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Prevalence and risk factors for hyperhomocysteinemia: a population-based cross-sectional study from Hunan, China

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ABSTRACT

Objectives Hyperhomocysteinemia is an independent risk factor for cardiovascular diseases. We aimed to investigate the prevalence and risk factors for hyperhomocysteinemia, especially modifiable lifestyle factors, such as smoking behaviour and dietary factors.

Design Population-based cross-sectional study.

Setting Hunan Province, China

Participants A total of 4012 participants completed the study, between July 2013 and March 2014. The median age is 55 (interquartile range: 45–63) years, with 1644 males (41%) and 2368 females (59%).

Main outcome measures Homocysteine level were measured by the microplate enzyme immunoassay method. Hyperthomocysteinemia was defined as ≥15 µmol/L. Questionnaire was used to investigate potential risk factors of hyperhomocysteinemia. Crude odd ratio (OR) or adjusted OR with 95% CI were determined by using univariable or multivariable logistic regression models.

Results The prevalence of hyperhomocysteinemia is 35.4% (45.4% vs 28.5% for men, women, respectively). One-year increase in age is significantly associated with 2% higher risk of hyperhomocysteinemia (OR=1.02, 95% CI: 1.01 to 1.03). One unit increase of BMI is associated with 5% higher risk of hyperhomocysteinemia (OR=1.05, 95% CI: 1.03 to 1.07). Compared with the non-smoker, smoking participants have a 24% higher risk of hyperhomocysteinemia (OR=1.24, 95% CI: 1.006 to 1.53), while the risk for those quitting smoking are not significantly different (OR=1.14, 95% CI: 0.85 to 1.54). compared with those consuming fruit and vegetable at least once every day, those consuming less than once every day had a significantly higher risk of hyperhomocysteinemia (OR=1.29, 95% CI:1.11 to 1.50). In addition, we found there were significant sex interaction with education level or alcohol drinking on the risk of hyperhomocysteinemia ($p_{interaction}$ <0.05).

Conclusions Higher BMI and older age are potential risk factors for hyperhomocysteinemia. Current smoking but not quitting smoking is associated with higher risk of hyperhomocysteinemia. Fruit and vegetable consumption may have protective effect against hyperhomocysteinemia. Alcohol consumption or education level might interact to influence the risk of hyperhomocysteinemia.

Strengths and limitations of this study

- Our study is a population-based cross-sectional study with a multistage cluster random sampling method, including a total of 4012 participants, most of previous related studies in China are hospital-based.
- The present study focused on modifiable lifestyle behaviours, the results have significant implications for public health policies or health education measures.
- The cross-sectional design limits us to draw causal relationships between the risk factors and hyperhomocysteinemia.

INTRODUCTION

About five decades ago, McCully first proposed the 'homocysteine theory of atherosclerosis'. It is based on the realisation that even a moderate increase in homocysteine can accelerate the development of atherosclerosis.[1] Since then, there has been ample evidence suggests that high circulating homocysteine (hyperhomocysteinemia) is associated with an increased risk of cardiometabolic risk factors and cardiovascular diseases, such as high blood pressure (BP), stroke, ischaemic heart disease and deep vein thrombosis.[2–4] In addition, hyperhomocysteinemia and hypertension have a potentiating effect on increasing the risk of stroke.[5–7] Therefore, if blood homocysteine can be controlled within a normal range, this could have beneficial effects on the risk of hypertension and stroke. A meta-analysis of 72 genetic *MTHFR* studies (a gene that increases homocysteine levels) and 20 prospective studies estimated that lowering serum homocysteine by just 3 µmol/L would reduce the risk of ischaemic heart disease by 16% (11%–20%) and stroke by 24% (15%–33%).[8] Also, dietary nutrients

intake (folate, Vitamin B_{12} and Vitamin D), or the presence of some disease (renal disease or hypothyroidism) are reportedly associated with hyperhomocysteinemia.[9–11]

There are few studies looking at underlying causes of elevated homocysteine concentrations in the blood in humans.[12] Most studies have focused on the reduction of nitric oxide (a potent vasodilator) bioavailability,[13] methoxistasis,[14] cytotoxic oxidative stress resulting from homocysteine imbalance[15] or reduction of endothelial cell relaxation.[16]

At the same time, many epidemiology studies have investigated the prevalence of hyperhomocysteinemia in different countries. In this context, it is noteworthy that the prevalence of hyperhomocysteinemia is much higher in China than in other countries.[17–20] The underlying reasons are yet unknown. It is also discussed debated whether the education level or BMI might have an influence on the risk of hyperhomocysteinemia.[20 21] Diet as a risk factor for hyperhomocysteinemia has been insufficiently studied.[22] Possible effects of smoking on the risk of developing hyperhomocysteinemia are also lacking.[23]

In addition, most epidemiological studies on hyperhomocysteinemia in China focused only on hypertensive patients or were hospital-based, and few population-based studies were presented, which may limit the interpretation of these data.

Therefore, the current study aimed to explore the epidemiological characteristics and multifaceted determinants of hyperhomocysteinemia using a population-based study design, with particular attention to modifiable lifestyle factors, such as dietary factors or smoking cessation, which are also crucial for the control and prevention of hyperhomocysteinemia and its consequences.

METHODS

Study design and population

This population-based cross-sectional study was conducted in Hunan Province, a developing province in central southern China, which is composed of 14 districts between July 2013 and March 2014. Our survey adopted a uniform and standardised investigation protocol in all the selected participants from the districts.

A multistage cluster random sampling technique was used to get the representative sample of adults ≥30 years. At the first stage, six districts were randomly selected from the 14 districts for the present study. Then, one urban and one rural community were randomly selected from each selected district at the second stage. Among all randomly selected communities, all adults aged ≥30 years old were invited for our study, and we recruited the adults signing a formal written consent and meeting the including criteria to participate in our study. The detailed flow chart of the sampling process is showed in figure 1. The details of this study were also described in our previous study.[24] We excluded subjects who lived in the community less than 5 years, suffered from any type of cancer or had a major surgery within the last 6 months. All participated subjects signed the informed consents. Randomisation in the sampling process was conducted by a staff member not involved in the investigation with a computer random number generator.

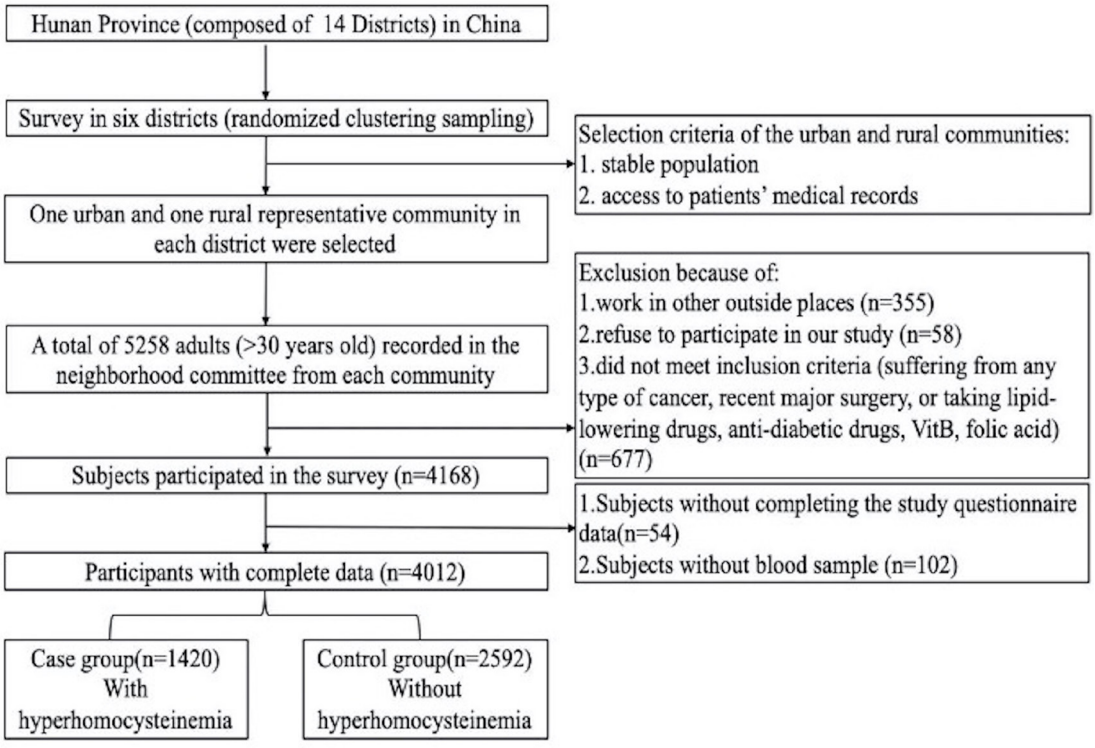

Figure 1 Flowchart of the sample selection.

Data collection and questionnaire survey

Data of subjects were collected with a standard and reliable questionnaire by face-to-face interviews. The questionnaire contains demographic information, cigarette smoking, alcohol drinking, dietary factors, walking and physical activity level. For the demographic factors, physical activity, alcohol drinking and smoking behaviour, we referred the questionnaire of the China Health and Retirement Longitudinal Study (CHARLS).[25] For the dietary factors, we referred to the 2007 Chinese Dietary Guidelines (Chinese Residents' Balanced Dietary Pagoda).[26 27] In addition, we did a pilot study to test the reliability of this questionnaire in 100 adult participants before the large-scale formal investigation. In the pilot study, we investigated the same 100 participants twice with the same questionnaire in 2 weeks by trained investigators. And there is a good test–retest reliability (*kappa*=0.778) for this questionnaire.

Demographic information included sex, age, marriage status, annual household income and education level. The status of marriage was categorised into four groups: unmarried, married, separation and divorce or widowhood. Education level was categorised into four groups: under primary school, primary school, junior school and college and above. The income of the family every year (annual household income, ¥) was asked for every participant.

Alcohol drinking, cigarette smoking and education level were defined as previous studies.[28–30] In brief, status of cigarette smoking was categorised into groups: non-smoker, smoking at past but not now and current smoking. Cigarette smoking is defined as have smoked at least 100 cigarettes in their lifetime.[29] Alcohol drinking is categorised into four groups: abstainer (non-drinker), light or moderate drinker (0–2 drinks/day), heavy drinker (≥2 drinks/day) and drinker who did not report the specific drinks.[30] And we used a set of simple and easy-to-understand photos to measure the drinks of different kinds of drinks (online supplemental figure S1), the participants reported their frequency (days) and amounts (drinks) of alcohol drinking in the past week. The average daily alcohol drinks were estimated as follows: average daily alcohol drinks = (days × (drinks in each of those days))/7.

For fruit and vegetable, it was categorised as two groups (variable values as 1 or 0): 1 means consumed ≥once of fruit and ≥once of vegetable every day, 0 means consumed <once of fruit or <once of vegetable every day. For fish or seafood, it was categorised as two groups (variable values as 1 or 0): 1 means≥once every week, 0 means<once every week. For sweet food, it was categorised as two groups (variable values as 1 or 0): 1 means≥once every week, 0 means<once every week. For pickled vegetables, it was categorised as two groups (variable values as 1 or 0): 1 means≥once every day, 0 means<once every day.

Walking is categorised as two groups: yes=having walked for at least 30 min every day, no=having not walking for at least 30 min every day. For physical activity, subjects were categorised as two groups (physically inactive or not physically inactive). Physical activity lasting for at least 10 continuous minutes per week was categorised as valid physical activity, less than 10 continuous minutes per week as invalid physical activity.[31] Subjects who had no valid physical activity in the past week were considered as physically inactive, otherwise not physical inactive.

Blood pressure measurement

Measurement of BP were conducted according to a standardised procedure with a standard mercury sphygmomanometer. All devices were calibrated before use (range: 0–300 mm Hg; unit: 2 mm Hg). The participants were required to avoid vigorous exercise, having drinks with caffeine and taking medicine which have an influence on BP for 1 hour before measurement. The participants were asked to stop smoking for 15 min before measurement. BP was measured after the participants sat for at least 5 min. Systolic BP (SBP) and diastolic BP (DBP) were recorded as the values where the first Korotkoff sound appeared and the fifth Korotkoff sound disappeared. Two independent BP measurements were performed. The average value of the BP readings was used for data analyses. Hypertension was defined as SBP ≥140 mm Hg and (or) DBP ≥90 mm Hg, or are taking antihypertensive medications or reported diagnosed hypertension by physician.

Biochemical analyses

The study participants were asked not to eat for at least 12 hours before taking the blood sample collection. Participants' venous blood samples were collected in an anticoagulation tube with heparin. Blood plasma was separated and stored at −80°C before measurement. The laboratory tests were conducted on a Hitachi 7600 Automatic Biochemistry Analyzer (Hitachi). The plasma homocysteine concertration was measured by the microplate enzyme immunoassay method,[24] with homocysteine Detection Kit of MedicalSystem Biotechnology Co., Ningbo, China (Reagent batch number, 13082408). Hyperhomocysteinemia was defined as homocysteine ≥15 µmol/L, since previous cohort studies found that homocysteine level (≥15 µmol/L) could significantly predict the risk of cardiovascular diseases and coronary heart disease.[3] Also, previous homocysteine study in Chinese population used the cut-off point of ≥15 µmol/L.[32]

Statistical analyses

Medians and IQR were calculated for numerical variables without normal distribution, and percentages were calculated for categorical variables. The differences between male and female subjects were compared by independent-samples t tests for continuous variables and by χ^2 tests for categorical variables. For association between continuous variables and plasma total homocysteine, figures showing concentration–response relationships with p value were obtained by linear regression. Z score of homocysteine were calculated for better comparison of associations

between different variables and homocysteine. Z score of homocysteine = (original value of homocysteine – mean value)/SD. So, the variable (homocysteine) was standardised to a mean of 0 and an SD of 1 before analysis. For categorical variables, means and 95% CI of Z score of plasma total homocysteine in different groups were presented with p value obtained by analysis of variance or independent t tests. Crude odds ratio (OR) and adjusted OR with 95% 95% CI of having hyperhomocysteinemia were determined by using univariable or multivariable logistic regression models. For the multivariable logistic regression model, we included all the potential variables (age, sex, BMI, marriage status, education level, annual household income, smoking, alcohol drinking, walking, physical activity, fruit and vegetable, sweet food and pickled vegetables) in the models to adjusted ORs for risk factors. Stratified analyses of sex of association between risk factors and hyperhomocysteinemia were conducted, then the sex interactions were tested with multivariable logistic regression models including the interaction term with age, BMI, sex, marriage status, education level, annual household income, smoking, alcohol drinking, walking, physical activity, fruit and vegetable, sweet food, pickled vegetables as covariates. In the stratified analyses, sex interaction was defined as different effect sizes in female and male groups, and the p value for interaction was statistically significant ($p_{interaction}$ <0.05).[33] The possible multicollinearity between the independent variables was quantified using the variance infiltration factor (VIF) of collinearity diagnostics. A VIF larger than 4.0 was considered an indication of severe multicollinearity in the regression model.[34] For the sensitivity analysis of the multivariable logistic regression, we also calculated a model with only including significant risk factors in the univariable analysis as covariates. All the statistical analyses were performed by IBM SPSS for Windows (V.20.0, SPSS, Chicago, IL, USA) and R software V.3.6.3.

Patient and public involvement

This study was conducted without patient involvement. Patients and public were not invited with regard to design of our study, measurement of outcomes, data analysis and interpretation of our results.

RESULTS

As illustrated in table 1, a total of 4012 participants was enrolled in the present study. Also, their general characteristics were shown in table 1. Of those, there are 1644 (41.0%) male participants and 2368 (59.0%) female participants. Male subjects have significantly higher age, height, weight, SBP, DBP, BMI and plasma homocysteine concentration than female subjects (p<0.001). Also, there are significant sex differences in education level, marriage status and cigarette smoking status (p<0.001). For other lifestyle factors, male subjects reported a higher rate of alcohol drinking, lower rate of adequate physical activity and lower rate of adequate fruit and vegetable intake than female subjects (p<0.001). Totally, 35.4% of the participants are categorised as having hyperhomocysteinemia (45.4% vs 28.5% for men, women, respectively). Male subjects have higher prevalence of hypertension, hyperhomocysteinemia and hypertension with hyperhomocysteinemia than female subjects (p<0.001). We categorised the homocysteine level into three groups (<10 µmol/L; 10–14.9 µmol/L and ≥15 umol/L), and then used the new categorical homocysteine variable to predict the risk of hypertension in different models with adjustment of potential covariates, we found in the full adjusted model only for participants with homocysteine ≥15 umol/L the risk of hypertension increased significantly (OR=3.74, 95% CI: 3.06 to 4.56) compared with participants with homocysteine <10 µmol/L, but not in the second group (10–14.9 µmol/L) with OR=1.114 (95% CI: 0.91 to 1.36) (online supplemental table S1).

The concentration–response relationships between plasma homocysteine level and related factors are shown in figures 2–5. There is an obvious increasing trend of homocysteine level with age, and male subjects have significantly higher homocysteine level (figure 2). Also, subjects with different education levels or marriage status have different homocysteine level (figure 3), subjects with adequate intake of fruit and vegetable have lower homocysteine level than those without (figure 4). Homocysteine level is also associated with different smoking status, different walking level, and alcohol drinking level (figure 5).

For better comparison of effect of different factors on homocysteine level, we used the Z score, which is calculated by (value−mean)/SD to show the relation between factors and homocysteine.[35] And also, the figure showing the relationship between factors and raw homocysteine level were presented in the supplementary materials (online supplemental figures S2-S5).

The univariable analysis of risk factors for hyperhomocysteinemia was conducted, the crude OR and 95% CI are also presented in table 2. Female participants have significantly lower risk of hyperhomocysteinemia (28.5% vs 45.4% for female and male, respectively). Similarly, smoking status, different alcohol drinking levels, higher BMI and older age are associated with higher risk of hyperhomocysteinemia (p<0.05). Whereas, adequate fruit and vegetable consumption, and physical activity are associated with lower risk of hyperhomocysteinemia (p<0.05). With the collinearity diagnosis between the independent variables, VIFs were all less than 2.0 and indicated that there was no significant collinearity between the independent variables (data not shown). For the sensitivity analysis of the multivariable logistic regression, we also calculated a model with only including significant risk factors in the univariable analysis as covariates, similar results were found (online supplemental table S2).

The results of risk factors for hyperhomocysteinemia under multivariable logistic analysis were also presented in table 2. Female participants have a 35% lower risk of hyperhomocysteinemia compared with male (OR=0.65,

Table 1 General characteristics of the study population

Categories	Variables		Male		Female		Total		P value
			N	Freq./median (IQR)	N	Freq./median (IQR)	N	Freq./median (IQR)	
Anthropometric variables	Height (cm)		1644	165 (159, 170)	2368	155 (148,1 59)	4012	158 (152, 165)	<0.001
	Weight (kg)		1644	65 (56.8, 73)	2368	54 (48, 59.3)	4012	57 (50, 65)	<0.001
	SBP (mm Hg)		1644	128 (118, 142)	2368	120 (110, 138)	4012	124 (110, 140)	<0.001
	DBP (mm Hg)		1644	80 (72, 90)	2368	78 (70, 84)	4012	80 (70, 88)	<0.001
	BMI (kg/m^2)		1644	23.81 (21.93, 26.04)	2368	22.67 (20.61, 24.67)	4012	23.13 (20.97, 25.28)	<0.001
Sociodemographic variables	Age (years)		1644	56 (47, 64)	2368	53 (45, 62)	4012	55 (45, 63)	<0.001
	Marriage	Unmarried	44	2.7%	21	0.9%	65	1.6%	<0.001
		Married	1557	94.7%	2251	95.1%	3808	94.9%	
		Separation	19	1.2%	34	1.4%	53	1.3%	
		Divorce or widowhood	24	1.5%	62	2.6%	86	2.1%	
	Education	Under primary school	352	21.4%	741	31.3%	1093	27.2%	<0.001
		Primary school	369	22.4%	571	24.1%	940	23.4%	
		Junior school	521	31.7%	633	26.7%	1154	28.8%	
		College and above	402	24.5%	423	17.9%	825	20.6%	
	Annual household income (10 000¥)		1644	3.00 (2.00, 4.60)	2368	3.00 (2.00, 4.00)	4012	3.00 (2.00, 4.00)	0.190

Continued

Table 1 Continued

Categories	Variables		Male		Female		Total		P value
			N	Freq./median (IQR)	N	Freq./median (IQR)	N	Freq./median (IQR)	
Behavioural variables	Smoking	Smoking	797	48.5%	45	1.9%	842	21.0%	<0.001
		Smoking at past but not now	216	13.1%	21	0.9%	237	5.9%	
		Not smoking	631	38.4%	2302	97.2%	2933	73.1%	
	Alcohol drinking	Abstainer (non-drinker)	894	54.4%	2148	90.7%	3042	75.8%	<0.001
		Light or moderate drinker	289	17.6%	164	6.9%	453	11.3%	
		Heavy drinker	156	9.5%	18	0.8%	174	4.3%	
		Drinker but not reported the specific drinks	305	18.60%	38	1.60%	343	8.5%	
	Walking at least half an hour per day	No	582	35.4%	837	35.3%	1419	35.4%	0.971
		Yes	1062	64.6%	1531	64.7%	2593	64.6%	
	Physical activity	0	502	30.6%	529	22.5%	1031	25.8%	<0.001
		1	1138	69.4%	1826	77.5%	2964	74.2%	
	Fruit and vegetable	0	1235	75.1%	1420	60.0%	2655	66.2%	<0.001
		1	409	24.9%	948	40.0%	1357	33.8%	
	Fish or seafood	0	849	51.6%	1161	49.0%	2010	50.1%	0.103
		1	795	48.4%	1207	51.0%	2002	49.9%	
	Sweet food	0	1330	80.9%	1924	81.3%	3254	81.1%	0.781
		1	314	19.1%	444	18.8%	758	18.9%	
	Pickled vegetables	0	1476	89.8%	2143	90.5%	3619	90.2%	0.452
		1	168	10.2%	225	9.5%	393	9.8%	
Disease-related variables	Hyperhomocysteinemia	No	898	54.6%	1694	71.5%	2592	64.6%	<0.001
		Yes	746	45.4%	674	28.5%	1420	35.4%	
	Hypertension with hyperhomocysteinemia	No	1181	71.8%	2001	84.5%	3182	79.3%	<0.001
		Yes	463	28.2%	367	15.5%	830	20.7%	
	Homocysteine (μmol/L)		1644	14.5 (11.4, 16.9)	2368	12.5 (9.8, 15.5)	4012	13.2 (10.3, 16.2)	<0.001
	Hypertension	No	878	53.4%	1596	67.4%	2474	61.7%	<0.001
		Yes	766	46.6%	772	32.6%	1538	38.3%	

For physical activity: 1=had moderate or high intensity physical activity for at least 10 min in the past week, 0=had no moderate or high intensity physical activity for at least 10 min in the past week. For fruit and vegetables: 1=consumed fruit once and vegetables once every day, 0=did not consume fruit once or vegetables once every day. For sweet food: 1=at least once every week, 0=less than once every week. For pickled vegetables: 1=at least once every day, 0=less than once every day. For fish or seafood: 1=consumed fish or seafood once every week, 0=did not consume fish or seafood once every week. Freq.: frequency.
BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.

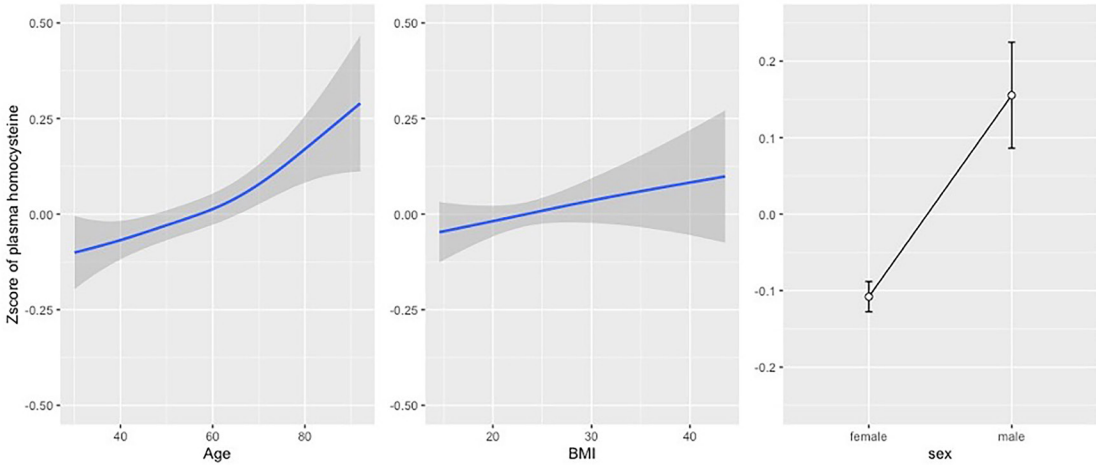

Figure 2 Association between age, BMI and sex and Z score of plasma total homocysteine. Means and 95% CI of Z score of plasma total homocysteine in different groups were presented with p values obtained by ANOVA or independent t tests. ANOVA, analysis of variance; BMI, body mass index.

95% CI: 0.54 to 0.78, p<0.001). Compared with the non-smoker, the smoking participants have a 24% higher risk of hyperhomocysteinemia (OR=1.24, 95% CI: 1.006 to 1.53, p=0.044), while the risk for the participants who smoked at past but not now are not significantly different (OR=1.14, 95% CI: 0.85 to 1.54, p=0.379). Compared with the unmarried participants, the participants who are on separation have a lower risk of hyperhomocysteinemia (OR=0.36, 95% CI:0.16 to 0.84), while those who are married or in divorce or in widowhood is not different from the reference group. BMI and age are both associated with higher risk of hyperhomocysteinemia (p<0.05). One-year increase in age is significantly associated with 2% higher risk of hyperhomocysteinemia (OR=1.02, 95% CI: 1.01 to 1.03). And one-unit increase of BMI is associated with 5% higher risk of hyperhomocysteinemia (OR=1.05, 95% CI: 1.03 to 1.07). Inadequate consumption of fruit and vegetable (consume fruit at least once and vegetable less than once every day) is associated with 29% higher risk of hyperhomocysteinemia with OR (95% CI) of 1.29 (1.11 to 1.50) compared with to those who with adequate consumption of fruit and vegetable.

For the interaction between sex and other factors on the risk of hyperhomocysteinemia, we did stratified analysis by sex, and also did the interaction analysis (table 3). In the female group, participants with college and above education have a significantly higher risk of hyperhomocysteinemia (OR=1.70, 95% CI: 1.18 to 2.46) compared with participants with under primary school education, while in the male group, participants with college and above education had a lower risk of hyperhomocysteinemia (OR=0.60, 95% CI: 0.41 to 0.87). When further interaction analysis was conducted, we identified interaction effects between sex and education level on risk of hyperhomocysteinemia ($p_{interaction}$=0.031).

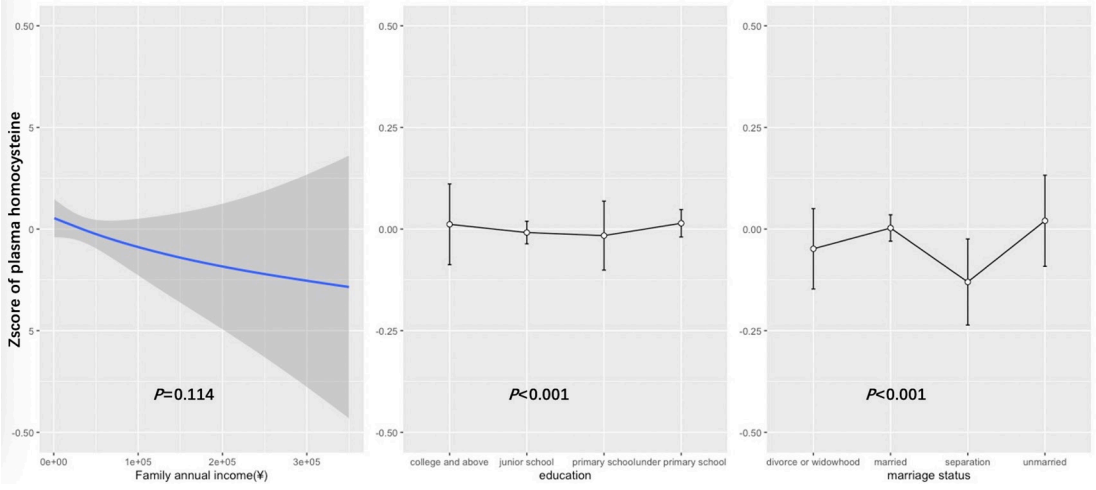

Figure 3 Association between education level, family income and marriage status and Z score of plasma total homocysteine. For family income, concentration–response relationships with p value was obtained by linear regression. For education and marriage status, means and 95% CI of Z score of plasma total homocysteine in different groups were presented with p values obtained by ANOVA or independent t tests. ANOVA, analysis of variance.

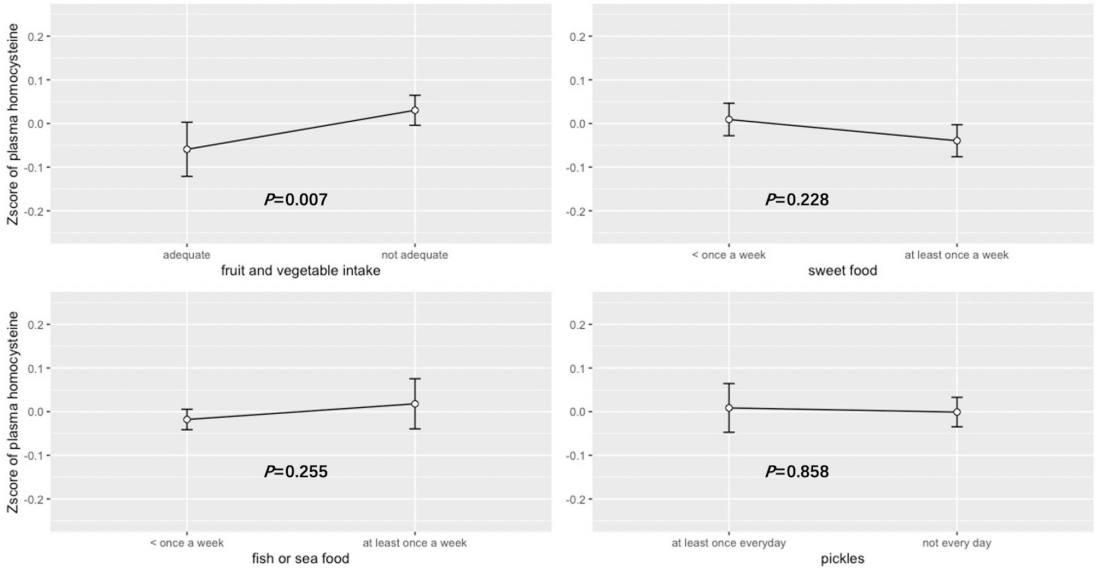

Figure 4 Association between fruit and vegetable intake, sweet food, fish or sea food and pickles and Z score of plasma total homocysteine. Means and 95% CI of Z score of plasma total homocysteine in different groups were presented with p values obtained by independent t tests. For fruit and vegetable: adequate=consumed fruit once and vegetable once every day; inadequate=not consumed fruit once and vegetable once every day.

Similar interaction effect between sex and alcohol drinking on risk of hyperhomocysteinemia was also found ($p_{interaction}$<0.001). In the female group, participants who were light or moderate alcohol drinker had a significantly lower risk of hyperhomocysteinemia (OR=0.47, 95% CI: 0.30 to 0.74) compared with participants who were not alcohol drinker, and female heavy alcohol drinker had a more than triple risk of hyperhomocysteinemia (OR=3.74, 95% CI: 1.41 to 9.88). While in the male group, compared with non-alcohol drinker, light or moderate male drinker have a significantly higher risk of hyperhomocysteinemia (OR=1.69, 95% CI: 1.28 to 2.23), heavy drinker also tended to have a higher risk of hyperhomocysteinemia, but the association did not reach statistical significance (p>0.05).

DISCUSSION

To the best of our knowledge, our study represents the only analyses of high homocysteine and its related risk factors in a large, population-based central southern Chinese population to date. The results showed that the prevalence of hyperhomocysteinemia is relatively high (total, 35.4%, and 45.4% vs 28.5% for men and women).

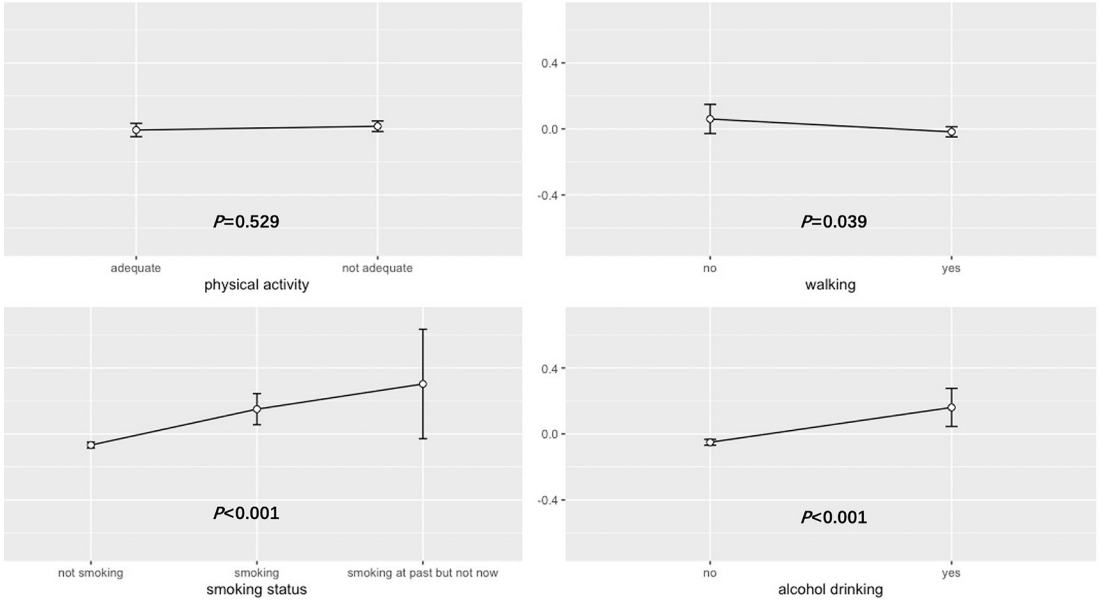

Figure 5 Association between smoking, alcohol drinking, physical activity and walking and Z score of plasma total homocysteine. Means and 95% CI of Z score of plasma total homocysteine in different groups were presented with p values obtained by ANOVA or independent t tests. ANOVA, analysis of variance.

Table 2 Univariable and multivariable logistic regression analysis of risk factors for high homocysteine

		Homocysteine <15 µmol/L		Homocysteine ≥15 µmol/L		Univariable logistic regression		Multivariable logistic regression	
Variables	**Category**	**N**	**Freq./median (IQR)**	**N**	**Freq./median (IQR)**	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age (years)		2592	53 (45, 62)	1420	56 (47, 64)	1.02 (1.01 to 1.02)	<0.001	1.02 (1.01 to 1.03)	<0.001
BMI (kg/m^2)		2592	22.98 (20.83, 24.97)	1420	23.52 (21.22, 25.82)	1.05 (1.03 to 1.08)	<0.001	1.05 (1.03 to 1.07)	<0.001
Sex	Male	898	54.6%	746	45.4%	Reference		Reference	
	Female	1694	71.5%	674	28.5%	0.48 (0.42 to 0.55)	<0.001	0.65 (0.54 to 0.78)	<0.001
Marriage	Unmarried	37	56.9%	28	43.1%	Reference		Reference	
	Married	2452	64.4%	1356	35.6%	0.73 (0.45 to 1.20)	0.215	0.77 (0.46 to 1.29)	0.314
	Separation	41	77.4%	12	22.6%	0.39 (0.17 to 0.87)	0.021	0.36 (0.16 to 0.84)	0.018
	Divorce or widowhood	62	72.1%	24	27.9%	0.51 (0.26 to 1.01)	0.053	0.64 (0.32 to 1.31)	0.224
Education	Under primary school	678	62.0%	415	38.0%	Reference		Reference	
	Primary school	627	66.7%	313	33.3%	0.82 (0.68 to 0.98)	0.029	0.93 (0.76 to 1.14)	0.484
	Junior school	744	64.5%	410	35.5%	0.90 (0.76 to 1.07)	0.230	1.03 (0.84 to 1.27)	0.770
	College and above	543	65.8%	282	34.2%	0.85 (0.70 to 1.03)	0.088	0.996 (0.77 to 1.28)	0.973
Annual income (10 000¥)		2592	3.00 (2.00, 4.20)	1420	3.00 (2.00, 4.10)	1.00 (1.00 to 1.00)	0.668	1.03 (0.999 to 1.05)	0.062
Smoking	Not smoking	2017	68.8%	916	31.2%	Reference		Reference	
	Smoking	445	52.9%	397	47.1%	1.96 (1.68 to 2.30)	<0.001	1.24 (1.006 to 1.53)	0.044
	Smoking at past but not now	130	54.9%	107	45.1%	1.81 (1.39 to 2.37)	<0.001	1.14 (0.85 to 1.54)	0.379
Alcohol drinking	Abstainer (non-drinker)	2046	67.3%	996	32.7%	Reference		Reference	
	Light or moderate drinker	272	60.0%	181	40.0%	1.37 (1.12 to 1.67)	0.003	1.11 (0.89 to 1.38)	0.349
	Heavy drinker	94	54.0%	80	46.0%	1.75 (1.29 to 2.38)	<0.001	1.23 (0.88 to 1.71)	0.230
	Drinker but not reported the specific drinks	180	52.5%	163	47.50%	1.86 (1.49 to 2.33)	<0.001	1.13 (0.88 to 1.45)	0.343
Walking at least half an hour per day	Yes	2035	65.4%	1075	34.6%	Reference		Reference	
	No	557	61.8%	345	38.2%	1.07 (0.93 to 1.22)	0.342	1.1 (0.95 to 1.27)	0.191
Physical activity	1	1952	65.9%	1012	34.1%	Reference		Reference	
	0	629	61.0%	402	39.0%	1.23 (1.07 to 1.43)	0.005	1.17 (1 to 1.36)	0.050

Continued

Table 2 Continued

Variables	Category	Homocysteine <15 µmol/L		Homocysteine ≥15 µmol/L		Univariable logistic regression		Multivariable logistic regression	
		N	Freq./median (IQR)	N	Freq./median (IQR)	OR (95% CI)	P value	OR (95% CI)	P value
Fruit and vegetable	1	960	70.7%	397	29.3%	Reference		Reference	
	0	1632	61.5%	1023	38.5%	1.52 (1.32 to 1.75)	<0.001	1.29 (1.11 to 1.5)	0.001
Fish or seafood	1	1305	65.2%	697	34.8%	Reference		Reference	
	0	1287	64.0%	723	36.0%	1.05 (0.92 to 1.20)	0.444	0.93 (0.81 to 1.08)	0.343
Sweet food	0	2081	64.0%	1173	36.0%	Reference		Reference	
	1	511	67.4%	247	32.6%	0.86 (0.73 to 1.01)	0.073	0.93 (0.78 to 1.11)	0.419
Pickled vegetables	0	2345	64.8%	1274	35.2%	Reference		Reference	
	1	247	62.8%	146	37.2%	1.09 (0.88 to 1.35)	0.443	0.97 (0.77 to 1.22)	0.801

For physical activity: 1=had moderate or high intensity physical activity for at least 10 min in the past week, 0=had no moderate or high intensity physical activity for at least 10 min in the past week. For fruit and vegetable: 1=consumed fruit once and vegetables once every day, 0=did not consume fruit once or vegetables once every day. For sweet food: 1=at least once every week, 0=less than once every week. For pickled vegetables: 1=at least once every day, 0=less than once every day. For fish or seafood: 1=consumed fish or seafood once every week, 0=did not consume fish or seafood once every week. Freq.: frequency.
BMI, body mass index.

Interestingly, we found that current smoking is a risk factor for high homocysteine, but not for smoking quitting. Notably, fruit and vegetable intake may decrease the risk of hyperhomocysteinemia with adjustment of potential covariates. Male sex, older age and higher BMI are risk factors for hyperhomocysteinemia. In addition, we examined significant interactions between sex and educational level, sex and alcohol drinking on the risk of hyperhomocysteinemia. But only associations, not causal relationships could be concluded in this cross-sectional study, future cohort studies or intervention studies should be done to verify these findings.

Our study population is a representative study conducted in the adult population in China using a multistage random sampling method. Both rural and urban areas were covered. In terms of age and sex distribution, our study is in line with other large Chinese population studies.[18 19 36] Reviewing studies on hyperhomocysteinemia, we found that it is relatively rare in developed countries, for example, 15.5% versus 3.9% for men and women in Israel, respectively,[17] with a threshold of 15µmol/L for hyperhomocysteinemia. The median homocysteine level was 9.3 umol (women 8.7 and men 10.0) in the USA.[37] A study from the Canary Islands showed that the prevalence of hyperhomocysteinemia (defined as ≥15 µmol/L) was 21.4 %.[38] With a low cut-off for the diagnosis of hyperhomocysteinaemia (≥14 µmol/L), a prevalence of hyperhomocysteinaemia of 11% was reported in a Finnish study.

In studies of Chinese population, the prevalence of hyperhomocysteinemia ranges substantially from 4% to 73.3 %.[18 20 39] Compared with studies in China, the estimates of hyperhomocysteinemia in our study (35.4%) are lower than in previous studies (67.7% in Shaanxi province),[18] but higher than in the study by Hao *et al* in Hebei, Shanxi and Jiangsu provinces (7% in southerner or southern provinces 28% in northern provinces).[36]

Biological or social factors and hyperhomocysteinemia

In agreement with previous studies, risk of hyperhomocysteinemia increases with age.[19 36 40] There is a significant sex difference in the prevalence of hyperhomocysteinemia.[18 36 41] The sex difference could be due to the different regulation of testosterone, which lead to higher renal cystathionine β-synthase (CBS) activity in men than women, and homocysteine is catalysed CBS.[42] Regarding the positive association between BMI and risk of hyperhomocysteinemia, few studies reported their relations with BMI as a continuous variable, but similar with previous studies. Wang *et al* reported that participants with BMI≥25 kg/m^2 have a significantly higher risk of hyperhomocysteinemia.[19] Jacques *et al* and Koehler *et al* also reported positive association between BMI and homocysteine concentration.[43 44] Education and socioeconomic status (annual family income) is not associated with risk of hyperhomocysteinemia, which is consistent with some previous studies.[19 45]

Table 3 Interaction between risk factors and sex on the risk of hyperhomocysteinemia

Variables	Group	Male		Female		$P_{interaction}$
		OR (95% CI)	P value	OR (95% CI)	P value	
Age (years)		1.01 (1.00 to 1.02)	0.046	1.03 (1.02 to 1.04)	<0.001	0.311
BMI (kg/m^2)		1.06 (1.03 to 1.10)	<0.001	1.04 (1.01 to 1.07)	0.004	0.246
Marriage	Unmarried	Reference		Reference		0.149
	Married	0.62 (0.33 to 1.16)	0.136	1.21 (0.39 to 3.74)	0.742	
	Separation	0.57 (0.19 to 1.78)	0.336	0.25 (0.05 to 1.32)	0.103	
	Divorce or widowhood	0.63 (0.22 to 1.79)	0.384	0.94 (0.26 to 3.37)	0.926	
Education	Under primary school	Reference		Reference		0.031
	Primary school	0.92 (0.68 to 1.26)	0.617	0.96 (0.72 to 1.26)	0.743	
	Junior school	0.83 (0.61 to 1.12)	0.223	1.27 (0.95 to 1.69)	0.109	
	College and above	0.60 (0.41 to 0.87)	0.007	1.70 (1.18 to 2.46)	0.004	
Annual income (10 000¥)		1.03 (1.00 to 1.07)	0.092	1.01 (0.97 to 1.05)	0.581	0.889
Smoking	Not smoking	Reference		Reference		0.966
	Smoking	1.12 (0.89 to 1.41)	0.322	1.37 (0.71 to 2.66)	0.348	
	Smoking at past but not now	1.13 (0.82 to 1.55)	0.459	1.48 (0.56 to 3.91)	0.431	
Alcohol drinking	Abstainer (non-drinker)	Reference		Reference		<0.001
	Light or moderate drinker	1.69 (1.28 to 2.23)	<0.001	0.47 (0.30 to 0.74)	0.001	
	Heavy drinker	1.24 (0.86 to 1.79)	0.250	3.74 (1.41 to 9.88)	0.008	
	Drinker but not reported the specific drinks	1.30 (0.99 to 1.71)	0.062	0.95 (0.45 to 2.02)	0.903	
Walking at least half an hour per day	Yes	Reference		Reference		0.258
	No	1.24 (1.00 to 1.54)	0.047	0.99 (0.81 to 1.21)	0.918	
Physical activity	1	Reference		Reference		0.966
	0	1.21 (0.97 to 1.51)	0.089	1.07 (0.86 to 1.34)	0.528	
Fruit and vegetable	1	Reference		Reference		0.606
	0	1.17 (0.92 to 1.48)	0.208	1.38 (1.13 to 1.70)	0.002	
Fish or seafood	1	Reference		Reference		0.651
	0	0.98 (0.79 to 1.21)	0.856	0.92 (0.76 to 1.12)	0.396	
Sweet food	0	Reference		Reference		0.904
	1	0.89 (0.68 to 1.15)	0.359	0.89 (0.70 to 1.14)	0.357	
Pickled vegetables	0	Reference		Reference		0.911
	1	1.00 (0.71 to 1.40)	0.979	0.91 (0.66 to 1.24)	0.538	

For physical activity: 1=had moderate or high intensity physical activity for at least 10 min in the past week, 0=had no moderate or high intensity physical activity for at least 10 min in the past week. For fruit and vegetables: 1=consumed fruit once and vegetables once every day, 0=did not consume fruit once or vegetables once every day. For sweet food: 1=at least once every week, 0=less than once every week. For pickled vegetables: 1=at least once every day, 0=less than once every day. For fish or seafood: 1=consumed fish or seafood once every week, 0=did not consume fish or seafood once every week.
BMI, body mass index.

Dietary factors or behavioural factors and hyperhomocysteinemia

Remarkably and interestingly, we could clearly show that smoking behaviour significantly influences the risk of hyperhomocysteinemia, which is in good agreement with the data of Wang *et al*.[19] Ogbebor *et al* also showed that smoking could increase homocysteine levels compared with non-smokers.[46] However, another study could not show this probably due to the rather small sample size in this study.[21] It should be emphasised, in addition to, that in our study, we analysed current smokers, smokers who had quit smoking and striked non-smokers separately. Our results therefore imply that smoking cessation is quite beneficial for reducing the risk of hyperhomocysteinaemia.

In addition, we found insufficient fruit and vegetable intake is related with an elevated risk of hyperhomocysteinemia. This is consistent with a study in Ethiopia and a study in Brazil, which found that low consumption of fruit and vegetable was major risk factors of

hyperhomocysteinemia.[47 48] However, a number of studies shown that there is a close association between serum folate levels, VitB2 or VitB6 and plasma homocysteine concentrations.[43 49] And the consumption of fruits and vegetables is associated with an increase in serum folate and VitB levels. The experimental study by Stea *et al* that focused on dietary changes of fruit, vegetables and bread found that plasma homocysteine decreased significantly after the intervention.[50]

Although, no significant association between alcohol consumption and physical activity and risk of hyperhomocysteinemia was found in the present study, for physical activity, the p value for this association is quite borderline (p=0.050), and the direction of association estimates is consistent with previous studies,[51 52] showing that higher level of physical activity is protective for high homocysteine. Similarly, the direction of association between alcohol drinking and risk of hyperhomocysteinemia is consistent with previous studies, but not reach the statistical significance.[53 54]

Interaction between sex and education level, or alcohol drinking

Interestingly, we also found that there are significant interactions between sex and education level, sex and alcohol drinking on the risk of hyperhomocysteinemia. In our study, the association between education level or alcohol drinking with risk of hyperhomocysteinemia is quite different in males and females. Higher education level (college and above) is a risk factor of hyperhomocysteinemia for females but seems to be a protective factor for male. Also, the association between heavy alcohol drinker and risk of hyperhomocysteinemia is much more pronounced in females than in males. And light alcohol drinker was a protective factor in women but a risk factor in men. These sex-different associations are independent of other potential covariates, including age, smoking, alcohol drinking and BMI *et al*. Actually, many studies have demonstrated the association between alcohol drinking and homocysteine, but inconsistent result were found. Some studies showed that alcohol drinking is associated with higher level of homocysteine,[55 56] while some other studies reported a U-shaped relationship and found that mild to moderate alcohol drinking was associated with lower homocysteine.[57 58] The association between alcohol drinking should be examined in future longitudinal or interventions studies. These sex interactions were first examined in our study, not reported in previous works. But sex–smoking interaction, and gene–environment interaction were examined in previous studies.[59 60]

Also, there are several limitations in our study. First, our study is a cross-sectional study, which could only provide associations but not causal relationships. Second, the lifestyle factors, for example, smoking and alcohol drinking were investigated by questionnaire, there could be some misclassifications of smoking or alcohol drinking since some subjects may lie about their status of smoking and alcohol drinking, but this misclassification could only attenuate the estimated value but not reverse it. Because of the design of the study, more women participated in the present study than men (59% vs 41% for females and males, respectively), this is an inevitable limitation for this study.

In conclusion, our study demonstrated that there is a relative high prevalence of hyperhomocysteinemia in a southern Chinese population. Age and gender are non-modifiable risk factors for hyperhomocysteinemia, whereas smoking behaviour, diet and BMI are modifiable risk factors for hyperhomocysteinemia. This is important, since modifiable risk factors for hyperhomocysteinemia is an independent risk factor for cardiovascular diseases and life-style interventions addressing modifiable risk factors for hyperhomocysteinemia thus might likewise reduce the risk for cardiovascular diseases. Additionally, we found significant sex interaction with education level, alcohol drinking on the risk of hyperhomocysteinemia, implying that sex-specific lifestyle modification or intervention should be conducted for the prevention or control of hyperhomocysteinemia.

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