Meta Gene 9 (2016) 70–75

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Relationship between *MTHFR* C677T and A1298C gene polymorphisms and complications of type 2 diabetes mellitus in an Emirati population

Sarah W. El Hajj Chehadeh [a], Herbert F. Jelinek [b], Wael A. Al Mahmeed [c], Guan K. Tay [d], Unini O. Odama [e], Gehad E.B. Elghazali [f], Habiba S. Al Safar [a,g,*]

[a] Khalifa University Center of Biotechnology, Abu Dhabi, United Arab Emirates
[b] School of Community Health and Centre for Research in Complex Systems, Charles Sturt University, Albury, New South Wales, Australia
[c] Institute of Cardiac Science, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates
[d] Centre for Forensic Science, University of Western Australia, Perth, Australia
[e] Landmark Nephrology and Hypertension Clinic, Talladega, AL, USA
[f] Institute of Laboratory Medicine, Sheikh Khalifa Medical City, Abu Dhabi, United Arab Emirates
[g] Faculty of Biomedical Engineering, Khalifa University of Science, Technology & Research, Abu Dhabi, United Arab Emirates

ARTICLE INFO

Article history:
Received 24 February 2016
Revised 11 April 2016
Accepted 15 April 2016
Available online 17 April 2016

Keywords:
MFTHR
A1298T
C677T
Emiratis
Type 2 diabetes mellitus

ABSTRACT

Purpose: Type 2 diabetes mellitus (T2DM) is the most common form of diabetes with clinical consequences giving rise to chronic multiple organ complications. Methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms are genetic variations that have been linked to T2DM, and micro/macrovascular complications. The link between *MTHFR* and T2DM however is strongly dependent on the ethnic group studied. The objective of this study was to investigate the possible association between two *MTHFR* polymorphisms (C677T and A1298C) and T2DM and specifically examine if there are any associations with clinical and demographic characteristics among patients in the United Arab Emirates (UAE).
Methods: The study included 169 T2DM patients and 209 healthy controls. Genomic DNA was isolated and genotyped using TaqMan real-Time PCR assays for the *MTHFR* C677T and A1298C polymorphisms.
Results: There were no significant differences in genotype and haplotype distributions observed between groups. A significant association was observed between the C677T polymorphism and history of cerebrovascular accident (CVA) ($p = 0.0330$), history of nephropathy ($p = 0.0280$) and levels of LDL cholesterol ($p = 0.0409$). Also, the A1298C polymorphism was associated with hypertriglyceridemia ($p = 0.0305$) in T2DM patients.
Conclusion: These findings demonstrate that the *MTHFR* gene polymorphisms are not related to T2DM in the Emirati population. However, these polymorphisms can be used as risk markers for CVA, nephropathy, high LDL cholesterol and triglycerides in T2DM patients and allow timely treatment.

1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM); a chronic metabolic disorder in older adults; is rapidly increasing worldwide (Chen et al., 2011). The World Health Organization (WHO) estimated the number of people with diabetes at approximately 171 million in 2000 and projected that the disease would affect more than 360 million people by the year 2030 (World Health Organization, 2007). The United Arab Emirates (UAE), a country which is experiencing rapid growth and development since formation in 1971, has one of the highest frequencies of diabetes in the world, particularly T2DM (International Diabetes Federation, 2013) with significant T2DM-related complications (Saadi et al., 2007). A study in the city of Al Ain, has estimated the prevalence of diabetes at 25% among nationals in 2000 (Malik et al., 2005). In 2007, two studies in the UAE showed that 35% of patients with diabetes suffered from hypertension, 19% from diabetic retinopathy, 35% from diabetic neuropathy, 4% from Cerebrovascular accident (CVA) and 14% from Coronary Artery Disease (CAD) (Al-Maskari and El-Sadig, 2007; Al-Maskari et al., 2007). T2DM is a complex disease, which involves environmental and genetic components, however gaps of knowledge still exist in the genetic polymorphic variations that occur with this disease. Therefore studies that focus on the genetics of T2DM are beneficial in providing additional knowledge that might aid in early disease detection and adequate management of complications that may arise.

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in folate metabolism. It catalyzes the reduction of 5, 10-

* Corresponding author at: Khalifa University Biotechnology Center, Khalifa University, PO BOX 127788, Abu Dhabi, United Arab Emirates.
E-mail address: habiba.alsafar@kustar.ac.ae (H.S. Al Safar).

http://dx.doi.org/10.1016/j.mgene.2016.04.002

methylene-tetrahydrofolate to 5-methyltetrahydrofolate (Frosst et al., 1995) a co-substrate for the transformation of homocysteine to methionine via a transmethylation pathway (Toffoli et al., 2003). The gene encoding MTHFR is located on chromosome 1 at 1p36.6 (Outinen et al., 1998). Reduction in the activity of MTHFR caused by congenital defects and/or deficiencies is associated with an increased blood level of homocysteine (Kluijtmans et al., 1996). Single nucleotide polymorphisms (SNPs) of the *MTHFR* gene, C677T and A1298C, cause decreased enzyme activity (Castro et al., 2003). The most common *MTHFR* polymorphism is a Cytosine (C) to Thymine (T) transition at nucleotide 677 (C677T) resulting in an alanine to valine substitution, which results in a decrease in enzymatic activity of 50% (Hankey and Eikelboom, 1999). The A1298C *MTHFR* variant is an Adenine (A) to Cytosine (C) transition at nucleotide 1298 resulting in a glutamate to alanine substitution. The enzyme activity is decreased to 40% when compared to the wild type enzyme (Weisberg et al., 1998). The frequency of the two mutations in the *MTHFR* gene (C677T and A1298C) and changes in enzyme activity differ among ethnic populations and geographical regions (Esfahani et al., 2003; Yang et al., 2013). The resultant decrease in enzyme activity reduces folate levels and consequently increases the blood level of homocysteine (van der Put and Blom, 2000; Weisberg et al., 1998). Some studies have shown that the elevated blood level of homocysteine is linked to insulin resistance (Scullion et al., 2012), which is the major cause of T2DM complications such as diabetic nephropathy (Mtiraoui et al., 2007; Ukinc et al., 2009).

Numerous studies have demonstrated associations between *MTHFR* polymorphisms (C677T and A1298C) and risk of T2DM (AbdRaboh et al., 2013; Settin et al., 2015; Zhang et al., 2014). However, the outcomes have not been consistent. Recent studies on patients of Arab and Asian ethnicities showed significant associations between *MTHFR* C677T as well as A1298C polymorphisms and risk of T2DM (Al-Rubeaan et al., 2013; Alghasham et al., 2012). Other studies found no association between these polymorphisms and T2DM in patients of Asian, Caucasian and African descent (Mtiraoui et al., 2007; Zhong et al., 2013). There is no data available on the frequencies of the *MTHFR* mutations (C677T and A1298C) and its relation to T2DM-related complications in the Emirati population. Therefore, this study was conceived to determine the frequencies of these mutations and evaluate if there are associations with T2DM and the range of complications linked to the disease among Emiratis. To our knowledge this study presents the first Arab MTHFR haplotype investigation in T2DM patients.

2. Research design and methods

2.1. Subjects and sample collection

The sample size was determined using the Power Calculator for Genetic Studies developed by Skol et al. (2006) applying the web-based program at http://www.sph.umich.edu/csg/abecasis/CaTS/index.html. The prevalence of 21% for T2DM in the adult population of the United Arab Emirates was used (Alsafar et al., 2012). We also predicted disease allele frequencies of ≥0.25, and assumed a multiplicative disease model (Zondervan and Cardon, 2007). From the power calculation: 200 cases and 150 controls were needed for this study to be able to reject the null hypothesis with an odds ratio (OR) of ≥1.5 reaching at least 85% power.

This study was a randomized case control study in which 209 (118 females and 91 males) unrelated diabetic patients and 169 (106 females and 63 males) healthy controls were enrolled during their routine visit to an endocrinology clinic in Abu Dhabi, United Arab Emirates between the period of June 2012 and December 2013.

Each volunteer agreed to take part in this study after a briefing session, with each signing an informed consent form that had been approved by the Institutional Ethics Committee of the Sheikh Khalifa Medical City (SKMC). There were several inclusion and exclusion criteria used in this study: inclusion criteria were UAE National, healthy individuals, T2DM patients with and without complications, able to give consent and above 18 years old. Exclusion Criteria were Non-UAE national, pregnant female, not be able to consent and less than 18 years old.

One milliliter of saliva was collected from each participant using the Oragene OGR-500 kit (DNA Genotek, Ottwa, Canada). Clinical assessments and a lifestyle questionnaire for each participant were completed at the clinic. These observations were required to study any correlation between lifestyle variables and genetic variation. An individual was classified as T2DM if the subject was (1) diagnosed with T2DM by a qualified physician, (2) on a prescribed drug treatment regimen for T2DM and (3) returned biochemical test results of a fasting plasma glucose level of at least 126 mg/dl based on the criteria outlined by the World Health Organization (WHO) consultation group report (Alberti and Zimmet, 1998).

The individuals with a Body Mass Index (BMI) score of greater than 30 were considered to be obese and those with a BMI less than 30 were grouped into the non-obese population. According to Eighth Joint National Committee (JNC 8) classification, all the individuals with a blood pressure of more than 140/90 mm Hg were considered to be hypertensive (James et al., 2014).

2.2. Genotyping

Genomic DNA was extracted from the saliva samples using the prepIT®L2P system (DNA Genotek, Ottwa, Canada) in accordance with the manufacturer's instructions. Pre-validated and designed allelic discrimination TaqMan real-Time PCR assays (Applied Biosystems, Foster City, CA) were used for detection of the respective SNPs in C677T (rs1801133) and A1298C (rs1801131) including appropriate primers and fluorescently labeled (FAM and VIC) minor groove binder (MGB™) probes to detect the alleles. For MTHFR C677T (rs1801133), forward primer 5′-GAAAAGCTGCGTGATGATG-3′, reverse primer 5′-TTGAAGGAGAAGGTGTC-3′, probe 1 (VIC-dye labeled) AATCGGCTCC CGC, probe 2 (FAM-dye labeled) AATCGACTCCCGC; for MTHFR A1298C (rs1801131), forward primer 5′-AAGAACGAAGACTTCAAA-3′, reverse primer 5′-TGGGGGGAGGAGCTGAC-3′, probe 1 (VIC-dye labeled) ACACTTGCTTCACT, probe 2 (FAM-dye labeled) ACACTTTCTTCA CT. All PCR reaction contained 10 ng of DNA, 5 μl TaqMan GTXpress Master Mix (Applied Biosystems, Foster City, CA), 0.5 μl primers and probes (20×) and water for a final volume of 10 μl including the appropriate negative controls in all assays. The Genotyping success rates were 99.9% for both SNPs. Amplification was performed in ViiA™ 7 Real-time PCR system (Applied Biosystems, Foster City, CA) with incorporated software for SNP genotyping (Applied Biosystems, Foster City, CA).

2.3. Statistical methods

All statistical tests performed in this study were two tailed and p values less than 0.05 were considered to be statistically significant, unless otherwise stated. Statistical analyses were performed using STATA version 13 (STATA Corp., TX, USA). All continuous variables were expressed as the mean ± SD or as percentages for categorical variables. The genotype frequencies were tested for Hardy–Weinberg equilibrium using a chi-square test. Fisher exact confidence intervals were drawn for relative risk estimates and calculated with a 95% confidence interval. The relationships between the C677T mutation or A1298C mutation and the demographic data and biochemical tests of the patients were analyzed by using the chi-square test and analysis of variance (ANOVA) followed by *post hoc* statistics, respectively. The following three genotype groups were considered: CC, CT, TT for *MTHFR* C677T and AA, AC, CC for *MTHFR* A1298C. The Student's t test was used to compare the continuous variables between the type 2 diabetes mellitus and healthy groups. For categorical variables, differences between the groups were tested by the chi-square test or by the Fisher's exact test.

Table 1
Demographic data and biochemical tests for patients with type 2 diabetes mellitus (n = 209) and their healthy controls (n = 169).

		Healthy controls	T2DM patients	p
Demographic data	Male, n (%)	63 (37.27)	91 (43.54)	
	Female, n (%)	106 (62.73)	118 (56.46)	
	Mean age (years)	44.52 ± 16.31	59.04 ± 12.47	<0.00001*
	BMI (kg/m^2)	29.33 ± 6.08	31.93 ± 6.39	0.0001*
	Systolic blood pressure (mm Hg)	122.69 ± 16.71	129.31 ± 18.99	0.0002*
	Diastolic blood pressure (mm Hg)	70.43 ± 11.64	69.22 ± 11.67	0.8224
	Family history of T2DM, n (%)	101 (59.76)	131 (62.68)	0.0080*
	Smoking, n (%)	31 (18.34)	46 (21.90)	0.4270
Complications	History of hypertension, n (%)	44 (26.03)	132 (62.85)	<0.0001*
	History of retinopathy, n (%)	0 (0.0)	27 (12.85)	<0.0001*
	History of neuropathy, n (%)	0 (0.0)	25 (11.90)	<0.0001*
	History of CVA, n (%)	0 (0.0)	5 (2.38)	0.1660
	History of nephropathy, n (%)	2 (0.95)	27 (12.85)	<0.0001*
	History of CAD, n (%)	0 (0.0)	16 (7.61)	0.0030*
Biochemical tests	HbA1c (%)	5.63 ± 0.65	7.56 ± 1.71	<0.00001*
	Triglyceride (mmol/l)	1.19 ± 0.73	1.41 ± 0.74	0.0138*
	Total cholesterol (mmol/l)	4.41 ± 0.97	4.07 ± 1.04	0.9952
	HDL-cholesterol (mmol/l)	1.29 ± 0.39	1.21 ± 0.33	0.9595
	LDL-cholesterol (mmol/l)	2.60 ± 0.92	2.24 ± 0.91	0.9988

All continuous variables are presented as mean ± standard deviation and all categorical variables as percentages. T2DM: type 2 diabetes mellitus, BMI: body mass index, CVA: cerebrovascular accident, CAD: coronary artery disease, HbA1c: glycosylated hemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, n: number of individuals.
* Significant p < 0.05.

The SHEsis online haplotype analysis software (http://analysis.bio-x.cn/myAnalysis.php) was used to test case control haplotypes.

3. Results

The demographic data from the T2DM patients studied and information collected from the biochemical tests for these volunteers and their corresponding healthy controls are shown in Table 1. The age mean ± standard deviation (SD) of the patients and healthy controls were 59.04 ± 12.47 and 44.52 ± 16.31 years, respectively (p < 0.00001). Furthermore, significant differences were observed between the groups for the following clinical indicators: HbA1c (p < 0.00001), history of hypertension (p < 0.0001), history of retinopathy (p < 0.0001), history of neuropathy (p < 0.0001) and history of nephropathy (p < 0.0001), BMI (p = 0.0001), systolic blood pressure (p = 0.0002), triglyceride (p = 0.0138) levels, family history with T2DM (p = 0.0080) and history of CAD (p = 0.0030). The differences in diastolic blood pressure (p = 0.8224), total cholesterol (p = 0.9952), high-density lipoprotein (HDL) cholesterol (p = 0.9595), low-density lipoprotein (LDL) cholesterol (p = 0.9988), smoking (p = 0.4270) and history of CVA (p = 0.1660) were not significant between the two groups.

The genotype and allele frequencies of *MTHFR* C677T and A1298C polymorphisms in patients and controls are shown in Tables 2 and 3, respectively. The most prevalent genotype for the C677T SNP was CC in both the patients and controls. The most prevalent genotype of the A1298C SNP was AC in both the patient and control groups. No significant differences were observed in the distributions of the genotype and allele frequencies of the *MTHFR* gene C677T (p = 0.0580 and p = 0.9342, odds ratio = 1.02, 95% confidence interval = 0.67–1.54) and A1298C (p = 0.5980 and p = 0.3182, odds ratio = 0.86, 95% confidence interval = 0.64–1.15) between the patients with T2DM and the control group. There were no differences found when the patient and control group were compared according to CC + CT versus TT and CC versus CT + TT genotypes (p = 0.0809 and p = 0.3725, respectively) or according to AA + AC versus CC and AA versus AC + CC genotypes (p = 0.4435 and p = 0.3793, respectively).

The demographic data of the subjects and their clinical parameters were stratified according to the *MTHFR* C677T and A1298C polymorphisms as shown in Tables 4 and 5, respectively. With respect to the *MTHFR* C677T genotype, there were no significant differences in the demographics and clinical parameters between T2DM patients with the CC genotype, when compared to the alternative allele (CT and TT) in terms of all baseline characteristics, except for those with a history of CVA (p = 0.0330), history of nephropathy (p = 0.0280) and LDL cholesterol (p = 0.0409). For the *MTHFR* A1298C polymorphism, there were no significant differences between patients with the AA genotype and those that carried the C allele (AC and CC) in terms of all baseline characteristics, except triglyceride levels (p = 0.0305).

The haplotypes of the *MTHFR* gene was represented in Table 6. Four haplotypes were detected in the Emirati population. No significant

Table 2
Genotype and allele frequency of *MTHFR* C677T gene polymorphism in patients with type 2 diabetes mellitus and healthy controls.

MTHFR	Healthy controls	T2DM patients	OR (CI 95%)	p
C677T	n = 169 (%)	n = 209 (%)		
CC	132 (78.11)	155 (74.16)		0.0580
CT	27 (15.98)	49 (23.44)		
TT	10 (5.91)	5 (2.40)		
CC + CT:TT	159 (94.09): 10 (5.91)	204 (97.60): 5 (2.40)	2.57 (0.86–7.66)	0.0809
CC:CT + TT	132 (78.11): 37 (21.89)	155 (74.16): 54 (25.84)	1.24 (0.77–2.00)	0.3725
C	291 (86.09)	359 (85.88)	1.02 (0.67–1.54)	0.9342
T	47 (13.91)	59 (14.12)		

MTHFR: methylenetetrahydrofolate reductase, n: number of individuals. T2DM: type 2 diabetes mellitus, OR: odds ratio, CI: confidence intervals.

Table 3
Genotype and allele frequency of *MTHFR* A1298C gene polymorphism in patients with type 2 diabetes mellitus and healthy controls.

MTHFR	Healthy controls	T2DM patients	OR (CI 95%)	p
A1298C	n = 169 (%)	n = 209 (%)		
AA	25 (14.79)	38 (18.18)		0.598
AC	81 (47.93)	101 (48.33)		
CC	63 (37.28)	70 (33.49)		
AA + AC:CC	106 (62.72): 63 (37.28)	139 (66.51): 70 (33.49)	1.18 (0.77–1.80)	0.4435
AA:AC + CC	25 (14.79): 144 (85.21)	38 (18.18): 171 (81.82)	0.78 (0.45–1.36)	0.3793
A	131 (38.75)	177 (42.35)	0.86 (0.64–1.15)	0.3182
C	207 (61.25)	241 (57.65)		

MTHFR: methylenetetrahydrofolate reductase, n: number of individuals. T2DM: type 2 diabetes mellitus, OR: odds ratio, CI: confidence intervals.

Table 4
Demographics and clinical parameters according to *MTHFR* C677T polymorphism in T2DM patients.

MTHFR C677T	CC n = 155	CT n = 49	TT n = 5	p
BMI (kg/m2)	31.82 ± 6.12	32.30 ± 7.24	31.29 ± 7.00	0.8852
Systolic blood pressure (mm Hg)	128.85 ± 19.80	131.29 ± 16.40	123.80 ± 18.07	0.5164
Diastolic blood pressure (mm Hg)	69.46 ± 11.87	68.81 ± 11.62	66.20 ± 5.54	0.7984
Family history of T2DM, n (%)	101 (65.16)	26 (53.06)	4 (80.0)	0.2100
History of hypertension, n (%)	92 (59.35)	36 (73.46)	4 (80.0)	0.2140
History of retinopathy, n (%)	24 (15.48)	3 (6.12)	0 (0.0)	0.1600
History of neuropathy, n (%)	22 (14.19)	2 (4.08)	1 (20.00)	0.1400
History of CVA, n (%)	3 (1.93)	1 (2.04)	1 (20.00)	0.0330*
History of nephropathy, n (%)	15 (9.67)	10 (20.40)	2 (40.00)	0.0280*
History of CAD, n (%)	10 (6.45)	6 (12.24)	0 (0.0)	0.3340
Smoking, n (%)	30 (19.35)	14 (28.57)	2 (40.0)	0.2550
HbA1c (%)	7.56 ± 1.76	7.50 ± 1.58	8.07 ± 1.64	0.8229
Triglyceride (mmol/l)	1.35 ± 0.65	1.59 ± 0.96	1.43 ± 0.45	0.2072
Total cholesterol (mmol/l)	4.00 ± 0.92	4.30 ± 1.31	4.09 ± 1.28	0.2663
HDL-cholesterol (mmol/l)	1.21 ± 0.31	1.20 ± 0.38	1.03 ± 0.09	0.5267
LDL-cholesterol (mmol/l)	2.15 ± 0.81	2.49 ± 1.12	2.65 ± 1.32	0.0409*
Fasting blood glucose (mmol/l)	7.87 ± 4.11	9.08 ± 3.30	8.40 ± 3.55	0.4080

All continuous variables are presented as mean ± standard deviation and all categorical variables as percentages. MTHFR: methylenetetrahydrofolate reductase, BMI: body mass index, CVA: cerebrovascular accident, CAD: coronary artery disease, HbA1c: glycosylated hemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, n: number of individuals.
* Significant $p < 0.05$.

difference was observed in any of the examined haplotypes between T2DM patients and their healthy controls. These findings suggest that the haplotypes of the *MTHFR* gene is not associated with susceptibility of T2DM in The Emirati population.

4. Discussion

In this study, the frequency of *MTHFR* C677T and A1298C genotypes and alleles in the Emirati population as well as their association with T2DM and its complications was determined for the first time. The study did not identify any significant associations between the *MTHFR* C677T and A1298C polymorphisms and T2DM per se, but there were significant associations between the polymorphisms investigated and T2DM-related complications such as history of CVA, history of nephropathy, LDL cholesterol and triglycerides in this population.

The findings of significant association between the *MTHFR* C677T and history of nephropathy in patients with T2DM is consistent with a recent meta-analysis of 29 studies which showed that 677TT genotype was associated with moderately elevated risk for diabetic nephropathy in different ethnic groups such as Japanese, Russian, Polish, Chinese and Arab groups (Niu and Qi, 2012) and with other studies, which have shown that the T allele was associated with diabetic nephropathy, but not with T2DM per se (Cui et al., 2012; Książek et al., 2004). These results suggests that the *MTHFR* C677T may be a risk factor for diabetic nephropathy in T2DM patients. In previous studies the *MTHFR* A1298C polymorphism was reported to be a risk factor for neural tube defects (van der Put et al., 1998), but not for diabetic nephropathy in patients with T2DM (Moczulski et al., 2003). The latter result is consistent with the current study showing that the frequency of A1298C polymorphism did not differ among patients with diabetic nephropathy to those without.

In a previous study, Biselli et al. (2009) did not show an association between C677T and A1298C polymorphisms and CAD risk. It has also been reported in the literature that the *MTHFR* C677T mutation was not a risk factor for CAD and T2DM in populations from western Iran (Rahimi et al., 2009). Our results are consistent with the reported results in the literature since no significant correlation between the risk of CAD and *MTHFR* genotypes in the Emirati population was found. However, the present study showed a significant correlation between C677T mutation and CVA risk in T2DM patients and are consistent with recent studies showing that the *MTHFR* C677T polymorphism was associated with a moderately increased risk of CVA (Kumar et al., 2015; Zhu et al., 2015). Regarding the *MTHFR* A1298C polymorphism, no association with the incidence of CVA in T2DM patients was found and agree

Table 5
Demographics and clinical parameters according to *MTHFR* A1298C polymorphism in T2DM patients.

MTHFR A1298C	AA n = 38	AC n = 101	CC n = 70	p
BMI (kg/m2)	31.75 ± 6.04	31.93 ± 6.38	32.01 ± 6.66	0.9801
Systolic blood pressure (mm Hg)	130.79 ± 17.81	129.09 ± 21.09	128.88 ± 16.36	0.8492
Diastolic blood pressure (mm Hg)	70.50 ± 11.20	69.57 ± 11.06	68.10 ± 12.77	0.5709
Family history of T2DM, n (%)	24 (63.16)	60 (59.40)	47 (67.14)	0.5740
History of hypertension, n (%)	20 (52.63)	62 (61.38)	50 (71.42)	0.1790
History of retinopathy, n (%)	5 (13.15)	15 (14.85)	7 (10.00)	0.6480
History of neuropathy, n (%)	5 (13.15)	9 (8.91)	11 (15.71)	0.3910
History of CVA, n (%)	0 (0.0)	3 (2.97)	2 (2.85)	0.5650
History of nephropathy, n (%)	2 (5.26)	16 (15.84)	9 (12.85)	0.2530
History of CAD, n (%)	3 (7.89)	5 (4.95)	8 (11.42)	0.2930
Smoking, n (%)	7 (18.42)	19 (18.81)	20 (28.57)	0.2780
HbA1c (%)	7.50 ± 1.26	7.54 ± 1.88	7.61 ± 1.67	0.9539
Triglyceride (mmol/l)	1.44 ± 0.71	1.27 ± 0.65	1.60 ± 0.82	0.0305*
Total cholesterol (mmol/l)	3.93 ± 0.93	4.06 ± 1.01	4.17 ± 1.14	0.5930
HDL-cholesterol (mmol/l)	1.17 ± 0.37	1.25 ± 0.33	1.16 ± 0.29	0.2555
LDL-cholesterol (mmol/l)	2.09 ± 0.74	2.27 ± 0.89	2.28 ± 1.03	0.6190
Fasting blood glucose (mmol/l)	7.34 ± 2.76	8.82 ± 4.90	7.65 ± 2.43	0.2483

All continuous variables are presented as mean ± standard deviation and all categorical variables as percentages. MTHFR: methylenetetrahydrofolate reductase, BMI: body mass index, CVA: cerebrovascular accident, CAD: coronary artery disease, HbA1c: glycosylated hemoglobin, HDL: high density lipoprotein, LDL: low density lipoprotein, n: number of individuals.
* Significant $p < 0.05$.

Table 6
Frequencies of haplotypes for MTHFR C677T/MTHFR A1208C polymorphisms in patients with type 2 diabetes mellitus (n = 209) and their healthy controls (n = 169).

Haplotypes[a]		Frequency		χ^2	p	OR (CI 95%)
		Healthy controls	T2DM patients			
C	A	0.383	0.423	1.154	0.2828	1.174 (0.876–1.574)
T	A	0.005	0.000	–	–	–
C	C	0.478	0.435	1.496	0.2213	0.835 (0.626–1.115)
T	C	0.135	0.141	0.056	0.8132	1.051 (0.693–1.595)

[a] The order of polymorphism in haplotypes: C677T and A1208C. p are calculated by chi-square test using the SHEsis online haplotype analysis software. T2DM: type 2 diabetes mellitus. OR: odds ratio, CI: confidence intervals.

with an earlier study showing that the MTHFR A1298C polymorphism could not be a risk factor for CVA in Caucasian populations (Lv et al., 2013).

The C677T polymorphism is associated with diabetic nephropathy and CVA, but not the A1298C polymorphism. This may be due to the difference of the location of these two variants. The C677T mutation is located in exon 4 of the catalytic N-terminal domain of MTHFR, while the A1298C polymorphism is located in exon 7 of the regulatory C-terminal domain. The greater effect of C677T is due to its location in the catalytic domain. In addition, a previous study has shown that the A1298C polymorphism reduced MTHFR activity, but to a lesser extent than the C677T polymorphism (Weisberg et al., 1998).

Many studies have investigated the relationship between the *MTHFR* gene polymorphisms and clinical parameters, which is related to T2DM. There were no significant differences in terms of age, BMI, systolic blood pressure, diastolic blood pressure, hypertension, smoking and plasma concentration of HbA1c, triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol and fasting blood glucose between 677T carriers and non-carriers (Bahadır et al., 2015; Pollex et al., 2005). In a Saudi population, no significant difference was found between *MTHFR* C677T and A1298C genotypes and the potential risk factors, involving age, smoking and family history (Alghasham et al., 2012). The results reported here show that BMI, systolic blood pressure, diastolic blood pressure, family history, hypertension, smoking, HbA1c, total cholesterol, HDL-cholesterol, and fasting blood glucose of the T2DM patients segregated according to the *MTHFR* C677T and A1298C genotypes were similar. However, LDL-cholesterol for *MTHFR* C677T polymorphism and triglyceride for *MTHFR* A1298C polymorphism were different. The 677T allele carriers had significantly elevated LDL-cholesterol than 677CC homozygote carriers in T2DM patients. Furthermore, the individuals with the CC genotype of the A1298C SNP had significantly higher triglyceride than 1298 AC heterozygote carriers in T2DM patients. These results suggest that both 677T and 1298C mutated alleles may be a risk factor for elevated LDL-cholesterol and higher triglyceride in T2DM patients, respectively.

With respect to an association with type 2 diabetes mellitus, many studies have shown that the *MTHFR* C677T polymorphism is associated with T2DM in the Czech population (Benes et al., 2001) and the *MTHFR* A1298C polymorphism has a significant association with diabetes in Asian population under a dominant model (CC/AC vs. AA: OR = 1.31, 95% CI = 1.003–1.72, p = 0.047) (Yan et al., 2014). Conversely, no significant association was observed between *MTHFR* C677T polymorphism and T2DM in the Iranian population (Fakhrzadeh et al., 2009). Similarly Yan et al. (2014) found no association between *MTHFR* A1298C polymorphism and diabetes susceptibility in Caucasian populations. Until now, no data are available about the association between haplotypes of *MTHFR* gene and the susceptibility to T2DM in populations living in the Gulf countries. We have not found any association between haplotypes of *MTHFR* gene and T2DM. This may be due to the presence of many civilizations and ethnic groups throughout history and/or consanguineous marriage in UAE.

In conclusion, these findings demonstrate that the *MTHFR* gene polymorphisms are not related to T2DM in the Emirati population. However, these polymorphisms can be used as markers for assessment of CVA, nephropathy, high LDL cholesterol and triglycerides in T2DM patients. The potential for targeted risk assessment, extensive counseling methods, initiation of early preventive measures and stricter management strategies may be enhanced with the ultimate goal of decreasing cardiovascular complications which accounts for the main cause of mortality in T2DM patients.

Author's contributions

Drs. Alsafar, Elghazali and El Hajj Chehadeh have designed the study, prepared the manuscript and performed all the data analyses with assistance from all co-authors. Specifically, Ms El Hajj Chehadeh who performed all laboratory work in the Molecular Cell Biology laboratory at Khalifa University, Drs Mirgani and Al Yafei provided endless support in Data Analysis. Dr. Almahameed provided endless support in recruiting volunteers at SKMC clinics and Drs Odama, Tay and Jelinek who conscientiously worked on manuscript preparation.

Conflict of interests

The authors declare that there were no conflicts of interest for financial interests associated with this manuscript.

Acknowledgments

We acknowledge the volunteers who made this study possible. We would also like to thank Shatha Alwahabi, Umalkhair Ahmed and Thuraia Alkhoori from Khalifa University for their specific technical support that allowed the genotyping work to be carried out for this study. Special Thank you to Dr Rajaa A. Mirgani for her input while preparing the manuscript.

References

AbdRaboh, N.R., Badr, S., Ali, S., 2013. Prevalence of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in Egyptian patients with type 2 diabetes mellitus. Egypt. J. Med. Hum. Genet. 14, 87–93.
Alberti, K.G.M.M., Zimmet, P., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet. Med. 15, 539–553.
Alghasham, A., Settin, A.A., Ali, A., Dowaidar, M., Ismail, H., 2012. Association of MTHFR C677T and A1298C gene polymorphisms with hypertension. Int. J. Health Sci. 6, 3–11.
Al-Maskari, F., El-Sadig, M., 2007. Prevalence of diabetic retinopathy in the United Arab Emirates: a cross-sectional survey. BMC Ophthalmol. 7, 11.
Al-Maskari, F., El-Sadig, M., Norman, J., 2007. The prevalence of macrovascular complications among diabetic patients in the United Arab Emirates. Cardiovasc. Diabetol. 6, 24.
Al-Rubeaan, K., Siddiqui, K., Saeb, A.T.M., Nazir, N., Al-Naqeb, D., Al-Qasim, S., 2013. ACE I/D and MTHFR C677T polymorphisms are significantly associated with type 2 diabetes in Arab ethnicity: a meta-analysis. Gene 520, 166–177.
Alsafar, H., Jama-Alol, K.A., Hassoun, A.A., Tay, G.K., 2012. The prevalence of type 2 diabetes mellitus in the United Arab Emirates: justification for the establishment of the Emirates family registry. Int. J. Diabetes Dev. Ctries. 32, 25–32.
Bahadır, A., Eroz, R., Türker, Y., 2015. Does the MTHFR C677T gene polymorphism indicate cardiovascular disease risk in type 2 diabetes mellitus patients? Anatol. J. Cardiol. 15, 524–530.
Benes, P., Kanková, K., Muzik, J., Groch, L., Benedik, J., Elbl, L., Izakovicová-Hollá, L., Vasků, A., Znojil, V., Vácha, J., 2001. Methylenetetrahydrofolate reductase polymorphism, type II diabetes mellitus, coronary artery disease, and essential hypertension in the Czech population. Mol. Genet. Metab. 73, 188–195.
Biselli, P.M., Guerzoni, A.R., Goloni-Bertollo, E.M., Godoy, M.F., Abou-Chahla, J.A.B., Pavarino-Bertelli, É.C., 2009. MTHFR genetic variability on coronary artery disease development. Rev. Assoc. Med. Bras. 55, 274–278.
Castro, R., Rivera, I., Ravasco, P., Jakobs, C., Blom, H.J., Camilo, M.E., de Almeida, I.T., 2003. 5,10-Methylenetetrahydrofolate reductase 677C → T and 1298A → C mutations are genetic determinants of elevated homocysteine. Q. J. Med. 96, 297–303.
Chen, L., Magliano, D.J., Zimmet, P.Z., 2011. The worldwide epidemiology of type 2 diabetes mellitus—Present and future perspectives. Nat. Rev. Endocrinol. 8, 228–236.
Cui, W.P., Du, B., Jia, Y., Zhou, W.H., Liu, S.M., Cui, Y.C., Ma, F.Z., Luo, P., Miao, L.N., 2012. Is C677T polymorphism in methylenetetrahydrofolate reductase Gene a risk factor for diabetic nephropathy or diabetes mellitus in a Chinese population? Arch. Med. Res. 43, 42–50.

Esfahani, S.T., Cogger, E.A., Caudill, M.A., 2003. Heterogeneity in the prevalence of methylenetetrahydrofolate reductase gene polymorphisms in women of different ethnic groups. J. Am. Diet. Assoc. 103, 200–207.
Fakhrzadeh, H., Mirarefin, M., Sharifi, F., Ghotbi, S., Amoli, M., Pourebrabrahim, R., Nouri, M., Larijani, B., 2009. Association of methylenetetrahydrofolate reductase gene polymorphism (C677T) with metabolic syndrome in an Iranian population: Tehran homocysteine survey. J. Diabetes Metab. Disord. 8, 6.
Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Matthews, R.G., Boers, G.J.H., den Heijer, M., Kluijtmans, L.A.J., van den Heuve, L.P., Rozen, R., 1995. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat. Genet. 10, 111–113.
Hankey, G.J., Eikelboom, J.W., 1999. Homocysteine and vascular disease. Lancet 354, 407–413.
International Diabetes Federation, 2013. International Diabetes Federation: IDF Diabetes Atlas. https://www.idf.org/sites/default/files/EN_6E_Atlas_Full_0.pdf.
James, P.A., Oparil, S., Carter, B.L., Cushman, W.C., Dennison-Himmelfarb, C., Handler, J., Lackland, D.T., LeFevre, M.L., MacKenzie, T.D., Ogedegbe, O., 2014. 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 311, 507–520.
Kluijtmans, L.A., van den Heuvel, L.P., Boers, G.H., Frosst, P., Stevens, E.M., van Oost, B.A., den Heijer, M., Trijbels, F.J., Rozen, R., Blom, H.J., 1996. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am. J. Hum. Genet. 58, 35–41.
Książek, P., Bednarek-Skublewska, A., Buraczyńska, M., 2004. The C677T methylenetetrahydrofolate reductase gene mutation and nephropathy in type 2 diabetes mellitus. Med. Sci. Monit. 10, BR47–BR51.
Kumar, A., Kumar, P., Prasad, M., Sagar, R., Yadav, A.K., Pandit, A.K., Jali, V.P., Pathak, A., 2015. Association of C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR gene) with ischemic stroke: a meta-analysis. Neurol. Res. 37, 568–577.
Lv, Q., Lu, J., Wu, W., Sun, H., Zhang, J., 2013. Association of the methylenetetrahydrofolate reductase gene A1298C polymorphism with stroke risk based on a meta-analysis. Genet. Mol. Res. 12, 6882–6894.
Malik, M., Bakir, A., Saab, B.A., Roglic, G., King, H., 2005. Glucose intolerance and associated factors in the multi-ethnic population of the United Arab Emirates: results of a national survey. Diabetes Res. Clin. Pract. 69, 188–195.
Moczulski, D., Fojcik, H., Zukowska-Szczechowska, E., Szydlowska, I., Grzeszczak, W., 2003. Effects of the C677T and A1298C polymorphisms of the MTHFR gene on the genetic predisposition for diabetic nephropathy. Nephrol. Dial. Transplant. 18, 1535–1540.
Mtiraoui, N., Ezzidi, I., Chaieb, M., Marmouche, H., Aouni, Z., Chaieb, A., Mahjoub, T., Vaxillaire, M., Almawi, W.Y., 2007. MTHFR C677T and A1298C gene polymorphisms and hyperhomocysteinemia as risk factors of diabetic nephropathy in type 2 diabetes patients. Diabetes Res. Clin. Pract. 75, 99–106.
Niu, W., Qi, Y., 2012. An updated meta-analysis of methylenetetrahydrofolate reductase gene 677C/T polymorphism with diabetic nephropathy and diabetic retinopathy. Diabetes Res. Clin. Pract. 95, 110–118.
Outinen, P.A., Sood, S.K., Liaw, P.C., Sarge, K.D., Maeda, N., Hirsh, J., Ribau, J., Podor, T.J., Weitz, J.I., Austin, R.C., 1998. Characterization of the stress-inducing effects of homocysteine. Biochem. J. 332, 213–221.
Pollex, R.L., Mamakeesick, M., Zinman, B., Harris, S.B., Hanley, A.J.G., Hegele, R.A., 2005. Methylenetetrahydrofolate reductase polymorphism 677C > T is associated with peripheral arterial disease in type 2 diabetes. Cardiovasc. Diabetol. 4, 17-17.
Rahimi, Z., Nomani, H., Mozafari, H., Vaisi-Raygani, A., Madani, H., Malek-Khosravi, S., Parsian, A., 2009. Factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate reductase polymorphism C677T are not associated with coronary artery disease and type 2 diabetes mellitus in western Iran. Blood Coagul. Fibrinolysis 20, 252–256.
Saadi, H., Carruthers, S.G., Nagelkerke, N., Al-Maskari, F., Afandi, B., Reed, R., Lukic, M., Nicholls, M.G., Kazam, E., Algawi, K., Al-Kaabi, J., Leduc, C., Sabri, S., El-Sadig, M., Elkhumaidi, S., Agarwal, M., Benedict, S., 2007. Prevalence of diabetes mellitus and its complications in a population-based sample in Al Ain, United Arab Emirates. Diabetes Res. Clin. Pract. 78, 369–377.
Scullion, S.M.J., Gurgul-Convey, E., Elsner, M., Lenzen, S., Flatt, P.R., McClenaghan, N.H., 2012. Enhancement of homocysteine toxicity to insulin-secreting BRIN-BD11 cells in combination with alloxan. J. Endocrinol. 214, 233–238.
Settin, A., El-Baz, R., Ismaeel, A., Tolba, W., Allah, W.A., 2015. Association of ACE and MTHFR genetic polymorphisms with type 2 diabetes mellitus: susceptibility and complications. J. Renin-Angiotensin-Aldosterone Syst. 16, 838–843.
Skol, A.D., Scott, L.J., Abecasis, G.R., Boehnke, M., 2006. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. Nat. Genet. 38, 209–213.
Toffoli, G., Russo, A., Innocenti, F., Corona, G., Tumolo, S., Sartor, F., Mini, E., Boiocchi, M., 2003. Effect of methylenetetrahydrofolate reductase 677C → T polymorphism on toxicity and homocysteine plasma level after chronic methotrexate treatment of ovarian cancer patients. Int. J. Cancer 103, 294–299.
Ukinc, K., Ersoz, H., Karahan, C., Erem, C., Eminagaoglu, S., Hacihasanoglu, A., Yilmaz, M., Kocak, M., 2009. Methyltetrahydrofolate reductase C677T gene mutation and hyperhomocysteinemia as a novel risk factor for diabetic nephropathy. Endocrine 36, 255–261.
van der Put, N.M.J., Blom, H.J., 2000. Neural tube defects and a disturbed folate dependent homocysteine metabolism. Eur. J. Obstet. Gynecol. Reprod. Biol. 92, 57–61.
van der Put, N.M., Gabreëls, F., Stevens, E.M., Smeitink, J.A., Trijbels, F.J., Eskes, T.K., van den Heuvel, L.P., Blom, H.J., 1998. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am. J. Hum. Genet. 62, 1044–1051.
Weisberg, I., Tran, P., Christensen, B., Sibani, S., Rozen, R., 1998. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. Mol. Genet. Metab. 64, 169–172.
World Health Organization, 2007. Country and regional data on diabetes: prevalence of diabetes worlwide. http://www.who.int/diabetes/facts/world_figures/en/index.html (accessed 2013 February 17).
Yan, Y., Liang, H., Yang, S., Wang, J., Xie, L., Qin, X., Li, S., 2014. Methylenetetrahydrofolate reductase A1298C polymorphism and diabetes risk: evidence from a meta-analysis. Ren. Fail. 36, 1013–1017.
Yang, B., Liu, Y., Li, Y., Fan, S., Zhi, X., Lu, X., Wang, D., Zheng, Q., Wang, Y., Wang, Y., Sun, G., 2013. Geographical distribution of MTHFR C677T, A1298C and MTRR A66G Gene polymorphisms in China: findings from 15357 adults of Han nationality. PLoS One 8, e57917.
Zhang, D., Zhou, Y., Han, L., Ji, H., Li, J., 2014. The effect of MTHFR C677T polymorphism on type 2 diabetes mellitus with vascular complications in Chinese Han population: a meta-analysis. Endocr. J. 61, 717–726.
Zhong, J.H., Rodríguez, A.C., Yang, N.N., Li, L.Q., 2013. Methylenetetrahydrofolate reductase gene polymorphism and risk of type 2 diabetes mellitus. PLoS One 8, e74521.
Zhu, X.Y., Hou, R.Y., Pan, X.D., Wang, Y.C., Zhang, Z.S., Guo, R.Y., 2015. Association between the methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism and ischemic stroke in the Chinese population: a meta-analysis. Int. J. Neurosci. 125, 885–894.
Zondervan, K.T., Cardon, L.R., 2007. Designing candidate gene and genome-wide case–control association studies. Nat. Protoc. 2, 2492–2501.