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Prevalence of hyperhomocysteinaemia and its major determinants in rural Chinese hypertensive patients aged 45–75 years

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Abstract

We aimed to investigate the prevalence of hyperhomocysteinaemia (total plasma homocysteine (tHcy) ≥10 μmol/l) and its major determinants in rural Chinese hypertensive patients. A cross-sectional investigation was carried out in Lianyungang of Jiangsu province, China. This analysis included 13 946 hypertensive adults. The prevalence of hyperhomocysteinaemia was 51·6 % (42·7 % in women and 65·6 % in men). The OR of hyperhomocysteinaemia were 1·52 (95 % CI 1·39, 1·67) and 2·32 (95 % CI 2·07, 2·61) for participants aged 55–65 and 65–75 *v.* 45–55 years; 1·27 (95 % CI 1·18, 1·37) for participants with a BMI ≥25 *v.* <25 kg/m^2; 1·14 (95 % CI 1·06, 1·23) for participants with *v.* without antihypertensive treatment; 1·09 (95 % CI 1·00, 1·18) for residents inland *v.* coastal; 0·89 (95 % CI 0·82, 0·97) and 0·83 (95 % CI 0·74, 0·92) for participants with moderate and high *v.* low physical activity levels; 1·54 (95 % CI 1·41, 1·68) and 2·47 (95 % CI 2·17, 2·81) for participants with a glomerular filtration rate 60–90 and <60 *v.* ≥90 ml/min per 1·73 m^2; and 1·20 (95 % CI 1·07, 1·35) and 3·81 (95 % CI 3·33, 4·36) for participants with CT and TT *v.* CC genotype at methylenetetrahydrofolate reductase 677C > T polymorphism, respectively. Furthermore, higher tHcy concentrations were observed in smokers of both sexes (men: geometric mean 12·1 (interquartile range (IQR) 9·2–14·5) *v.* 11·9 (IQR 9·3–14·0) μmol/l, *P*=0·005; women: geometric mean 10·3 (IQR 8·3–13·0) *v.* 9·6 (IQR 7·8–11·6) μmol/l, *P*=0·010), and only in males with hypertension grade 3 (*v.* grade 1 or controlled blood pressure) (geometric mean 12·1 (IQR 9·2–14·4) *v.* 11·7 (IQR 9·2–14·0), *P*=0·016) and in male non-drinkers (yes *v.* no) (geometric mean 12·3 (IQR 9·4–14·8) *v.* 11·7 (IQR 9·1–13·9), *P*=0·014). In conclusion, there was a high prevalence of hyperhomocysteinaemia in Chinese hypertensive adults, particularly in the inlanders, who may benefit greatly from tHcy-lowering strategies, such as folic acid supplementation and lifestyle change.

Key words: Prevalence: Hyperhomocysteinaemia: Hypertension: Determinants: Chinese

Traditional risk factors are estimated to account for only part of the CVD risk[1]. Non-traditional risk factors, such as increased fasting total plasma homocysteine (tHcy), have received great attention. Abundant studies have established elevated tHcy as a potent independent risk factor for coronary artery disease, stroke and deep vein thrombosis[2–4]. Furthermore, our previous meta-analysis[5] suggested that tHcy-lowering therapy (mainly folic acid supplementation) could reduce the risk of stroke by 18–23 %. Our recent meta-analysis[6], which included fifteen randomised trials with prevention of stroke as one of the study endpoints and represented the largest number of subjects included in previously published papers from meta-analysis, also proved that folic acid supplementation significantly reduced the risk

Abbreviations: CSPPT, China Stroke Primary Prevention Trial; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HTN, hypertension; *MTHFR*, methylenetetrahydrofolate reductase; SBP, systolic blood pressure; tHcy, total plasma homocysteine; WC, waist circumference.

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of stroke by 8 % (n 55 764; relative risk (RR) 0·92; 95 % CI 0·86, 1·00; P=0·038). In the ten trials with no or partial folic acid fortification (n 43 426), the risk of stroke was reduced by 11 % (RR 0·89; 95 % CI 0·82, 0·97; P=0·010). Folic acid supplementation[7] also was found to reduce CVD risk in patients with end-stage renal disease or advanced chronic kidney disease (creatinine clearance <30 ml/min) by 15 and by 17 % in trials that showed a larger decrease in the concentration of tHcy. Furthermore, folic acid supplementation[8] could significantly reduce the progression of carotid intima-media thickness (weighted mean difference −0·04; 95 % CI −0·07, −0·02; P<0·001) and the percentage reduction of tHcy was positively related to the effect size. Similarly, a recent meta-analysis proved that the methylenetetrahydrofolate reductase (*MTHFR*) 677C > T variant was associated with a larger effect on tHcy concentration in regions of low folate consumption than in regions with high dietary folate intake or with established programmes of folic acid fortification of flour for the prevention of neural tube defects. A similar pattern was noted for the genetic association with stroke risk[9]. However, a previous report[10] from meta-analysis with eight randomised trials found that dietary supplementation with folic acid had no significant effects within 5 years on cardiovascular events. Therefore, additional large, randomised studies, particularly in regions with insufficient folic acid supplementation and high tHcy levels, should provide further important evidence for confirming the effect of tHcy-lowering therapy on stroke prevention.

There were significant concentration–response associations between plasma tHcy and mortality, and CVD with no apparent threshold concentration[11], which means that it is difficult to define a range of safe tHcy concentrations. However, in a community-based prospective cohort study of 2009 participants in China, participants with tHcy >9·47 μmol/l had a 2·3-fold higher risk for cardiovascular events (95 % CI 1·24, 4·18; P=0·008)[12]. So, to be consistent with previous reports[13,14], we defined hyperhomocysteinaemia as a tHcy concentration ≥10 μmol/l in the present study.

Stroke has been the leading cause of death in China, and its morbidity and mortality have been rapidly rising, particularly in rural areas[15]. Previous studies have reported that hypertension (HTN) and hyperhomocysteinaemia are the two most important modifiable risk factors for stroke. More importantly, it has been reported that an increased tHcy concentration showed a more than multiplicative effect on CVD risk in hypertensive subjects[16,17]. In a recent study[18], individuals with a combination of elevated tHcy (≥10 μmol/l) and HTN were substantially more likely to have prevalent stroke compared with individuals without either condition (men: OR 12·02, 95 % CI 6·36, 22·73; women: OR 17·34, 95 % CI 10·49, 28·64). However, to our knowledge, no previous publication has studied the prevalence of hyperhomocysteinaemia in Chinese hypertensive adults, particularly in rural areas. The present study was conducted to investigate the prevalence of hyperhomocysteinaemia and its major determinants in rural Chinese hypertensive patients.

Subjects and methods

Study population

The study subjects were participants of an ongoing China Stroke Primary Prevention Trial (CSPPT, clinicaltrials.gov identifier: NCT00794885). The CSPPT is a multi-centre randomised controlled trial designed to confirm that enalapril maleate and folic acid tablets combined is more effective in preventing stroke among patients with HTN when compared with enalapril maleate alone. Details regarding inclusion/exclusion criteria, treatment assignment and outcome measures of the trial have been described elsewhere (http://clinicaltrials.gov/ct2/show/NCT00794885). In the present study, we included subjects from Lianyungang who participated in the double-blinded treatment phase of the trial in which tHcy measurements were performed.

Briefly, we conducted a community-based screening in twenty townships within two counties (Ganyu, which is coastal, and Donghai, which is inland) in Lianyungang of Jiangsu province, China, from October 2008 to September 2009. The inclusion criteria were as follows: (1) aged 45–75 years and (2) seated systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg at both of the two screening visits (with at least 24 h between the visits) or currently under antihypertensive treatment. Participants were excluded if they reported a history of myocardial infarction, stroke, heart failure, cancer or serious mental disorders; or they were unwilling to participate in the survey. The present study was approved by the Ethics Committee of the Institute of Biomedicine, Anhui Medical University, Hefei, China. Written informed consent was obtained from each participant before data collection.

Data collection procedures

In the screening phase, researchers and village doctors travelled to participating communities to screen local residents for hypertensive patients. Candidate hypertensive patients were then invited to the local study centres for a formal recruitment visit. At the beginning of the recruitment visit, each participant was asked to provide written informed consent in compliance with the Declaration of Helsinki and the requirements of the ethics committee.

Baseline data were collected by trained research staff according to the standard operating procedure. Each participant was interviewed using a standardised questionnaire designed specifically for the present study. The question about standard of living was phrased as follows: 'How does your standard of living compare to others?'; and a choice of three responses was given as follows: poor, fair and good. The question about physical activity was phrased as follows: 'How do you describe your daily physical activity level?'; and a choice of three responses was provided as follows: low, moderate and high. Finally, the question about fruit and green vegetable consumption was phrased as follows: 'How much fruit and green vegetables do you eat (count the annually averaged weekly intake of fruits and green vegetables)?'; and a choice of three responses regarding weekly intake was given as follows: <1 jin (<500 g), 1–3 jin (500–1500 g) and ≥3 jin (≥1500 g).

Anthropometric measurements, including height, weight and waist circumference (WC), were taken using the standard operating procedure. Height was measured without shoes to the nearest 0·1 cm on a portable stadiometer. Weight was measured in light indoor clothing without shoes to the nearest 0·1 kg. BMI was calculated as weight (kg)/height (m^2). WC was measured as the minimum circumference between the inferior margin of the ribcage and the crest of the ileum.

Seated blood pressure measurements were obtained by trained research staff after subjects had been seated for 10 min using a mercury manometer, and using the standard method of calibration and appropriately sized cuffs, according to the standard operating procedure. Triplicate measurements on the same arm were taken, with at least 2 min between readings. Each patient's SBP and DBP were calculated as the mean of three independent measures. Blood pressure measured at visit 2 was used for analysis.

Blood sample collection and laboratory methods

After 12–15 h of fasting, a venous blood sample was obtained from each subject. Serum or plasma samples were separated within 30 min of collection and were stored at −70°C. Plasma tHcy was measured by an enzyme cycling method using a Hitachi 7020 Automatic Analyzer (Hitachi). Serum creatinine was measured by a modified kinetic rate Jaffe reaction method using a Dade Dimension Chemistry Analyzer (Siemens). The *MTHFR* 677C > T genotype was determined by the Taqman assay designed and manufactured by Applied Biosystems.

Statistical analysis

HTN was categorised into three grades: grade 1, SBP 140–159 and/or DBP 90–99 mmHg; grade 2, SBP 160–179 and/or DBP 100–109 mmHg; grade 3, SBP ≥180 and/or DBP ≥110 mmHg. Treated HTN was defined as receiving antihypertensive medication within the past 2 weeks. Current smoker was defined as having smoked at least one cigarette per d or eighteen packs or more in the last year. Current drinking was defined as drinking alcohol at least twice per week in the last year. Glomerular filtration rate (GFR) was estimated by using the Cockcroft–Gault equation adjusted for body surface area and corrected for the bias in the Modification of Diet in Renal Disease (MDRD) Study sample[19]:

$$\text{GFR (ml/min per } 1{\cdot}73\,\text{m}^2) = 0{\cdot}8 \times ((140 - \text{age}) \times \text{weight (kg)}) \times 0{\cdot}85\ \text{(if female)} \times 1{\cdot}73/(72 \times \text{body surface area} \times \text{serum creatinine}).$$

Body surface area was calculated using the DuBios method[20]:

$$\text{Body surface area (m}^2) = 0{\cdot}007184 \times \text{height (cm)}^{0{\cdot}725} \times \text{weight (kg)}^{0{\cdot}425}.$$

Hyperhomocysteinaemia was defined as a tHcy concentration ≥10 μmol/l.

Means and proportions were calculated for population characteristics by sex. The difference in population characteristics was compared using Student's t tests or χ^2 test. The adjusted OR and 95 % CI of having hyperhomocysteinaemia were determined from logistic regression models that included age group (45–54, 55–64 and 65–75 years), sex, BMI (≥25 *v.* <25 kg/m^2), cigarette smoking, alcohol drinking, GFR (>90, 60–90 and <60 ml/min per 1·73 m^2), antihypertensive treatment status (treated and untreated), HTN grades (controlled blood pressure or grade 1 HTN, grade 2 HTN and grade 3 HTN), geographic region (coastal and inland), season, standard of living (poor, fair and good), fruit and green vegetable consumption (<1 jin (<500 g), 1–3 jin (500–1500 g) and ≥3 jin (≥1500 g)), education level (illiterate, primary level, and elementary or higher level), physical activity level (low, moderate and high) and *MTHFR* 677C>T polymorphism (CC, CT and TT). Both sex-specific and sex-combined regression analyses were performed using the above model. In the sex-combined model, an interaction term between sex and the genotype was added. Similar linear models for ln-transformed tHcy concentrations were also analysed. All statistical analyses were performed using SAS 8.2 (SAS Institute).

Results

Overall, plasma tHcy was measured in 16 441 participants aged 45–75 years with HTN. In the present study, study participants with vitamin use (*n* 256), CVD (*n* 474), cancer (*n* 36), diabetes (*n* 542), dyslipidaemia (*n* 371), or with any missing data (*n* 816) on antihypertensive treatment status, age, sex, height, weight, WC, smoking status, drinking status, standard of living, fruit and green vegetable consumption, education, physical activity level and *MTHFR* 677C > T polymorphism were excluded. The final analysis included 13 946 participants (5421 men and 8525 women).

The population characteristics by sex are listed in Table 1. Men had significantly higher values for age, DBP, percentage of cigarette smoking and alcohol drinking, and levels of living standard and education, and had lower values for SBP, BMI and percentage of antihypertensive treatment.

The geometric mean of tHcy was 12·0 (interquartile range (IQR) 9·3–14·2) μmol/l in men and 9·6 (IQR 7·8–11·6) μmol/l in women. The prevalence of hyperhomocysteinaemia (≥10 μmol/l) was 51·6 % (women 42·7 % and men 65·6 %) (Table 2).

The results from the logistic regression analyses for hyperhomocysteinaemia are presented in Table 2. In general, age, male sex, higher GFR, T-allele of *MTHFR* 677C > T, BMI, inlanders and HTN treatment (but not HTN grade) were associated with an increased risk of hyperhomocysteinaemia, whereas the physical activity level was associated with decreased risk. Similar association patterns were observed in men and women. Similar findings were also observed in the regression analysis of ln-transformed tHcy (Table 3). In addition, smokers in both men and women, men with grade 3 HTN and male non-drinkers had higher tHcy. Most importantly, a significant interaction between sex and the *MTHFR* 677C > T genotype was observed for both tHcy and hyperhomocysteinaemia. While the TT genotype was a significant

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Table 1. Population characteristics by sex

(Mean values and standard deviations; number of participants and percentages; geometric means and interquartile ranges (IQR))

	Total		Men		Women		
	n	%	*n*	%	*n*	%	*P*
n	13 946		5421		8525		
Age (years)							< 0·001
Mean	59·4		60·2		59·0		
SD	7·6		7·7		7·5		
Age group (years)							
45–55	4270	30·6	1489	27·5	2781	32·6	< 0·001
55–65	6030	43·2	2301	42·4	3729	43·7	
65–75	3646	26·1	1631	30·1	2015	23·6	
SBP (mmHg)							< 0·001
Mean	168·5		167·0		169·5		
SD	20·8		20·7		20·8		
DBP (mmHg)							< 0·001
Mean	95·5		97·4		94·3		
SD	11·9		12·3		11·6		
HTN grade							
Controlled BP or grade 1*	3905	28·0	1501	27·7	2404	28·2	0·574
Grade 2	5602	40·2	2167	40·0	3435	40·3	
Grade 3	4439	31·8	1753	32·3	2686	31·5	
GFR (ml/min per 1·73 m^2)							< 0·001
Geometric mean	77·2		74·1		79·2		
IQR	64·6–92·2		62·5–87·5		66·1–95·1		
GFR group (ml/min per 1·73 m^2)							
≥ 90	3911	28·0	1155	21·3	2756	32·3	< 0·001
60–90	7635	54·7	3184	58·7	4451	52·2	
< 60	2400	17·2	1082	20·0	1318	15·5	
Homocysteine (μmol/l)							< 0·001
Geometric mean	10·5		12·0		9·6		
IQR	8·2–12·6		9·3–14·2		7·8–11·6		
BMI (kg/m^2)							< 0·001
Mean	25·6		24·9		26·0		
SD	3·6		3·3		3·7		
Waist circumference (cm)							0·075
Mean	85·4		85·5		85·2		
SD	9·7		9·8		9·6		
Current smoking	3200	22·9	2875	53·0	325	3·8	< 0·001
Current drinking	3261	23·4	2983	55·0	278	3·3	< 0·001
Antihypertensive treatment, treated	6443	46·2	2315	42·7	4128	48·4	< 0·001
County							
Ganyu (coastal)	5667	40·6	2300	42·4	3367	39·5	0·001
Donghai (inland)	8279	59·4	3121	57·6	5158	60·5	
Season							
Spring and winter	9605	68·9	3847	71·0	5758	67·5	< 0·001
Summer and autumn	4341	31·1	1574	29·0	2767	32·5	
Living standards							
Poor	1535	11·0	506	9·3	1029	12·1	< 0·001
Fair	10 822	77·6	4183	77·2	6639	77·9	
Good	1589	11·4	732	13·5	857	10·1	
Fruit and vegetable consumption							
< 500 g/week	240	1·7	100	1·8	140	1·6	0·315
500–1500 g/week	2659	19·1	1060	19·6	1599	18·8	
≥ 1500 g/week	11 047	79·2	4261	78·6	6786	79·6	
Education							
Illiterate	9066	65·0	2035	37·5	7031	82·5	< 0·001
Primary level	2064	14·8	1256	23·2	808	9·5	
Elementary or higher levels	2816	20·2	2130	39·3	686	8·0	
Physical activity							
Low	5458	39·1	2069	38·2	3389	39·8	0·169
Moderate	5490	39·4	2173	40·1	3317	38·9	
High	2998	21·5	1179	21·7	1819	21·3	
MTHFR 677C > T polymorphism							
CC	3247	23·3	1274	23·5	1973	23·1	0·196
CT	7014	50·3	2677	49·4	4337	50·9	
TT	3685	26·4	1470	27·1	2215	26·0	

SBP, systolic blood pressure; DBP, diastolic blood pressure; HTN, hypertension; BP, blood pressure, GFR, glomerular filtration rate; *MTHFR*, methylenetetrahydrofolate reductase.

* A total of 388 subjects with antihypertensive treatment and controlled BP were included.

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Table 2. Adjusted* OR of having hyperhomocysteinaemia (total plasma homocysteine ≥10 μmol/l) in different subgroups
(Adjusted odds ratios and 95 % confidence intervals)

	Total					Men					Women				
	Prevalence		Adjusted			Prevalence		Adjusted			Prevalence		Adjusted		
	n	%	OR	95 % CI	*P*	*n*	%	OR	95 % CI	*P*	*n*	%	OR	95 % CI	*P*
Sex															
Women	3642	42·7	1·00	Reference											
Men	3558	65·6	2·25	1·89, 2·67	<0·001										
MTHFR 677CT*Men			1·22	1·01, 1·46	0·034										
MTHFR 677TT*Men			1·34	1·07, 1·68	0·012										
MTHFR 677C > T polymorphism															
CC	1322	40·7	1·00	Reference		672	52·7	1·00	Reference		650	32·9	1·00	Reference	
CT	3242	46·2	1·20	1·07, 1·35	0·002	1650	61·6	1·46	1·27, 1·68	<0·001	1592	36·7	1·21	1·07, 1·36	0·002
TT	2636	71·5	3·81	3·33, 4·36	<0·001	1236	84·1	4·98	4·14, 5·98	<0·001	1400	63·2	3·89	3·40, 4·45	<0·001
GFR (ml/min per 1·73 m^2)															
≥90	1455	37·2	1·00	Reference		626	54·2	1·00	Reference		829	30·1	1·00	Reference	
60–90	4079	53·4	1·54	1·41, 1·68	<0·001	2059	64·7	1·44	1·24, 1·68	<0·001	2020	45·4	1·59	1·42, 1·78	<0·001
<60	1666	69·4	2·47	2·17, 2·81	<0·001	873	80·7	2·80	2·25, 3·49	<0·001	793	60·2	2·31	1·97, 2·71	<0·001
Age (years)															
45–55	1669	39·1	1·00	Reference		867	58·2	1·00	Reference		802	28·8	1·00	Reference	
55–65	3120	51·7	1·52	1·39, 1·67	<0·001	1457	63·3	1·13	0·97, 1·32	0·108	1663	44·6	1·79	1·59, 2·01	<0·001
65–75	2411	66·1	2·32	2·07, 2·61	<0·001	1234	75·7	1·78	1·48, 2·15	<0·001	1177	58·4	2·71	2·34, 3·14	<0·001
BMI (kg/m^2)															
<25	3367	52·5	1·00	Reference		1899	64·7	1·00	Reference		1468	42·1	1·00	Reference	
≥25	3833	50·9	1·27	1·18, 1·37	<0·001	1659	66·7	1·28	1·13, 1·46	<0·001	2174	43·1	1·26	1·14, 1·39	<0·001
Current smoking															
No	5158	48·0	1·00	Reference		1680	66·0	1·00	Reference		3476	42·4	1·00	Reference	
Yes	2044	63·9	1·09	0·97, 1·22	0·101	1878	65·3	1·07	0·94, 1·22	0·297	166	51·1	1·18	0·92, 1·51	0·203
Current drinking															
No	5162	48·3	1·00	Reference		1638	67·2	1·00	Reference		3524	42·7	1·00	Reference	
Yes	2038	62·5	0·99	0·88, 1·11	0·907	1920	64·4	1·00	0·88, 1·14	0·994	118	42·4	0·91	0·69, 1·20	0·505
Antihypertensive treatment															
Untreated	3745	49·9	1·00	Reference		1977	63·7	1·00	Reference		1768	40·2	1·00	Reference	
Treated	3455	53·6	1·14	1·06, 1·23	0·001	1581	68·3	1·14	1·01, 1·29	0·040	1874	45·4	1·14	1·04, 1·25	0·007
HTN grade															
Controlled BP or grade 1†	2006	51·4	1·00	Reference		976	65·0	1·00	Reference		1030	42·8	1·00	Reference	
Grade 2	2877	51·4	1·01	0·92, 1·11	0·826	1428	65·9	1·10	0·95, 1·28	0·204	1449	42·2	0·95	0·84, 1·06	0·359
Grade 3	2317	52·2	1·00	0·91, 1·10	0·998	1154	65·8	1·09	0·94, 1·28	0·256	1163	43·3	0·93	0·82, 1·05	0·267
County															
Ganyu (coastal)	2698	47·6	1·00	Reference		1414	61·5	1·00	Reference		1284	38·1	1·00	Reference	
Donghai (inland)	4502	54·4	1·09	1·00, 1·18	0·047	2144	68·7	1·09	0·96, 1·24	0·200	2358	45·7	1·09	0·98, 1·22	0·094
Season															
Spring and winter	4526	47·1	1·00	Reference		2388	62·1	1·00	Reference		2138	37·1	1·00	Reference	
Summer and autumn	2674	61·6	1·91	1·75, 2·08	<0·001	1170	74·3	1·81	1·56, 2·10	<0·001	1504	54·4	1·96	1·76, 2·18	<0·001
Living standards															
Poor	770	50·2	1·00	Reference		343	67·8	1·00	Reference		427	41·5	1·00	Reference	
Fair	5588	51·6	1·10	0·97, 1·24	0·130	2753	65·8	1·00	0·81, 1·24	0·976	2835	42·7	1·15	0·99, 1·33	0·067
Good	842	53·0	1·08	0·92, 1·27	0·322	462	63·1	0·89	0·68, 1·16	0·389	380	44·3	1·21	0·99, 1·48	0·069

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Table 2. *Continued*

	Total					Men					Women				
	Prevalence					Prevalence					Prevalence				
	n	%	Adjusted OR	95 % CI	*P*	*n*	%	Adjusted OR	95 % CI	*P*	*n*	%	Adjusted OR	95 % CI	*P*
Fruit and vegetable consumption															
<500 g/week	116	48·3	1·00	Reference		60	60·0	1·00	Reference		56	40·0	1·00	Reference	
500–1500 g/week	1363	51·2	1·20	0·90, 1·61	0·190	692	65·3	1·49	0·95, 2·34	0·082	671	42·0	1·06	0·72, 1·55	0·760
≥1500 g/week	5721	51·8	1·16	0·87, 1·54	0·284	2806	65·9	1·34	0·86, 2·07	0·194	2915	43·0	1·08	0·74, 1·56	0·690
Education															
Illiterate	4471	49·3	1·00	Reference		1366	67·1	1·00	Reference		3105	44·2	1·00	Reference	
Primary level	1153	55·9	1·00	0·89, 1·11	0·941	830	66·1	1·01	0·86, 1·19	0·902	323	40·0	1·04	0·89, 1·23	0·612
Elementary or higher levels	1576	56·0	1·02	0·91, 1·14	1·000	1362	63·9	1·07	0·92, 1·24	0·976	214	31·2	0·87	0·72, 1·05	0·136
Physical activity															
Low	3047	55·8	1·00	Reference		1436	69·4	1·00	Reference		1611	47·5	1·00	Reference	
Moderate	2733	49·8	0·89	0·82, 0·97	0·009	1367	62·9	0·82	0·71, 0·95	0·007	1366	41·2	0·95	0·85, 1·06	0·323
High	1420	47·4	0·83	0·74, 0·92	<0·001	755	64·0	0·91	0·76, 1·07	0·250	665	36·6	0·78	0·68, 0·89	<0·001

MTHFR, methylenetetrahydrofolate reductase; GFR, glomerular filtration rate; HTN, hypertension; BP, blood pressure.
* All variables were included in the same model.
† A total of 388 subjects with antihypertensive treatment and controlled BP were included.

independent risk factor in both sexes, TT in men was associated with an additional 22 % increase in tHcy and 34 % increase in OR for hyperhomocysteinaemia (Tables 2 and 3; Fig. 1).

Discussion

In the present study, the geometric mean tHcy concentration was 10·5 μmol/l (women 9·6 and men 12·0) and the median tHcy concentration was 10·1 μmol/l (women 9·4 and men 11·2). The prevalence of hyperhomocysteinaemia was 51·7 % (women 42·8 % and men 65·8 %). These estimates are lower than those in a previous report of hypertensive adults in different Chinese populations (Ha'erbin, Shanghai, Shenyang, Beijing, Xi'an and Nanjing; median tHcy: 12·2 μmol/l)[21,22]), and lie in the middle between the southerners and the northerners with respect to another study of 2471 Chinese adults aged 35–64 years[23]. The differences in tHcy levels described in various reports could be due to different population inclusion criteria, genetic backgrounds, as well as differences in risk factor profiles across regional areas.

Consistent with previous studies[11,24], the present study found that a higher prevalence of hyperhomocysteinaemia or tHcy was associated with men, older age, overweight (BMI ≥25 kg/m^2) and current smoking. However, the effect size of current smoking and current drinking on tHcy, about 4 % increase and 3 % decrease in tHcy, respectively, is relatively small. The relationship between alcohol consumption and tHcy concentration from previous reports had been inconclusive[25,26]. In the present study, current alcohol drinkers were significantly associated with decreased tHcy only in men. This is partly due to the percentage of current drinkers in women being very low (approximately 3 %), hence a larger sample size is needed to detect such a small effect size. Since BMI and WC were highly correlated (Pearson's r 0·79), we did not include BMI and WC in the same regression model.

The present study also observed a 3 % decrease in tHcy and an approximately 20 % decrease in OR for hyperhomocysteinaemia in subjects with high physical activities. A recent meta-analysis reported that moderately or highly physically active people had a lower risk of stroke incidence or mortality than those with a low level of activity[27]. Given the fact that elevated tHcy was more strongly associated with stroke than with IHD[2], it would be interesting to test whether physical activity is an independent risk factor after taking into consideration of tHcy.

It is noteworthy that, in the present study, the inlanders had a higher tHcy and prevalence of hyperhomocysteinaemia, which is contrary to the results from a small study (n 208) in coastal West Africa[28]. A possible explanation for the observation in the present study could be a higher betaine[29] and vitamin B_{12}[30] (rich in seafood) intake in coastal areas. Unfortunately, we did not have such data in the present study.

In the present study, although participants with antihypertensive treatment had slightly lower SBP (167·9 *v.* 169·1 mmHg, P=0·001) and DBP (95·1 *v.* 95·9 mmHg, P<0·001) than those without antihypertensive treatment,

Table 3. Relationships between homocysteine concentrations* and related factors in different subgroups†
(Geometric means, interquartile ranges (IQR, first to third quartiles) and 95 % confidence intervals)

	Total					Men					Women				
	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy‡ (%)	95 % CI	*P*	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy (%)	95 % CI	*P*	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy (%)	95 % CI	*P*
Sex															
Women	9·6	7·8–11·6	Reference												
Men	12·0	9·3–14·2	41·9	37·8, 46·1	<0·001										
MTHFR 677CT*Men			0·7	−2·6, 4·1	0·683										
MTHFR 677TT*Men			22·2	17·8, 26·9	0·000										
MTHFR 677C > T polymorphism															
CC	9·4	7·8–11·2	Reference			10·3	8·5–12·2	Reference			8·8	7·5–10·6	Reference		
CT	9·7	8·1–11·7	3·9	1·8, 6·1	<0·001	10·8	9·0–12·7	4·6	1·7, 7·6	0·002	9·1	7·7–11·0	3·9	2·0, 6·0	<0·001
TT	13·3	9·6–17·4	30·2	27·2, 33·3	<0·001	16·5	11·1–24·0	58·9	53·9, 64·1	0·000	11·5	8·8–14·5	30·2	27·4, 33·1	<0·001
GFR (ml/min per 1·73 m^2)															
≥90	9·3	7·5–11·1				10·8	8·4–12·7				8·7	7·2–10·4			
60–90	10·6	8·4–12·6	8·4	6·7, 10·1	<0·001	11·9	9·2–14·0	7·8	4·6, 11·1	<0·001	9·8	8·1–11·7	8·4	6·5, 10·4	<0·001
<60	12·2	9·5–14·7	19·0	17·1, 20·9	<0·001	13·8	10·5–16·4	21·3	17·7, 25·0	<0·001	11·0	8·9–13·4	17·1	15·0, 19·2	<0·001
Age (years)															
45–55	9·6	7·6–11·4	Reference			11·4	8·7–13·4	Reference			8·7	7·3–10·3	Reference		
55–65	10·4	8·3–12·4	5·3	3·6, 7·0	<0·001	11·8	9·1–13·8	0·7	−2·3, 3·7	0·662	9·7	7·9–11·7	7·7	5·7, 9·7	<0·001
65–75	11·6	9·3–14·0	10·7	8·5, 12·9	<0·001	12·9	10·0–15·4	5·5	1·8, 9·3	0·003	10·7	8·7–12·9	13·8	11·0, 16·6	<0·001
BMI (kg/m^2)															
<25	10·6	8·3–12·8	Reference			11·9	9·2–14·1	Reference			9·6	7·8–11·7	Reference		
≥25	10·4	8·2–12·6	3·8	2·4, 5·2	<0·001	12·1	9·3–14·3	4·2	1·7, 6·7	0·001	9·6	7·8–11·6	3·4	1·8, 5·1	<0·001
Current smoking															
No	10·1	8·1–12·1	Reference			11·9	9·3–14·0	Reference			9·6	7·8–11·6	Reference		
Yes	11·9	9·1–14·3	3·8	1·8, 5·8	<0·001	12·1	9·2–14·5	3·5	1·0, 6·0	0·005	10·3	8·3–13·0	4·9	0·6, 9·4	0·010
Current drinking															
No	10·2	8·1–12·2	Reference			12·3	9·4–14·8	Reference			9·6	7·8–11·6	Reference		
Yes	11·6	8·9–13·7	−2·7	−4·6, −0·7	0·007	11·7	9·1–13·9	−3·0	−5·3, −0·6	0·014	9·5	7·9–11·4	−2·8	−7·1, 1·6	0·207
Antihypertensive treatment															
Untreated	10·4	8·2–12·4	Reference			12·0	9·3–14·2	Reference			9·5	7·7–11·4	Reference		
Treated	10·5	8·3–12·7	−0·2	−1·5, 1·2	0·806	12·0	9·4–14·2	−1·5	−3·7, 0·9	0·224	9·7	7·9–11·9	0·6	−0·9, 2·2	0·423
HTN grade															
Controlled BP or grade 1§	10·3	8·2–12·5	Reference			11·7	9·2–14·0	Reference			9·5	7·8–11·6	Reference		
Grade 2	10·5	8·2–12·5	1·0	−0·6, 2·6	0·217	12·1	9·3–14·3	2·6	−0·3, 5·5	0·078	9·6	7·7–11·6	−0·2	−2·1, 1·7	0·838
Grade 3	10·6	8·3–12·7	1·7	0·0, 3·4	0·055	12·1	9·2–14·4	3·7	0·7, 6·8	0·016	9·7	7·9–11·7	0·0	−2·0, 2·1	0·969
County															
Ganyu (coastal)	10·1	8·0–12·1	Reference			11·5	8·9–13·7	Reference			9·2	7·6–11·1	Reference		
Donghai (inland)	10·8	8·4–12·9	3·5	2·0, 5·0	<0·001	12·4	9·4–14·7	3·5	1·0, 6·2	0·006	9·9	8·0–11·9	3·6	1·8, 5·4	<0·001
Season															
Spring and winter	10·1	8·0–12·1	Reference			11·7	9·0–13·8	Reference			9·2	7·5–11·1	Reference		
Summer and autumn	11·2	9·0–13·4	9·4	7·8, 11·0	<0·001	12·7	9·9–15·0	7·5	4·7, 10·4	<0·001	10·5	8·6–12·6	10·4	8·5, 12·4	<0·001
Living standards															
Poor	10·5	8·2–12·6	Reference			12·6	9·3–15·6	Reference			9·6	7·7–11·5	Reference		
Fair	10·4	8·2–12·6	0·4	−1·7, 2·5	0·702	12·0	9·3–14·2	−1·7	2·2, −5·4	0·329	9·6	7·8–11·7	1·7	−0·8, 4·1	0·182
Good	10·6	8·3–12·3	0·2	−2·6, 3·1	0·878	11·7	9·1–13·7	−3·3	4·7, −10·7	0·114	9·7	8·0–11·5	2·7	−0·7, 6·2	0·119
Fruit and vegetable consumption															
<500 g/week	10·7	8·2–12·8	Reference			12·3	8·7–15·2	Reference			9·7	7·8–11·6	Reference		
500–1500 g/week	10·4	8·2–12·6	−0·9	−5·8, 4·3	0·736	11·8	9·2–14·3	0·3	−8·1, 9·4	0·948	9·6	7·8–11·5	−1·4	−7·3, 5·0	0·669

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Table 3. *Continued*

	Total					Men					Women				
	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy‡ (%)	95 % CI	P	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy (%)	95 % CI	P	Geometric mean (μmol/l)	IQR (μmol/l)	Change in tHcy (%)	95 % CI	P
≥1500 g/week	10·5	8·3–12·6	−1·5	−6·3, 3·5	0·551	12·0	9·3–14·2	0·2	−8·0, 9·0	0·972	9·6	7·8–11·6	−2·2	−8·0, 3·9	0·470
Education															
Illiterate	10·2	8·1–12·3	Reference			12·1	9·4–14·6	Reference			9·7	7·9–11·8	Reference		
Primary level	10·9	8·4–13·1	−0·8	−2·8, 1·1	0·403	12·1	9·2–14·4	−0·6	−3·6, 2·4	0·683	9·3	7·6–11·2	−0·3	−2·9, 2·3	0·808
Elementary or higher levels	11·0	8·5–13·1	0·1	−1·8, 2·1	0·903	11·8	9·1–13·8	0·6	−2·2, 3·5	0·681	8·9	7·4–10·6	−1·7	−4·6, 1·2	0·245
Physical activity															
Low	10·8	8·5–13·0	Reference			12·4	9·5–14·9	Reference			9·9	8·1–12·0	Reference		
Moderate	10·3	8·1–12·3	−2·1	−3·6, −0·6	0·006	11·7	9·1–13·9	−3·3	−5·8, −0·7	0·014	9·5	7·7–11·4	−1·2	−3·0, 0·6	0·189
High	10·2	8·0–12·2	−3·3	−5·1, −1·6	<0·001	11·8	9·1–13·9	−3·1	−6·1, 0·1	0·056	9·2	7·6–11·1	−3·5	−5·5, −1·3	0·002

tHcy, total plasma homocysteine; *MTHFR*, methylenetetrahydrofolate reductase; GFR, glomerular filtration rate; HTN, hypertension; BP, blood pressure.
* Ln-transformed homocysteine concentrations were used in the regression model.
† All variables were included in the same models.
‡ Calculated as (antilog β coefficient − 1) × 100.
§ A total of 388 subjects with antihypertensive treatment and controlled BP were included.

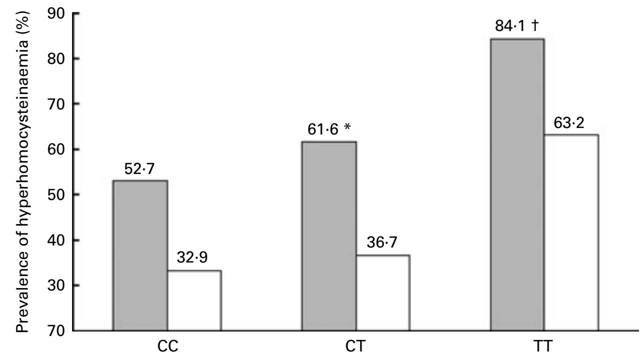

Fig. 1. Prevalence of hyperhomocysteinaemia (total plasma homocysteine ≥10 μmol/l) by methylenetetrahydrofolate reductase 677C > T polymorphism and sex (men (▩), *n* 5421; women (□), *n* 8525). * There was a significant interaction effect between sex and 677CT genotype ($P<0·05$). † There was a significant interaction effect between sex and 677TT genotype ($P<0·05$).

participants with antihypertensive treatment had a higher prevalence of hyperhomocysteinaemia. Whether this was a treatment side effect is still inconclusive[31,32].

The *MTHFR* 677C > T polymorphism is the most important genetic determinant of plasma tHcy[33]. Furthermore, previous studies[34,35] suggested that age and sex may modify the contribution of the *MTHFR* 677C > T polymorphism to tHcy concentrations under conditions of lower folate status. For example, Papoutsakis *et al.*[34] reported that only males with the *MTHFR* 677TT genotype had tHcy that was significantly higher than tHcy levels of C-allele carriers ($P=0·001$). Russo *et al.*[35] observed that only in younger men (<55 years old), TT subjects had significantly higher tHcy concentrations than those with the CT or CC genotype ($P<0·05$ for either of these genotypes). In the present study, subjects with *MTHFR* 677TT and CT genotypes (*v.* CC genotype) had higher tHcy concentrations in men or women. Also, there were significant interaction effects between the *MTHFR* C677T polymorphism and sex on the prevalence of hyperhomocysteinaemia. Most importantly, the frequency of the *MTHFR* 677TT genotype was 26·4 %, similar to the result (24·8 %) found in a previous hypertensive adult study in different Chinese regions[21,22], but higher than that in other countries[36]. Another study has found that the *MTHFR* 677C > T polymorphism was one of the major determinants of stroke risk[9]. Thus, the high prevalence of the *MTHFR* 677TT genotype and its interaction effects with sex on the prevalence of hyperhomocysteinaemia may partly explain the high incidence of stroke, even with strict HTN control, in Chinese hypertensive adults, particularly in men[15,37].

In summary, we simultaneously evaluated the effect of a major genetic polymorphism, lifestyle, region, socio-economic status and diet on the prevalence of hyperhomocysteinaemia in Chinese hypertensive adults. The inclusion or exclusion of participants with known CVD, diabetes and dyslipidaemia in the present analyses had little effect on the results. There are limitations of the present study. The data collected on food intake and lifestyle habit are quite limited; plasma levels of folic acid and other B vitamins are not available at this time; and our sample was from hypertensive adults in one region. So, the findings from the present study may not be directly generalisable to the general population. We mainly focused on the effect of lifestyle factors (smoking, alcohol drinking, physical activity, etc.), socio-economic status and genetic background on the prevalence of hyperhomocysteinaemia.

In conclusion, there was a high prevalence of hyperhomocysteinaemia in Chinese hypertensive adults, particularly in participants in inland (*v.* coastal) areas. Sex, age and the *MTHFR* genotype were the major risk factors, while BMI, GFR, smoking, alcohol drinking and physical activity were also significant determinants. Folic acid supplementation and the concomitant lifestyle change, including smoking cessation, obesity control and improvement in physical activity levels may help to decrease tHcy and reduce the CVD risk in this population.

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