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Relationship of *MTHFR* Gene 677C→T Polymorphism, Homocysteine, and Estimated Glomerular Filtration Rate Levels With the Risk of New-Onset Diabetes

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Abstract: East Asian patients with diabetes have a higher risk for renal complications and strokes than Europeans. We aimed to evaluate the effect of methylenetetrahydrofolate reductase (*MTHFR)* gene 677C→T polymorphism, which was associated with a higher stroke risk and was common in the Chinese population, as well as homocysteine and estimated glomerular filtration rate (eGFR) levels on the risk of new-onset diabetes (NOD).

A total of 2422 subjects without diabetes were followed-up for 7 years. NOD was defined as fasting plasma glucose ≥7.0 mmol/L or self-reported physician diagnosis of diabetes.

Compared with subjects with *MTHFR* 677CC genotype, those with TT genotype had a higher risk of NOD in females (odds ratio 2.78, 95% confidence interval 1.39–5.56) but not in males (0.80, 0.40–1.61, *P* for interaction = 0.008). Furthermore, *MTHFR* 677C→T polymorphism was more strongly associated with the risk of NOD among females with higher body mass index (BMI, ≥23 vs <23 kg/m^2, *P* for interaction = 0.009) or lower high-density lipoprotein-cholesterol (HDL-C, <1.3 vs ≥1.3 mmol/L, *P* for interaction = 0.015) levels. Hyperhomocysteinemia (≥16 vs <10 μmol/L) was not significantly associated with NOD in males (0.88, 0.42–1.85) or females (1.52, 0.65–3.57). However, mildly decreased eGFR (<90 vs 90–120 mL/min/1.73 m^2) was associated with NOD mainly in males (1.96, 1.01–3.78; females, 0.74, 0.32–1.72, *P* for interaction = 0.134).

Females with *MTHFR* 677TT genotype had a significantly higher risk of NOD, particularly those with higher BMI or low HDL-C levels. The higher risk of NOD associated with mildly decreased eGFR also warrants more investigation. Our results provide insights into the ethnic differences of diabetic complications between East Asian patients and Europeans.

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Abbreviations: BMI = body mass index, CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = homeostasis model assessment of insulin resistance, MTHFR = methylenetetrahydrofolate reductase, NAFLD = nonalcoholic fatty liver disease, NOD = new-onset diabetes, OR = odds ratio, TC = total cholesterol, TG = triglyceride.

INTRODUCTION

Diabetes is now recognized as a worldwide public health problem. A recent national cross-sectional survey[1] showed that the overall prevalence of diabetes was estimated to be 11.6% in the Chinese adult population. Diabetes is a progressive disease, due in part to the loss of β-cell function, with the reduction in function probably commencing 10 to 12 years prior to diagnosis and aggravated by increasing fasting plasma glucose (FPG) levels.[2] Furthermore, East Asian patients with type 2 diabetes have a higher risk of developing renal complications than Europeans, and, with regard to cardiovascular complications, a predisposition for developing strokes.[3] These results denote that more studies are needed to explain these interethnic differences, and, most importantly, effective strategies are required to facilitate earlier identification and prevention to combat these growing disease burdens, particularly in China.

Methylenetetrahydrofolate reductase (*MTHFR*) is the main regulatory enzyme for folate/homocysteine metabolism. *MTHFR* converts 5,10-methylene-tetrahydrofolate (THF) into 5-methyl-THF, the dominant circulating form of folate. The 5-methyl-THF product donates a methyl group to homocysteine in the generation of *S*-adenosylmethionine, a major source of methyl groups used for DNA methylation. Polymorphism of *MTHFR* 677C→T leads to a reduction in enzyme activity, resulting in increased concentrations of plasma homocysteine

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and lower levels of serum folate, and thereby confers a higher risk for stroke, particularly in those with low folate intake.[4] A recent meta-analysis of case–control studies[5] found that the homocysteine concentration in individuals with type 2 diabetes was significantly higher than that in control subjects (0.94 μmol/L, 95% confidence interval [CI] 0.40–1.48), and the odds ratio (OR) associated with type 2 diabetes for *MTHFR* 677TT relative to 677CC was 1.38 (95% CI 1.18–1.62).[5] Our recent study also showed that participants with *MTHFR* 677TT genotype had a higher prevalence of diabetes than those with CC genotype.[6] Furthermore, plasma homocysteine levels were significantly inversely associated with estimated glomerular filtration rate (eGFR), and *MTHFR* 677TT genotype was associated with a higher risk for decreased kidney function independent of homocysteine levels.[7]

However, no prospective studies investigating the association between *MTHFR* 677C→T polymorphism, homocysteine, renal function, and new-onset diabetes (NOD) have been conducted. Furthermore, the frequency of the MTHFR 677 TT genotype, which showed marked ethnic variations, was more common in China than in most of the European countries.[8] The Chinese also had higher homocysteine levels,[9] lower folate concentrations,[10] and did not have a policy of mandatory folic acid fortification in food. These characteristics make the Chinese population particularly suited for testing the possible genotype (*MTHFR* 677C→T polymorphism) and phenotype (homocysteine and eGFR levels)-disease association using the same analytical framework. Therefore, in the current study, we aimed to evaluate the effect of *MTHFR* 677C→T polymorphism, homocysteine, and eGFR levels on the risk of NOD in a rural Chinese cohort, and to identify the possible effect modifiers. Our findings may possibly provide insights into the ethnic differences in diabetic complications seen between East Asian patients and Europeans.

MATERIALS AND METHODS

This report followed the STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) statement for cohort studies.

STUDY POPULATION AND DATA COLLECTION

Study participants were from an epidemiological study of metabolic syndrome conducted during 2003 to 2005 in rural communities (Dongzhi and Wangjiang) in Anqing, Anhui province of China. Detailed protocol and the details regarding ''study population'' and ''data collection'' have been previously described.[11,12] Briefly, 6301 of the study subjects from Dongzhi community who received baseline screening examination were invited for a follow-up visit in 2011, and 2901 (46%) of them responded. The nonresponders did not differ from the responders substantially with respect to baseline characteristics (data not shown). This study was approved by the institutional review boards from the Nanfang Hospital in Guangzhou and the Institute of Biomedicine in the Anhui Medical University. Written informed consent was obtained from each study participant.

Baseline data was ascertained by trained research staff according to the standard operating procedures. Venous blood was drawn from the forearm of each participant in the fasting status. FPG, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and homocysteine were measured on the Hitachi 7020 Automatic Analyzer (Hitachi, Tokyo, Japan). Serum creatinine concentrations were determined using an enzymatic method (sarcosine oxidase-peroxidase–anti-peroxidase). Plasma insulin was measured using an enhanced chemiluminescence method on an Elecsys 2010 system (Roche, Basel, Switzerland). DNA was extracted from leukocytes in peripheral blood using standard techniques. *MTHFR* 677C→T genotype was determined by Taqman assay designed and manufactured by Applied Biosystems (Foster City, CA).

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated from the fasting concentrations of insulin and glucose using the following formula: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5.[13] GFR was estimated using the equation according to the Chronic Kidney Disease Epidemiology Collaboration.[14]

OUTCOMES

We excluded participants with self-reported physician diagnosis of diabetes or FPG ≥7.0 mmol/L at baseline in the final analysis. NOD was defined as FPG ≥7.0 mmol/L or self-reported physician diagnosis of diabetes at follow-up year.

STATISTICAL ANALYSIS

Current smoking was defined as having smoked ≥10 packs in the last year. Current alcohol drinking was defined as drinking alcohol at least once per week in the last year. The question about insomnia was phrased as follows: ''Do you frequently suffer from insomnia?'', and a choice of 3 responses (frequent: almost every week, medium: 1–3 times per month, and seldom) was provided.

Baseline characteristics are presented as mean or percentage, except for fasting insulin and homocysteine, which are presented as median (Q1–Q3) because of the skewed distribution. Between-group differences in baseline characteristics were tested using the Student *t* test, the signed rank test, or the χ^2 test, accordingly. The effects of *MTHFR* 677C→T polymorphism (CC, CT, and TT), homocysteine (<10, 10–16, and ≥16 μmol/L), and eGFR (≥120, 90–120, <90 mL/min/1.73 m^2) on the risks of NOD in males and females were estimated using logistic regression models with adjustment for baseline covariates including age (year), baseline FPG (≥5.6 vs <5.6 mmol/L), body mass index (BMI, ≥23 vs <23 kg/m^2), blood pressure (<130/85, 130/85–140/90, ≥140/90 mm Hg), TG (≥1.7 vs <1.7 mmol/L), TC (≥5.2 vs <5.2 mmol/L), HDL-C (<1.3 [females]/1.0 [males] vs ≥1.3/1.0 mmol/L), current cigarette smoking, current alcohol drinking, and insomnia (frequent, medium, and seldom). We tested for effect modification with stratified analyses of the above major covariates. A 2-tailed $P < 0.05$ was considered statistically significant in all analyses. R software, version 2.15.1 (http://www.R-project.org) was used to perform all statistical analyses.

RESULTS

Overall, 2901 subjects were revisited. In this report, study participants with self-reported physician diagnosis of cardiovascular disease (CVD, n = 28), diabetes (n = 10), hypertension (n = 124), or with any missing data (n = 186) regarding baseline values for age, cigarette smoking status, alcohol drinking status, fasting glucose, homocysteine, *MTHFR* 677C→T polymorphism or insomnia, or with any missing data (n = 61) for FPG or self-reported physician diagnosis of diabetes at follow up, or

who had an FPG value of ≥7.0 mmol/L at baseline(n = 70) were excluded. Our final analysis included 2422 participants.

The prevalence of *MTHFR* 677 CC, 677 CT, and 677 TT genotypes was 36.8%, 47.4%, and 15.8%, respectively. This population had no significant deviations in genotype distributions from expected Hardy–Weinberg equilibrium. Those with *MTHFR* 677 TT genotype had significantly higher homocysteine levels (median, 12.0 in males and 10.1 μmol/L in females) than those with CT (10.4 and 9.1 μmol/L) or CC (10.4 and 8.6 μmol/L) genotype ($P < 0.05$ for either of these genotypes in males or females). Furthermore, there was an inverse association between homocysteine and eGFR levels in males ($r = -0.43$, $P < 0.001$) and females ($r = -0.63$, $P < 0.001$).

The follow-up time ranged from 5.81 to 7.57 years, with a mean of 7.02 (SD 0.31) years. There were no significant differences in the follow-up time between subjects with and without NOD in males (6.97 [0.31] vs 7.02 [0.31], $P = 0.137$) and females (7.05 [0.34] vs 7.03 [0.32], $P = 0.506$). The baseline characteristics of the study subjects stratified by NOD status and sex are summarized in Table 1. NOD patients had significantly higher TG, BMI, insulin levels, and HOMA-IR

TABLE 1. Baseline Characteristics According to NOD Status by Sex

Variables	Males			Females		
	Nondiabetes	NOD	*P*	Nondiabetes	NOD	*P*
N	1150	100		1090	82	
Age, y	50.9 (6.0)	51.1 (6.4)	0.705	48.8 (5.9)	50.4 (6.0)	0.015
FPG, mmol/L	5.4 (0.5)	5.8 (0.6)	<0.001	5.4 (0.5)	5.9 (0.6)	<0.001
Systolic BP, mm Hg	125.1 (17.7)	129.4 (18.2)	0.020	123.8 (18.1)	127.4 (18.4)	0.086
Diastolic BP, mm Hg	79.4 (11.4)	81.8 (11.1)	0.040	77.2 (10.5)	78.3 (11.4)	0.348
TC, mmol/L	4.5 (0.8)	4.5 (1.0)	0.389	4.5 (0.9)	4.8 (0.9)	0.012
TG, mmol/L	1.1 (0.7)	1.4 (1.4)	<0.001	1.4 (1.1)	1.8 (1.4)	0.005
HDL-C, mmol/L	1.5 (0.4)	1.5 (0.4)	0.944	1.4 (0.3)	1.4 (0.3)	0.590
BMI, kg/m^2	21.3 (2.2)	22.1 (3.3)	<0.001	22.1 (2.7)	22.8 (3.0)	0.028
Homocysteine, μmol/L*	10.6 (8.6–13.7)	10.9 (8.8–13.9)	0.607	9.0 (7.1–12.0)	9.4 (7.4–12.6)	0.241
eGFR, mL/min/1.73 m^2	105.2 (17.8)	101.9 (20.3)	0.076	106.7 (20.3)	104.5 (22.4)	0.352
Insulin, μU/mL*,†	2.4 (1.6–3.6)	2.9 (1.8–4.6)	0.020	4.3 (3.0–6.1)	5.6 (3.6–8.1)	0.002
HOMA-IR*,†	0.58 (0.36–0.88)	0.78 (0.45–1.14)	<0.001	1.04 (0.71–1.48)	1.45 (0.97–2.02)	<0.001
Categorical variables						
BP, mm Hg			0.046			0.594
<130/85	658 (57.2)	45 (45.0)		684 (62.8)	47 (57.3)	
130/85–140/90	209 (18.2)	26 (26.0)		172 (15.8)	14 (17.1)	
≥140/90	283 (24.6)	29 (29.0)		234 (21.5)	21 (25.6)	
FPG ≥5.6 mmol/L	359 (31.2)	68 (68.0)	<0.001	342 (31.4)	59 (72.0)	<0.001
BMI ≥23 kg/m^2	224 (19.5)	32 (32.0)	0.003	367 (33.7)	35 (42.7)	0.097
TC ≥5.2 mmol/L	164 (14.3)	20 (20.0)	0.120	214 (19.6)	25 (30.5)	0.019
TG ≥1.7 mmol/L	128 (11.1)	21 (21.0)	0.003	214 (19.6)	30 (36.6)	<0.001
HDL-C <1.0 (males)/1.3 (females) mmol/L	79 (6.9)	10 (10.0)	0.243	460 (42.2)	30 (36.6)	0.320
Homocysteine, μmol/L			0.778			0.695
<10	495 (43.0)	40 (40.0)		661 (60.6)	47 (57.3)	
10–16	465 (40.4)	44 (44.0)		314 (28.8)	24 (29.3)	
≥16	190 (16.5)	16 (16.0)		115 (10.6)	11 (13.4)	
eGFR, mL/min/1.73 m^2			0.128			0.963
≥120	162 (14.1)	13 (13.0)		212 (19.4)	15 (18.3)	
90–120	850 (73.9)	68 (68.0)		743 (68.2)	57 (69.5)	
<90	138 (12.0)	19 (19.0)		135 (12.4)	10 (12.2)	
Insomnia			0.069			0.008
Frequent	111 (9.7)	16 (16.0)		163 (15.0)	21 (25.6)	
Medium	477 (41.5)	33 (33.0)		466 (42.8)	23 (28.0)	
Seldom	562 (48.9)	51 (51.0)		461 (42.3)	38 (46.3)	
MTHFR 677C→T polymorphism			0.536			0.001
CC	416 (36.2)	36 (36.0)		420 (38.5)	19 (23.2)	
CT	538 (46.8)	51 (51.0)		518 (47.5)	41 (50.0)	
TT	196 (17.0)	13 (13.0)		152 (13.9)	22 (26.8)	
Current smoking	813 (70.7)	68 (68.0)	0.571	31 (2.8)	1 (1.2)	0.384
Current drinking	532 (46.3)	40 (40.0)	0.228	24 (2.2)	4 (4.9)	0.126

Data are presented as mean (SD) or N (%), unless stated otherwise. BMI = body mass index, BP = blood pressure, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein-cholesterol, HOMA-IR = insulin resistance index of homeostasis model assessment, MTHFR = methylenetetrahydrofolate reductase, NOD = new-onset diabetes, TC = total cholesterol, TG = triglyceride.

* Homocysteine, insulin, and HOMA-IR are presented as median (Q1–Q3) because of skewed distribution.

† Insulin and HOMA-IR are available only for 2144 of the participants.

compared with those without in both males and females. However, NOD patients had significantly higher *MTHFR* 677 TT genotype rates in females only (Table 1).

The incidence rates of NOD in males and females were 8.0% and 7.0%, respectively. Compared with subjects with *MTHFR* 677 CC genotype, those with TT genotype had a higher risk of NOD in females (OR 2.78, 95% CI 1.39–5.56) but not in males (0.80, 0.40–1.61, *P* for interaction = 0.008). Hyperhomocysteinemia (≥16 vs <10 μmol/L) was not associated with NOD in males (0.88, 0.42–1.85) or females (1.52, 0.65–3.57). However, mildly decreased eGFR (<90 vs 90–120 mL/min/1.73 m^2) was associated with NOD mainly in males (1.96, 1.01–3.78; females, 0.74, 0.32–1.72, *P* for interaction = 0.134) (Table 2).

In the full models, we observed that higher TG, BMI, FPG levels, and insomnia, in addition to *MTHFR* C677T polymorphism in females, and higher FPG levels and insomnia in addition to lower eGFR levels in males remained independent risk factors for the risk of NOD (data not shown).

In the stratified analyses, the *MTHFR* 677C→T polymorphism (CC, CT, and TT genotypes) was more strongly associated with the risk of NOD among females with higher BMI (≥23 vs <23 kg/m^2, *P* for interaction = 0.009) or lower HDL-C levels (<1.3 vs ≥1.3 mmol/L, *P* for interaction = 0.015). However, the association of *MTHFR* 677C→T polymorphism with increased risks of NOD in females appeared to be similar among subgroups classified according to baseline homocysteine (≥10 vs <10 μmol/L, *P* for interaction = 0.061), eGFR (<110 vs ≥110 mL/min/1.73 m^2, *P* for interaction = 0.229), FPG (≥5.6 vs <5.6 mmol/L, *P* for interaction = 0.635), TG levels (≥1.7 vs <1.7 mmol/L, *P* for interaction = 0.189), or insomnia (frequent vs medium or seldom, *P* for interaction = 0.831). Furthermore, similar trends for eGFR category and increased risk of NOD in males were observed among subgroups stratified by insomnia (*P* for interaction = 0.946), baseline FPG (*P* for interaction = 0.977), homocysteine levels (*P* for interaction = 0.573), 0.573), or *MTHFR* 677C→T polymorphism (*P* for interaction = 0.880) (Table 3).

Further adjustment for HOMA-IR (n = 2144) did not substantially change the relationship of *MTHFR* 677C→T polymorphism (TT vs CC, 2.20; 1.02–4.73) in females or eGFR levels (<90 vs 90–120 mL/min/1.73 m^2, 1.85; 0.92–3.71) in males with the risk of NOD.

DISCUSSION

Previous studies have shown conflicting results regarding the homocysteine levels in patients with diabetes. A recent meta-analysis of 14 case–control studies found that the mean homocysteine concentration was greater in patients with type 2 diabetes than in control subjects.[5] However, the 3 studies[15–17] that contributed most to the overall estimate included patients with type 2 diabetes accompanied by varying degrees of kidney disorders. These results suggest that it may be renal dysfunction but not diabetes that mostly explains the difference in homocysteine levels between type 2 diabetes patients and control subjects in this meta-analysis.[5] In fact, in our present prospective study, we did not find significant associations between homocysteine levels and NOD in males or females.

However, consistent with this meta-analysis,[5] female subjects with TT genotype in the current study had a higher risk of NOD (TT vs CC genotype, 2.78; 1.39–5.56). To explain the significant relationship of *MTHFR* gene 677C→T polymorphism (but not homocysteine levels) with the risk of NOD, we speculate that, first, the homocysteine levels (median 10.7 μmol/L in males and 9.1 μmol/L in females) in the present study was possibly not high enough to cause obvious organ damage. Second, plasma homocysteine may just serve as a reliable functional marker of folate status, and folate may have other actions, including antioxidant actions, effects on cofactor availability, or direct interactions with the enzyme endothelial NO synthase, in addition to homocysteine lowering that influence health status.[18] A previous report[19] showed that folic acid given to patients with coronary heart disease resulted in improvement in endothelial function without any change in

TABLE 2. Relationship of *MTHFR* 677C→T Polymorphism, Baseline Homocysteine Levels, and eGFR With the Risk of NOD

	Males				Females			
	Events (%)	Age-Adjusted OR	Multivariate-Adjusted OR*	Full Model-Adjusted OR†	Events (%)	Age-Adjusted OR	Multivariate-Adjusted OR*	Full Model-Adjusted OR†
MTHFR C677T genotypes								
CC	36 (8.0)	1.00	1.00	1.00	19 (4.3)	1.00	1.00	1.00
CT	51 (8.7)	1.10 (0.70–1.71)	1.07 (0.67–1.71)	1.06 (0.66–1.69)	41 (7.3)	1.67 (0.95–2.92)	1.47 (0.82–2.65)	1.47 (0.81–2.65)
TT	13 (6.2)	0.77 (0.40–1.48)	0.76 (0.38–1.50)	0.80 (0.40–1.61)	22 (12.6)	3.11 (1.63–5.91)	2.99 (1.51–5.90)	2.78 (1.39–5.56)
Homocysteine, μmol/L								
<10	40 (7.5)	1.00	1.00	1.00	47 (6.6)	1.00	1.00	1.00
10–16	44 (8.6)	1.17 (0.75–1.82)	1.11 (0.69–1.78)	1.06 (0.65–1.73)	24 (7.1)	1.00 (0.60–1.67)	0.93 (0.54–1.61)	0.97 (0.55–1.71)
≥16	16 (7.8)	1.04 (0.57–1.90)	1.13 (0.60–2.12)	0.88 (0.42–1.85)	11 (8.7)	1.24 (0.62–2.48)	1.42 (0.68–3.00)	1.52 (0.65–3.57)
eGFR, mL/min/1.73 m^2								
≥120	13 (7.4)	1.02 (0.54–1.92)	1.21 (0.61–2.38)	1.20 (0.60–2.40)	15 (6.6)	1.26 (0.66–2.40)	1.48 (0.75–2.94)	1.39 (0.69–2.82)
90–120	68 (7.4)	1.00	1.00	1.00	57 (7.1)	1.00	1.00	1.00
<90	19 (12.1)	1.71 (1.00–2.95)	1.89 (1.06–3.36)	1.96 (1.01–3.78)	10 (6.9)	0.97 (0.48–1.95)	0.85 (0.40–1.80)	0.74 (0.32–1.72)

BMI = body mass index, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein-cholesterol, MTHFR = methylenetetrahydrofolate reductase, NOD = new-onset diabetes, OR = odds ratio, TC = total cholesterol, TG = triglyceride.

* Adjustment for age (year), FPG (≥5.6 vs <5.6 mmol/L), BMI (≥23 vs <23 kg/m^2), blood pressure (<130/85, 130/85–140/90, and ≥140/90 mm Hg), TGs (≥1.7 vs <1.7 mmol/L), TC (≥5.2 vs <5.2 mmol/L), HDL-C (<1.3/1.0 vs ≥1.3/1.0 mmol/L), current smoking (yes vs no), current alcohol drinking (yes vs no), and insomnia (frequent, medium, and seldom).

† The full model included *MTHFR* 677C→T polymorphism (CC, CT, and TT), homocysteine (<10, 10–16, and ≥16 μmol/L), eGFR (≥120, 90–120, and <90 mL/min/1.73m^2), and all the above variables.

TABLE 3. Stratified Analysis of Multivariate ORs for NOD Among 1172 Women According to *MTHFR* 677C→T Polymorphism*

Variables	CC		CT		TT		*P* for trend	*P* for interaction
	Events (%)	OR	Events (%)	OR	Events (%)	OR		
FPG, mmol/L								
<5.6	5 (1.6)	1.00	12 (3.4)	1.99 (0.67–5.90)	6 (5.8)	3.45 (0.97–12.28)	0.052	0.635
≥5.6	14 (10.9)	1.00	29 (14.4)	1.33 (0.65–2.71)	16 (22.9)	2.52 (1.09–5.82)	0.036	
eGFR,† mL/min/1.73 m^2								
≥110	11 (4.6)	1.00	17 (6.2)	1.05 (0.46–2.38)	10 (11.4)	1.79 (0.68–4.71)	0.279	0.229
<110	8 (4.0)	1.00	24 (8.4)	2.34 (0.94–5.80)	12 (14.0)	5.36 (1.82–15.83)	0.002	
Homocysteine, μmol/L†								
<10	15 (5.3)	1.00	23 (6.8)	1.21 (0.60–2.45)	9 (10.5)	1.74 (0.69–4.41)	0.262	0.061
≥10	4 (2.6)	1.00	18 (8.1)	2.95 (0.91–9.59)	13 (14.8)	6.92 (2.00–24.01)	0.001	
BMI, kg/m^2								
<23	13 (4.6)	1.00	26 (6.9)	1.20 (0.58–2.48)	8 (7.1)	1.06 (0.39–2.90)	0.824	0.009
≥23	6 (3.8)	1.00	15 (8.3)	2.52 (0.87–7.28)	14 (22.6)	9.43 (2.94–30.22)	<0.001	
HDL-C, mmol/L								
≥1.3	15 (6.1)	1.00	28 (8.3)	1.16 (0.57–2.36)	9 (9.0)	1.27 (0.49–3.30)	0.600	0.015
<1.3	4 (2.1)	1.00	13 (5.9)	3.06 (0.93–10.05)	13 (17.6)	9.22 (2.62–32.45)	<0.001	

BMI = body mass index, eGFR = estimated glomerular filtration rate, FPG = fasting plasma glucose, HDL-C = high-density lipoprotein-cholesterol, MTHFR = methylenetetrahydrofolate reductase, NOD = new-onset diabetes, OR = odds ratio, TC = total cholesterol, TG = triglyceride.

* Adjusted, if not stratified, for age (year), *MTHFR* 677C→T polymorphism (CC, CT, and TT), homocysteine (10, 10–16, and ≥16 μmol/L), eGFR (≥120, 90–120, and <90 mL/min/1.73 m^2), FPG (≥5.6 vs <5.6 mmol/L), body mass index (≥23 vs <23 kg/m^2), blood pressure (<130/85, 130/85–140/90, and ≥140/90 mm Hg), TGs (≥1.7 vs <1.7 mmol/L), TC (≥5.2 vs <5.2 mmol/L), HDL-C (<1.3 vs ≥1.3 mmol/L), and insomnia (frequent, medium, and seldom).

† The median of the distribution for eGFR and homocysteine was found to be 110 and 10, respectively; therefore, this value was used as the cut off point for stratification.

plasma homocysteine concentration. Unfortunately, we were not able to directly examine the association between *MTHFR* 677C→T polymorphism, folate, and the risk of NOD in the current study due to the lack of baseline folate data. Additional studies are required to further address this topic and to evaluate the role of folic acid supplementation in reducing the risk of diabetes, particularly in populations without folic acid fortification. Meanwhile, we did not have enough information to explain the sex-specific effect of *MTHFR* 677C→T polymorphism, which may relate to the possible role of hormones in folate/homocysteine metabolism.[20] This topic also needs to be confirmed and investigated in future studies.

Our current study detected a detrimentally interactive effect between the *MTHFR* 677C→T polymorphism and higher BMI (≥23 vs <23 kg/m^2), or lower HDL-C (<1.3 vs ≥1.3 mmol/L) levels on the risk of NOD among females. Obese subjects without diabetes have been shown to exhibit an enhanced rate of glucose production.[21] We hypothesize that ''a higher BMI state'' may augment the *MTHFR* 677C→T polymorphism-mediated risk of NOD. Furthermore, *MTHFR* 677C→T polymorphism appeared to modify the efficacy of the drug pravastatin in reducing risk of cardiovascular events. A significantly protective effect against coronary heart disease (hazard ratio [HR] 0.71, 95% CI 0.58–0.87) was shown in subjects with CC genotype but not in subjects with CT (HR 1.25, 95% CI 0.97–1.61) or TT genotype (HR 0.80, 95% CI 0.50–1.28, *P* for interaction = 0.004).[22] Consistently, we also observed an interaction between the *MTHFR* 677C→T polymorphism and HDL-C levels on the risk of NOD. These results suggest that an investigation of the possible modifying effect of *MTHFR* 677C→T polymorphism on CVD associated with HDL-C increasing therapy maybe provide some clues regarding the conflicting results gathered from these kinds of trials.[23] Overall, our results indicate that the *MTHFR* 677TT genotype, along with homocysteine, BMI, and HDL-C levels, may help to identify apparently healthy females at increased risk for diabetes.

Menon et al[24] reported that hyperhomocysteinemia did not appear to be a risk factor for all-cause or CVD mortality, and prior studies demonstrating an association between homocysteine and CVD risk may have inadequately adjusted for the confounding effects of kidney function. We also observed that mildly decreased eGFR (<90 mL/min/1.73 m^2), but not homocysteine, was associated with the risk of new-onset disease. Nevertheless, more studies are needed to verify if our findings can be generalized to other populations or ethnicities, particularly individuals with lower eGFR levels or obvious chronic kidney disease.

The strengths of our study include the 7-year prospective follow-up of middle-aged rural Chinese men and women, and the comprehensive adjustments for the major traditional risk factors for NOD, including baseline FPG and IR. Our study also has several limitations. First, we did not measure glycosylated hemoglobin levels or perform glucose-tolerance tests. Our analyses were mainly based on the diabetes defined by FPG levels. Second, we did not have information regarding family history of diabetes. However, further adjustment for family history of diabetes did not change the significant association between *MTHFR* 677C→T polymorphism and prevalence of diabetes in our previous study.[6] Lastly, previous studies have shown that nonalcoholic fatty liver disease (NAFLD) was strongly associated with IR and diabetes.[25] Unfortunately, we were not able to examine this effect in the current study due to the lack of data on NAFLD. Additional studies are required to further address this topic.

In conclusion, we found that individuals with *MTHFR* 677TT genotype had a significantly higher risk of NOD among

females, particularly in those with higher BMI or lower HDL-C levels. The higher risk of NOD associated with mildly decreased eGFR ($<90\,mL/min/1.73\,m^2$) also warrants more investigation. Our study findings, if further confirmed, will provide new strategies to identify apparently healthy population at increased risk for diabetes. Furthermore, considering the higher frequency of *MTHFR* 677TT genotype in China and the higher stroke risk associated with TT genotype, our results also provide some explanations for the ethnic differences in diabetic complications seen between East Asian patients and Europeans.

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