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**Original Paper**

# Associations of Common Variants in Methionine Metabolism Pathway Genes with Plasma Homocysteine and the Risk of Type 2 Diabetes in Han Chinese

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**Key Words**
Methionine · Homocysteine · Type 2 diabetes mellitus · Single nucleotide polymorphisms

**Abstract**
***Background/Aims:*** An association of genetic variants of homocysteine (Hcy) metabolic genes with type 2 diabetes mellitus (T2DM) has been reported. The objective of the present study was to investigate the relationship between the genetic variants in Hcy metabolism-related genes and plasma Hcy levels and T2DM susceptibility in Han Chinese. ***Methods:*** A total of 774 patients with T2DM and 500 healthy individuals were recruited. Single-nucleotide polymorphism was determined by standard methods. ***Results:*** The Hcy-increasing allele score was positively associated with plasma Hcy levels in both T2DM patients and healthy subjects (r = 0.171 and 0.247, respectively). Subjects with the genotype CC of *MTHFR* (rs1801131) had a significantly higher likelihood of T2DM compared with subjects with the AA or AA+AC genotypes (OR = 1.93 for CC vs. AA, p = 0.041; OR = 3.13 for CC vs. AA+AC, p = 0.017, respectively). Subjects with the genotype AA of the *MTHFD* variant (rs2236225) had a significantly lower likelihood of T2DM compared with subjects with the GG or GG+GA genotypes (OR = 0.36 for AA vs. GG, p = 0.027; OR = 0.36 for AA vs. GG+GA, p = 0.017, respectively). In addition, the genotype CT+TT of the *PEMT* (rs4646356) variants displayed a significant association with an increased risk of T2DM (OR = 1.52 for CT+TT vs. CC, p = 0.042). ***Conclusions:*** *MTHFR* rs1801131 C allele and *PEMT* rs4646356 T allele were associated with a high risk of T2DM in these Han Chinese.


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## Introduction

Hyperhomocysteinemia (HHcy) is associated with cardiovascular disease and diabetic complications [1, 2]. The etiology of HHcy involves both nutritional (e.g. folate, vitamin $B_{12}$, or vitamin $B_6$) and genetic factors [1]. Rare mutations of methylenetetrahydrofolate reductase *(MTHFR)*, methionine synthase *(MS)* and cystathionine β-synthase *(CBS)* that control homocysteine (Hcy) concentrations can result in a complete loss of enzyme activity and consequent homocystinuria (fig. 1). In particular, the *MTHFR* 677C>T genotype influences folate and Hcy concentrations [3, 4]. It has been suggested that other polymorphisms located in the genes coding for glutamate carboxypeptidase II (*GCP2* 1561C>T; H475Y), methionine adenosyltransferase I, alpha *(MAT1A)* variants, and for reduced folate carrier 1 (*RFC1* 80G>A; R27H) are associated with alterations in Hcy and folate metabolism [5–7]. Furthermore, a polymorphism in the transcobalamin II gene *(TCN2)* (776C>G; P259R) affects the transcobalamin plasma concentration and, thus, may interfere with cellular vitamin $B_{12}$ availability and Hcy metabolism [8]. *MTHFR* 1298A>C and *MTHFR* 677C>T were associated with plasma Hcy in Puerto Rican adults [3]. *MAT1A* genotypes interact with plasma folate and vitamin $B_6$ status on plasma Hcy [9]. Genetic polymorphisms *MTHFR* 677C>T, *FOLH1* 1561C>T, *FOLH1* rs647370, and *PCFT* 928A>G interacted significantly with smoking for Hcy [10].

Cardiovascular complications are the leading cause of death in type 2 diabetes mellitus (T2DM). HHcy has been demonstrated to affect the clinical course, metabolic control, and possible complications of diabetes mainly by its influence on the development of macro- and microangiopathy [11], and by elicitation of oxidative stress, systemic inflammation, and/or endothelial dysfunction [2]. These factors are known to promote insulin resistance and β-cell dysfunction, two important underlying causes of T2DM [12]. The biological relevance of Hcy metabolism and its association with metabolic disorders makes it an important factor in the development of T2DM.

The Chinese are at a high risk for metabolic disorders, as reflected by their high prevalence of T2DM and metabolic syndrome [13, 14]. Furthermore, there is no evidence of an association between the genetic variants involved in Hcy metabolism and a risk of T2DM in the Chinese. The genetic causes of HHcy and the role of the genetic variants in the diabetes risk in the Chinese have not been fully explained yet.

The aim of the present study was to assess the plasma Hcy concentration, genetic polymorphisms in the methionine metabolic pathway, and their possible contribution to T2DM susceptibility in Han Chinese.

## Research Design and Methods

*Study Design and Participants*

The study protocol was approved by the Ethics Committee, College of Biosystem Engineering and Food Science, Zhejiang University, China, and the Institutional Review Board of Huadong Hospital. All subjects were volunteers who gave their written consent prior to participation in the study. Diabetic patients were from the outpatient clinic. Participants were considered eligible if they met the WHO criteria for the diagnosis of diabetes mellitus (World Health Organization, 1999). Exclusion criteria included: (1) abnormal vitamin and mineral absorption due to gastrointestinal disease; (2) administration of vitamin and mineral supplements for 3 months; (3) severe renal, liver, heart, or psychiatric diseases (apart from the complications of diabetes); (4) history of cancer, thyroid disease, or alcohol abuse, and (5) pregnancy or lactation. After careful screening, 774 T2DM outpatients (348 males, 426 females), aged 58.25 ± 12.31 years, with blood sugar levels stabilized between 7.0 and 10.0 mmol/l were recruited from 30 hospitals in 20 provinces in China.

Healthy control subjects were recruited through a health check program during the period from March 2011 through October 2011 in the Zhejiang Hospital, Hangzhou, China. After careful screening for hyper-

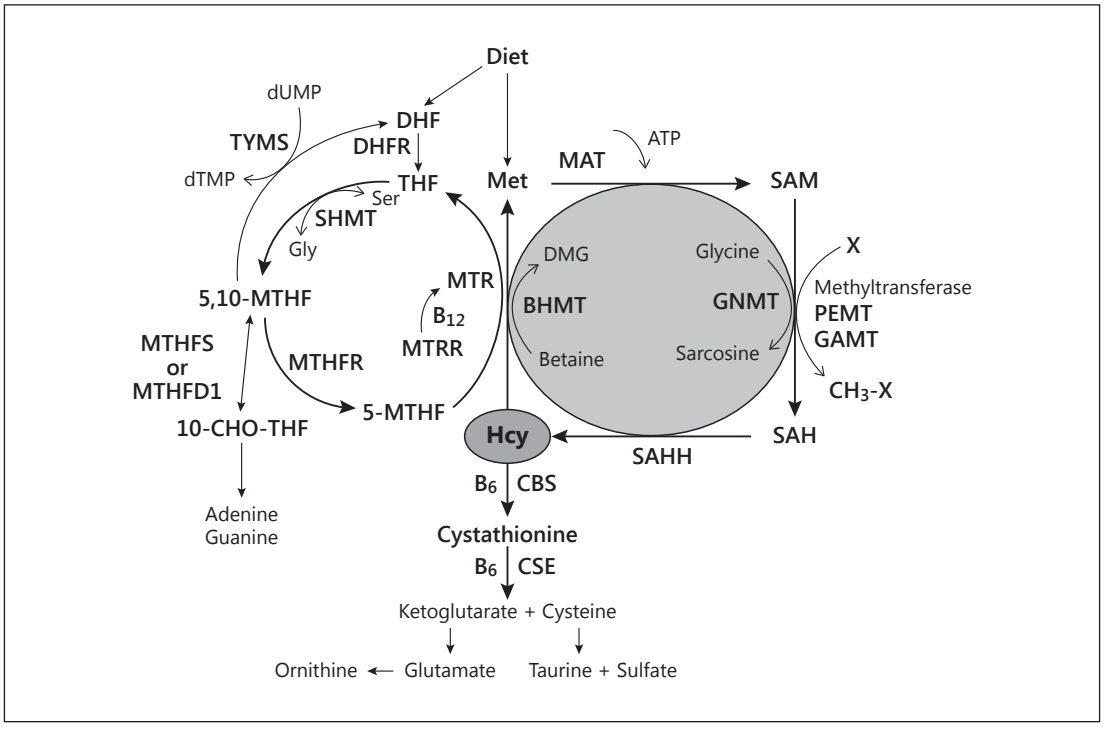


**Fig. 1.** Hcy metabolism. Met = Methionine; MAT = methionine adenosyltransferase; SAHH = S-adenosylhomocysteine hydrolase; CBS = cystathionine β-synthase; CSE = cystathionine γ-lyase; MTHFR = methylenetetrahydrofolate reductase; BHMT = betaine-homocysteine methyltransferase; DMG = dimethylglycine; 5,10-MTHF = 5,10-methylenetetrahydrofolate; 5-MTHF = 5-methyltetrahydrofolate; THF = tetrahydrofolate; SH = serine hydroxymethyltransferase; MTR/MS = methionine synthetase; MTRR = methionine synthase reductase; GAMT = guanidinoacetate N-methyltransferase; PEMT = phosphatidylethanolamine N-methyltransferase; GNMT = glycine N-methyltransferase; SHMT = serine hydroxymethyltransferase; MTHFS = 5,10-methenyltetrahydrofolate synthetase; MTHFD1 = 5,10-methylenetetrahydrofolate dehydrogenase; TYMS = thymidylate synthase; DHFR = dihydrofolate reductase; SAM = S-adenosylmethionine; SAH = S-adenosylhomocysteine; 10-CHO-THF = 10-formyltetrahydrofolate .

tension, renal disease, hyperlipemia, hematological disorders, diabetes, a family history of cardiovascular disease and diabetes, excessive alcohol intake, and drug use, 500 healthy subjects (256 males, 244 females, aged 51.99 ± 8.41 years) were accepted.

*Blood Collection and Plasma Biochemical Parameters*

Subjects attended the hospitals in the morning following an overnight fast. They were allowed to sit relaxed for 10 min, before their weight, height, waist-to-hip ratio and blood pressure were measured by a research assistant trained in standardized procedures. Then, venous blood was collected with 21-gauge needles in plain and EDTA vacuum tubes; plasma samples were prepared during the 2 h after blood had been drawn, aliquoted into separate tubes and stored at –20°C until analysis. Plasma total Hcy was measured using HPLC with fluorescence detection as described [15]. Plasma folate and vitamin $B_{12}$ were measured using immulite chemiluminescent kits according to the manufacturer's instructions (Diagnostic Products Corporation/Siemens, Los Angeles, Calif., USA).

*Genotyping*

DNA was isolated from blood samples using QIAamp DNA Blood Mini kits according to the manufacturer's instructions (Qiagen, Valencia, Calif., USA). We selected Hcy single nucleotide polymorphisms (SNPs) that were previously associated with plasma Hcy. *MTHFR* (rs1801131, rs1801133), *MAT1A* (rs4933327, rs3851059), phosphatidylethanolamine N-methyltransferase *(PEMT)* (rs4646356, rs4646406), methylene-

**Table 1.** Characteristics of the genotyped SNPs in the gene region

| Gene | SNP accession | Chr | mRNA ID No. | Nucleotide change | Location | Amino acid change | Common name | HGVS name | MAF |
|---|---|---|---|---|---|---|---|---|---|
| *MTHFR* | rs1801131 | 1p36.3 | NM_005957.3 | 1470 A>C | exon 8 | E429A | MTHFR_E429A, A1298C | NP_005948.3p.Glu429Ala | 0.32 |
| *MTHFR* | rs1801133 | 1p36.3 | NM_005957.3 | 849 C>T | exon 5 | A222V | MTHFR_A222V, C677T | NP_005948.3p.Ala222Val | 0.26 |
| *MAT1A* | rs4933327 | 10q22 | NM_000429.2 | 1341G>A | intron 8 | NA | MAT1A_i15752 | NM_000429.2c.1341–11G>A | 0.48 |
| *MAT1A* | rs3851059 | 10q22 | NM_000429.2 | 18777G>A | downstream | NA | MAT1A_d18777 | NT_030059.12g.779174G>A | 0.48 |
| *PEMT* | rs4646356 | 17p11.2 | NM_148172.1 | NA | intron 2 | NA | NA | NM_007169.2:c.93+9059C>T | 0.36 |
| *PEMT* | rs4646406 | 17p11.2 | NM_148172.1 | NA | intron 3 | NA | NA | NM_007169.2:c.210-1201A>T | 0.16 |
| *MTHFD* | rs2236225 | 14q24 | NM_005956.3 | 1958G>A | NA | Arg653Gln | MTHFD 1958G>A | NP_005947.3:p.Arg653Gln | 0.31 |
| *MTHFD* | rs1950902 | 14q24 | NM_005956.3 | T401C | intron 5 | Lys134Arg | MTHFD T401C | NP_005947.3:p.Lys134Arg | 0.37 |
| *MTRR* | rs1801394 | 5p15.31 | NM_002454.2 | 66 A>G | NA | Ile22Met | MTRR 66 A>G | NP_002445.2:p.Ile22Met | 0.32 |
| *MTRR* | rs1532268 | 5p15.31 | NM_024010.2 | 524 C>T | exon 9 | Ser175Leu | MTRR 524 C>T | NP_002445.2:p.Ser175Leu | 0.22 |
| *MTRR* | rs162036 | 5p15.31 | NM_024010.2 | 1049 A>G | NA | Lys350Arg | MTRR 1049 A>G | NP_002445.2:p.Lys350Arg | 0.26 |
| *MTR* | rs16834521 | 1q43 | NM_000254.2 | 3144A>G | NA | Ala1048Ala | MTR 3144A>G | NT_167186.1:g.30572348A>G | 0.49 |
| *MTR* | rs1805087 | 1q43 | NM_000254.2 | 2756A>G | NA | Asp919Gly | MTR 2756A>G | NP_000245.2:p.Asp919Gly | 0.16 |

All SNPs are in Hardy-Weinberg equilibrium ($\chi^2$ test). ID = Identification; MAF = minor allele frequency; NA = not available.

tetrahydrofolate dehydrogenase *(MTHFD)* (rs2236225, rs1950902), methionine synthase reductase *(MTRR)* (rs1801394, rs1532268, rs162036), and methionine synthase *(MTR)* (rs16834521, rs1805087) which are involved in the Hcy metabolism pathway were genotyped using the TaqMan SNP genotyping kits with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif., USA) (fig. 1) [16]. The average genotyping success rate was 98%.

*Linkage Disequilibrium and Haplotype Analysis*

Pair-wise linkage disequilibria among SNPs were estimated as correlation coefficients ($r^2$) using the haploview program. For haplotype analysis, the association between haplotypes and plasma Hcy, and estimated haplotype frequencies, we used the R software (haplo.stats package).

*Statistical Analyses*

Statistical analyses were performed using SAS 9.1. (Cary, N.C., USA). The power of the study was calculated assuming 10% disease risk under additive model using Quanto (http://hydra.usc.edu/gxe/). All continuous dependent variables that were not normally distributed were Box-Cox transformed prior to statistical analysis [17]. To ensure adequate statistical power, men and women were analyzed together when there was no gender-specific influence on phenotypes. The association of SNPs with T2DM was determined by logistic regression assuming additive model, recessive model and dominant model. The association of haplotypes with the genetic risk score with T2DM was determined by logistic regression. The analyses were adjusted for age and sex. Odds ratios (ORs) and 95% confidence intervals were calculated with respect to a homozygote wild type. $\chi^2$ tests were conducted to examine whether genotype frequencies of the selected SNPs were in the Hardy-Weinberg equilibrium. Differences between groups were considered to be statistically significant at $p \leq 0.05$.

## Results

*Clinical Characteristics of Populations and Genetic Variants at MTHFR*

All 13 SNPs, where the minor allele frequencies ranged from 0.16 to 0.49, were in Hardy-Weinberg equilibrium (by $\chi^2$ test) (table 1). The distribution of the genotype and the frequency of alleles in T2DM and healthy subjects are reported in table 2. We did not observe a significant association of plasma Hcy with T2DM (data not shown).

*Genetic Variants and Haplotype Analysis*

To examine the combined effect of genetic variants on plasma Hcy, we performed haplotype analysis based on the selected variants (table 3). For these 13 SNPs in 6 genes, we observed the following: four haplotypes for SNPs of *MTHFD:* C-A (0.02), C-G (0.28), T-A (0.20),

**Table 2.** Distribution of the genotype and alleles in T2DM and healthy subjects

| dbSNP | Alleles (major/minor) 1/2 | SNP accession | Distribution of genotype | | |
|---|---|---|---|---|---|
| | | | 11 | 12 | 22 |
| MTHFR A1298C | A/C | rs1801131 | 521/256 (68) | 203/108 (28) | 30/10 (4) |
| MTHFR C677T | C/T | rs1801133 | 271/143 (37) | 357/169 (47) | 125/61 (16) |
| MAT1A i15752 | G/A | rs4933327 | 213/100 (28) | 370/179 (49) | 171/97 (23) |
| MAT1A d18777 | G/A | rs3851059 | 216/100 (28) | 367/178 (48) | 171/98 (24) |
| PEMT1 | C/T | rs4646356 | 379/178 (51) | 293/144 (39) | 80/33 (10) |
| PEMT2 | A/T | rs4646406 | 617/302 (81) | 134/69 (18) | 4/3 (1) |
| MTHFD G1958A | G/A | rs2236225 | 369/142 (56) | 278/111 (43) | 5/5 (1) |
| MTHFD T401C | T/C | rs1950902 | 332/129 (50) | 255/108 (40) | 68/29 (10) |
| MTRR A66G | A/G | rs1801394 | 368/146 (56) | 234/100 (36) | 53/17 (8) |
| MTRR C524T | G/A | rs1532268 | 473/194 (73) | 158/63 (24) | 24/6 (3) |
| MTRR A1049G | A/G | rs162036 | 434/175 (67) | 188/78 (29) | 31/9 (4) |
| MTR A3144G | A/G | rs16834521 | 203/70 (30) | 321/137 (50) | 129/56 (20) |
| MTR A2756G | A/G | rs1805087 | 538/204 (81) | 112/56 (18) | 5/3 (1) |

The $\chi^2$ test was used to test the distribution of the genotype and alleles.

T-G (0.49); two haplotypes for SNPs of *MAT1A:* A-A (0.47), G-G (0.51); three haplotypes for *MTHFR:* A-C (0.41), A-T (0.39), C-C (0.17); three haplotypes for SNPs of *MTR:* A-A (0.45), A-G (0.10), G-A (0.45); four haplotypes for SNPs of *PEMT:* C-A (0.66), C-T (0.03), T-A (0.23), T-T (0.06), and five haplotypes for SNPs of *MTRR:* A-A-A (0.07), A-G-A (0.09), G-A-A (0.49), G-A-G (0.19), G-G-A (0.17).

*Genetic Variants in Hcy Metabolism and Plasma Hcy Levels*

*MTHFR* (rs1801133) was significantly associated with plasma Hcy in T2DM patients (table 4). Participants homozygous for the T allele (*MTHFR*, rs1801133) had significantly higher plasma Hcy compared to carriers of the C allele. *MTR* (rs1805087, rs16834521) was also significantly associated with plasma Hcy in T2DM patients. *PEMT* (rs4646406) was significantly associated with plasma Hcy in a recessive model (i.e., 11 + 12 vs. 22) in T2DM patients.

*MTHFR* (rs1801133) was significantly associated with plasma Hcy in healthy subjects (table 4). Plasma Hcy concentrations were significantly higher in participants homozygous for the T allele (*MTHFR*, rs1801133) compared to carriers of the C allele (p = 0.001). *MTRR* (rs162036, rs1532268) was significantly associated with plasma Hcy in healthy subjects. *MTR* (rs16834521) was also significantly associated with plasma Hcy in healthy subjects. *MAT1A* (rs3851059, rs4933327) was significantly associated with plasma Hcy in a recessive model (i.e., 11 + 12 vs. 22) in healthy subjects (table 4). Participants homozygous for the A allele (*MTHFR*, rs1801133) had significantly higher plasma Hcy compared to carriers of the G allele. However, we did not observe any associations of *PEMT* genotypes with plasma Hcy concentrations in healthy subjects.

*Association between Genotype and T2DM*

Subjects with the genotype CC of *MTHFR* (rs1801131) had a significantly higher likelihood of T2DM compared with subjects with the AA or AA+AC genotypes (table 5, OR = 1.93 for CC vs. AA, p = 0.041; OR = 3.13 for CC vs. AA+AC, p = 0.017, respectively). Participants homozygous for the minor allele (CC) had more than a 3-fold higher likelihood of T2DM than did carriers of the A allele (AA+AC). They had a 93% higher likelihood of having T2DM than did homozygotes (AA).

**Table 3.** The haplotypes of the critical gene involved in Hcy metabolism

| Gene | Haplotypes | Carrier/ noncarrier, n | Genotypes | | | Frequency |
|---|---|---|---|---|---|---|
| *MTHFD*[1] | | | rs2195090 | rs2236225 | | |
| | C-A | 13/490 | C | A | | 0.02 |
| | C-G | 248/255 | C | G | | 0.28 |
| | T-A | 206/297 | T | A | | 0.20 |
| | T-G | 382/121 | T | G | | 0.49 |
| *MAT1A*[2] | | | rs4933327 | rs3851059 | | |
| | A-A | 435/168 | A | A | | 0.47 |
| | G-G | 471/131 | G | G | | 0.51 |
| *MTHFR*[3] | | | rs1801131 | rs1801133 | | |
| | A-C | 396/207 | A | C | | 0.41 |
| | A-T | 389/214 | A | T | | 0.39 |
| | C-C | 176/427 | C | C | | 0.17 |
| *MTR*[4] | | | rs16834521 | rs1805087 | | |
| | A-A | 348/155 | A | A | | 0.45 |
| | A-G | 91/412 | A | G | | 0.10 |
| | G-A | 349/154 | G | A | | 0.45 |
| *PEMT*[5] | | | rs4646356 | rs4646406 | | |
| | C-A | 538/65 | C | A | | 0.66 |
| | C-T | 30/573 | C | T | | 0.03 |
| | T-A | 227/376 | T | A | | 0.23 |
| | T-T | 84/519 | T | T | | 0.06 |
| *MTRR*[6] | | | rs1532268 | rs1801394 | rs162036 | |
| | A-A-A | 56/447 | A | A | A | 0.07 |
| | A-G-A | 96/407 | A | G | A | 0.09 |
| | G-A-A | 371/132 | G | A | A | 0.49 |
| | G-A-G | 174/329 | G | A | G | 0.19 |
| | G-G-A | 134/369 | G | G | A | 0.17 |

[1] *MTHFD* haplotypes were estimated based on two SNPs in the order: rs2195090, rs2236225.
[2] *MAT1A* haplotypes were estimated based on two SNPs in the order: rs4933327, rs3851059.
[3] *MTHFR* haplotypes were estimated based on two SNPs in the order: rs1801131, rs1801133.
[4] *MTR* haplotypes were estimated based on two SNPs in the order: rs16834521, rs1805087.
[5] *PEMT* haplotypes were estimated based on two SNPs in the order: rs4646356, rs4646406.
[6] *MTRR* haplotypes were estimated based on three SNPs in the order: rs1532268, rs1801394, rs162036.

Subjects with the genotype AA of the *MTHFD* variant (rs2236225) had a significantly lower likelihood of T2DM compared with subjects with the GG or GG+GA genotypes (OR = 0.36 for AA vs. GG, p = 0.027; OR = 0.36 for AA vs. GG+GA, p = 0.017, respectively). In addition, the genotype CT+TT of the *PEMT* (rs4646356) variants displayed a significant association with an increased risk of T2DM (OR = 1.52 for CT+TT vs. CC, p = 0.042). However, the genotype TT of the *PEMT* (rs4646406) variants was significantly associated with a decreased risk of T2DM (OR = 0.48 for TT vs. AA, p = 0.048). But other variants were not associated with T2DM.

*MTHFR* haplotypes correlated significantly with T2DM. Consistent with this, carriers of the haplotype A-T were associated with a higher likelihood of T2DM (OR = 1.73, p = 0.020). Moreover, carriers of the *PEMT* haplotypes C-A (OR = 2.71, p = 0.001) and T-A (OR = 2.71, p = 0.001) had a significantly higher likelihood of T2DM (OR = 1.73, p = 0.029) when compared to noncarriers (fig. 2).

**Table 4.** Association between the Hcy metabolic gene variants and plasma Hcy in T2DM and healthy subjects

| Gene | SNP | Alleles | | T2DM | | | | | | Healthy subjects | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | genotype | | | p | | | genotype | | | p | | |
| | | | | 11 | 12 | 22 | $p^1$ | $p^2$ | $p^3$ | 11 | 12 | 22 | $p^1$ | $p^2$ | $p^3$ |
| *MTHFR* | rs1801131 | A | C | 12.64 ± 6.31 | 12.85 ± 6.28 | 11.12 ± 5.03 | 0.321 | 0.120 | 0.210 | 14.77 ± 8.60 | 13.26 ± 5.26 | 15.02 ± 6.00 | 0.345 | 0.164 | 0.858 |
| | rs1801133 | C | T | 12.18 ± 6.62 | 12.24 ± 5.93 | 13.07 ± 6.60 | 0.483 | 0.643 | **0.049** | 13.34 ± 5.30 | 13.17 ± 4.81 | 19.58 ± 14.21 | **0.001** | 0.102 | **0.001** |
| *MTRR* | rs162036 | A | G | 11.70 ± 5.45 | 12.86 ± 7.60 | 11.31 ± 5.68 | 0.139 | 0.117 | 0.532 | 15.82 ± 7.38 | 18.22 ± 9.09 | 10.20 ± 3.89 | **0.049** | 0.139 | 0.172 |
| | rs1532268 | G | A | 12.13 ± 6.44 | 11.81 ± 5.66 | 11.03 ± 4.11 | 0.69 | 0.493 | 0.493 | 15.23 ± 4.96 | 20.27 ± 12.87 | 13.85 ± 2.05 | **0.001** | **0.001** | 0.460 |
| | rs1801394 | A | G | 12.02 ± 5.80 | 12.21 ± 6.82 | 11.05 ± 5.79 | 0.565 | 0.964 | 0.307 | 16.72 ± 8.94 | 15.84 ± 7.19 | 17.45 ± 3.63 | 0.708 | 0.634 | 0.624 |
| *MTR(MS)* | rs1805087 | A | G | 12.32 ± 6.25 | 10.39 ± 5.04 | 16.38 ± 15.72 | **0.013** | **0.015** | **0.018** | 16.56 ± 8.10 | 16.10 ± 7.71 | 16.22 ± 3.63 | 0.948 | 0.744 | 0.977 |
| | rs16834521 | A | G | 11.46 ± 6.10 | 11.92 ± 5.56 | 13.08 ± 7.58 | 0.12 | 0.19 | **0.05** | 19.19 ± 12.80 | 15.77 ± 4.33 | 14.03 ± 4.71 | **0.010** | **0.004** | **0.072** |
| *MTHFD* | rs1950902 | T | C | 12.04 ± 6.49 | 12.27 ± 5.66 | 10.58 ± 6.57 | 0.26 | 0.913 | 0.112 | 15.75 ± 6.67 | 17.22 ± 8.99 | 17.59 ± 10.46 | 0.448 | 0.209 | 0.522 |
| | rs2236225 | G | A | 11.71 ± 5.62 | 12.42 ± 6.86 | 11.51 ± 4.85 | 0.439 | 0.208 | 0.888 | 16.52 ± 8.06 | 16.20 ± 8.11 | 21.66 ± 1.77 | 0.632 | 0.879 | 0.356 |
| *MAT1A* | rs3851059 | G | A | 12.61 ± 7.01 | 12.49 ± 6.23 | 11.61 ± 5.21 | 0.331 | 0.516 | 0.059 | 13.87 ± 7.32 | 13.79 ± 5.27 | 15.81 ± 10.92 | 0.179 | 0.527 | **0.044** |
| | rs4933327 | G | A | 12.64 ± 7.07 | 12.47 ± 6.21 | 11.61 ± 5.21 | 0.327 | 0.466 | 0.056 | 13.87 ± 7.32 | 13.78 ± 5.25 | 15.86 ± 10.99 | 0.166 | 0.527 | **0.050** |
| *PEMT* | rs4646356 | C | T | 12.49 ± 5.88 | 12.27 ± 6.03 | 11.56 ± 8.62 | 0.57 | 0.48 | 0.324 | 14.46 ± 8.59 | 14.57 ± 7.30 | 13.11 ± 4.32 | 0.652 | 0.820 | 0.358 |
| | rs4646406 | A | T | 12.15 ± 5.92 | 13.21 ± 7.68 | 10.45 ± 0.55 | 0.24 | 0.601 | **0.031** | 14.53 ± 7.69 | 13.64 ± 8.08 | 8.68 ± 5.91 | 0.461 | 0.380 | 0.300 |

The associations of genotypes with plasma Hcy was examined using the general linear model controlled for confounding factors. Bold values represent p value ≤0.05.
[1] p for the additive model; [2] p for the dominant model (11 vs. 12 + 22); [3] p for the recessive model (11 + 12 vs. 22).

**Table 5.** Association of gene variants with T2DM

| Gene and SNP name | OR (95% interval) for T2DM | | | p[1] | | |
|---|---|---|---|---|---|---|
| | additive model | dominant model | recessive model | p[2] | p[3] | p[4] |
| *MTRR* (rs162036) | 1.47 (0.68–3.17) | 1.03 (0.76–1.40) | 1.48 (0.69–3.17) | 0.312 | 0.848 | 0.315 |
| *MTRR* (rs1532268) | 1.40 (0.56–3.51) | 1.10 (0.79–1.53) | 1.38 (0.55–3.44) | 0.515 | 0.564 | 0.489 |
| *MTRR* (rs1801394) | 1.23 (0.68–2.20) | 0.96 (0.71–1.28) | 1.27 (0.72–2.26) | 0.392 | 0.761 | 0.412 |
| *MTR* (rs1805087) | 0.60 (0.14–2.59) | 0.77 (0.54–1.10) | 0.62 (0.14–2.70) | 0.598 | 0.154 | 0.527 |
| *MTHFR* (rs1801131) | 1.93 (0.85–3.42) | 0.80 (0.48–1.33) | 3.13 (0.65–5.17) | **0.041** | 0.387 | **0.017** |
| *MTHFR* (rs1801133) | 1.43 (0.67–3.02) | 1.23 (0.76–2.01) | 1.30 (0.65–2.60) | 0.445 | 0.399 | 0.461 |
| *MTHFD* (rs1950902) | 0.96 (0.59–1.57) | 0.93 (0.69–1.24) | 1.00 (0.63–1.60) | 0.987 | 0.605 | 0.990 |
| *MTHFD* (rs2236225) | 0.36 (0.10–1.27) | 0.93 (0.70–1.25) | 0.36 (0.10–1.29) | 0.115 | 0.247 | 0.117 |
| *MAT1A* (rs3851059) | 0.79 (0.41–1.52) | 1.02 (0.58–1.77) | 0.71 (0.42–1.21) | 0.261 | 0.953 | 0.206 |
| *MAT1A* (rs4933327) | 0.85 (0.44–1.66) | 1.08 (0.62–1.87) | 0.75 (0.44–1.28) | 0.357 | 0.797 | 0.291 |
| *PEMT* (rs4646356) | 1.18 (0.50–2.77) | 1.52 (0.75–2.97) | 1.08 (0.48–2.48) | 0.877 | **0.042** | 0.848 |
| *PEMT* (rs4646406) | 0.48 (0.10–2.22) | 0.97 (0.70–1.36) | 0.48 (0.10–2.22) | **0.048** | 0.878 | 0.347 |

Bold values represent p value ≤0.05.
[1] Adjusted for age and sex; [2] p for the additive model; [3] p for the dominant model (12 + 22 vs. 11); [4] p for the recessive model (22 vs. 11 + 12).

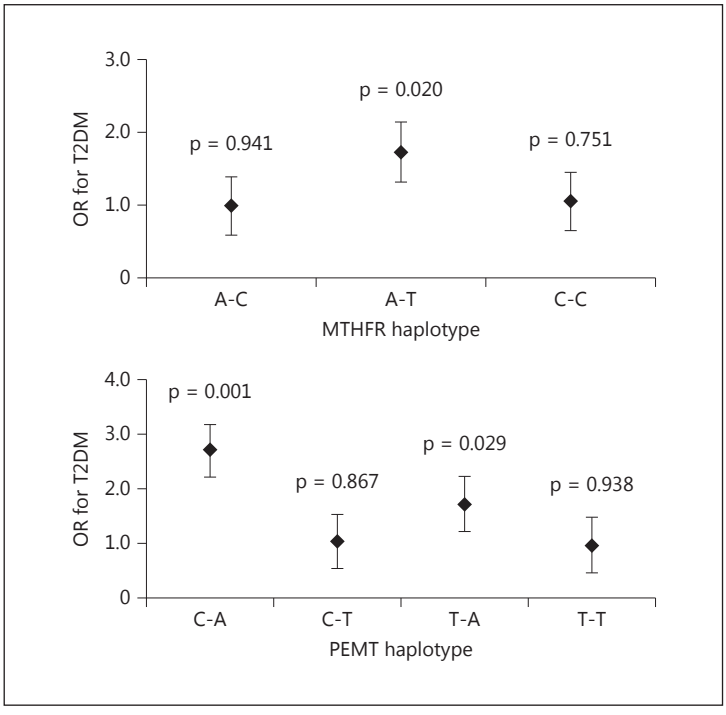


**Fig. 2.** Association between haplotypes and T2DM. *MTHFR* haplotype were estimated based on two SNPs in the order: rs1801131, rs1801133. *PEMT* haplotype were estimated based on two SNPs in the order: rs4646356, rs4646406. Logistical regression model was used to test the associations of *MTHFR* and *PEMT* haplotypes (noncarriers as references) with T2DM adjusted for age, sex.

## Discussion

The present study included a large consecutive series of patients with T2DM recruited at the time of clinical assessment. To the best of our knowledge, it evaluates most comprehensively the associations of the variants of Hcy metabolic pathway genes with Hcy levels and T2DM. Our study showed that the Hcy-increasing allele score was positively associated with plasma Hcy levels in T2DM patients and healthy subjects. Subjects with the genotype CC of

*MTHFR* (rs1801131) had a significantly higher likelihood of T2DM compared with subjects with the AA or AA+AC genotypes. Subjects with the genotype AA of the *MTHFD* variant (rs2236225) had a significantly lower likelihood of T2DM compared with subjects with the GG or GG+GA genotypes. In addition, the genotype CT+TT of the *PEMT* (rs4646356) variants displayed a significant association with an increased risk of T2DM.

The metabolism of Hcy requires the contribution of a number of enzyme pathways and the availability of vitamin cofactors [1]. HHcy is caused by a low intake of folate and other B vitamins and by genetic factors [18, 19], including polymorphisms of the genes encoding the enzymes involved in Hcy remethylation, such as *MTHFR*, *MTR*, *MTRR*, and *CBS*.

*MTHFR* C677T is a strong determinant of Hcy in individuals with impaired folate status [20]. Previous studies identified the *MTHFR* 677TT genotype as being associated with increased plasma Hcy and decreased plasma folate. *MTHFR* 1298A>C, *SLC19A1* intron 5A>G, and *FPGS* 2006A>G polymorphisms were associated with significantly altered plasma Hcy concentrations [21]. In the present study, we have been able to confirm that *MTHFR* C677T and *MTHFR* 1298A>C affect plasma Hcy levels in Chinese subjects with T2DM, while similar to our previous findings in Puerto Ricans, dietary PUFA intake may modulate the effect of two *MTHFR* variants on plasma Hcy [3]. Furthermore, *MTR* (rs1805087, rs16834521) was significantly associated with plasma Hcy in T2DM. *MTRR* (rs162036, rs1532268) was significantly associated with plasma Hcy in healthy subjects. These findings are consistent with previous results [19, 22]. It has previously been reported that the *MAT1A* 3U1510 genotype was associated with plasma Hcy [9]. Furthermore, *MAT1A* SNP i15173 also displayed an association with Hcy that narrowly failed to meet the significance criterion, but it should be borne in mind that these associations can be modulated by plasma folate, vitamin $B_6$, or vitamin $B_{12}$ status [9]. Unfortunately, we did not measure these blood nutrients; however, in future studies this should be done to clarify their role in the development of T2DM. In the present study, we found that *MAT1A* (rs3851059, rs4933327) was associated with plasma Hcy concentrations in T2DM patients and healthy subjects. Dietary fatty acids may modulate these effects of the *MAT1A* variants on plasma Hcy concentrations [6], while other lifestyle factors may also influence the effects of the genetic variants of the Hcy metabolism genes in affecting plasma Hcy concentrations [10].

Elevated Hcy levels are associated with vascular complications in diabetic patients [23, 24]. Of note, several studies have demonstrated that elevated Hcy levels predict the risk of death or coronary events in patients with T2DM [25]. In recent years, elevated fasting serum Hcy levels have emerged as an independent risk factor for the development of diabetes. The proposed mechanism that was reported associated elevated Hcy levels with endothelial dysfunction, insulin resistance, the prothrombotic state, macroangiopathy, and nephropathy [25]. However, plasma Hcy levels have been reported to be increased [25–27], unchanged [28, 29], or decreased [30] in patients with T2DM. In the present study, we found that the plasma Hcy level in T2DM patients was significantly lower than in healthy subjects (not shown), which is consistent with previous results [30, 31]. Wollesen et al. [30] found lower plasma Hcy and cysteine concentrations in 50 type 1 diabetes mellitus and 30 T2DM patients than in nondiabetic subjects with a negative association with the glomerular filtration rate. Smulders et al. [31] found that the plasma Hcy level was lower in patients with complications, and the Hcy levels correlated with creatinine clearance. We speculated that the patients have been counseled (at diagnosis) to make diet/lifestyle changes, or to take a micronutrient supplement that would decrease the blood Hcy level. However, an animal study showed a 30% reduction of Hcy in untreated diabetic rats [32] and found an increase in the activities of hepatic transsulfuration enzymes in untreated diabetic rats. The decrease in Hcy was prevented when diabetic rats

received insulin. This confirms that insulin is involved in the regulation of the metabolism of the plasma Hcy concentration by affecting the hepatic transsulfuration pathway, which is involved in the catabolism of Hcy. This may be the reason why serum/plasma Hcy in diabetic patients is lower than in nondiabetic patients. Conflicting results regarding the circulating levels of Hcy in patients with diabetes may relate to the heterogeneity of the patients included, particularly with regard to their renal function status and the presence of vascular arterial disease.

Mutations of the *MTHFR* gene together with an elevated plasma Hcy were shown to be associated with a predisposition to developing T2DM complications, including diabetic retinopathy [33] and diabetic nephropathy [34]. The latter association was exemplified by the finding that the increased frequency of the 677T allele and the CT and TT genotypes in T2DM patients correlated with a progression to renal failure [35]. This prompted the conclusion that the *MTHFR* C677T gene polymorphism associated with a predisposition to increased plasma Hcy levels may represent a genetic risk factor for diabetic complications [36]. Therefore, further investigation into the role of the genetic variants involved in Hcy metabolism in diabetes will help to elucidate the pathogenic physiological mechanism of diabetes.

Previous studies demonstrated a significantly higher frequency of the *MTHFR* 1298C allele in macroalbuminuric patients compared with normoalbuminuric patients [37]. The *MTHFR* 1298C allele increases the risk of macroalbuminuria 2.5-fold in T2DM patients carrying this allele. Also, the presence of this allele increases the risk of progression from microalbuminuria to macroalbuminuria 2.08-fold [37]. Sun et al. [38] reported that among the Chinese population with T2DM, *MTHFR* C677T polymorphism may be a genetic risk factor for diabetic nephropathy. However, some studies did not demonstrate any association between diabetic nephropathy and *MTHFR* A1298C polymorphism [36, 39]. Furthermore, it was demonstrated in Lebanese T2DM patients that the 677T allele but not the 1298C allele was a risk factor for diabetic nephropathy [39]. In our study population, *MTHFR* A1298C showed a significant association with T2DM. Participants homozygous for the minor allele (CC) had more than a 3-fold higher likelihood of T2DM than did carriers of the allele A (AA+AC). Participants homozygous for the minor allele (CC) had a 93% higher likelihood of T2DM than did homozygotes (AA). However, we did not observe the association of *MTHFR* C677T with T2DM in the Chinese. It should be noted here that the contribution of inherited and environmental risk factors may vary significantly according to ethnicity [36]. In our study, some SNPs seem to confer protective effects against the risk of T2DM [*MTHFD* 1958 G>A (rs2236225) and *PEMT* (rs4646406) variant], whereas the *PEMT* (rs4646356) variant seems to be associated with a greater risk of T2DM.

The present study has limitations in exposure assessment and consideration of potential confounding factors. We did not collect data on antidiabetic medication or the plasma status of folate, vitamins $B_6$ and $B_{12}$ that subjects took. The causes of T2DM are multifactorial, and in some populations a genetic makeup may be protective against the development of T2DM despite increased Hcy levels due to genetic mutations. In addition, the ethnic variations in terms of these relationships between either or both *MTHFR* mutations with T2DM have often yielded conflicting results about the role of these mutations, which further highlights the need for a careful assessment of any contributing lifestyle factors. It also demonstrated the necessity of follow-up studies of T2DM patients from different ethnic groups.

In conclusion, our study provides evidence that these common variants in *MTHFR, MTR, MAT1A, PEMT*, and *MTRR* genes associated with alterations in plasma Hcy confer the risk for T2DM in the Han Chinese. Studies involving a larger sample size and different ethnic groups are required before ruling out the role of these important candidate genes in the development of T2DM.

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## Disclosure Statement

The authors have no financial or commercial conflicts of interest to report in respect of this work.

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