Distribution characteristics of circulating homocysteine and folate and related factors in agriculture, stock-raising and urban populations: a cross-sectional survey

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Abstract

Objective: To investigate homocysteine (Hcy) and folate levels, prevalence of hyperhomocysteinaemia (HHcy) and folate deficiency, which are affected by lifestyles in urban, agricultural and stock-raising populations.

Design: This is a cross-sectional study.

Setting: Urban, agricultural and stock-raising regions in Emin, China.

Participants: Totally 1926 subjects – 885 (45·9 %) from urban, 861 (44·7 %) from agricultural and 180 (9·4 %) from stock-raising regions – were obtained using multistage stratified random sampling. Inclusion criteria encompassed inhabitants aged ≥15 years who resided at the current address for ≥6 months and agreed to participate in the study. Surveys on health behaviour questionnaires and physical examinations were conducted and blood samples collected.

Results: The folate level of subjects from the stock-raising region was the lowest, followed by those from the agricultural region, and the highest in those from the urban region (3·48 *v.* 6·50 *v.* 7·12 ng/ml, $P < 0.001$), whereas mean Hcy showed no significant difference across regions. The OR for HHcy in stock-raising regions was 1·90 (95 % CI 1·11, 3·27) compared with the urban region after adjusting for all possible covariates. The OR for folate deficiency in stock-raising and agriculture regions was 11·51 (95 % CI 7·09, 18·67) and 1·91 (95 % CI 1·30, 2·82), respectively, compared with the urban region after adjusting for all possible covariates.

Conclusions: HHcy and folate deficiency are highly prevalent in stock-raisers, which is of important reference for HHcy control in Xinjiang, with a possibility of extension to others with approximate lifestyles.

CVD has a high prevalence and huge burden worldwide, and has become one of the leading causes of human death, and 80 % of CVD takes place in the developing world[1]. Numerous factors are related to CVD, of which circulating homocysteine (Hcy) and folate levels are the focus of much attention in recent years. Related research shows that folate deficiency and elevated Hcy levels are associated with human health, CVD and mortality[2–4]. Hcy can also be used to predict diabetes, dementia and pregnancy disorders[5–7]. Folate deficiency is also associated with chronic diseases such as CVD, cancer and cognitive dysfunction; maternal folate nutritional status is related to the risk of neural tube defects in the offspring[8]. Folate is also one of the main determinants of circulating Hcy[9]. Folate supplementation may prevent CVD and some cancers and lower circulating Hcy[10].

For this purpose, some developed countries have carried out several large-scale population-based surveys to evaluate circulating Hcy and folate status and taken evidence-based actions such as folate fortification[11]. They have established policies for folate fortification of cereals and flour to decrease the prevalence of hyperhomocysteinaemia (HHcy), and observed some plausible changes including in CVD prevention[12–14], which is of valuable reference for developing countries. Folate is a vitamin that cannot

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be synthesised by human body and only obtained from microbes that break down food during digestion[15]. Therefore, folate and Hcy levels vary widely due to high geographical, lifestyle, dietary and racial/ethnic diversity[16–18]. Accurate estimates of the prevalence of a condition and relevant determinants are essential as a source of primary information and for rational planning of health services, and would allow public health policymakers to assign sufficient priority and resources to its management and prevention[19], which is also the same for folate deficiency and HHcy, especially in resource-constricted areas and developing countries. Nonetheless, there is a paucity of equivalent data from developing countries.

Based on previous studies, the prevalence of HHcy in Chinese population is 27·5 %[20], and the prevalence and the risk factors may also vary due to geographical, ethnic, social and dietary diversity[20]. Xinjiang, a multi-ethnic province with a population of various lifestyles and dietary habits living in urban, agricultural and stock-raising regions[21], is located in northwest China and Central Asia and provides an ideal setting for this type of study, where folate fortification is not mandatory as well. Local nomads have been residing in stock-raising areas like villages, forests and mountains; move around depending on seasonal changes; and spend most of their time in remote areas, and thus their living is characterised by much dependence on animal products, including animal oil, meat, milk and dairy products, and by limited access to fresh fruit and vegetables[21,22]. Only an existing study conducted in rural Xinjiang showed a high prevalence of HHcy, but failed to analyse the effects of different living environments and folate concentrations on Hcy[23].

In countries where folate fortification is not mandatory and where resource is constricted, it is of significant value to identify the target population that would most benefit from folate supplementation. Therefore, the main purpose of the current study was to comprehend folate and Hcy levels and their potential determinants in agricultural, stock-raising and urban regions using a population-based, cross-sectional survey, and to provide clues to other regions with approximate conditions.

Subjects and methods

Site

Emin county, northern Xinjiang, is home to >133 000 people aged ≥15 years from urban, agricultural and stock-raising regions, which makes it an ideal setting for a study of the effects of different lifestyles on Hcy and folate metabolism. The survey was conducted from January to December 2014.

Study population

Multistage stratified random sampling was used to obtain a sample of the population aged ≥15 years. At the first stage, the whole county was divided into three regions as urban, agricultural and stock-raising regions, based on the urban and rural area code issued by the National Bureau of Statistics of the People's Republic of China, as well as on the regional production mode and the main source of economic income. At the second stage, two townships were randomly selected in each region using simple random sampling. At the third stage, two villages were randomly selected as survey villages in each of the extracted townships. In the final stage of sampling, a given number of participants from each site were selected from communities or villages using lists compiled from local government registers of households. Inclusion criteria encompassed: (i) local inhabitants aged ≥15 years; (ii) residing at current address for ≥6 months; (iii) agreeing to participate and sign an informed consent form. Exclusion criteria included renal insufficiency; pernicious anaemia; malignant tumours; pregnancy; and taking anticonvulsants, anti-tuberculosis drugs, birth control pills, dopamine drugs, folate and/or vitamin B_{12} supplementation in the past month.

Data collection

Questionnaire, physical examination and biochemical examination

Population health behaviour questionnaires and physical examinations were conducted using onsite surveys to collect detailed information from all participants via a face-to-face interview by trained investigators, which included demographic characteristics (name, gender, age, ethnicity and current address), socioeconomic status (educational status), lifestyle risk factors (cigarette consumption and alcohol intake) and individual and family medical history. Physical examination included measurements of height, body weight, waist circumference (WC) and blood pressure (BP). Laboratory examination included measurement of plasma Hcy and folate.

Measurement of blood pressure, height, weight and waist circumference

BP was presented as the mean of three measurements using an Omron HEM-1000 electronic sphygmomanometer via a standardised procedure[24]. All participants were advised to avoid cigarette smoking, alcohol, caffeinated beverages, tea and exercise for at least 30 min prior to measurements. Three BP measurements were taken, after a rest of at least 5 min, from the unclothed right arm of the person in a sitting position at an interval of at least 1 min. Body weight, height and WC were measured using standard methods[25]. Height and weight were measured to the nearest 0·1 cm and 0·1 kg, respectively, with the participants in lightweight clothing and without shoes. WC was measured at the midpoint between the lower rib and upper margin of the iliac crest to the nearest 0·1 cm at the end of a normal expiration. BMI was calculated by dividing weight by height-squared (kg/m^2).

Laboratory measurements

Venous blood samples were obtained by the same trained nurses in the morning after overnight fasting. Plasma was separated within 30 min of collection by centrifugation (4°C, 20 min, at 1509·3g); stored at onsite refrigerators temporarily; transported in a cooler to the clinical laboratory at the People's Hospital of Xinjiang Uygur Autonomous Region; and stored at –80°C until assayed. Plasma Hcy and folate were measured by the same staff blinded to the aim and design of the study. Plasma Hcy was measured by an enzymatic cycling assay method using a DIRUI equipment of the type CS-T300, with a plasma Hcy precision of 0·1 μmol/l. The kit purchased from Shenzhen Tailed Medical Co. (batch number 20190104) was used. Folate was measured by chemiluminescence method with an UniCel DxI 800 automatic biochemical analyser (Beckman Coulter), with a plasma folate precision of 0·01 ng/ml and with the detectable concentration ranging from 1·0 to 24·2 ng/ml. The folate assay kit was purchased from Beckman Coulter, Inc. (batch number 832212). All measurements were performed in strict accordance with the instrument and reagent instructions.

Diagnostic criteria

HHcy is defined as circulating Hcy ≥10 μmol/l[26]. Folate deficiency is defined as a concentration <3 ng/ml[12]. Hypertension is defined as systolic BP ≥140 mmHg, and/or diastolic BP ≥90 mmHg, and/or use of antihypertensive medication within previous 2 weeks. Overweight and obesity is defined as BMI ≥25 kg/m² according to WHO classifications[27]. Abdominal obesity is defined as WC ≥90 cm for men and ≥85 cm for women. Cigarette consumption is defined as cumulative smoking in the past reaching more than twenty packs of cigarettes and is currently smoking. Alcohol intake is defined as at least once a week within the previous 30 d of the survey.

Statistical analysis

Continuous variables, if normally distributed, were presented as means ± SD and analysed using t test or ANOVA, followed by *post hoc* analysis (least significant difference test). Categorical variables were expressed as proportion (%) and frequency (n) and analysed using the χ^2 test. Hcy and folate levels were compared by covariance analysis among and between groups after adjusting for gender, age, BMI, alcohol intake, cigarette consumption, hypertension, education and ethnicity. Partial correlation analysis was performed between Hcy and folic acid. Univariate logistic regression analysis was performed to select the potential factors that have been speculated to influence Hcy and folate concentrations with $P < 0·1$ to enter the equation (data not reported). Multivariable logistic regression was performed for Hcy after adjusting for significant variables in univariate regression analysis such as gender, age, BMI, alcohol intake, cigarette consumption, hypertension, folate tertile and regions; and for folate deficiency by adjusting for gender, age, ethnicity, education and regions. OR and 95 % CI were calculated. Tertile of folate was used in the multivariable logistic regression analysis. A P value <0·05 was considered statistically significant. All statistical analyses were performed with SPSS, version 23.0.

Results

Characteristics of the study populations

A total of 1946 participants volunteered to complete the study, of whom 1934 provided blood samples. An additional eight subjects were excluded from the analysis because of insufficient blood sample. Finally, data of 1926 participants with 885 (45·9 %) from urban, 861 (44·7 %) from agricultural and 180 (9·4 %) from stock-raising regions were evaluated. The proportion of subjects enrolled is approximate to that of the local population[28].

As in Table 1, study populations from urban, agricultural and stock-raising regions were similar in terms of age and gender. The prevalence of hypertension, systolic BP and diastolic BP levels, education attainment, cigarette consumption, alcohol intake, overweight and obesity proportion, and ethnicity were significantly different among the three regions (P for all <0·05). In the stock-raising region, Kazakh subjects accounted for 79·1 %, and the prevalence of hypertension was higher compared with agricultural and urban regions (38·3 v. 24·8 v. 18·5 %, $P < 0·001$). In the urban region, the majority was Han ethnicity, accounting for 63 %, and the proportion of cigarette consumers, alcohol intake and overweight and obese subjects were significantly higher than in rural and pastoral regions (P for all <0·05). In the agricultural region, the majority was also Han ethnicity, accounting for 56·2 %, and the prevalence of hypertension was lower than in urban and stock-raising regions (18·5 v. 24·8 v. 38·3 %, $P < 0·001$).

Level of homocysteine and folate

As shown in Table 2, after adjusting for gender, age, BMI, alcohol intake, cigarette consumption, hypertension, education and ethnicity, the folate level of subjects from the stock-raising region was the lowest, followed by those from the agricultural region, and the highest in those from the urban region with statistical significance (3·48 v. 6·50 v. 7·12 ng/ml, $P < 0·001$). Nonetheless, the mean concentration of plasma Hcy showed no significant differences among subjects from different regions.

Prevalence of hyperhomocysteinaemia and folate deficiency

As given in online Supplemental Table S1, the prevalence of HHcy in the stock-raising region was significantly higher than in agricultural and urban regions (82·2 v. 69·7

Table 1 Characteristics of the study population*

Characteristics	Urban n	Urban %	Agriculture n	Agriculture %	Stock-raising n	Stock-raising %	P	P1	P2	P3
	885	45·9	861	44·7	180	9·4				
Age (years)										
Mean		51·7		50·7		50·6	0·316	0·159	0·933	0·363
SD		14·1		14·4		14·2				
≤44	285	32·2	276	32·1	61	33·9	0·884	0·635	0·827	0·897
45–59	323	36·5	331	38·4	65	36·1				
≥60	277	31·3	254	29·5	54	30·3				
Gender										
Men	382	43·2	388	45·1	72	40·0	0·420	0·424	0·213	0·434
Ethnicity										
Han	469	63·0	455	56·2	26	15·1	<0·001	<0·001	<0·001	<0·001
Kazakh	171	23·0	262	32·3	136	79·1				
Mongolian	30	4·0	32	4·0	8	4·7				
Others	75	10·1	61	7·5	2	1·2				
Education										
Primary and lower	560	86·7	399	52·2	50	29·1	<0·001	<0·001	<0·001	<0·001
Junior	76	11·8	310	40·5	118	68·6				
Senior and higher	10	1·5	56	7·3	4	2·3				
Cigarette consumption	180	28·3	169	23·2	34	21·1	0·046	0·032	0·572	0·068
Cigarette consumption in men	173	60·7	160	48·5	34	54·0	0·010	0·002	0·425	0·325
Alcohol intake	175	27·5	157	21·5	36	22·4	0·033	0·011	0·818	0·189
Alcohol intake in men	174	61·1	151	45·8	35	55·6	0·001	<0·001	0·153	0·420
BMI (kg/m^2)										
Mean		25·7		25·2		25·5	0·011	0·003	0·232	0·571
SD		3·6		3·5		4·2				
BMI ≥25 kg/m^2	462	54·9	414	49·1	90	51·1	0·049	0·015	0·615	0·349
Abdominal obesity	499	59·7	452	53·6	98	54·7	0·035	0·011	0·783	0·216
Women's WC ≥85 cm	268	56·4	246	53·6	60	56·1	0·652	0·366	0·643	0·930
Men's WC ≥ 90 cm	231	64·0	206	53·6	38	52·8	0·011	0·004	0·892	0·073
Prevalence of hypertension	219	24·8	159	18·5	69	38·3	<0·001	0·001	<0·001	<0·001
Systolic blood pressure (mmHg)										
Mean		128·7		125·0		129·5	<0·001	<0·001	0·002	0·572
SD		17·8		17·5		17·4				
Diastolic blood pressure (mmHg)										
Mean		79·1		78·0		81·4	<0·001	0·038	<0·001	0·010
SD		11·2		10·7		9·8				

WC, waist circumference.

*We conducted among-group and between-group comparisons using ANOVA followed by *post hoc* analysis (least significant difference test) and χ^2 test and provided specific P-values as P for among-group comparison and as P1 (urban *v.* agriculture), P2 (agriculture *v.* stock-raising) and P3 (stock-raising *v.* urban) for between-group comparisons.

Table 2 Homocysteine and folate levels in different populations*

	Urban Mean	Urban SD	Agriculture Mean	Agriculture SD	Stock-raising Mean	Stock-raising SD	F/P	P1	P2	P3
Hcy (μmol/l)	13·48	8·92	13·48	7·92	14·37	7·91	1·001/0·368	0·994	0·170	0·193
Folate (ng/ml)	7·12	4·46	6·50	3·81	3·48	4·02	54·058/<0·001	0·003	<0·001	<0·001

Hcy, homocysteine.

*Adjusted for gender, age, BMI, alcohol intake, cigarette consumption, hypertension, education and ethnicity. F is the statistic value of covariance analysis; P represents the P value for among-group comparison; P1, urban *v.* agriculture; P2, agriculture *v.* stock-raising; P3, stock-raising *v.* urban.

and 68·4 %, P < 0·001). The prevalence of HHcy in female, Kazakh, non-drinking, non-smoking, overweight and obese subjects and those with lower education attainment from the stock-raising region was significantly higher than those of other regions (P for all <0·05).

The prevalence of folate deficiency in subjects from the stock-raising region was significantly higher than in those from the agricultural and urban regions (54·8 *v.* 8·3 and 2·6 %, P < 0·001), which was also the same in the subgroup analysis (P for all <0·001) as shown in online Supplemental Table S2.

Relationship between homocysteine and folate

The partial correlation analysis showed a significant inverse relationship between Hcy and folate concentration ($r = -0·200$, $P < 0·001$) after controlling for age, gender, alcohol and cigarette consumption, hypertension and region as shown in Fig. 1.
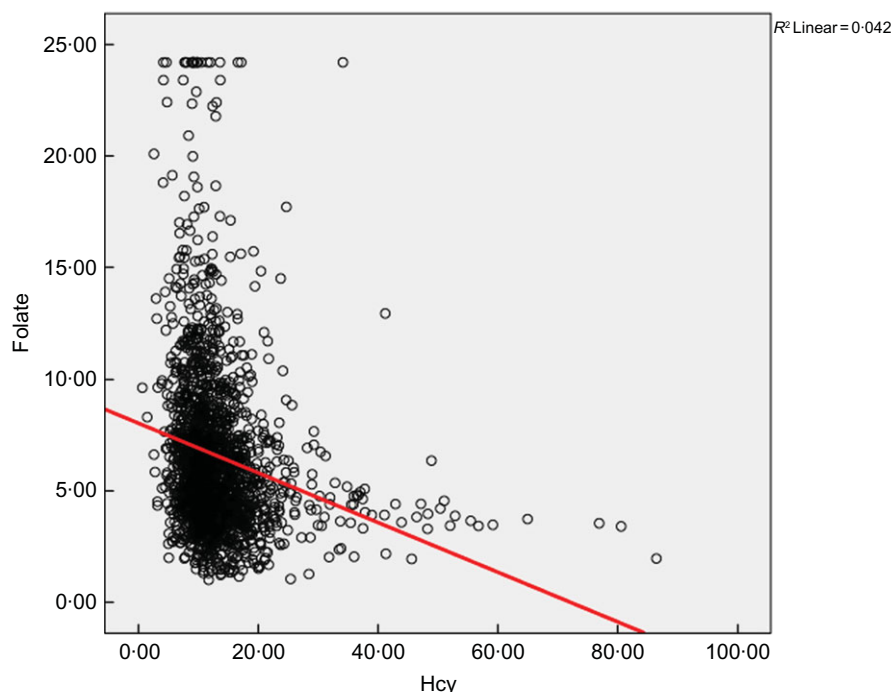
Fig. 1 (colour online) Relationship between circulating homocysteine and folate in the study population, adjusted for age, gender, cigarette consumption, alcohol intake and BMI

Factors associated with hyperhomocysteinaemia and folate deficiency

Univariable and multivariate logistic regression analyses of risk factors for HHcy and folate deficiency are given in Table 3. A multivariate logistic regression analysis of risk factors for HHcy was conducted after adjusting for gender, age, BMI, alcohol and cigarette consumption, hypertension, folate tertile and regions. The OR for HHcy in the stock-raising region was 1·90 (95 % CI 1·11, 3·27) compared with the urban region after adjusting for all possible covariates. A multivariate logistic regression analysis of risk factors for folate deficiency was conducted after adjusting for gender, age, region, ethnicity and education. The OR for folate deficiency in the stock-raising and agriculture regions were 11·51 (95 % CI 7·09, 18·67) and 1·91 (95 % CI 1·30, 2·82),

respectively, compared with the urban region after adjusting for all possible covariates.

Discussion

CVD is the leading cause of human death[1], for which folate deficiency and elevated Hcy are independent risk factors[2,3,8]. Hcy is also a sensitive marker of folate deficiency[2,29]. Folate and Hcy levels vary widely due to geographical, racial/ethnic, lifestyle and dietary diversity. Xinjiang in northwest China provides an ideal setting for a study on the effects of diverse lifestyles and dietary habits across populations from urban, agricultural and stock-raising regions. The main observations of the current study

Table 3 Univariable and multivariate logistic regression analyses of risk factors for HHcy and folate deficiency*

	Univariable analysis			Multivariable analysis		
	OR	95 % CI	P	OR	95 % CI	P
HHcy deficiency						
Urban	1			1		
Agriculture	1·064	0·869, 1·303	0·550	0·949	0·731, 1·231	0·691
Stock-raising	2·140	1·424, 3·217	<0·001	1·902	1·106, 3·271	0·020
Folate deficiency						
Urban	1			1		
Agriculture	2·684	1·928, 3·735	<0·001	1·909	1·295, 2·815)	0·001
Stock-raising	23·168	15·456, 34·727	<0·001	11·511	7·091, 18·686)	<0·001

*Hyperhomocysteinaemia (HHcy) deficiency was adjusted for gender, age, BMI, alcohol intake, cigarette consumption, hypertension, folate tertile and region. Folate deficiency was adjusted for gender, age, region, ethnicity and education.

encompassed[1]: levels of circulating folate were the lowest in the stock-raising region[2]; the prevalence of HHcy and folate deficiency was higher in the stock-raising region[3]; stock-raising and agricultural regions were the major related factors for elevated Hcy and folate deficiency.

The current study extended previous findings to stock-raising regions[23]. That is, the stock-raising region showed a significantly higher HHcy prevalence and higher OR for the presence of HHcy and folate deficiency. Indeed, it may be indicative of the dietary habits of the population living in the stock-raising region. As evidenced previously, stock-raisers reside in villages, forests and mountains and move around based on seasonal changes, which makes them dependent on animal products with limited access to enough vegetables and fruits[21]. Therefore, populations from stock-raising regions may benefit most from Hcy lowering possibly by folate supplementation.

The current study also provides a direct evidence on the coexistence of HHcy and folate deficiency in populations with different lifestyles, especially in stock-raisers, from developing countries. Folate fortification is not mandatory in China, making the results possibly representative of this type of study. Consistent with previous studies[29], coexistence of elevated Hcy and folate deficiency and a significant inverse relationship between the two in a population from the same background may further confirm the importance of folate in terms of lowering elevated Hcy. Hcy is an intermediate product in the metabolism of methionine and cysteine. The circulation of methionine requires the participation of folate, a vitamin that human body cannot synthesise. Methylenetetrahydrofolate reductase catalyses the irreversible conversion of 5, 10-methylene tetrahydrofolate to 5-methyl-tetrahydrofolate, committing one-carbon units to the methionine cycle. When folate and/or B vitamins are deficient, methionine cycle is blocked, generating the accumulation of plasma Hcy in the blood. HHcy further induces oxidative stress, endothelium dysfunction, inflammation, smooth muscle cell proliferation and endoplasmic reticulum stress, which are the key pathogenesis of CVD[30]. At present, most scholars are optimistic for the trend of folate intervention on the risk of HHcy and CVD reduction[31]. With folate fortification implemented in the United States and Canada since 1998, circulating folate concentrations in the population increased from 4·6 to 10·0 ng/ml, and Hcy decreased from 10·1 to 9·4 μmol/l[32]. Folate supplementation reduces the risk of stroke by about 10 %, and the risk of CVD by about 4 %, which is more effective in populations with lower baseline folate and at a high risk[33,34]. Consistent with previous studies[35–37], the presence of HHcy is also associated with male gender and older age. In contrast, current alcohol intake is not associated with elevated Hcy in our data, which may be due to some bias in population selection. Therefore, folate fortification, cigarette cessation and alcohol abstinence may be still the target lifestyle modification measures to lower elevated Hcy[20,23,36].

Moreover, this observation could be extended to settings of populations with approximate lifestyles and dietary habits. Xinjiang, located in northwest China, is close to Central Asian and Eastern European countries such as Kazakhstan, Kyrgyzstan, Mongolia and Russia. Most of the population there still share similar lifestyles (stock-raising) and dietary habits (more animal products and limited fruit and vegetables)[38,39], where the burden of CVD seems to be huge. For instance, CVD is estimated to account for more than a half (53 %) of all deaths in Kazakhstan. Age-standardised CVD mortality was 636 in Kazakhstan and 531 in the Russia Federation per 100 000 population in 2010, almost 5–6 times higher than in the United Kingdom (112 per 100 000)[40].

The current study has following innovations: First, it extended previous findings to stock-raising regions. The prevalence of HHcy and folate deficiency was higher in stock-raisers and so they are likely the target population for folate supplementation, which may be of valuable reference to other stock-raising regions. Second, the current data included both Hcy and folate analyses, which may provide a direct evidence for disease prevention. Nevertheless, some limitations to the current study need to be acknowledged. This is a cross-sectional study that did not show a causal relationship between Hcy and folate, whereas it has been well established. Thus, our data provide a direction for the prevention and control of public health-related diseases. In addition, we failed to assess the contribution of vitamin B12 on Hcy, which may have brought some bias to our results and explanations.

In conclusion, the prevalence of HHcy and folate deficiency is unacceptably high in underdeveloped regions, particularly among stock-raisers and thus a possible target for folate supplementation. The results may be of important reference for the prevention and control of HHcy in Xinjiang, with a possibility of extension to populations that share lifestyles and dietary habits.

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and manuscript writing. All authors have read and approved the final manuscript. *Ethics of human subject participation:* The current study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving study participants were approved by the ethics committee at People's Hospital of Xinjiang Uygur Autonomous Region China. Written informed consent was obtained from all participants.

Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980019004841

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