Enrichment of MTHFR 677 T in a Chinese long-lived cohort and its association with lipid modulation

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Abstract

Background: Variants in the Methylenetetrahydrofolate reductase (MTHFR) gene may result in a lowered catalytic activity and associate with subsequent elevated serum homocysteine (Hcy) concentration, abnormal DNA synthesis and methylation, cardiovascular risk, and unhealthy aging. Several investigations on the relationship of MTHFR C677T polymorphism with serum lipid profile and longevity have been conducted in some populations, but the findings remain mixed. Herein, we sought to look at the association between MTHFR C677T and lipid profile in a longevous cohort in Bama, a well-known home of longevity in China.

Methods: Genotyping of MTHFR C677T was undertaken in 516 long-lived inhabitants (aged 90 and older, long-lived group, LG) and 493 healthy controls (aged 60–75, non-long-lived group, non-LG) recruited from Bama area. Correlation between MTHFR genotypes and lipids was then evaluated.

Results: T allele and TT genotype were significantly more prevalent in LG (P = 0.001 and 0.002, respectively), especially in females, than in non-LG. No difference in the tested lipid measures among MTHFR C677T genotypes was observed in LG, non-LG and total population (P > 0.05 for all). However, female but not male T carriers exhibited higher TC and LDL-C levels than did T noncarriers in the total population and in LG after stratification by sex (P < 0.05 for each). These differences did not however remain through further subdivision by hyperlipidemia and normolipidemia.

Conclusion: The higher prevalence of MTHFR 677 T genotypes and its modest unfavorable impact on lipids in Bama long-lived individuals may imply an existence of other protective genotypes which require further determination.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl donor which remethylates homocysteine (Hcy) to methionine [1]. Methionine is the immediate precursor of S-adenosylmethionine (SAM), the universal methyl donor of numerous biological methylation reactions that are essential to the synthesis of DNA, proteins, phospholipids and various neurotransmitters [2]. Recently, the relation between Hcy and lipid metabolism has been underpinned, and the proposed biochemical link between the two is the SAM-dependent formation of phosphatidylcholine from phosphatidylethanolamine [3]. Phospholipids are associated with very low-density lipoprotein assembly, thus affecting the high-density lipoprotein (HDL) pool [4]. Many studies have demonstrated that insufficient MTHFR activity confers higher serum Hcy level which has been increasingly linked to the pathogenesis of several age-related disorders, such as inherited thrombophilias [5], ischemic heart disease, stroke [6], Alzheimer’s disease (AD) [7], cognition impairment [8], and diabetes [9]. The underlying mechanism of age-related phenotypes under higher Hcy condition may point to its detrimental damage on vascular endothelial cells due to uncontrolled lipid peroxidation, enhanced oxidative stress and inflammation [10,11]. Elevated serum...
Hcy concentration (hyperhomocysteinemia) can in the main be ascribed to vitamin deficiency (e.g. folate, B12 and B6), renal dysfunction, aging or a common variance to the MTHFR gene, in which cytosine is replaced by thymidine (C > T) at base position 677. This single nucleotide polymorphism (rs1801133) causes an alanine-to-valine substitution in the MTHFR protein at polypeptide position 222 that renders the enzyme more thermolabile and less active as compared to the wild-type enzyme [12].

Collectively, a complex relationship is potentially existed among Hcy, lipid levels, MTHFR C677T polymorphism, aging, and ultimately, lifespan. The exploration of the relation between the MTHFR C677T and lipid profiles in long-lived individuals may thus help to provide insight into the biology of human aging and aging-related diseases. However, available literatures from this subfield are limited and the results are mixed. Bama long-lived individuals, a unique cohort reside along the midstream of Hongshuihe River in Guangxi Province, P.R. China, has emerged as an optimal cohort for human aging/longevity study in view of its relatively uniform genetic background over the past decades [13]. We designed the present study to test the hypothesis that the MTHFR C677T polymorphism is associated with different serum lipid profiles and may partially account for the longevity in Bama nonagenarians/centenarians of Zhuang ethnic origin.

Materials and methods

Study subjects

We screened common age-related disorders including coronary heart disease (CHD), stroke, hypertension, diabetes, cancer, gout, asthma, chronic bronchitis and chronic sinusitis for 574 nonagenarians/centenarians who have been physically living in Bama area (Bama, Fengshan, Donglan, and Du'an County) along the midstream of Hongshuihe River in Guangxi Province, P.R. China, has emerged as an optimal cohort for human aging/longevity study in view of its relatively uniform genetic background over the past decades [13]. We designed the present study to test the hypothesis that the MTHFR C677T polymorphism is associated with different serum lipid profiles and may partially account for the longevity in Bama nonagenarians/centenarians of Zhuang ethnic origin.

Epidemiological survey

Socio-demographic information was obtained using a standardized questionnaire. Anthropometric measures including height, weight and waist were measured in all groups. Body mass index (BMI) was calculated as weight (kg)/height\(^2\) (m). Sitting blood pressure measures (average of three readings) were collected using a standard mercury sphygmomanometer with the subject resting for at least 5 minutes before measurement. Systolic blood pressure was determined by the first Korotkoff sound; and diastolic, by the fifth Korotkoff sound. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg. Normal weight, overweight, and obesity were defined as a BMI <24, 24 to 28, and >28 kg/m\(^2\), respectively [14].

Biochemical measurements

A blood sample of 8 mL was collected by venipuncture from each subject after an overnight fast of >8 h, 4 mL of which was for serum separation and subsequent lipid determination while the remaining was transferred to an anticoagulant tube (4.80 g/L citric acid, 14.70 g/L glucose, and 13.20 g/L trisodium citrate) for DNA isolation. Total cholesterol (TC), triglycerides (TG), LDL-C and high density lipoprotein cholesterol (HDL-C) concentrations were measured by standard enzymatic methods using commercially available kits (Daichi Pure Chemicals Co, Ltd., Tokyo, Japan) on a biochemical analyzer (Type 7170A; Hitachi Ltd, Tokyo, Japan) at our Clinical Science Experimental Center. The normal ranges of serum TC, TG, HDL-C, and LDL-C levels in the Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, and 1.70-3.20 mmol/L, respectively. The individuals with TC >5.17 mmol/L and/or TG >1.70 mmol/L were defined as dyslipidemia [15].

Genotyping

Genomic DNA was isolated from nucleated blood cells using standard methods [16]. The nt 677 C>T variance was determined by use of the polymerase chain reaction (PCR) and HinfI restriction enzyme digestion as described by Froost et al. [17]. Briefly, a 198-bp fragment in the exon 4 of the MTHFR gene was amplified by using primers 5'-TGA AGG AGA AGG TGT CTG CCG GA-3' (forward) and 5'-AGG ACG GTG CCG TGA GAG TG-3' (reverse) (Sangon Biotech, China). PCR was
performed in a volume of 20 μL containing 200 ng of genomic DNA, 10 μL of 2× Taq MasterMix (Beijing CoWin Bioscience, China), 6.25 μM (1.0 μL) of each primer, 5 μL ddH2O and 1 U of DNA polymerase (Takara Biotechnology, DaLian, China). The mixture was initially denatured at 94°C for 2 min, followed by 33 cycles of 95°C for 30 sec, 61°C 30 sec, and 72°C 30 sec, with a final 7 min extension at 72°C. The amplified PCR products (13 μL) were digested with Hinf I (5 U) restriction endonuclease (New England Biolabs, Beijing, China) at 37°C for 4 h, and the restriction digestion products were separated on a 3% agarose gel and visualized by ethidium bromide staining. The mutant allele (677 T) gives Hinf I restriction fragments of 175 bp and 23 bp, whereas the normal allele (C677), gives a single fragment of 198 bp. To assess genotyping reliability, six randomly selected DNA samples (two for each genotype) were directly sequenced and the sequencing results were all consistent with that of genotyping. Laboratory technicians who performed genotyping were blinded to clinical and biochemical data.

Statistical analysis
Data were analyzed with the statistical package SPSS 13.0 (SPSS Inc, Chicago, IL). Levels of the quantitative variables are presented as mean ± SD. Allelic and genotypic frequencies were calculated directly. Comparison of values of general characteristics between study groups and test for Hardy-Weinberg equilibrium were performed with the Pearson chi-square test or analysis of covariance (ANCOVA). The statistical evaluation for the categorical variables was based on the calculation of the Student t-test. The association between the MTHFR C677T polymorphism and lipid variables was tested by ANCOVA. Multiple logistic analyses with stepwise modeling were used to evaluate the association of serum lipid levels with genotypes (CC = 1, CT = 2, TT = 3) and several environment factors. In all hypothesis tests, two-tailed levels with genotypes (CC = 1, CT = 2, TT = 3) and several environment factors were used to evaluate the association of serum lipid levels with genotypes (CC = 1, CT = 2, TT = 3) and several environment factors in the entire population and in LG, indicating a possible detrimental nature of the C > T transition at 677 MTHFR in lipid modulation. Considering that hyperlipidemia may be more persuasive than lipid level in interpreting the association between the lipid metabolism and thrombophilic phenotypes, we further analyzed the influence of MTHFR C677T on lipids according to lipid status. Again, no significantly different lipid level was observed among the three genotypes in the hyperlipidemic and normolipidemic subgroup of LG and the hyperlipidemic subgroup of non-LG, with an exception of a moderate lower TC and lower LDL-C were noted in subjects harboring

Table 1 Subject characteristics

|                        | LG (n = 516) | non-LG (n = 493) | F (t or χ²) | P  |
|------------------------|-------------|-----------------|------------|----|
| Age (year)             | 93.23 ± 2.95 | 67.19 ± 4.70    | 104.823    | 0.000 |
| Male/female            | 127/389     | 142/351         | 2.265      | 0.132 |
| BMI (kg/m²)            | 20.40 ± 3.62 | 21.40 ± 3.11    | 5.161      | 0.023 |
| SBP (mmHg)             | 166.72 ± 27.65 | 135.87 ± 23.25 | 1.927      | 0.165 |
| DBP (mmHg)             | 89.36 ± 13.58 | 83.15 ± 11.10  | 0.931      | 0.335 |
| TC (mmol/L)            | 5.15 ± 1.03   | 4.97 ± 0.96     | 0.522      | 0.470 |
| TG (mmol/L)            | 0.98 (0.48)   | 0.91 (0.59)     | 1.979      | 0.160 |
| HDL-C (mmol/L)         | 1.60 ± 0.38   | 1.71 ± 0.41     | 0.841      | 0.359 |
| LDL-C (mmol/L)         | 3.05 ± 0.87   | 2.73 ± 0.88     | 0.018      | 0.892 |
| Dyslipidemia n (%)     | 261 (50.58)   | 227 (46.04)     | 2.078      | 0.149 |
| Hypertension n (%)     | 456 (88.37)   | 231 (46.86)     | 200.000    | 0.000 |

Note: LG, long-lived group; non-LG, non-long-lived group; BMI: body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. TG values were presented as median (interquartile range) while other measures were presented as mean ± SD. The difference between the two groups was determined by analysis of covariance.

Results
General characteristics and serum lipid levels
The basic demographic, clinical and biochemical data of long-lived individuals and controls are presented in Table 1. After adjusting for covariates, BMI was significantly lower while the hypertension rate was significantly higher in LG as compared to non-LG group (P = 0.002 and 0.001, respectively), with T allele and TT genotype presenting more highly in LG than in non-LG. These differences persist in females but not in males after sex stratification. In LG, almost equal genotypic and allelic frequency of MTHFR C677T was observed between sexes, while in non-LG, men presented higher T allele and TT genotype than women.

MTHFR C677T polymorphism
Allele and genotype frequencies in both LG and non-LG are described in Table 2. The genotypes within each group were distributed in accordance with the Hardy-Weinberg equilibrium. The overall prevalence of genotypes and alleles were significantly different between the two groups (P = 0.002 and 0.001, respectively), with T allele and TT genotype presenting more highly in LG than in non-LG. These differences persist in females but not in males after sex stratification. In LG, C677T was observed between sexes, while in non-LG, men presented higher T allele and TT genotype than women.
CT or TT genotype as compared with CC genotype in the normolipidemic subgroup of non-LG (p = 0.001 and 0.005, respectively, Table 4).

Correlation between serum lipid parameters and genotypes
Multiple linear regression analyses showed that TC, TG and LDL-C were positively while HDL-C was negatively correlated in the main with age, diastolic blood pressure and BMI but not with MTHFR C677T genotypes in the overall population, i.e. the older of age and the higher of diastolic blood pressure and BMI, the higher of unfavorable lipids (Table 5). After sex stratification, lipids, TC and TG in particular, correlated with not only the above mentioned factors, but also with MTHFR C677T genotypes in both sexes. When LG and non-LG were analyzed separately, the correlation between lipids and MTHFR C677T genotype exist mainly in the females of LG. These findings are basically in line with that in Table 1 and Table 3.

Discussion
In the present study, the prevalence of MTHFR 677 T was 17.44% and 14.50% in the combined samples and control population respectively, with a lower frequency of TT homozygote (=4%). These allelic and genotypic frequencies are close to that of Bai Ku Yao (22.6%) whereas much lower than that of Guangxi Han Chinese (39.1%), two neighboring populations we reported recently [18].

It has been noted that the distribution of the T allele vary substantially across general populations worldwide, with a north-to-south increase in European continent but a reverse gradient in China mainland [19-21]. Zhuang and Bai Ku Yao are typical aboriginal ethnic groups in Southern China, our results thus defer somewhat to this pattern. The underlying mechanism for this great geographical diversity remains unclear, adaptation to external conditions such as climate or nutritional status could be one explanation [22]. Further investigation of gene-gene and gene-environment interaction might help to determine the

| Group | N  | Genotype n (%) | Allele n (%) |
|-------|----|----------------|--------------|
|       |    | CC  | CT  | TT | C  | T  |
| All   | 1009 | 700 (69.38) | 266 (26.36) | 43 (4.26) | 1666 (82.56) | 352 (17.44) |
| LG    | 516  | 332 (64.34) | 159 (30.81) | 26 (4.84)  | 823 (79.75)  | 209 (20.25)  |
| non-LG| 493  | 368 (74.65) | 107 (21.70) | 18 (3.65)  | 843 (85.50)  | 143 (14.50)  |

\[ \chi^2 \]
- 12.639
- 0.002

\[ P \]
- 0.001

| Group | N  | Genotype n (%) | Allele n (%) |
|-------|----|----------------|--------------|
|       |    | CC  | CT  | TT | C  | T  |
| LG    | 127  | 83 (65.35) | 35 (27.56) | 9 (7.09)  | 201 (79.13)  | 53 (20.87)  |
| Female| 389  | 249 (64.01) | 124 (31.88) | 16 (4.11) | 622 (79.95) | 156 (20.05) |

\[ \chi^2 \]
- 2.353
- 0.308

\[ P \]
- 0.779

| Group | N  | Genotype n (%) | Allele n (%) |
|-------|----|----------------|--------------|
|       |    | CC  | CT  | TT | C  | T  |
| LG    | 142  | 95 (66.90) | 38 (26.76) | 9 (6.32)  | 228 (80.28)  | 56 (19.72)  |
| Female| 351  | 273 (77.78) | 69 (19.66) | 9 (2.56)  | 615 (87.61)  | 87 (12.39)  |

\[ \chi^2 \]
- 7.896
- 0.198

\[ P \]
- 0.741

| Group | N  | Genotype n (%) | Allele n (%) |
|-------|----|----------------|--------------|
|       |    | CC  | CT  | TT | C  | T  |
| LG    | 127  | 83 (65.35) | 35 (27.56) | 9 (7.09)  | 201 (79.13)  | 53 (20.87)  |
| non-LG| 142  | 95 (66.90) | 38 (26.76) | 9 (6.34)  | 228 (80.28)  | 56 (19.72)  |

\[ \chi^2 \]
- 0.096
- 0.953

\[ P \]
- 0.741

| Group | N  | Genotype n (%) | Allele n (%) |
|-------|----|----------------|--------------|
|       |    | CC  | CT  | TT | C  | T  |
| LG    | 389  | 249 (64.01) | 124 (31.88) | 16 (4.11) | 622 (79.95) | 156 (20.05) |
| non-LG| 351  | 273 (77.78) | 69 (19.66) | 9 (2.56)  | 615 (87.61) | 87 (12.39)  |

\[ \chi^2 \]
- 16.830
- 2.22E-4

\[ P \]
- 2.22E-5
Table 3 Impact of MTHFR C677T on serum lipid levels (mmol/L)

| Group | Genotype | n  | TC (mmol/L) | TG (mmol/L) | HDL-C (mmol/L) | LDL-C (mmol/L) |
|-------|----------|----|-------------|-------------|----------------|---------------|
| All   | CC       | 700| 5.04 ± 0.97 | 0.94 (0.52) | 1.66 ± 0.39    | 2.86 ± 0.90   |
|       | CT       | 266| 5.15 ± 1.01 | 0.98 (0.53) | 1.63 ± 0.39    | 3.01 ± 0.84   |
|       | TT       | 43 | 4.86 ± 1.31 | 0.88 (0.41) | 1.61 ± 0.46    | 2.70 ± 0.98   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
|       | CC       | 700| 5.04 ± 0.97 | 0.94 (0.52) | 1.66 ± 0.39    | 2.86 ± 0.90   |
|       | CT/TT    | 309| 5.11 ± 1.06 | 0.96 (0.50) | 1.64 ± 0.41    | 2.97 ± 0.87   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
| Male  | CC       | 178| 5.00 ± 1.04 | 0.95 (0.70) | 1.61 ± 0.41    | 2.84 ± 0.96   |
|       | CT       | 73 | 4.78 ± 0.97 | 0.87 (0.48) | 1.59 ± 0.42    | 2.72 ± 0.83   |
|       | TT       | 18 | 4.70 ± 0.98 | 0.83 (0.45) | 1.68 ± 0.57    | 2.58 ± 0.73   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
| Female| CC       | 522| 5.05 ± 0.95 | 0.93 (0.49) | 1.68 ± 0.38    | 2.87 ± 0.88   |
|       | CT       | 193| 5.29 ± 0.99 | 1.02 (0.62) | 1.65 ± 0.38    | 3.12 ± 0.82   |
|       | TT       | 25 | 4.97 ± 1.52 | 1.01 (0.43) | 1.61 ± 0.46    | 2.79 ± 1.13   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
|       | CC       | 522| 5.05 ± 0.95 | 0.93 (0.49) | 1.68 ± 0.38    | 2.87 ± 0.88   |
|       | CT/TT    | 218| 5.26 ± 1.06 | 1.02 (0.53) | 1.65 ± 0.39    | 3.08 ± 0.86   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
| LG    | CC       | 332| 5.12 ± 1.00 | 0.97 (0.49) | 1.59 ± 0.37    | 3.04 ± 0.87   |
|       | CT       | 159| 5.22 ± 1.06 | 0.99 (0.50) | 1.61 ± 0.38    | 3.09 ± 0.86   |
|       | TT       | 25 | 5.13 ± 1.31 | 1.07 (0.44) | 1.56 ± 0.51    | 3.01 ± 0.96   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
|       | CC       | 332| 5.12 ± 1.00 | 0.97 (0.49) | 1.59 ± 0.37    | 3.04 ± 0.87   |
|       | CT/TT    | 184| 5.20 ± 1.09 | 1.00 (0.48) | 1.61 ± 0.40    | 3.08 ± 0.88   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
| Male  | CC       | 83 | 5.00 ± 1.11 | 0.94 (0.39) | 1.57 ± 0.41    | 2.96 ± 0.95   |
|       | CT       | 35 | 4.67 ± 1.11 | 0.87 (0.29) | 1.54 ± 0.39    | 2.67 ± 0.97   |
|       | TT       | 9  | 4.71 ± 1.09 | 0.84 (0.53) | 1.55 ± 0.64    | 2.76 ± 0.77   |
|       | F        | —  | —            | —            | —               | —             |
|       | p        | —  | —            | —            | —               | —             |
### Table 3 Impact of MTHFR C677T on serum lipid levels (mmol/L) (Continued)

|     | CC     | CT/TT  | F     | p     |
|-----|--------|--------|-------|-------|
|     | 83     | 44     | 0.100 | 0.120 |
| **CC** | **5.00 ± 1.11** | **4.67 ± 1.09** | **2.749** | **2.749** |
| **CT/TT** | **5.37 ± 1.00** | **5.37 ± 1.40** | **2.179** | **2.179** |
| **F** | 0.94 (0.39) | 0.87 (0.34) | 1.398 | 2.148 |
| **p** | 1.57 ± 0.41 | 1.54 ± 0.44 | 0.158 | 0.605 |
| **Female** | **1.57 ± 0.41** | **1.54 ± 0.44** | **0.546** | **0.306** |
| **CT** | 249    | 124    | 0.037 | 0.130 |
| **TT** | 16     | 16     | 1.017 | 0.010 |
| **F** | 5.15 ± 0.96 | 5.15 ± 0.96 | 2.135 | 0.728 |
| **p** | 0.98 (0.52) | 0.98 (0.52) | 0.119 | 0.483 |
| **Non-LG** | **1.60 ± 0.35** | **1.63 ± 0.38** | **0.483** | **0.010** |
| **CC** | 368    | 107    | 0.137 | 0.128 |
| **CT** | 18     | 18     | 1.017 | 0.010 |
| **TT** | 16     | 16     | 1.017 | 0.010 |
| **F** | 4.97 ± 0.95 | 5.06 ± 0.92 | 2.135 | 0.728 |
| **p** | 0.91 (0.60) | 0.95 (0.59) | 0.119 | 0.483 |
| **Male** | **1.73 ± 0.40** | **1.75 ± 0.49** | **0.483** | **0.010** |
| **CC** | 95     | 38     | 0.037 | 0.130 |
| **CT** | 9      | 9      | 0.050 | 0.384 |
| **TT** | 18     | 18     | 0.050 | 0.384 |
| **F** | 4.99 ± 0.99 | 4.89 ± 0.83 | 1.372 | 1.096 |
| **p** | 0.97 (0.91) | 0.89 (0.70) | 0.257 | 0.337 |
| **Female** | **1.63 ± 0.41** | **1.81 ± 0.49** | **0.337** | **0.010** |
| **CC** | 273    | 69     | 0.415 | 0.646 |
| **CT** | 69     | 69     | 0.415 | 0.646 |
| **TT** | 9      | 9      | 0.415 | 0.646 |
| **F** | 4.96 ± 0.94 | 5.15 ± 0.96 | 1.372 | 1.096 |
| **p** | 0.90 (0.52) | 1.00 (0.49) | 0.257 | 0.337 |

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### Table 4 Impact of MTHFR C677T on serum lipid levels according to lipid status (mmol/L)

| Group       | Genotype | n   | TC          | TG          | HDL-C       | LDL-C       |
|-------------|----------|-----|-------------|-------------|-------------|-------------|
| **LG**      |          |     |             |             |             |             |
| **Dyslipidemia** | CC       | 167 | 5.84 ± 0.76 | 1.14 (0.69) | 1.68 ± 0.39 | 3.57 ± 0.74 |
|             | CT       | 84  | 5.96 ± 0.75 | 1.16 (0.71) | 1.70 ± 0.41 | 3.66 ± 0.70 |
|             | TT       | 10  | 6.30 ± 1.14 | 1.13 (1.10) | 1.72 ± 0.66 | 3.80 ± 0.89 |
|             | F        | —   | 1.265       | 0.120       | 0.501       | 0.431       |
|             | p        | —   | 0.284       | 0.887       | 0.607       | 0.605       |
|             | CC       | 167 | 5.84 ± 0.76 | 1.14 (0.69) | 1.68 ± 0.39 | 3.57 ± 0.74 |
|             | CT/TT    | 89  | 5.99 ± 0.81 | 1.16 (0.72) | 1.71 ± 0.44 | 3.67 ± 0.72 |
|             | F        | —   | 1.001       | 0.041       | 0.232       | 0.625       |
|             | p        | —   | 0.318       | 0.839       | 0.631       | 0.430       |
| **Normolipidemia** | CC       | 165 | 4.38 ± 0.58 | 0.85 (0.33) | 1.51 ± 0.32 | 2.49 ± 0.62 |
|             | CT       | 75  | 4.38 ± 0.65 | 0.85 (0.28) | 1.51 ± 0.32 | 2.45 ± 0.52 |
|             | TT       | 15  | 4.35 ± 0.69 | 0.94 (0.47) | 1.45 ± 0.36 | 2.48 ± 0.57 |
|             | F        | —   | 0.021       | 0.228       | 0.240       | 0.222       |
|             | p        | —   | 0.979       | 0.796       | 0.786       | 0.801       |
|             | CC       | 165 | 4.38 ± 0.58 | 0.85 (0.33) | 1.51 ± 0.32 | 2.49 ± 0.62 |
|             | CT/TT    | 90  | 4.38 ± 0.65 | 0.85 (0.34) | 1.50 ± 0.33 | 2.46 ± 0.53 |
|             | F        | —   | 0.029       | 0.350       | 0.109       | 0.149       |
|             | p        | —   | 0.805       | 0.555       | 0.742       | 0.700       |
| **Non-LG**  |          |     |             |             |             |             |
| **Dyslipidemia** | CC       | 168 | 5.65 ± 0.92 | 1.16 (0.98) | 1.71 ± 0.45 | 3.19 ± 1.00 |
|             | CT       | 53  | 5.77 ± 0.59 | 1.11 (1.06) | 1.68 ± 0.43 | 3.43 ± 0.61 |
|             | TT       | 6   | 5.85 ± 0.39 | 0.85 (0.55) | 2.19 ± 0.49 | 3.12 ± 0.46 |
|             | F        | —   | 0.307       | 1.270       | 1.880       | 0.501       |
|             | p        | —   | 0.736       | 0.283       | 0.155       | 0.606       |
|             | CC       | 146 | 5.71 ± 0.66 | 1.16 (0.98) | 1.71 ± 0.41 | 3.43 ± 0.64 |
|             | CT/TT    | 58  | 5.76 ± 0.58 | 1.08 (1.13) | 1.70 ± 0.42 | 3.45 ± 0.58 |
|             | F        | —   | −0.530      | −0.443      | 0.121       | −0.148      |
|             | p        | —   | 0.597       | 0.658       | 0.903       | 0.883       |
| **Normolipidemia** | CC       | 200 | 4.40 ± 0.50 | 0.80 (0.34) | 1.74 ± 0.36 | 2.29 ± 0.52 |
|             | CT       | 54  | 4.36 ± 0.58 | 0.69 (0.32) | 1.65 ± 0.39 | 2.37 ± 0.56 |
|             | TT       | 12  | 3.79 ± 0.90 | 0.79 (0.24) | 1.53 ± 0.33 | 1.86 ± 0.70 |
|             | F        | —   | 7.662       | 0.987       | 1.272       | 5.337       |
|             | p        | —   | 0.001       | 0.374       | 0.282       | 0.005       |
|             | CC       | 200 | 4.40 ± 0.50 | 0.80 (0.34) | 1.74 ± 0.36 | 2.29 ± 0.52 |
|             | CT/TT    | 66  | 4.26 ± 0.68 | 0.75 (0.33) | 1.63 ± 0.38 | 2.27 ± 0.61 |
|             | F        | —   | 1.478       | 1.202       | 1.339       | 0.156       |
|             | p        | —   | 0.225       | 0.274       | 0.248       | 0.694       |
| Group/Lipid | Relative factor | Unstandardized coefficients | Standardized coefficients | t       | P        |
|------------|----------------|----------------------------|---------------------------|---------|----------|
|            |                | B  | Std. error | Beta  |          |         |
| All        | TC             | 0.10 | 0.03  | 0.123 | 3.802  | 1.52E-4 |
|            | BMI            | 0.035 | 0.009 | 0.121 | 3.794  | 1.57E-4 |
|            | Sex            | 0.207 | 0.070 | 0.091 | 2.960  | 0.003   |
|            | Age            | 0.005 | 0.002 | 0.067 | 2.073  | 0.038   |
|            | TG             | 0.016 | 0.002 | 0.265 | 8.754  | 8.59E-18 |
|            | BMI            | 0.001 | 0.000 | 0.094 | 3.099  | 0.002   |
|            | Sex            | 0.068 | 0.028 | 0.076 | 2.437  | 0.014   |
|            | Age            | 0.011 | 0.002 | 0.169 | 5.247  | 1.88E-7 |
|            | BMI            | 0.008 | 0.002 | 0.115 | 3.599  | 3.35E-4 |
|            | Sex            | 0.027 | 0.008 | 0.102 | 3.228  | 0.001   |
|            | Age            | 0.137 | 0.062 | 0.068 | 2.230  | 0.026   |
| Male       | TG             | 0.010 | 0.004 | 0.157 | 2.593  | 0.010   |
|            | Age            | -0.002 | 0.001 | -0.142 | -2.343 | 0.020   |
|            | Sex            | -0.042 | 0.020 | -0.127 | -2.136 | 0.034   |
|            | HDL-C          | -0.029 | 0.008 | -0.225 | -3.700 | 2.62E-4 |
|            | Age            | -0.005 | 0.002 | -0.153 | -2.522 | 0.012   |
| Female     | TC             | 0.009 | 0.003 | 0.117 | 3.052  | 0.002   |
|            | BMI            | 0.046 | 0.011 | 0.160 | 4.311  | 1.85E-5 |
|            | Age            | 0.009 | 0.003 | 0.118 | 3.078  | 0.002   |
|            | BMI            | 0.019 | 0.001 | 0.327 | 9.317  | 1.35E-19|
|            | Age            | 0.002 | 0.001 | 0.169 | 4.822  | 1.73E-6 |
|            | HDL-C          | -0.005 | 0.001 | -0.183 | -5.013 | 6.71E-7 |
|            | BMI            | -0.015 | 0.004 | -0.132 | -3.601 | 3.38E-4 |
|            | Age            | 0.013 | 0.002 | 0.199 | 5.176  | 2.94E-7 |
|            | BMI            | 0.030 | 0.009 | 0.117 | 3.184  | 0.002   |
|            | DBP            | 0.007 | 0.003 | 0.105 | 2.759  | 0.006   |
|            | Genotype       | 0.139 | 0.069 | 0.072 | 2.006  | 0.045   |
| LG         | TC             | 0.012 | 0.003 | 0.160 | 3.699  | 3.40E-4 |
|            | Sex            | 0.323 | 0.103 | 0.135 | 3.130  | 0.002   |
|            | TG             | 0.007 | 0.002 | 0.160 | 3.708  | 2.32E-4 |
|            | Sex            | 0.046 | 0.016 | 0.120 | 2.779  | 0.006   |
|            | HDL-C          | -0.015 | 0.005 | -0.145 | -3.312 | 0.001   |
|            | BMI            | 0.001 | 0.001 | 0.103 | 2.359  | 0.019   |
|            | DBP            | 0.008 | 0.003 | 0.124 | 2.857  | 0.004   |
|            | Sex            | 0.232 | 0.088 | 0.115 | 2.639  | 0.009   |
| Male       | TC             | 0.015 | 0.007 | 0.181 | 2.056  | 0.042   |
evolutionary pressures favouring a high prevalence of this variant in certain areas and ethnic groups. Intriguingly, MTHFR 677 T and its relevant genotypes (CT, TT) were significantly more prevalent in our nonagenarian and centenarian populations, especially in females, as compared with the elder control group (60–75 years). These observations implicate a potential association between MTHFR C677T polymorphism and longevity in Bama area. A question of whether this variant is favorable or deleterious for Bama long-lived individuals therefore emerges. According to most but not all reported data, MTHFR 677 T genotypes have been linked to unfavorable lipid profiles, including greater concentrations of TC, TG, and LDL-C [18,23-25] and lower level of HDL-C [26,27], all known risk factors for cardiovascular and metabolic diseases. Herein, the female but not male T-allele carriers in the pooled population and in LG presented greater level of TC, TG and LDL-C than did T noncarriers (Table 3 and Table 5), in agreement with prevailing data as aforementioned, indicating that MTHFR 677 T genotypes may play detrimental rather than beneficial role in lipid modulation and survivorship. However, this influence seems to be limited because the impact of MTHFR 677 T on lipid metabolism remained only in the normolipidemic subgroup of non-LG after further analysis according to lipid status categorization (Table 4). Together, these data suggest that although MTHFR C677T affects the TC and LDL-C

| Table 5 Correlation between serum lipid parameters and the MTHFR C677T polymorphism (Continued) |
|---------------------------------------------------------------|
| **HDL-C** | **BMI** | −0.026 | 0.011 | −0.217 | −2.480 | 0.014 |
| **LDL-C** | **DBP** | 0.014 | 0.006 | 0.200 | 2.277 | 0.024 |
| **Female** | | | | | | |
| **TC** | **DBP** | 0.011 | 0.004 | 0.154 | 3.072 | 0.002 |
| **Genotype** | | 0.215 | 0.103 | 0.104 | 2.087 | 0.038 |
| **TG** | **BMI** | 0.029 | 0.007 | 0.197 | 3.974 | 8.45E-5 |
| **Genotype** | | 0.117 | 0.047 | 0.124 | 2.511 | 0.012 |
| **HDL-C** | **Age** | 0.014 | 0.006 | 0.111 | 2.204 | 0.028 |
| **Non-LG** | | | | | | |
| **TC** | **BMI** | 0.081 | 0.013 | 0.263 | 6.047 | 2.92E-9 |
| **TG** | **BMI** | 0.097 | 0.013 | 0.324 | 7.598 | 1.54E-13 |
| **HDL-C** | **BMI** | −0.019 | 0.006 | −0.149 | −3.314 | 0.001 |
| **SBP** | **BMI** | −0.002 | 0.001 | −0.118 | −2.636 | 0.009 |
| **Sex** | **BMI** | 0.084 | 0.040 | 0.094 | 2.133 | 0.033 |
| **LDL-C** | **BMI** | 0.067 | 0.013 | 0.237 | 5.304 | 1.72E-7 |
| **Male** | | | | | | |
| **TC** | **BMI** | 0.061 | 0.027 | 0.189 | 2.307 | 0.023 |
| **Age** | | −0.046 | 0.020 | −0.186 | −2.265 | 0.025 |
| **TG** | **Age** | −0.064 | 0.021 | −0.247 | −3.066 | 0.003 |
| **BMI** | **Age** | 0.064 | 0.027 | 0.188 | 2.336 | 0.021 |
| **HDL-C** | **BMI** | −0.033 | 0.012 | −0.222 | −2.698 | 0.008 |
| **LDL-C** | **BMI** | 0.064 | 0.025 | 0.212 | 2.569 | 0.011 |
| **Female** | | | | | | |
| **TC** | **BMI** | 0.087 | 0.016 | 0.285 | 5.553 | 5.57E-8 |
| **TG** | **BMI** | 0.103 | 0.014 | 0.364 | 7.294 | 2.03E-12 |
| **HDL-C** | **SBP** | −0.003 | 0.001 | −0.166 | −3.126 | 0.002 |
| **BMI** | **SBP** | −0.015 | 0.007 | −0.121 | −2.281 | 0.023 |
| **LDL-C** | **BMI** | 0.060 | 0.015 | 0.216 | 3.983 | 8.28E-5 |
| **Age** | **BMI** | 0.029 | 0.009 | 0.162 | 3.093 | 0.002 |
| **DBP** | **BMI** | 0.012 | 0.004 | 0.139 | 2.632 | 0.009 |

Note: DBP, Diastolic blood pressure; SBP, Systolic blood pressure; BMI, Body mass index.
metabolism of LG and the overall population studied to varying degree, particularly of the females in LG, these impacts may not be sufficient to cause extremely lipid abnormality.

With regard to the enrichment of deleterious genotypes in long-lived females, it might be partially interpreted by the Buffering Mechanisms in Aging hypothesis proposed recently by Bergman and colleagues who reasoned that in a subpopulation endowed with a favorable longevity genotype(s), the prevalence of a deleterious genotype is expected not to vary or even increase with age because the longevity genotype may buffer out or modulate the harmful effect of deleterious ones, while in a subpopulation lacking longevity genotypes, the prevalence of a deleterious genotype will decrease with age since subjects with this genotype are weeded out due to mortality [28,29]. Therefore, screening for potential longevity genotypes such as CETP (VV) (rs5882), APOC3 (CC) (rs2542052), AdipoQ (del/del APM1 + 2019) (rs56354395), and FOXO3a (GG), which have been demonstrated in other populations [30-33], will be one of our next efforts in the near future.

Due to the established link among MTHFR C677T polymorphism, Hcy, cardiovascular risk and aging, several investigations have been conducted in some elder cohorts to look at the possible contribution of MTHFR to longevity. However, findings are still inconsistent thus far. For instance, lower frequency of MTHFR C677T +/+ genotype, with raised Hcy level, was observed in French cohort with longevity trait (> 90 yrs, n = 564) than in controls (< 70 yrs, n = 374), albeit no statistical significance was reached [34]. Similar observations were made in Swiss (106 elderly, 68–95 yrs vs 118 younger, 21–64 yrs) [35], Japanese (148 oldest, > 80 yrs including 22 nonagenarians vs 311 younger, < 55 yrs) [36] and Jerusalem Ashkenazi (224 elderly, > 75 yrs vs 441 controls, < 22 yrs) [37] populations, while almost equal prevalence of MTHFR C677T genotypes were seen between elderly and younger group in Swede (222 elderly, 80–108 yrs vs 220 newborn) [38], British (282 elderly, > 84 yrs vs 200 younger, < 17 yrs) [39], and Jordanian (130 elderly, > 85 yrs, mean age 90.01 yrs vs 135 younger, 20–50 yrs, mean age 33.34 yrs) [40] population. Conversely, we detected a higher prevalence of MTHFR 677 T in our oldest olds. Although it is known that MTHFR TT might be a risk biomarker against longevity from other perspectives, we cannot exclude the possibility that it may also be in favor of good health or long life span as implicated by Le Marchand et al. that there might be an inverse association between MTHFR 677TT genotype and the development of colorectal cancer [41]. To the best of our knowledge, the nonagenarian population here is the only long-lived cohort that enriches this variant, whose significance deserves further clarification. These discrepancies may arise from factors related to differences in ethnic background of the population studied, recruitment strategy for long-lived and control group, assessment methods or sample sizes.

Despite strengths such as large sample size, highly population genetic homogeneity and well-characterized cases and controls, all the difference of lipid profiles between long-lived individuals and controls cannot completely attribute to MTHFR C677T. The current study should be viewed in the light of some limitations: (1) no determination of folate status, serum MTHFR activity and Hcy levels on baseline data collection due to insufficient funding, which would be more significantly conclusive and would help to interpret the selection and the outcome of MTHFR 677 T [22,42]; (2) lack of the evaluation of other modifying genetic variants, risk factors such as smoking and alcohol consumption and lifestyle which may interact with MTHFR C677T and change its association with longevity; (3) we could not completely exclude asymptomatic disorders such as atherosclerosis which may create a potentially significant bias due to poor field study condition; (4) it is very much speculative to imagine the controls would have a short life span because of a specific family history, this may also be a significant bias factor. (5) longitudinal follow up is warranted in further interpreting the potential effect of MTHFR C677T polymorphism on lipid metabolism.

Conclusions
Overall, our results show that MTHFR 677 T allele and TT genotype are accumulative in Bama long-living individuals in a gender-specific manner and are associated with unfavorable lipid profile although this impact on lipid modulation seems limited. Potential longevity gene(s) which may interact with or buffer out the deleterious effect of this variant needs to be determined.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
NYC participated in the design, undertook genotyping, and drafted the manuscript. CWL, LLD, LPX, LG, YYW and ZW helped with genotyping. HYW, CYL, LL, JHP, XQL, RXY and CPN took part in the epidemiological survey, collected the samples, and helped to draft the manuscript. SLP conceived the study, participated in the design, carried out the epidemiological survey, collected the samples, and helped to draft the manuscript. All authors read and approved the final manuscript.

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References

1. Selhub J, Miller JW: The pathogenesis of homocysteinemia: interruption of the coordinate regulation by S-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1991, 55:131–138.

2. Almeida OP, Flicker L, Lautenschlager NT, Leedman P, Vasikaran S, van Stee R: Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life. Neurobiol Aging 2005, 26:251–257.

3. Obeid R, Herrmann W: Homocysteine and lipids: S-adenosyl methionine as a key intermediate. FEBS Lett 2000, 538:1215–1225.

4. Chapman MJ, Ginsberg HN, Amarencu P, Andreotti F, Boren J, Catapano AL, Descamps OS, Fisher E, Kovanen PT, Kuivenhoven JA, Lensink P, Masana L, Nordengaard B, Ray KK, Reiner Z, Tskhovrebov MR, Tokgozoglu L, Tytgat-Hansen A, Watts GF: European Atherosclerosis Society Consensus Panel, European Atherosclerosis Society Consensus Panel, Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. Eur Heart J 2011, 32:1345–1361.

5. Jarvenpaa J, Pakkila M, Savolainen ER, Perheentupa A, Jarvela I, Ryynanen M: Evaluation of factor V Leiden, prothrombin and methylenetetrahydrofolate reductase gene mutations in patients with severe pregnancy complications in northern Finland. Gynecol Obstet Invest 2006, 62:28–32.

6. Kerk M, Verhoeft P, Clarke R, Blom HJ, Kool FC, Schouten EG, MTHFR Studies Collaboration Group: MTHFR 677CT polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 2002, 288:2033–2031.

7. Wang B, Jin F, Kan R, Ji S, Zhuang C, Lu Z, Zheng C, Yang Z, Wang L: Association of MTHFR gene polymorphism C677T with susceptibility to late-onset Alzheimer’s disease. J Mol Neurosci 2005, 27:23–27.

8. Ford AH, Flicker L, Hankey GJ, Norman P, van Bockxmeer FM, Almeida OP: Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism and cognitive impairment: the health in men study. Mol Psychiatry 2012, 17:559–566.

9. Ramirez M, Hasanvand A, Rahimi Z, Vaisi-Raygani A, Mozaffari H, Rezaei M, Zargoschi J, Najafi F, Shakiba E: Synergistic Effects of the MTHFR C677T polymorphism and risk of coronary artery disease: evidence and guidance for management. Eur J Clin Invest 2010, 40:1335–1339.

10. Okawa S, Murakami K, Kawanishi S: Oxidative damage to cellular and isolated DNA by homocysteine: implications for carcinogenesis. Oncogene 2003, 22:3530–3538.

11. Jamaluddin MS, Yang X, Wang H: Hyperhomocysteinemia, DNA methylation and vascular disease. Clin Chem Lab Med 2007, 45:1660–1666.

12. Kang SS, Zhou J, Wang PW, Kowalsky J, Strokosch G: Intermediate homocysteinemia: a heritable variant of methylenetetrahydrofolate reductase. Am J Hum Genet 1988, 43:414–421.

13. Pan SL, Luo XQ, Lu ZP, Lu SH, Luo H, Liu CW, Hu CY, Yang M, Du LL, Song Z, Pang GF, Wu HY, Huang JB, Peng JH, Yin RX: Microsomal triglyceride transfer protein gene -493GT polymorphism and its association with serum lipid levels in Bama Zhuang long-living families in ethnic minority of China. Lipids Health Dis 2011, 10:177.

14. Cooperative Meta-analysis Group of China Obesity Task Force: Predictive values of body mass index and waist circumference to risk factors of related diseases in Chinese adult population. Chin J Epidemiol 2002, 23:5–10.

15. Ruixing Y, Yuming C, Shangling P, Fengbing H, Tangwei L, Deshai Y, Jianwen W, Limei Y, Weixong L, Rongshan L, Jiandong H: Effects of demographic, dietary, and other lifestyle factors on the prevalence of hyperlipidemia in Guangxi Hei Yi Zhuang and Han populations. Eur J Cardiovasc Prev Rehabil 2006, 13:977–984.

16. Miller SA, Dykes DD, Polesky HF: A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988, 16:2115.
36. Matsushita S, Muramatsu T, Arai H, Matsui T, Higuchi S: The frequency of the methylenetetrahydrofolate reductase-gene mutation varies with age in the normal population. Am J Hum Genet 1997, 61:1459–1460.

37. Stessman J, Maaravi Y, Hammerman-Rozenberg R, Cohen A, Nemanov L, Gritsenko I, Gruberman N, Ebstein RP: Candidate genes associated with ageing and life expectancy in the Jerusalem longitudinal study. Mech Ageing Dev 2005, 126:333–339.

38. Brattström L, Zhang Y, Hurtig M, Refsum H, Ostensson S, Fransson L, Jonés K, Landgren F, Brudin L, Ueland PM: A common methylenetetrahydrofolate reductase gene mutation and longevity. Atherosclerosis 1998, 141:315–319.

39. Galinsky D, Tysoe C, Brayne CE, Easton DF, Huppert FA, Dening TR, Paykel ES, Rubinsztein DC: Analysis of the apo E/apo C-I, angiotensin converting enzyme and methylenetetrahydrofolate reductase genes as candidates affecting human longevity. Atherosclerosis 1997, 129:177–183.

40. Khabour OF, Abdelhalim ES, Abu-Wardeh A: Association between SOD2 T-9C and MTHFR C677T polymorphisms and longevity: a study in Jordanian population. BMC Genet 2009, 9:57.

41. Le Marchand L, Wilkens LR, Kolonel LN, Henderson BE: The MTHFR C677T polymorphism and colorectal cancer: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2005, 14:1198–1203.

42. Jennings BA, Willis GA, Skinner J, Retel CL: Genetic selection? A study of individual variation in the enzymes of folate metabolism. BMC Med Genet 2010, 11:18.

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