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Homocysteine, vitamin B12, and folate levels in patients with multiple sclerosis in Chinese population: A case-control study and meta-analysis

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ABSTRACT

Background: Current studies suggested discrepancies on the correlations between multiple sclerosis (MS) and blood levels of homocysteine (Hcy), vitamin B12 (VB12), and folate. We performed a case-control study and meta-analysis to help resolve the controversy of these lab values in Chinese patients with MS.

Methods: We recruited 80 Chinese MS patients, 86 age/sex matched neurological controls (patients with peripheral vertigo or sleep disorders), and 80 age- and sex-matched healthy controls. Serum Hcy levels were measured using flourimetric high-performance liquid chromatography, serum levels of VB12 and folate using immune assay. A literature search of PubMed, Embase, Web of Science, Chinese National Knowledge Infrastructure, Wanfang, and SinoMed was conducted for case-control studies with pure Chinese populations published up to March 16, 2019. The effective size was estimated by the pooled standardized mean difference (SMD) and associated 95% confidence interval (CI).

Results: The case-control study results suggest higher Hcy levels (mean ± SD) and frequency of hyperhomocysteinemia in the Chinese MS cases than control groups (all $p < 0.001$), lower for VB12 levels (mean ± SD, $p = 0.043$ or 0.039). No significant difference was observed for levels of folate (mean ± SD, both $p > 0.05$), and for frequency of folate or VB12 deficiency (all $p > 0.05$). Analysis of pooled SMDs and 95% CIs suggested increased Hcy levels in Chinese MS patients (SMD: 2.31, 95% CI: 1.33–3.28, $p < 0.001$), and in relapsing or remitting cases relative to controls (SMD: 0.94 or 0.85, 95% CI: 0.49–1.39 or 0.35–1.34, both $p < 0.001$). The meta-analysis results also suggested reduced VB12 levels in Chinese MS patients (SMD: −0.30, 95% CI: −0.46–0.14, $p < 0.001$), and in relapsing MS patients compared to controls (SMD: −0.31, 95% CI: −0.47–0.15, $p < 0.001$), while no statistical difference for cases in remission. No significant difference was observed for levels folate in all comparisons.

Conclusion: Patients with MS tend to have increased blood Hcy levels compared to controls. MS patients of Chinese origin and those in relapse may have decreased levels of VB12. Hcy and VB12 may contribute to pathogenesis of the disease, and VB12 may correlate with MS relapse.

1. Introduction

Multiple sclerosis (MS) is a neurologic disease characterized by diffuse inflammatory demyelinating throughout the central nervous system (CNS). The exact etiology of MS is unclear, but complex interactions between genetic and environmental factors are considered to be involved in the autoimmune inflammatory process (Karussis, 2014). Among these factors, homocysteine (Hcy), vitamin B12 (VB12), and

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folate have been considered to be possible participants of MS in previous studies. Hcy is a sulfur-containing amino acid, an important intermediate product in the methionine metabolism. Furthermore, modifications involving methylation could destabilize myelin structures by triggering hypomethylation of myelin basic protein, a cardinal component of myelin in the CNS (Kim et al., 2003). Deficiency of VB12 and/or folate can affect the conversion of homocysteine to methionine essential for DNA and RNA synthesis, thereby causing a neurotoxin effect of activating aspartate receptor and cell death (Diaz-Arrastia, 2000; Reynolds, 2006). These results suggest that Hcy, VB12, and folate may contribute to MS pathogenesis.

However, the findings of case-control studies are controversial. While some investigations showed increased Hcy levels and decreased levels of VB12 and folate in MS patients compared to controls (Besler and Comoglu, 2003; Chen et al., 2016; Moghaddasi et al., 2013; Ramsaransing et al., 2006), other studies reported no differences between patients with MS and their controls in levels of Hcy (Kocer et al., 2009; Teunissen et al., 2008; Triantafyllou et al., 2008), VB12, or folate (Basil et al., 1965; Kocer et al., 2009; Najafi et al., 2012; Russo et al., 2008; Zoccolella et al., 2012). A meta-analysis by Zhu et al., in 2011 revealed higher levels of Hcy and lower VB12, while no significant difference for folate (Zhu et al., 2011). Another meta-analysis in 2017 showed that MS patients display elevated Hcy blood levels relative to the controls, but no significant difference in either VB12 or folate (Dardiotis et al., 2017). However, these two meta-analysis studies failed to take some critical factors into account, including sex, age, disease phase and/or severity, and/or race of study population.

In our attempt to help resolve these discrepancies and limitations, we performed a case-control study on concentrations of Hcy, VB12, and folate in Chinese MS patients and controls. An updated meta-analysis was conducted to evaluate the levels of Hcy, VB12, and folate in pure Chinese MS patients. We also meta-analyzed these lab values of MS patients in relapse or remission and controls.

2. Methods

2.1. Case-control study

2.1.1. Study population

Patients were retrospectively recruited between July 16, 2013 and March 16, 2019 from the in-patient neurology department of the People's Hospital of Yichun City (n = 28), the Fifth Central Hospital of Tianjin (n = 28), the People's Hospital of Taizhou City (n = 13), and the Second Affiliated Hospital of Guangzhou Medical University (n = 11). All the patients were in relapse. We collected the data of the first visit for all the patients. Eligibility criteria for cases were as follows: (1) according to the revised McDonald's criteria of 2005 (Polman et al., 2005), 2010 (Polman et al., 2011), and 2017 (Thompson et al., 2018); (2) no corticosteroid treatment for at least 2 months prior to the inclusion in the study. Eighty-six patients with peripheral vertigo (n = 45) or sleep disorders (n = 39) and 80 health subjects were recruited as neurological or healthy controls, respectively, from the neurology department of the People's Hospital of Yichun City (n = 58 or 40) and the Fifth Central Hospital of Tianjin (n = 28 or 40). The exclusion criteria for all cases and control subjects were as follows: (1) abnormal blood pressure or lipid profile, (2) cardiac, liver, or renal dysfunction, (3) atherosclerosis risk factors except for smoking; (4) history of atrophic gastritis or gastrectomy; (5) rheumatic or autoimmune diseases; (6) suffering from any infection disease or unidentified cause fever; (7) suffering from any disease which may correlate with Hcy, VB12, or folate; (8) having received any medication impacting serum homocysteine levels, VB12, or folate supplementary products, or corticosteroids in the past 2 months; (9) including subjects of non-Chinese origin. The demographic information, past medical history, smoking, family history, and clinical data were collected. Patients' disability was evaluated using the expanded disability status scale (EDSS) score.

2.1.2. Laboratory assessment

Peripheral vein blood samples were taken after overnight fasting. Serum Hcy levels were quantified using flourimetric high-performance liquid chromatography, serum levels of VB12 and folate using immune assay following the procedures published elsewhere (Ubbink et al., 1991). The cut-off values of the diagnostic kit for hyperhomocysteinemia (HHcy), VB12, or folate deficiency were higher than 15 μmol/L, lower than 170 pg/mL or 4 nmol/L, respectively.

2.1.3. Statistical analysis

Data normality was estimated using the Komolgorov–Smirnov test. Analysis of variance (ANOVA) and Pearson correlation analysis were used for continuous and normally distributed data with homogeneity of variance, Mann–Whitney U test and Spearman correlation analysis for continuous variables of non-normal distribution and/or heterogeneity of variance. The data of MS patients were compared with neurological and healthy controls, respectively. Chi-square (χ^2) or Fisher's exact test was applied to analyze categorical data.

In all statistical analyses, the level of statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

2.2. Meta-analysis

2.2.1. Literature search strategy

Eligible studies were identified by systematically searching PubMed, Embase, Web of Science, Chinese National Knowledge Infrastructure, Wanfang, and SinoMed. The following terms were used for literature search: multiple sclerosis *and* Hcy, cobalamin or VB12, or folate. No language restriction was imposed. Last literature search was conducted on March 16, 2019. To identify studies that might have been missed by the database search, reference lists of all articles that met the inclusion criteria and of relevant review articles were examined.

2.2.2. Selection criteria

To be included in the meta-analysis, studies had to (1) evaluate the association between levels of Hcy, VB12, or folate and MS; (2) provide sufficient data for assessing a standardized mean difference (SMD) with 95% confidence interval (CI); and (3) apply a case-control design. The mean and standard deviation (SD) of the values were estimated according to the established methods (Hozo et al., 2005) when they were not available but median, range, and the sample size provided in an eligible study. Studies were excluded if they did not report original research or were published only as abstracts or letters to the editor, or if they recruited cases or controls having disease which may correlate with Hcy, VB12, or folate.

2.2.3. Data extraction

Data were extracted by two of the authors (L Pan and Y Yin), and discrepancies were resolved by discussion with a third reviewer (K Wu). The following data were extracted: the first author's name, year of publication, country or region, race of study population, gender distribution, past medication history, family history, mean age at onset with SD, sample size, disease duration and/or severity, and blood levels of Hcy, VB12, and folate in cases and/or controls.

2.2.4. Statistical analysis of the meta-analysis

All data analyses were conducted using Stata 12.0 (www.stata.com). The effective size was estimated by pooled SMD and associated 95% CI. Subgroup analysis was conducted according to disease phase (relapse or remission). A p value less than 0.05 was considered the threshold for statistical significance in all analyses.

Prior to meta-analysis, heterogeneity among studies was evaluated using the Q text and was quantified using I^2 (Zhang and Zhong, 2012).

Table 1
Demographic features and laboratory analysis of patients with MS and control subjects.

	MS	Neurological controls	p^*	Healthy controls	p^{**}
Number of subjects	80	86		80	
Sex (M/F)	21/59	32/54	0.17[a]	28/52	0.303[a]
Age (y), mean ± SD	35.34 ± 9.25	37.92 ± 8.59	0.064 [b]	37.24 ± 8.02	0.167[b]
Age at onset (y), mean ± SD	31.14 ± 7.77	–	–	–	–
Clinical phenotype (RR:SP:PP)	57:07:06	–	–	–	–
Smoking (%)	19 (23.75)	18 (20.93)	0.71[a]	21 (26.25)	0.855[a]
Serum Hcy (μmol/L), mean ± SD (range)	13.39 ± 4.94 (4.2–32.0)	10.32 ± 5.38 (5.1–46.8)	< 0.001[b]	8.97 ± 5.64 (4.1–36.8)	< 0.001[c]
Freq of HHcy (%), > 15 μmol/L	31 (38.75)	10 (11.63)	< 0.001[a]	6 (7.50)	< 0.001[a]
Serum VB12 (pg/mL), mean ± SD (range)	351.18 ± 94.44 (105–611)	385.55 ± 114.65 (162–772)	0.043[b]	385.51 ± 107.79 (99–651)	0.039[b]
Freq of VB12 deficiency (%), < 170 pg/mL	5 of 73 (9.59)	1 (1.16)	0.095[a]	3 (3.75)	0.48[a]
Serum folate (nmol/L), mean ± SD (range)	6.46 ± 3.36 (2.73–29.86)	7.61 ± 4.27 (1.42–22.54)	0.245[c]	6.74 ± 4.77 (2.27–36.6)	0.838[c]
Freq of folate deficiency (%), < 4 nmol/L	6 of 73 (8.22)	9 (10.47)	0.787[a]	2 (2.50)	0.152[a]

Freq frequency, Hcy homocysteine, HHcy Hyperhomocysteinemia, M/F male/female, MS multiple sclerosis, ND neurological disorder, PP primary progressive, RR relapsing remitting, SD standard deviation, SP secondary progressive, VB12 vitamin B12.

* MS versus neurological control group.

** MS versus healthy control group.

[a] Fisher's exact test.

[b] Analysis of variance.

[c] Mann–Whitney *U* test.

An I^2 value below 25% was considered to indicate homogeneity; values of 25% to just under 50%, to indicate low heterogeneity; values of 50% to just under 75%, moderate heterogeneity, and values of at least 75%, substantial heterogeneity (Higgins et al., 2003). We planned to use a fixed-effect model to meta-analyze pooled data classified as homogeneous or of low heterogeneity, and a random-effect model to meta-analyze data classified as of moderate or substantial heterogeneity according to established guidelines (Zhang and Zhong, 2012). Test for publication bias were conducted using Egger's and/or Begg's tests if there were at least 10 studies were included the meta-analysis, or not conducted if less than 10 studies included because of low power (Zhang and Zhong, 2012). Sensitivity analysis was conducted by removing one single study each time (Zhang and Zhong, 2012).

3. Results

3.1. Case-control study

Table 1 summarizes the key characteristics of the case-control study. Quantitative data were normally distributed except serum Hcy levels of the healthy controls and folate levels in all groups. There is no significant difference in age, sex, and smoking habits between MS patients and neurological/healthy controls. Data of EDSS score were available in 51 of 80 MS cases. Hcy concentrations (mean ± SD) and frequency of HHcy were significantly higher in the Chinese MS cases than both control groups (all $p < 0.001$), lower for VB12 levels (mean ± SD, $p = 0.043$ or 0.039). No significant difference was observed for levels of folate (mean ± SD, both $p > 0.05$), and for frequency of folate or VB12 deficiency (all $p > 0.05$).

Correlation analysis showed that serum Hcy levels in Chinese MS patients were associated with VB12 (Pearson correlation coefficient $r = -0.328$, $p = 0.005$), and folate concentrations (Spearman correlation coefficient $\rho = 0.289$, $p = 0.013$). No correlation was found between Hcy levels and age, age at disease onset, smoking, and EDSS score (all $p > 0.05$).

3.2. Meta-analysis

3.2.1. Literature search and included studies

After screening titles or abstracts of 190 potentially eligible publications, 38 articles were retained for read in full. Through retrieving the full-text version of the above 38 articles, we excluded 15 of them because the studies did not apply a case-control design or included cases and/or controls that had diseases potentially correlated with Hcy, VB12, or folate (Matthews et al., 1965; Simpson, 1964); or the studies did not provide sufficient data for assessing means and/or SDs of Hcy, VB12, or folate levels (Isager, 1970; Reynolds et al., 1991; Rio et al., 1994). Of the 23 remaining publications, 5 were Chinese studies (Chen et al., 2016; Luo et al., 2015; Zhang et al., 2014, 2007; Zou et al., 2012), which were not included in the meta-analysis by Dardiotis et al. (2017). The 2 comparisons between MS patients and neurological/healthy controls of the present study were regarded as 2 independent eligible studies in the meta-analysis. Therefore, 7 case-control studies, including the present study, were included in the final meta-analysis of pure Chinese population (Table 2 and Fig. 1).

Of the above 23 articles, 5 recruited MS patients in relapse (Besler and Comoglu, 2003; Chen et al., 2016; Luo et al., 2015; Russo et al., 2008; Zou et al., 2012), and 6 included cases in remission (Aksungar et al., 2008; Moghaddasi et al., 2013; Najafi et al., 2012; Ramsaransing et al., 2006; Salemi et al., 2010; Triantafyllou et al., 2008). Zoccolella et al. reported Hcy levels in relapsing and remitting patients respectively (Zoccolella et al., 2012), which were treated as data from two independent case-control studies in the subgroup meta-analysis (Table 3). Therefore, stratified by disease phase, the final subgroup meta-analysis included 8 studies in the comparison between MS patients in relapse and controls ($n = 8$, 6, or 6 for Hcy, VB12, or folate, respectively; Table 3 and Fig. 2), and 7 studies comparing cases in remission and controls ($n = 6$, 4, or 3 for Hcy, VB12, or folate, respectively; Table 3 and Fig. 2).

3.2.2. Characteristics of included studies

Table 2 summarizes the key characteristics of the Chinese studies included in the meta-analysis. Each separate study suggested that the Chinese MS patients may have higher Hcy levels (mean ± SD) than controls (Table 2). VB12 deficiency were found in the present study and one publication (Chen et al., 2016). The results also showed that patients tend to have lower folate levels than controls (mean ± SD). MS is more common in females than males (females/males: 230/92).

Table 3 summarizes the key characteristics of the studies included in the subgroup meta-analysis stratified according to the disease phase. All MS patients of the included Chinese studies were in relapse. All included studies suggested, significantly or not significantly, increased VB12 levels (mean ± SD) in the MS cases relative to controls except the one by Najafi et al. (2012).

Table 2
Characteristics of the studies included in the meta-analysis of Hcy, VB12, and folate in Chinese MS patients and controls.

	First author	Year	MS				Controls				Sig
			n =	M/F	Age, y	Lab values	*n* =	M/F	Age, y	Lab values	
Lab value: Hcy, μmol/L	Zhang**	2007	37	12/25	38.19 ± 10.9	23.76 ± 1.659	40	15/25	33.82 ± 19.81	15.5 ± 1.764	yes
	Zou**	2012	45	16/29	37.36 ± 13.75	17.25 ± 5.65	35	12/23	33.47 ± 10.91	10.29 ± 3.41	yes
	Zhang**	2014	41	14/27	36.47 ± 8.6	25.82 ± 1.724	42	16/26	37.82 ± 10.31	13.6 ± 1.517	yes
	Luo**	2015	68	20/48	34.8 ± 19.2	16.75 ± 8.82	100	30/70	39.2 ± 18.6	7.52 ± 5.58	yes
	Chen*	2016	21	9/12	38.7 ± 10.2	16.95 ± 3.08	21	10/11	40.2 ± 6.3	14.76 ± 2.89	yes
	Present study*	–	80	21/59	35.34 ± 9.25	13.39 ± 4.94	86	32/54	37.92 ± 8.59	10.32 ± 6.3	yes
	Present study**	–	80	21/59	35.34 ± 9.25	13.39 ± 4.94	80	52/28	37.24 ± 8.02	8.97 ± 5.64	yes
Lab value: VB12, pg/mL	Zhang**	2007	37	12/25	38.19 ± 10.9	361.9 ± 38.9	40	15/25	33.82 ± 19.81	367.8 ± 23.6	not
	Zou**	2012	45	16/29	37.36 ± 13.75	523.82 ± 200.16	35	12/23	33.47 ± 10.91	527.17 ± 156.42	not
	Zhang**	2014	41	14/27	36.47 ± 8.6	352.6 ± 26.4	42	16/26	37.82 ± 10.31	359.7 ± 24.8	not
	Chen*	2016	21	9/12	38.7 ± 10.2	361.04 ± 73.4	21	10/11	40.2 ± 6.3	454.33 ± 119.99	yes
	Present study*	–	80	21/59	35.34 ± 9.25	13.39 ± 4.94	86	32/54	37.92 ± 8.59	385.55 ± 114.65	yes
	Present study**	–	73	20/53	35.34 ± 9.25	351.18 ± 94.44	80	52/28	37.24 ± 8.02	385.51 ± 107.79	yes
Lab value: Folate, ng/mL	Zhang**	2007	37	12/25	38.19 ± 10.9	6.081 ± 1.406	40	15/25	33.82 ± 19.81	5.982 ± 0.378	not
	Zou**	2012	45	16/29	37.36 ± 13.75	6.93 ± 3.32	35	12/23	33.47 ± 10.91	6.37 ± 2.26	not
	Zhang**	2014	41	14/27	36.47 ± 8.6	6.329 ± 1.224	42	16/26	37.82 ± 10.31	6.425 ± 1.325	not
	Chen*	2016	21	9/12	38.7 ± 10.2	4.76 ± 2.61	21	10/11	40.2 ± 6.3	6.9 ± 2.5	yes
	Present study*	–	80	21/59	35.34 ± 9.25	13.39 ± 4.94	86	32/54	37.92 ± 8.59	7.61 ± 4.27	not
	Present study**	–	73	20/53	35.34 ± 9.25	6.46 ± 3.36	80	28/52	37.24 ± 8.02	6.74 ± 4.77	not

Hcy homocysteine, SD standard deviation, M/F male/female, MS multiple sclerosis, Sig: significance, VB12 vitamin B12.
* MS patients versus neurological controls.
** MS patients versus healthy controls.

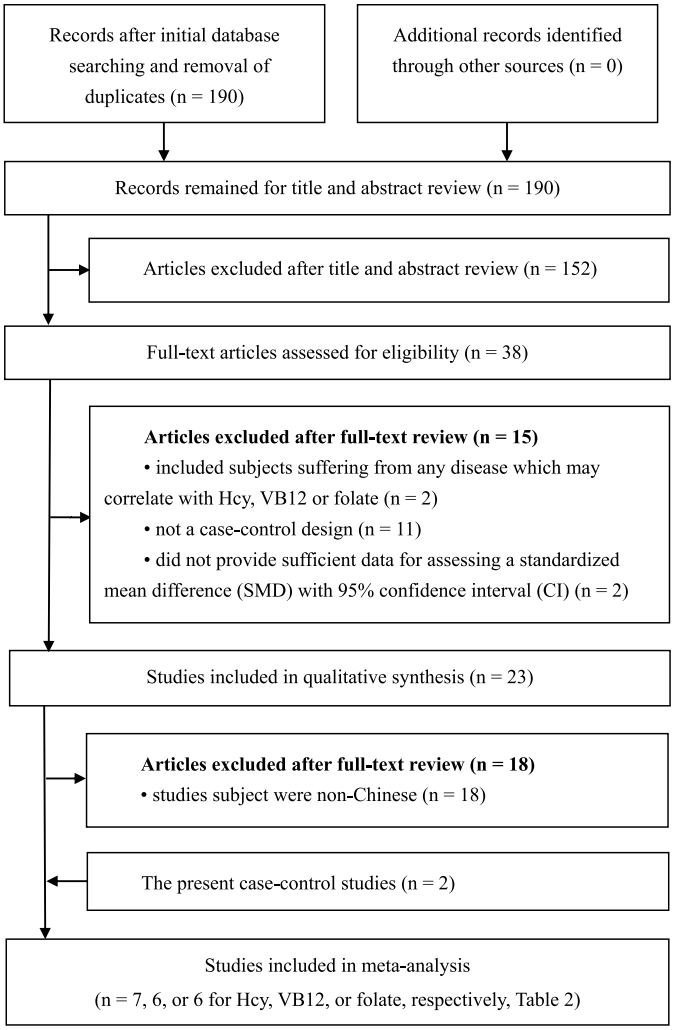

Fig. 1. Flowchart presenting the selection of eligible Chinese studies.
Hcy homocysteine, VB12 vitamin B12.

3.2.3. Meta-analysis results

Table 4 and Fig. 3 summarizes the key results of the meta-analysis. Overall, the meta-analysis results suggested higher levels of Hcy in all comparisons (all $p < 0.001$) and lower levels of VB12 in patients of Chinese origin and cases in relapse (both $p < 0.001$).

Considering that corticosteroids have multiple side effects such as increased hunger, peptic ulcers, etc., which may increase or decrease intake of vitamin B12 or folate and therefore may influence levels of these values, we further meta-analyzed 4 studies (2 previous publications (Besler and Comoglu, 2003; Russo et al., 2008) and the other 2 from our own data), of which MS patients treated with corticosteroids for relapse prior to VB12 test were not included. The results still suggested reduced VB12 in relapsing MS patients relative to controls ($I^2 = 0.0\%$, SMD: −0.30, 95% CI: −0.48 to −0.10, $p = 0.001$, Fig. 3. F).

3.2.4. Assessment of publication bias and sensitivity analysis

Because there were less-than 10 studies in each comparison (Table 2), tests for publication bias were not carried out according the established principles (see Methods section) in the present meta-analysis. The results of sensitivity analysis indicated no significant differences after removing any single study (data not shown).

4. Discussion

We performed the present case-control study and meta-analysis to address the blood levels of Hcy, VB12, and folate in Chinese MS patients. With a combined population of pure Chinese origin, the findings should be particularly useful because of greater statistical power than separate studies they included (Higgins et al., 2003) or than meta-analysis with combined multiracial populations. In the recent meta-analysis with mixed-race samples by Dardiotis et al., it has been suggested that patients with MS tend to have increased Hcy levels compared to controls (SMD: 0.70, 95% CI: 0.06–1.34), while no significant difference in VB12 (SMD: −0.09, 95% CI: −0.29–0.10) or folate (SMD: −0.06, 95% CI: −0.17–0.05) (Dardiotis et al., 2017). With a combined sample of pure Chinese population, our meta-analysis results showed that MS patients tend to have lower VB12 levels in comparison with controls (SMD: −0.30, 95% CI: −0.46–0.14, $p < 0.001$, Table 4 and Fig. 3. D). The differences between the present and the published meta-analysis (Dardiotis et al., 2017) may reflect a potential racial

Table 3
Characteristics of the studies included in the meta-analysis stratified by relapse or remission.

	First author	Year/country	MS				Controls			
			n =	Hcy, μmol/L	VB12	Folate	*n* =	Hcy, μmol/L	VB12	Folate
RP	Besler*	2003/Turkey	24	17.08 ± 2.25	223 ± 32	18.8 ± 4.08	24	12.34 ± 1.98	243 ± 42	20.8 ± 3.81
	Russo*	2008/Italy	94	13.19 ± 5.58	273 ± 112	14.3 ± 12.7	53	9.81 ± 2.53	291 ± 109	13.8 ± 8.2
	Zoccolella	2012/Italy	61	8.6 ± 5.9	NA	NA	219†	8.6 ± 3.98	NA	NA
	Zou**	2012/China	45	15.13 ± 6.79	523.82 ± 200.16	6.62 ± 2.63	35	10.29 ± 3.41	527.17 ± 156.42	6.37 ± 2.26
	Luo	2015/China	68	16.75 ± 8.82	NA	NA	100	7.52 ± 5.58	NA	NA
	Chen**	2016/China	21	16.95 ± 3.08	361.04 ± 73.4	4.76 ± 2.61	21†	14.76 ± 2.89	454.33 ± 119.99	6.9 ± 2.5
	Present study**	-/China	80	13.39 ± 4.94	351.18 ± 94.44	6.46 ± 3.36	86†	10.32 ± 5.38	385.55 ± 114.65	7.61 ± 4.27
	Present study**	-/China	80	13.39 ± 4.94	351.18 ± 94.44	6.46 ± 3.36	80	8.97 ± 5.64	385.51 ± 107.79	6.74 ± 4.77
RM	Ramsaransing	2006/Netherlands	88	13.8 ± 4.9	NA	NA	57	10.1 ± 4.9	NA	NA
	Aksungar**	2008/Turkey	42	15.56 ± 3.74	317.26 ± 78.23	5.15 ± 1.92	31	10.53 ± 2.85	300.68 ± 66.24	5.44 ± 1.67
	Triantafyllou	2008/Greece	65	13.5 ± 4.7	NA	NA	60	8.5 ± 3.1	NA	NA
	Salemi**	2010/Italy	40	9.3 ± 20.63	666.42 ± 354.16	7.3 ± 5.55	80	6.3 ± 1.97	493.7 ± 229.16	5.9 ± 2.47
	Zoccolella	2012/Italy	156	9.2 ± 5.42	NA	NA	219†	8.6 ± 3.98	NA	NA
	Najafi**	2012/Iran	60	NA	108.9 ± 45.3	NA	38	NA	98.9 ± 44.3	NA
	Moghaddasi**	2013/Iran	75	22.73 ± 11.63	342.64 ± 210.66	9.74 ± 4.77	75	11.04 ± 4.71	426.48 ± 284.65	11.64 ± 5.76

MS multiple sclerosis, NA not available, RM remission, RP Relapse, VB12 vitamin B12.
* Unit for VB12 or folate: pmol/L or nmol/L.
** Unit for VB12 or folate: pg/mL or ng/mL.
† Neurological controls.

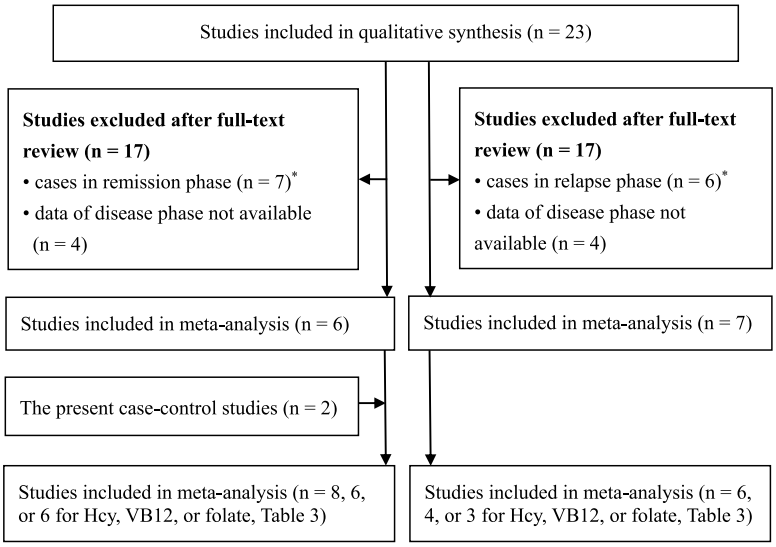

Fig. 2. Flowchart presenting the selection of eligible studies according to relapse or remission.
Hcy homocysteine, VB12 vitamin B12.
*Hcy levels were reported in the patients in relapse and those in remission respectively in one study and were treated as data from two independent case-control studies.

Table 4
Results of meta-analysis.

	Lab values	Sample size		No. of studies	Heterogeneity		Model	Effect size		
		Cases	Controls		*p*	I^2 (%)		SMD	95% CIs	*p*
MS_Chinese vs. Controls	Hcy	372	404	7	< 0.001	96.8	R	2.31	1.33, 3.28	< 0.001
	VB12	304	304	6	0.333	12.8	F	−0.30	−0.46, 0.14	< 0.001
	Folate	304	304	6	0.104	45.3	F	−0.12	−0.29, 0.04	0.135
MS_relapse vs. controls	Hcy	629	618	8	< 0.001	92.2	R	0.94	0.49, 1.39	< 0.001
	VB12	344	299	6	0.240	25.9	F	−0.31	−0.47, 0.15	< 0.001
	Folate	344	299	6	0.056	53.6	R	−0.14	−0.42, 0.06	0.290
MS_remission vs. controls	Hcy	487	442	6	< 0.001	92.4	R	0.85	0.35, 1.34	< 0.001
	VB12	177	144	4	0.002	79.4	R	0.17	−0.26, 0.61	0.428
	Folate	354	276	3	0.016	76.0	R	−0.05	−0.51, 0.40	0.819

CI confidence interval, F fixed model, Hcy homocysteine, MS multiple sclerosis, R random model, SMD standardized mean difference, VB12 vitamin B12.

discrepancy or environmental factors underlying MS.

According to stratification of disease phase, the subgroup meta-analysis results suggested increased Hcy levels in relapsing or remitting MS patients relative to controls but no difference for folate (Table 4, Fig. 3. B and C). The results also showed decreased VB12 levels in relapsing MS patients compared to controls (I^2 = 25.9%, SMD: −0.31,

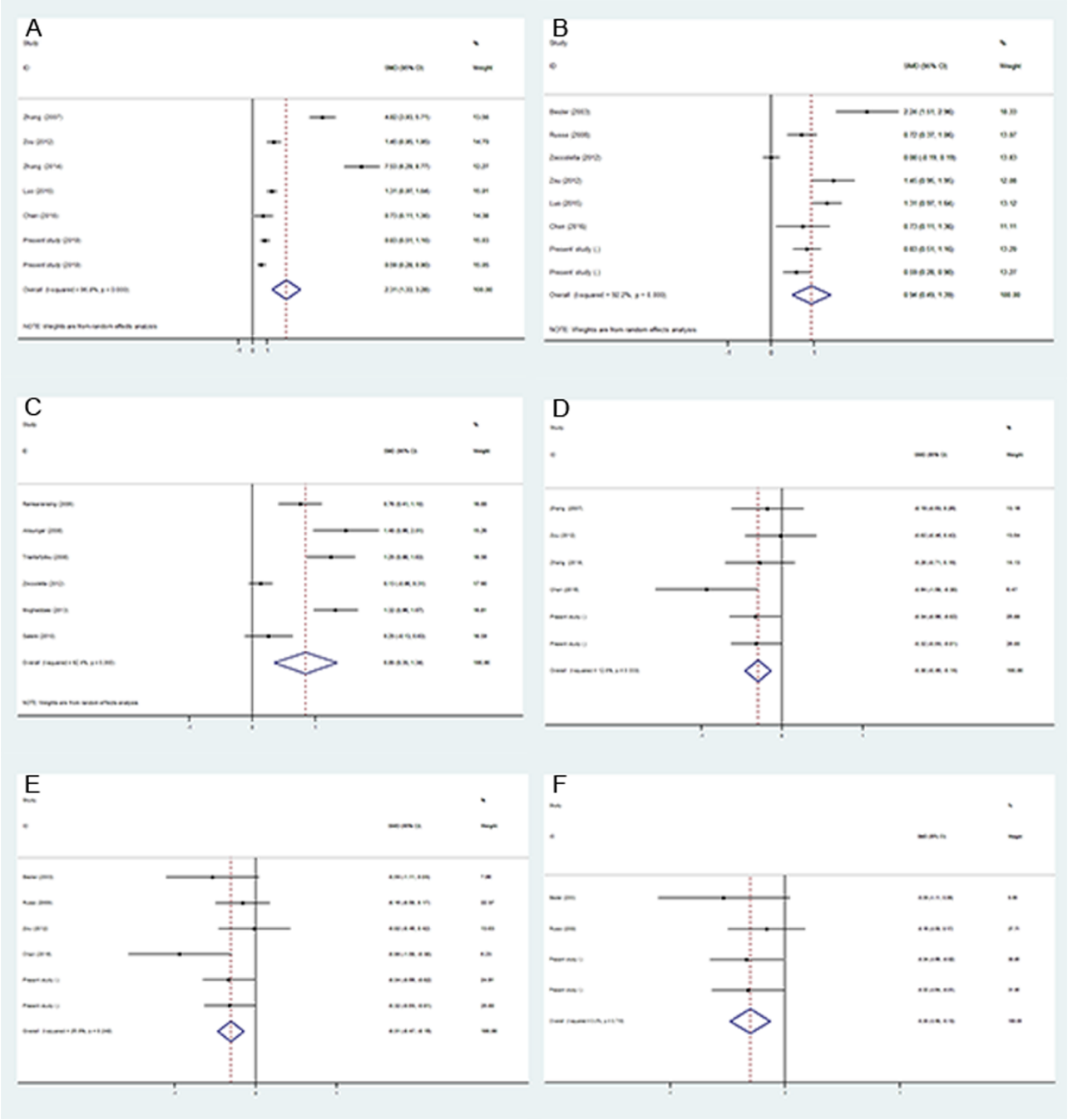

Fig. 3. Forest plots of the meta-analysis.
A. Forest plot: SMD of Hcy levels between Chinese MS patients and controls. B. Forest plot: SMD of Hcy levels between relapsing MS patients and controls. C. Forest plot: SMD of Hcy levels between remitting MS patients and controls. D. Forest plot: SMD of vitamin B12 levels between Chinese MS patients and controls. E. Forest plot: SMD of vitamin B12 levels between relapsing MS patients and controls. F. Forest plot: SMD of vitamin B12 levels between relapsing MS patients and controls, subjects of both group not receiving steroid therapy.

95% CI: −0.47–0.15, $p < 0.001$), while no difference for cases in remission ($I^2 = 79.4\%$, SMD: 0.17, 95% CI: −0.26–0.61, $p = 0.428$) (Table 4 and Fig. 3. E). The reduction trend of VB 12 was still observed, with a higher homogeneity ($I^2 = 0.0\%$), in the further meta-analysis including studies of which MS patients treated with corticosteroids for relapse were not included (SMD: −0.30, 95% CI: −0.48 to −0.10, $p = 0.001$, Fig. 3. F), which may reflect the potential contribution of the lab value to MS relapse. To our knowledge, this is the first stratified meta-analysis by relapse or remission phase of MS focusing on the associations between Hcy, VB12, and folate levels and the disease.

The present case-control study results suggest higher frequency of hyperhomocysteinemia in the Chinese MS cases than controls, consistent with some (Ramsaransing et al., 2006; Sahin et al., 2007; Triantafyllou et al., 2008; Vrethem et al., 2003), but inconsistent with other, previous studies (Kararizou et al., 2013; Rio et al., 1994; Teunissen et al., 2008). Our case-control study found no significant difference for frequency of VB12 deficiency in Chinese patients with MS in comparison with control groups, which is in line with previous studies from England (Basil et al., 1965) and Sweden (Vrethem et al., 2003), while discordant with other studies from England (Reynolds et al., 1992) and Turkey (Kocer et al., 2009). These differences may reflect different sample size and/or the regional or

environmental factors of the disease.

While our meta-analysis offers the comprehensive evaluation of the levels of Hcy, VB12, and folate in MS patients, the results should be interpreted with caution in view of several limitations. First, obvious heterogeneity among studies in the comparison models for Hcy (Table 4) may influence the validity of the conclusion, though we applied a random-effect for the meta-analysis in these comparison models and our sensitivity analysis showed that the pooled SMDs and 95% CIs were not significantly influenced by any separate study. Second, the publication bias risk always exists, though we searched a range of international and Chinese databases without language constraints. Finally, because of insufficiency of original information for each included subject, data were not adjusted by risk factors of gender, mean age at disease onset, disease duration and disease severity.

Future studies with larger populations are needed to verify the findings in the present study. Studies should also assess the differences between MS patients in relapse and those in remission.

5. Conclusion

Patients with MS tend to have increased blood Hcy levels compared to controls. MS patients of Chinese origin and those in relapse may have decreased levels of VB12. Hcy and VB12 may contribute to pathogenesis of the disease, and VB12 may correlate with MS relapse

Declaration of Competing Interest

The authors declare no conflicts of interest.

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