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

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A B S T R A C T

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.

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methylene-tetrahydrofolate to 5-methyltetrahydrofolate (Frost 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 (Klujtimans 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 (Mitraoui et al., 2007; Uckun et al., 2009).

Numerous studies have demonstrated associations between MTHFR polymorphisms (C677T and A1298C) and risk of T2DM (AbdRaboh et al., 2013; Sellin 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 (Mitraoui 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/CatTS/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 able to consent and less than 18 years old.

One milliliter of saliva was collected from each participant using the Oragene GOR-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 preplant 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 (MG™) probes to detect the alleles. For MTHFR C677T (rs1801133), forward primer 5′-GAAAGTCTGGTATGATG-3′, reverse primer 5′-TTGAGGAAAGGCAGC-3′, probe 1 (VIC-dye labeled) AATCGGCTCCGC, probe 2 (FAM-dye labeled) AATCGACTCCGC, for MTHFR A1298C (rs1801131), forward primer 5′-AAAGAGACATCCTA-3′, reverse primer 5′-TGTCGGGAGGAGCCTAC-3′, probe 1 (VIC-dye labeled) ACACCTGGCTTACT, probe 2 (FAM-dye labeled) ACACCTGGCTTACT. All PCR reaction contained 10 ng of DNA, 5 μl TaqMan iGXpress 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 99.5% 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.
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 BMI (p < 0.0001), systolic blood pressure (p = 0.0001), diastolic blood pressure (p = 0.0001), smoking (p = 0.0001), history of CVA (p = 0.0001), history of nephropathy (p = 0.0001), history of neuropathy, n (%) 0 (0.0) 25 (11.90) 0.1660

The differences in diastolic blood pressure (p = 0.0001), systolic blood pressure (p = 0.0001), smoking (p = 0.0001), history of CVA (p = 0.0001), history of nephropathy, n (%) 0 (0.0) 25 (11.90) 0.1660

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

| Demographic data | Healthy controls | T2DM patients | p      |
|------------------|-----------------|--------------|--------|
|                  | Healthy controls | T2DM patients | p      |
|                  |                  |              |        |
| Sex              | Male, n (%)      | 63 (37.27)   | 91 (43.54) | 0.1660 |
|                  | Female, n (%)    | 106 (62.73)  | 118 (56.46) |        |
| Mean age (years) | 44.52 ± 16.31    | 59.04 ± 12.47 | <0.0001 * |
| BMI (kg/m²)      | 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 (66.28) | 0.0808 |
| Smoking, n (%)   | 31 (18.34)       | 46 (21.90)   | 0.4270 |
| 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) | 0.0300 |
| Biochemical tests |                  |              |        |
| HbA1c (%)        | 5.63 ± 0.65      | 7.56 ± 1.71  | <0.0001 |
| Triglyceride (mmol/l) | 1.19 ± 0.73 | 1.41 ± 0.74 | 0.0136 |
| 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.

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 |                  |              |            |        |
| 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) | 204 (95.86) | 2.57 (0.86–7.66) | 0.0809 |
| CC:CT + TT | 132 (78.11) | 155 (74.16) | 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|                  |              |            |        |
| AA    | 25 (14.79)      | 38 (18.18)    | 0.598      |        |
| AC    | 87 (47.93)      | 101 (48.33)   |            |        |
| CC    | 68 (37.38)      | 70 (33.49)    |            |        |
| AA + AC:CC | 106 (62.72) | 139 (68.15) | 1.18 (0.77–1.80) | 0.4435 |
| AA:AC + CC | 70 (43.70) | 84 (41.67) | 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.
demographic and clinical parameters according to Table 5.

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 C677T 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 suggest 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 (Moczulska 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, Riselli 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
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 C677T carriers and non-carriers (Bahadur et al., 2015; Pollex et al., 2005). In a Saudi population, no significant difference was found between 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 C677T allele carriers had significantly elevated LDL-cholesterol than 1298C 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 C677T and 1298C mutated alleles may be a risk factor for elevated LDL-cholesterol and higher triglyceride in T2DM patients, respectively.

Table 6

| Haplotypes | Frequency | χ² | p | OR (95% CI) |
|-----------|----------|----|---|------------|
| 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) |

*The order of polymorphism in haplotypes: C677T and A1298C. χ² are calculated by chi-square test using the SHEsis online haplotype analysis software. T2DM: type 2 diabetes mellitus. OR: odds ratio, CI: confidence intervals.

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

Dr. Alsafr, Elghazali and El Haji Chehadeh have designed the study, prepared the manuscript and performed all the data analyses with assistance from all co-authors. Specifically, Ms El Haji 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.

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