meta-analysis.

studies (15, 19) with RR reported in the form of the lowest vs. the highest, the method of Hamling et al. (42) was used to convert risk estimates. We adopted the I^2 to assess heterogeneity (43). The DerSimonian and Laird random effect model was utilized to combine study-specific RRs (95% CIs) (44). Subgroup analysis was used to examine the potential interactions. Meta-regression was performed to explore the potential origin of heterogeneity (45). Leave-one-out sensitivity analysis was conducted to evaluate the pivotal studies that have substantial impacts on between-study heterogeneity (46). The influence analysis assessed whether a single study had an obvious effect on the results. The Egger's test and funnel plot were adopted to evaluate the publication bias (47).

For dose-response analysis, a 2-stage random-effects dose-response meta-analysis (48) was performed. First, a restricted cubic spline model with 3 knots at the 25th, 50th, and 75th percentiles (49) of the levels of dietary folic acid was estimated using generalized least square regression, taking into account the correlation within each set of published RRs (50). Second, the study-specific estimates were combined using the restricted maximum likelihood method in a multivariate random-effects meta-analysis (51). A p value for nonlinearity was calculated by testing the null hypothesis that the coefficient of the second spline is equal to 0.

We adopted Stata 15.0 to perform all analysis, and the results were considered statistically significant, when probabilities (*P*-values) reported 2-sided ≤ 0.05.

3 Results

3.1 Literature search and study characteristics

Following an exhaustive search and evaluation, 24 articles remained (9, 14–36), including 12 observational studies (9, 14–24) and 12 RCTs (25–36). Figure 1 and Supplementary Tables 1, 2 displayed the workflow for filtering accessible articles. In the observational studies, 16 results were pooled, because four publication (16, 20, 21, 24) containing two results, one for ischemic stroke and one for hemorrhagic stroke. Among the 12 studies, four (19, 21, 23, 24) with six studies were undertaken in North America, three (9, 15, 17) in Asia, and five (14, 16, 18, 20, 22) with seven studies were conducted in Europe (Table 1). Of these 12 RCTs, four (25, 27, 34, 35) were conducted in Europe, six (26, 29–33) in North America, one (28) in Oceania, and one (36) in Asia (Table 2).

3.2 Quality assessment

The mean score for case-control studies was 8.67 (range: 8 to 9) and for cohort studies was 8.00 (range: 6 to 9) after utilizing the Newcastle-Ottawa Scale to evaluate the quality of the 12 publications in this investigation. The mean score for RCTs was 3.83 (range: 3 to 5). The findings of the quality evaluation of the included studies were displayed in Supplementary Tables 3, 4 and Table 2.

3.3 The association between folic acid and the primary prevention of stroke

3.3.1 The association between dietary folic acid and the primary prevention of stroke

Twelve observational publications, which consisted of four case-control studies (9, 16, 17) and twelve cohort studies (14, 15, 18–24), were analyzed to explore the association between dietary folic acid and stroke primary prevention. These studies had 312,320 individuals and 8,816 stroke instances. Seven research (9, 14, 15, 17, 20, 21, 23) found a substantial link between dietary folic acid and stroke incidence, while nine studies (16, 18–22, 24) showed no connection. The combined RR (95%CI) of stroke incidence was 0.83 (95% CI: 0.73–0.94; I^2 = 49.10; $p_{\text{heterogeneity}}$ = 0.014; Figure 2), for the highest vs. lowest category of dietary folic acid intake. Seven research (9, 15, 16, 19–21, 24) examined the association between dietary folic acid and the occurrence of ischemic stroke, while four studies (16, 20, 21, 24) investigated the connection between dietary folic acid and hemorrhagic stroke. The pooled RRs (95%CIs) for the highest vs. lowest consumption categories of dietary folic acid were 0.82 (95% CI: 0.74–0.91; I^2 = 43.60%; $p_{\text{heterogeneity}}$ = 0.100) for ischemic stroke and 0.93 (95% CI: 0.73–1.18; I^2 = 2.90%; $p_{\text{heterogeneity}}$ = 0.378) for hemorrhagic stroke (Supplementary Figure 1).

The 12 observational studies were conducted in seven countries, and the background of grain folic acid fortification for each country is detailed in Supplementary Table 5. The integrated RRs (95%CIs) for the highest vs. the lowest category of dietary folic acid were 0.89 (95% CI: 0.76–1.05; I^2 = 4.10%; $p_{\text{heterogeneity}}$ = 0.383) for studies performed in areas with folic acid fortification and 0.80 (95% CI: 0.67–0.95; I^2 = 59.10%; $p_{\text{heterogeneity}}$ = 0.007) in areas without folic acid fortification (Supplementary Figure 2).

Analysis of nine articles (9, 14, 15, 17, 20–24) containing twelve research revealed a nonlinear relationship between folic acid consumption and stroke incidence ($p_{\text{nonlinearity}}$ = 0.007) (Figure 3). The RRs with 95% CIs of stroke incidence were 0.99 (95% CI: 0.96–1.02), 0.97 (95% CI: 0.93–1.02), 0.92 (95% CI: 0.88–0.96), 0.82 (95% CI: 0.74–0.89) and 0.77 (95% CI: 0.68–0.87) for 241, 330, 410, 547 and 611 ug/day of dietary folic acid intake, respectively.

3.3.2 The association between folic acid supplementation and the stroke primary prevention

Twelve RCTs were analyzed to explore the association between folic acid supplementation and the primary prevention of stroke. These studies included 75,042 individuals and 2,036 stroke cases. One study (36) reported an inverse association between folic acid supplementation and stroke incidence, while the other 11 studies (25–35) found no association between folic acid supplementation and stroke. The combined RR (95%CI) of stroke primary prevention was 0.92 (95% CI: 0.80–1.05; I^2 = 37.30%; $p_{\text{heterogeneity}}$ = 0.093; Figure 4), for the highest vs. lowest category of folic acid supplementation. Of these 12 RCTs, four studies evaluated the association between folic acid supplementation and ischemic stroke incidence (26, 31, 32, 36), and three studies assessed the association between folic acid supplementation and hemorrhagic stroke incidence. The integrated RRs (95% CIs) for the highest vs. lowest categories of folic acid supplementation were 0.98 (95%CI: 0.74–1.29; I^2 = 73.40%; $p_{\text{heterogeneity}}$ = 0.010) for ischemic stroke and 1.03 (95% CI: 0.80–1.34; I^2 = 0.00%; $p_{\text{heterogeneity}}$ = 0.544) for hemorrhagic stroke (Supplementary Figure 3).

The 12 RCTs were conducted in nine countries. The pooled RRs (95%CIs) for the highest vs. the lowest category of supplementation were 1.06 (95% CI: 0.95–1.19; I^2 = 0.00%; $p_{\text{heterogeneity}}$ = 0.898) for studies performed in areas with folic acid fortification and 0.78 (95% CI: 0.68–0.89; I^2 = 0.00%; $p_{\text{heterogeneity}}$ = 0.968) in areas without folic acid fortification (Supplementary Figure 4).

3.4 Subgroup analysis

The subgroup results of dietary folic acid and primary prevention of stroke were shown in Table 3. In subgroup analysis stratified by geographic region, higher folic acid intake was significantly associated with decreased stroke incidence among studies conducted in Europe (OR: 0.85 95% CI: 0.77–0.95) and North America (OR: 0.86 95% CI: 0.76–0.98), but not in Asia (OR: 0.18 95% CI: 0.03–1.06). Increased folic acid intake was linked to a decreased risk of stroke in cohort studies (OR: 0.84 95% CI: 0.78–0.90), but not in case-control studies (OR: 0.21 95% CI: 0.04–1.23).

TABLE 1 Summary of design characteristics in observational studies included in this meta-analysis.

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
Zhang et al. (14)	Britain (2010–2018)	40–70	C-S	24-h ditery recall questionnaire (ug/day)	Quartile 1: 167.45	115664(1402)	1 (ref)	Age, sex, ethnic, physical activity, smoking status, employment, coffee, HDL-C, LDL-C, TC, adiposity	Total stroke	No
					Quartile 2: 221.52		0.94 (0.80–1.11)			
					Quartile 3: 321.41		0.90 (0.76–1.06)			
					Quartile 4: 432.81		0.86 (0.75–0.99)			
Van Guelpen et al. (16)	Sweden (1986–2000)	25–74	C-C-S	Food frequency questionnaire (mg/1000kcal/day)	Quartile 1	563 (139)	1 (ref)	BMI, current smoking, cholesterol, diabetes, hypertension, and plasma homocysteine	IS	No
					Quartile 2		1.14 (0.64–2.03)			
					Quartile 3		0.94 (0.48–1.8)			
					Quartile 4		1.19 (0.64–2.20)			
Van Guelpen et al. (16)	Sweden (1986–2000)	25–74	C-C-S	Food frequency questionnaire (mg/1000kcal/day)	Quartile 1	102 (25)	1 (ref)	BMI, current smoking, cholesterol, diabetes, hypertension, and plasma homocysteine	HS	No
					Quartile 2		0.13 (0.01–1.26)			
					Quartile 3		0.16 (0.02–1.21)			
					Quartile 4		0.16 (0.02–1.23)			
He et al. (21)	America (1986–2000)	40–75	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 262	43732 (455)	1 (ref)	BMI, physical activity, history of hypertension and hypercholesterolemia, smoking status, aspirin use, alcohol, total calorie, intake of fiber, potassium, and vitamin E	IS	Yes
					Quintile 2: 336		1.00 (0.74–1.36)			
					Quintile 3: 413		0.75 (0.53–1.06)			
					Quintile 4: 547		0.96 (0.68–1.35)			
					Quintile 5: 821		0.66 (0.45–0.98)			

(Continued)

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TABLE 1 (Continued)

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
He et al. (21)	America (1986–2000)	40–75	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 262	43732 (125)	1 (ref)	BMI, physical activity, history of hypertension and hypercholesterolemia, smoking status, aspirin use, alcohol, total calorie, intake of fiber, potassium, and vitamin E	HS	Yes
					Quintile 2: 336		1.28 (0.71–2.32)			
					Quintile 3: 413		1.49 (0.79–2.83)			
					Quintile 4: 547		1.31 (0.67–2.55)			
					Quintile 5: 821		0.86 (0.40–1.88)			
Weng et al. (15)	China (1990–1992)	40+	C–S	Food frequency questionnaire (ug/day)	<297.33	1772 (132)	1 (ref)	Age, sex, age*sex,hypertension, use of antihypertensive drugs, diabetes mellitus, area, central obesity, alcohol consumption habits, smoking habit, sex-smoking habit interaction, BMI, self-report heart disease, hypercholesterolemia, hypertriglyceridemia, physical activity, fibrinogen, apolipoprotein B, and plasminogen	IS	No
					297.33–369.45		1.15 (0.63–2.11)			
					>369.48		0.63 (0.40–0.98)			
Luu et al. (19)	America (1989–2005)	45–64	C-S	Food frequency questionnaire (ug/day)	Quartile 1: <155	12926 (594)	1 (ref)	Age, sex, current smoking status, diabetes, caloric intake, and hypertension	IS	Yes
					Quartile 2: 156–211		0.98 (0.68–1.43)			
					Quartile 3: 212–218		0.77 (0.54–1.12)			
					Quartile 4: ≥ 279		0.84 (0.64–1.10)			

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TABLE 1 (Continued)

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
Larsson et al. (20)	Finland (1985–2004)	50–69	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 262	27111 (2702)	1 (ref)	Age, supplementation group, total number of cigarettes smoked daily, BMI, systolic and diastolic blood pressure, serum TC, serum HDL-C, histories of diabetes and coronary heart disease, leisure-time physical activity, and intakes of alcohol and total energy	IS	No
					Quintile 2: 300		0.99 (0.88–1.11)			
					Quintile 3: 330		1.09 (0.97–1.23)			
					Quintile 4: 360		0.98 (0.87–1.10)			
					Quintile 5: 410		0.80 (0.70–0.91)			
Larsson et al. (20)	Finland (1985–2004)	50–69	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 262	27111 (579)	1 (ref)	Age, supplementation group, total number of cigarettes smoked daily, BMI, systolic and diastolic blood pressure, serum total cholesterol, serum HDL-C, histories of diabetes and coronary heart disease, leisure-time physical activity, and intakes of alcohol and total energy	HS	No
					Quintile 2: 300		1.16 (0.84–1.59)			
					Quintile 3: 330		0.90 (0.69–1.18)			
					Quintile 4: 360		0.98 (0.58–1.67)			
					Quintile 5: 410		0.93 (0.69–1.26)			
Choe et al. (9)	Korea (2011–2012)	65+	C-C-S	Food frequency questionnaire (ug/day)	<400	120 (60)	1 (ref)	Age, sex, smoking, TC, HDL-C, fasting blood glucose, hypertension, and regular exercise	IS	No
					≥ 400		0.16 (0.03-0.94)			

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TABLE 1 (Continued)

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
Bazzano et al. (23)	America (1971–1992)	25-74	C-S	24-h ditery recall questionnaire (ug/day)	Quartile 1: <136.0	9764 (926)	1 (ref)	Age, race, sex, systolic blood pressure, serum cholesterol, BMI, history of diabetes, physical activity, level of education, regular alcohol consumption, current cigarette smoking, saturated fat intake, and total energy intake	Total stroke	No
					Quartile 2: 136.0-203.7		0.93 (0.74–1.16)			
					Quartile 3: 203.7–300.6		0.85 (0.71–1.02)			
					Quartile 4: >300.6		0.80 (0.64–0.99)			
Al-Delaimy et al. (24)	America (1980–1998)	34–59	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 30–210	83896 (924)	1 (ref)	Age, time period, smoking history, BMI, hormone use and menopausal status, currently taking aspirin, vitamin E supplements, physical activity, alcohol use, history of high blood pressure, history of diabetes, history of hypercholesterolemia, parental history of myocardial infarction, at or before the age of 65 years, total caloric intake	IS	Yes
					Quintile 2: 211–271		1.09 (0.89–1.33)			
					Quintile 3: 272–354		1.17 (0.95–1.44)			
					Quintile 4: 355–526		1.03 (0.81–1.30)			
					Quintile 5: >526		1.03 (0.80–1.33)			

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TABLE 1 (Continued)

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
Al-Delaimy et al. (24)	America (1980–1998)	34–59	C-S	Food frequency questionnaire (ug/day)	Quintile 1: 30–210	83896 (390)	1 (ref)	Age, time period, smoking history, BMI, hormone use and menopausal status, currently taking aspirin, vitamin E supplements, physical activity, alcohol use, history of high blood pressure, history of diabetes, history of hypercholesterolemia, parental history of myocardial infarction, at or before the age of 65 years, total caloric intake	HS	Yes
					Quintile 2: 211–271		1.29 (0.89–1.88)			
					Quintile 3: 272–354		0.98 (0.64–1.49)			
					Quintile 4: 355–526		1.23 (0.79–1.88)			
					Quintile 5: >526		1.05 (0.65–1.70)			
Park (17)	Korea (2007–2009)	57.4/57.9	C-C-S	Food frequency questionnaire (ug/day)	Quartile 1: ≤ 286.38	138 (69)	1 (ref)	Age, sex, BMI, and family history of stroke	Total stroke	No
					Quartile 2: 286.38–412.38		0.36 (0.12–1.10)			
					Quartile 3: 412.38–520.28		0.09 (0.02–0.39)			
					Quartile 4: >520.28		0.04 (0.008–0.23)			

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TABLE 1 (Continued)

References	Country (Study period)	Age range Mean age (Case/ control)	Study design	Method of assessing folate intake (Unit)	The exposure of folate intake Mean level or range	Sample (cases)	RR(95%CI)	Adjustment for Covariates	Types of stroke	Grain fortification
Dalmeijer et al. (22)	the Netherlands (1997–2004)	49–70	C-S	Food frequency questionnaire (ug/day)	Quartile 1: ≤ 169	16165 (224)	1 (ref)	Hypertension, cholesterolemia, mean systolic blood pressure, age, total physical activity, BMI, smoking, diabetes, intake of energy, proteins, saturated fats, monounsaturated fats, polyunsaturated fats, alcohol, vitamin B2, vitamin B6, vitamin B12, betaine and choline.	Total stroke	No
					Quartile 2: 169–191		0.99 (0.64–1.54)			
					Quartile 3: 191–215		1.12 (0.66–1.89)			
					Quartile 4: >215		1.38 (0.67–2.81)			
Marniemi et al. (18)	Finland (1987–1997)	65–99	C-S	dietary history interviews (ug/day)	Tertile 1	660 (70)	1 (ref)	Age, gender, smoking, functional capacity and weight adjusted energy intake	Total stroke	No
					Tertile 2		0.83 (0.46–1.48)			
					Tertile 3		0.75 (0.38–1.46)			

RR, relative risk; CI, confidence interval; C-C-S, case-control study; C-S, cohort study; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; TC, total cholesterol; IS, Ischemic stroke; HS, Hemorrhagic stroke.

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TABLE 2 Summary of design characteristics in RCTs included in this meta-analysis.

References	Country (Study period)	Mean age	Number (Treatment/control)	Cases (Treatment/control)	Intervention regimen			Control	RR(95%CI)	Duration (months)	Grain fortification	Data quality
					Folic acid	Vitamin 12	Vitamin B6					
Ebbing et al. (25)	Norway (1999–2006)	61.4	1540/779	28/19	0.8mg/d	0.4mg/d	40mg/d	placebo	0.75 (0.42–1.33)	38.4	No	5
Wrone et al. (26)	America (1998–1999)	61.2	166/168	3/1	15mg/d	0.006mg/d	12.5mg/d	Folic acid 1 mg/d, vitamin B12 0.006 mg/d and vitamin B6 12.5 mg/d	3.04 (0.32–28.93)	24	Yes	3
Heinz et al. (27)	Germany (2002–2008)	61.0	327/323	11/15	5 mg/time three times a week	0.05 mg/time three times a week	20 mg/time three times a week	Folic acid 0.2 mg/time, vitamin B12 0.004 mg/time and vitamin B6 1 mg/time; three times a week	0.72 (0.34–1.55)	25.2	No	4
Zoungas et al. (28)	Australia; New Zealand (1998–2000)	57.0	156/159	8/18	15 mg/d	no	no	placebo	0.45 (0.20–1.01)	43.2	Partial	3
Bostom et al. (29)	America, Canada, Brazil (2002–2007)	52.0	2056/2054	35/32	5 mg/d	1 mg/d	50 mg/d	vitamin B12 0.002 mg/d, Vitamin B6 1.4 mg/d, folic acid 0 mg/d	1.09 (0.68–1.76)	48	Yes	3
Jamison et al. (30)	America (2001–2002)	65.8	1032/1024	37/41	40 mg/d	2 mg/d	100 mg/d	placebo	0.90 (0.58–1.39)	38.4	Yes	5
Albert et al. (31)	America (1998–2006)	62.8	2721/2721	79/69	2.5 mg/d	1 mg/d	50 mg/d	placebo	1.14 (0.83–1.57)	87.6	Yes	3

(Continued)

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TABLE 2 (Continued)

References	Country (Study period)	Mean age	Number (Treatment/ control)	Cases (Treatment/ control)	Intervention regimen			Control	RR(95%CI)	Duration (months)	Grain fortifica-tion	Data quality
					Folic acid	Vitamin 12	Vitamin B6					
Sesso et al. (32)	America (1997–2011)	64.3	7317/7324	332/311	The intervention contains folic acid but the dosage of folic acid is unknown	The intervention contains vitamin B12 but the dosage of vitamin B12 is unknown	The intervention contains vitamin B6 but the dosage of vitamin B6 is unknown	placebo	1.07 (0.92–1.24)	134.4	Yes	4
Sesso et al. (33)	America (2015–2020)	72.1	10720/10720	121/116	The intervention contains folic acid but the dosage of folic acid is unknown	The intervention contains vitamin B12 but the dosage of vitamin B12 is unknown	The intervention contains vitamin B6 but the dosage of vitamin B6 is unknown	placebo	1.04 (0.81–1.34)	43.2	Yes	4
Righetti et al. (34)	Italy (2001–2003)	64.5	63/51	7/10	5 mg/d or 5 mg every other day	500 mg every other day	500 mg every other day	placebo	0.57 (0.23–1.39)	29.03	No	2
van Dijk et al. (35)	Netherlands (2008–2011)	74.0	1461/1458	46/60	0.4 mg/d	0.5 mg/d	no	placebo	0.77 (0.52–1.12)	24	No	5
Huo et al. (36)	China (2008–2013)	60.0	10348/10354	282/355	0.8 mg/d	no	no	Usual care	0.79 (0.68–0.93)	54	No	5

RCT, randomized controlled trial; RR, relative risk; CI, confidence interval.

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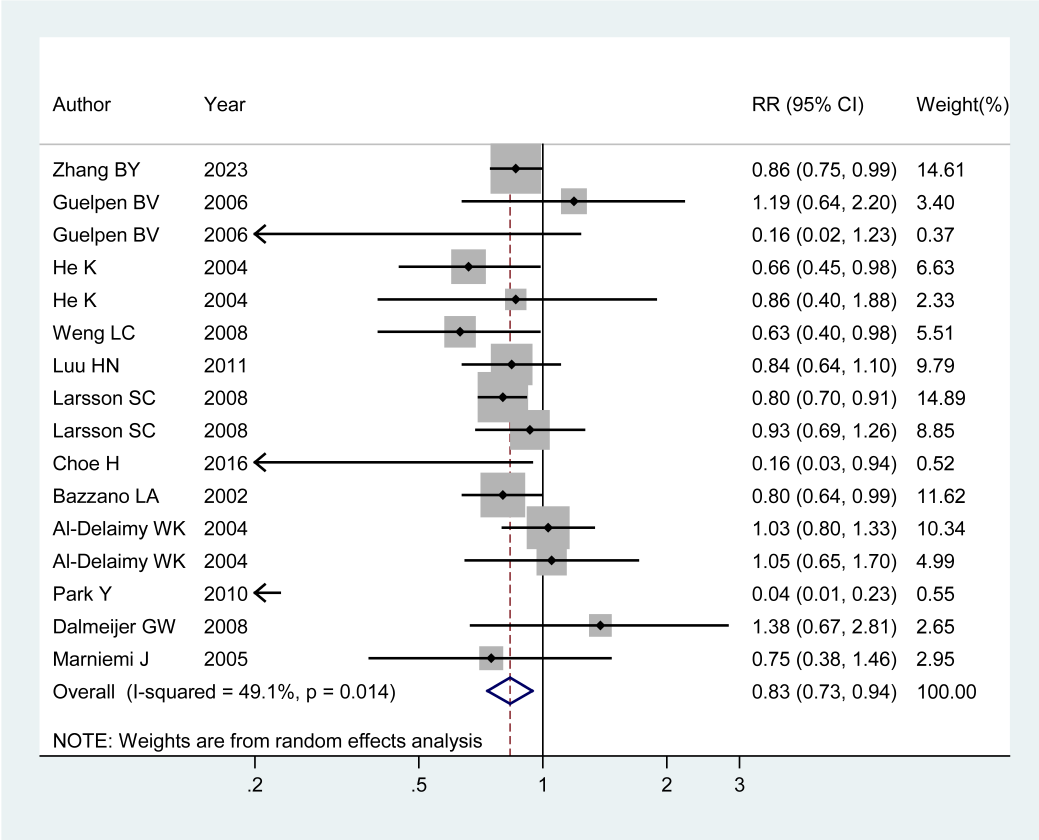

FIGURE 2
Forest plot of dietary folic acid and the primary prevention of stroke. The size of gray box is positively proportional to the weight assigned to each study, and horizontal lines represent the 95% confidence intervals. The RR (95% CI) in every article is the relative risk (95% confidence interval) of stroke incidence for the highest vs. the lowest stratification of dietary folic acid.

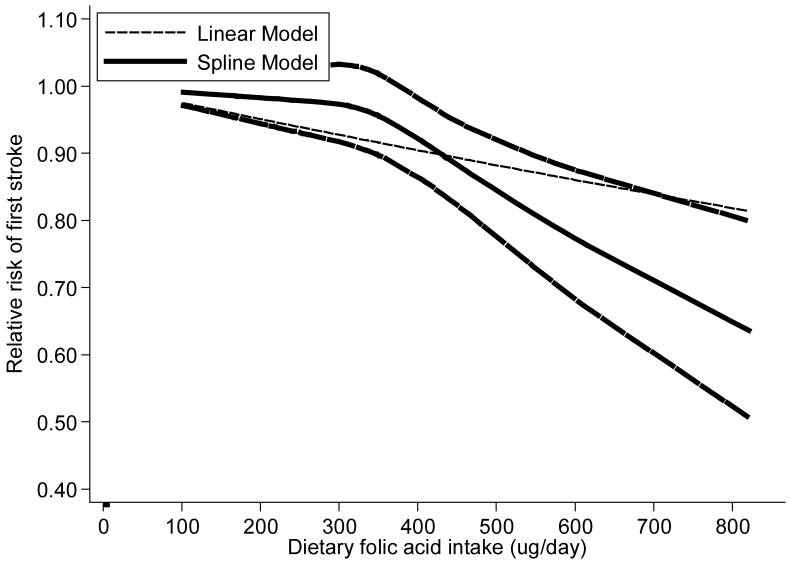

FIGURE 3
The dose-response analysis between dietary folic acid and the primary prevention of stroke with restricted cubic splines in a multivariate random-effects dose-response model. The solid line and the long dash line represent the estimated relative risks and its 95% CIs. Short dash line represents the linear relationship.

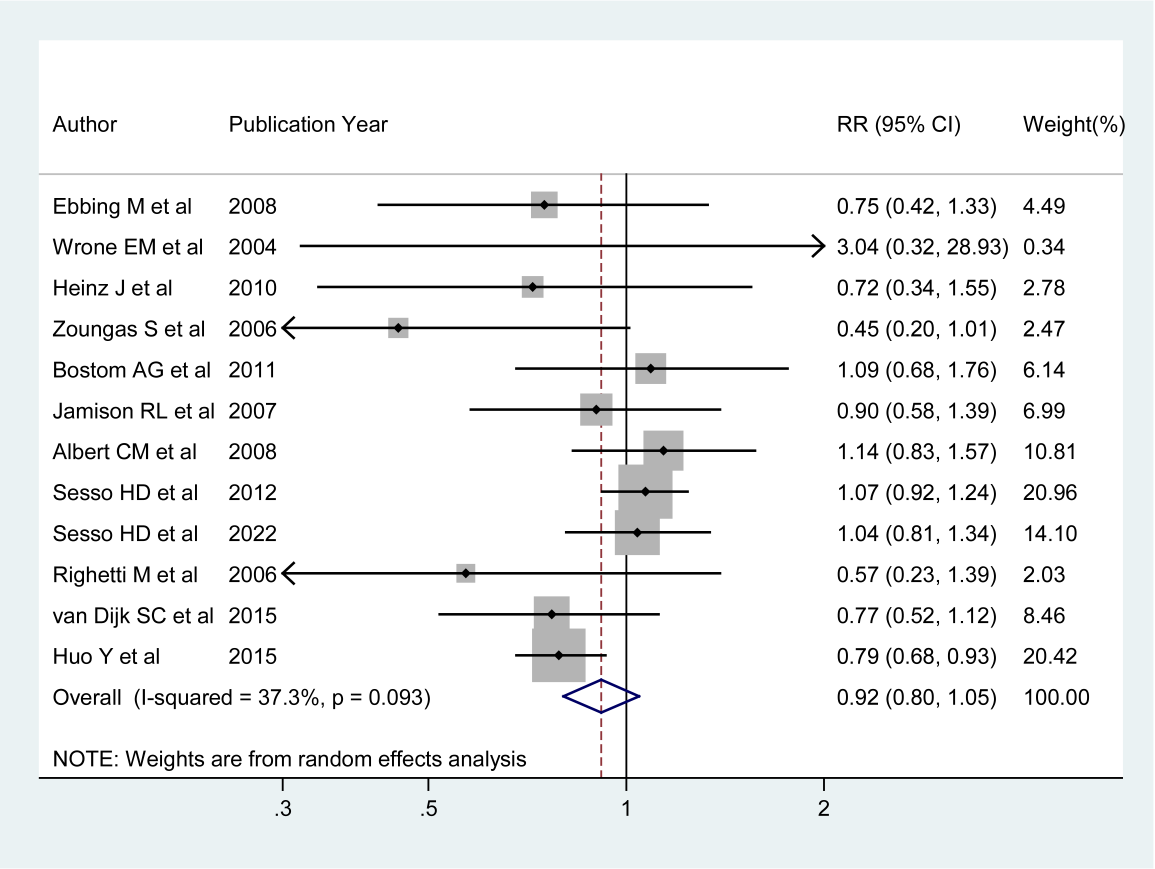

FIGURE 4
Forest plot of folic acid supplementation and the primary prevention of stroke. The size of gray box is positively proportional to the weight assigned to each study, and horizontal lines represent the 95% confidence intervals. The RR (95%CI) in every article is the relative risk (95% confidence interval) of stroke incidence for the highest vs. the lowest stratification of dietary folic acid.

With respect to major confounding factors, this inverse association remained after adjusting for BMI (OR: 0.84 95% CI: 0.70–0.99) and diabetes (OR: 0.86 95% CI: 0.77–0.97). Our analysis revealed that folic acid intake was inversely associated with stroke incidence in men (OR: 0.81 95% CI: 0.72–0.90), but not in women (OR: 1.06 95% CI: 0.86–1.32).

The subgroup results of folic acid supplementation and primary prevention of stroke were shown in Table 4. We found that folic acid supplement intervention was significantly associated with decreased stroke incidence among studies conducted in Europe (OR: 0.85 95% CI: 0.77–0.95) and Asia (OR: 0.86 95% CI: 0.76–0.98), and it was not associated with stroke in any of the other subgroups.

3.5 Meta-regression and sensitive analysis

A univariate meta-regression analysis was performed to investigate the origin of heterogeneity. The findings showed that whether adjusted for the history of hypertension (p = 0.730), the history of diabetes (p = 0.821), and BMI (p = 0.230), study design (p = 0.704), publication year (p = 0.896), and geographic region (p = 0.882) did not have a significant effect on the heterogeneity in the process of exploring the association between dietary folic acid and stroke primary prevention. In the process of exploring the association between folic acid supplementation and stroke primary prevention, geographic region (p = 0.928), data quality (p = 0.445), whether folic acid supplementation alone (p = 0.568), folic acid dosage (p = 0.615) and duration of intervention (p = 0.129) did not have a significant effect on the heterogeneity.

The leave-one-out sensitivity analysis revealed that the study conducted by Park (17) had a significant impact on the observed heterogeneity of the results pooled from observational studies. The between-study heterogeneity dropped to 16.96% after removal ($P_{\text{for heterogeneity}}$ = 0.264), and the OR (95%CI) was still significant with 0.84 (0.77–0.92). Considering the low heterogeneity of results synthesized from RCTs, we did not conduct leave-one-out sensitivity analysis.

3.6 Influence analysis and publication bias

The influence analysis showed that no observational study or RCT had a significant impact on the outcomes. No evidence of obvious small-study effect was found by the visual inspection of the funnel plot and Egger's test in the included observational studies,

TABLE 3 Summary of RRs with 95% CIs for association between dietary folic acid and the primary prevention of stroke.

Stratification	Number of studies	OR (95% CI)	I^2%	P for heterogeneity
All studies	16	0.83 (0.73–0.94)	49.10%	0.014
Continent where the study was conducted				
North America	6	0.86 (0.76–0.98)	0.00%	0.438
Asia	3	0.18 (0.03–1.06)	82.40%	0.003
Europe	7	0.85 (0.77–0.95)	10.80%	0.347
Whether the results were adjusted for the history of hypertension or not				
Yes	10	0.86 (0.70–1.05)	40.60%	0.087
No	6	0.81 (0.68–0.96)	63.70%	0.017
Whether the results were adjusted for the history of diabetes or not				
Yes	10	0.86 (0.77–0.97)	22.00%	0.240
No	6	0.60 (0.39–0.94)	71.60%	0.004
Whether the results were adjusted for BMI or not				
Yes	12	0.84 (0.70–0.99)	57.10%	0.007
No	4	0.83 (0.70–0.99)	20.40%	0.287
The type of study design				
Cohort studies	12	0.84 (0.78–0.90)	0.00%	0.568
Case-control studies	4	0.21 (0.04–1.23)	83.80%	<0.001
Gender				
Women	3	1.06 (0.86–1.32)	0.00%	0.752
Men	4	0.81 (0.72–0.90)	0.00%	0.589

RR, relative risk; CI, confidence interval.

TABLE 4 Summary of RRs with 95% CIs for association between folic acid supplementation and the primary prevention of stroke.

Stratification	Number of studies	RR (95% CI)	I^2%	P for heterogeneity
All studies	12	0.92 (0.80–1.05)	37.30%	0.093
Continent where the study was conducted				
North America	6	1.06 (0.95–1.19)	0.00%	0.898
Europe	4	0.74 (0.56–0.97)	0.00%	0.946
Oceania	1	0.45 (0.20–1.01)	–	–
Asia	1	0.79 (0.68–0.92)	–	–
Folic acid supplementation alone				
Yes	2	0.69 (0.43–1.11)	44.10%	0.181
No	10	1.01 (0.91–1.12)	0.00%	0.562
Folic acid dosage, mg/d				
≤0.8	5	0.91 (0.77–1.08)	59.10%	0.044
>0.8	7	0.92 (0.71–1.18)	21.90%	0.262
Duration of intervention, months				
≤36	4	0.75 (0.55–1.03)	0.00%	0.600
>36	8	0.94 (0.81–1.10)	49.30%	0.055
Data quality				
<4	5	0.91 (0.62–1.34)	42.40%	0.139
≥4	5	0.91 (0.79–1.04)	41.20%	0.116

RR, relative risk; CI, confidence interval.

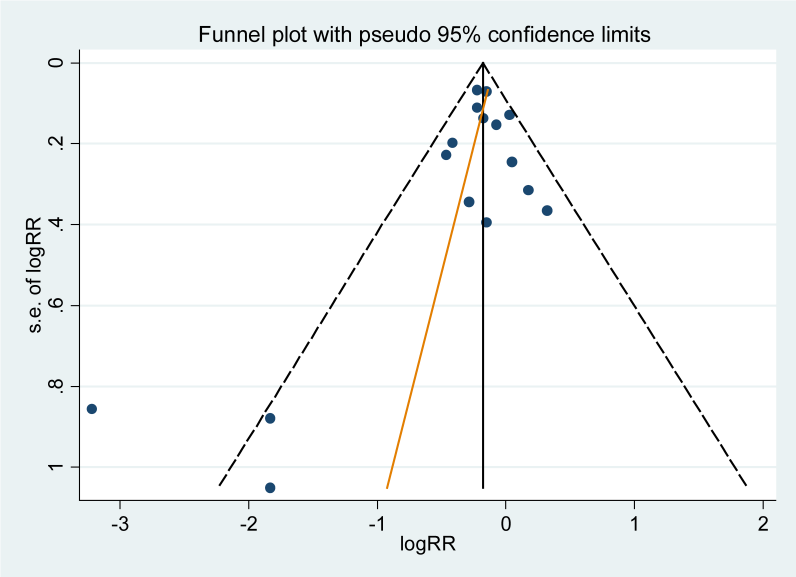

FIGURE 5
Funnel plot with pseudo 95% confidence limits for the analysis of dietary folic acid and he primary prevention of stroke. The RR (95%CI) in every article is the relative risk (95% confidence interval) of stroke incidence for the highest vs. the lowest stratification of dietary folic acid.

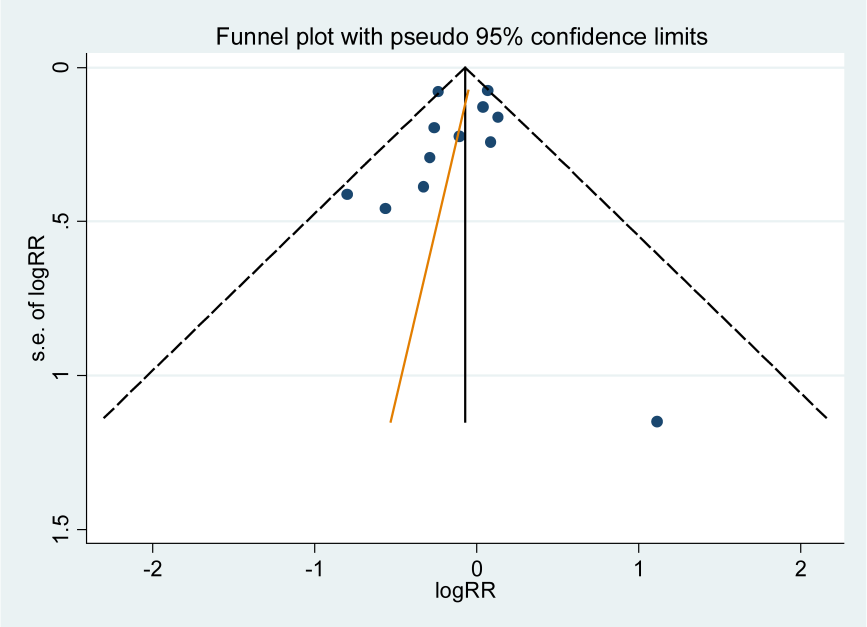

FIGURE 6
Funnel plot with pseudo 95% confidence limits for the analysis of folic acid supplementation and he primary prevention of stroke. The RR (95%CI) in every article is the relative risk (95% confidence interval) of stroke incidence for the highest vs. the lowest stratification of dietary folic acid.

with the p values of Egger's test were 0.179 and 0.508 respectively. The results of funnel plot were displayed in Figures 5, 6.

4 Discussion

We included 24 studies in this meta-analysis, including 12 observational studies to assess the association between dietary folic acid intake and primary prevention of stroke, and 12 RCTs to assess the association between folic acid supplementation and primary prevention of stroke. The summaries from these studies disclosed that high dietary folic acid intake was associated with a 17% reduction in stroke incidence, and the effect of dietary folic acid was greater in areas without grain fortification. The negative link was significant for dietary folic acid and the occurrence of ischemic stroke, but not for

hemorrhagic stroke. Moreover, folic acid supplementation was not associated with the stroke primary prevention, but folic acid supplementation was effective in areas without grain fortification.

The results of our meta-analysis aligned with those of other studies. An review of 22 randomized controlled trials revealed a 20% decrease in stroke risk and a 0.72umol/L decrease in Hcy levels in the group that took folic acid supplements compared to the control group (52). Two more studies (53, 54) also found a negative correlation between using folic acid supplements and the stroke events. The study showed that dietary folic acid and folic acid supplementation had a significant preventive effect on stroke in individuals without grain fortification, which aligned with other research indicating that folic acid supplement could decrease the risk of stroke, especially in regions without folic acid fortification (39, 55, 56). These results suggested that individuals with folic acid deficiency might gain more protection on stroke by increasing their dietary folic acid consumption or take folic acid supplements as appropriate. Encouraging the nutritional supplementation of folic acid through food should be advocated in countries where food is not fortified with folic acid due to its cost-effectiveness, safety, and wide applicability.

A negative association between folic acid and stroke incidence is biologically plausible. The mechanism by which folic acid reduces stroke incidence may be through its role as a substrate for the remethylation of Hcy to methionine, thereby reducing plasma Hcy concentration (57). Elevated amounts of Hcy can cause damage to blood vessels by accumulating endothelial cell toxicity and generating free radicals, which can contribute to the development of atherosclerosis (7, 58). Additionally, Hcy can enhance clotting function and disrupt the regulation of endothelium-dependent vasomotor (7). Folic acid not only regulates Hcy but also possesses antioxidant and vascular protecting properties, which are crucial in preventing stroke formation (59, 60).

Heterogeneity is prevalent in the meta-analysis, so it is principal to exploring the sources of heterogeneity. In this meta-analysis, I^2 = 49.10% from observational studies indicated moderate heterogeneity among the studies on the association between folic acid intake and stroke primary prevention. However, the P-values of all variables in the meta-regression model were greater than 0.05, and the source of heterogeneity could not be found through the meta-regression. By leaving one out, the result of sensitivity analysis indicated that one study (17) contributed a lot to heterogeneity. When this study was deleted, the heterogeneity of the results was reduced to 16.96%, and the results between dietary folic acid and stroke incidence were still significant, strengthening the stability and reliability of our results.

This meta-analysis had several strengths. First, we analyzed the association of dietary folic acid intake, folic acid supplementation with the primary prevention of stroke. Second, by enlarging the sample size and only including studies with first stroke as the reporting endpoints, we had a high statistical power to conclude the relationship between folic acid and the primary prevention of stroke. Third, we investigated the association between folic acid and the primary prevention of stroke in areas with and without acid fortification. Fourth, RRs (95% CIs) from observational studies were the results with adjusting for the most confounding factors, and we investigated the association of dietary folic acid with ischemic stroke and hemorrhagic stroke.

Several shortcomings need to be taken into account when interpreting the results. First, in the observational studies, results may have recall and selection biases, and the confounding factors adjusted in each article are different, so we could not completely eliminate the uncontrolled factors that may be inherent in these articles. Furthermore, the category of dietary folic acid is different, with some studies using four-point classifications and same using five-point classifications, which may underestimate the association between dietary folic acid and stroke. Second, in the RCTs, the dose of folic acid in the intervention group was slightly different, which may weaken the association between folic acid supplementation and stroke incidence. Third, populations with specific genetic backgrounds may respond differently to folic acid, but we don't have enough data to analyze it. Finally, different methods of stroke diagnosis in the included studies may also have influenced the overall results.

5 Conclusion

The comprehensive review and meta-analysis indicated a substantial association between dietary folic acid intake and the primary prevention of stroke, and an association between folic acid supplementation and the primary prevention of stroke in areas without folic acid fortification. Our research indicated that increasing folic acid consumption in the diet or take folic acid supplements as appropriate can be an effective way in the primary prevention of stroke, particularly in countries that do not fortify foods with folic acid. This comprehensive analysis improved the current knowledge base and supplied pertinent data for clinical guidance and public health policies.

Data availability statement

The original contributions presented in this study are included in this article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

JY: Conceptualization, Data curation, Formal analysis, Writing—original draft. JW: Investigation, Project administration, Writing—review and editing. BL: Formal analysis, Software, Validation, Writing—review and editing. YZ: Methodology, Software, Validation, Writing—original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2024.1288417/full#supplementary-material

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