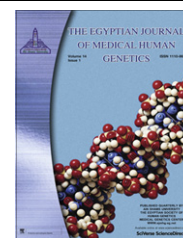




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ORIGINAL ARTICLE

Prevalence of methylenetetrahydrofolate reductase C677T and A1298C polymorphisms in Egyptian patients with type 2 diabetes mellitus

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KEYWORDS

T2DM;
(MTHFR) gene;
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Abstract Type 2 diabetes mellitus (T2DM) is a major public health problem around the world. The C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene have been reported to be associated with T2DM and its complications. This study is a case-control study which was performed to clarify the association between polymorphisms in these two genes and T2DM among Egyptians. Study population ($n = 120$) consists of 60 Egyptian diabetic patients and 60 healthy controls. The MTHFR C677T and A1298C polymorphisms were genotyped by polymerase chain reaction, followed by enzymatic digestion with *HinfI* and *MboII* enzymes, respectively. C677T and A1298C genetic polymorphisms conveyed an increase in T2DM risk (OR = 3.5, 95% CI = 1.1–11.6, $p = 0.032$ and OR = 2.2, 95% CI = 0.7–6.9, $p = 0.004$ respectively). Additionally, no significant associations between lipid/glucose metabolic indexes with MTHFR genotypes among diabetic patients were observed. Combined MTHFR gene polymorphisms revealed higher T2DM risk in homozygous and heterozygous forms compared to single gene polymorphism with pronounced risk in C677T/CT-A1298C/CC combined form (OR = 6.56, 95% CI = 0.76–56.2, $p = 0.041$). In conclusion, our data suggest that MTHFR C677T and A1298C polymorphisms are risk factor for T2DM in Egyptian patients. Also, the two gene polymorphisms may act synergistically to increase the risk of diabetes. Furthermore, it

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should be noted that the size of the studied population was relatively small and therefore, large-scale prospective studies are needed to confirm these findings.

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1. Introduction

The incidence of Type 2 diabetes mellitus (T2DM) has been increasing throughout the world [1]. It accounts for over 90–97% of all DM cases in developed countries with higher prevalence rates in developing countries [2,3]. It is characterized by a decreased production of insulin, together with insulin resistance [4,5]. This multifactorial disease or metabolic syndrome results from a complex interaction of genetic and environmental factors which influence beta-cell mass, insulin secretion, insulin action, fat distribution, and obesity [6]. With the increase in DM, there is also a corresponding increase in the related complications such as cardiovascular disease, retinopathy, neuropathy, and nephropathy [7].

Elevated homocysteine (Hcy) levels are associated with endothelial dysfunction [8], insulin resistance [9], prothrombotic state [10], macroangiopathy [11,12] and nephropathy [6,7] in diabetic patients. Several studies have demonstrated that elevated Hcy levels predict the risk of death or coronary events in T2DM patients [13–14]. MTHFR gene, located on chromosome 1 (1p36.3), encodes for methylenetetrahydrofolate reductase enzyme and catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine [15].

Two of the most investigated polymorphisms in the MTHFR gene are C677T and A1298C. The C677T polymorphism converts an alanine residue to a valine (ALA222VAL), leading to lower enzymatic activity [16]. Compared with the 677CC wild genotype, the 677TT homozygous and 677CT heterozygous genotypes decrease enzyme activity by approximately 70% and 40%, respectively [17,18]. The prevalence of this polymorphism differs among ethnic groups, ranging from 2% to 54.5% [19]. The second polymorphism of the MTHFR gene, A1298C, causes the substitution of glutamate by an alanine residue (GLU429ALA). 1298AA is the “normal” homozygous, 1298AC the heterozygous, and 1298CC the homozygous for the “variant”. The functional relevance in vivo of the A1298C allele is less well defined. A1298C affects enzyme function in vitro to a lesser degree, and individuals carrying the variation have frequently normal homocysteine and plasma folate concentrations [18,20]. The aim of this study was to investigate the effect of genetic polymorphism at MTHFR C677T as well as its potential interaction with A1298C on susceptibility to diabetes in a cohort of Egyptians.

2. subjects and methods

2.1. Subjects

A total of 60 adult Egyptian T2DM patients (mean age \pm SD 46.53 \pm 6.53 years) were recruited from the outpatient diabetes clinic of Ain Shams University Hospital in addition to 60 age and sex matched healthy unrelated Egyptian adult subjects (mean age \pm SD 44.76 \pm 7.21 years). Informed consent was obtained from all subjects with approval granted by Ain Shams Research and Ethics Committee “Ain-Shams Faculty

of Medicine federal number IRB00006444”, prior to sample collection. Inclusion criteria: fasting plasma glucose \geq 126 mg/dl, Hemoglobin A1c \geq 6.5% and/or treatment for diabetes including diet and/or oral antidiabetic drugs to achieve glycemic control. Duration of the disease on diabetic patients was 7.8 \pm 2.1 years. Recruitment of patients was restricted by the following criteria: the presence of hypertension/or taking antihypertensive drugs, diabetic nephropathy defined by persistent microalbuminuria (Albumin: Creatinine Ratio in spot urine sample: 2.5–25 mg/mmol in males, 3.5–35 mg/mmol in females) checked at least on two consecutive occasions over the previous 6 months. Renal or liver failure, retinopathy diagnosed by funduscopy examination, cardiovascular disease and intake of hormonal replacement therapy. Taking hypoglycemic drugs and lipid lowering drugs were not considered as exclusion criteria.

Detailed medical history for each group was obtained. Weight and height were measured to calculate the body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in a sitting position using a mercury column sphygmomanometer after at least 5 min of rest; two readings of SBP and DBP were taken. Using NICE hypertension guideline 2011, standards for hypertension was SBP \geq 140 mmHg and DBP \geq 90 mmHg [21]. Clinical and biochemical measurements of patients with type 2 diabetes were performed after 8 h of fasting. Diagnosis of diabetes was according to the criteria of American diabetes association (Fasting plasma glucose; FPG \geq 126 mg/dl. Fasting is defined as no caloric intake for at least 8 h) [22]. Control subjects did not have any abnormalities regarding their physical examination, blood pressure, family history, urine analysis, and routine laboratory blood tests; none of them were receiving any medications at the time of participation.

2.2. Methods

Venous blood samples were collected from each subject in three separate test tubes: one was used for biochemical analysis. The others were collected on EDTA for hemoglobin A1C and DNA extraction.

2.2.1. Biochemical measurements

Serum urea, creatinine, total Cholesterol, High density lipoprotein, low density lipoprotein, Triglyceride hemoglobin A1C and fasting blood sugar were done using C111 analyser Commercial kits (Roch-Diagnostics, Switzerland).

2.2.2. DNA Extraction

Genomic DNA was extracted from white blood cell pellets by salting out extraction method [15] using a wizard genomic DNA extraction kit from Promega. Red blood cell lysis was done by using red cell lysis buffer (20 mM tris-HCl pH 7.6) followed by centrifugation. Nuclei lysis was carried out by cell lysis buffer (10 mM tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 0.1% (w/v) SDS) and proteinase K (20 mg/mL) followed by centrifugation. Protein was precipitated by protein

precipitation solution (60 mL of 5 M potassium acetate, 11.5 mL of glacial acetic acid, 28.5 mL of water) followed by centrifugation. Finally DNA was precipitated by isopropanol and then ethanol 70% and rehydrated in Tris EDTA buffer (10 mM tris, 1 mM EDTA pH 8.0) and stored at -20°C . DNA purity and concentration were determined by the spectrophotometer measurement of absorbance at 260 and 280 nm.

2.2.3. Detection of MTHFR C677T and A1298C genetic polymorphism by restriction fragment length polymerase chain reaction

0.5–2.0 μg of human genomic DNA was amplified by polymerase chain reaction on Gene Amp PCR System 9700 thermocycler (Applied Biosystems). Genotyping was based on the methods described by Frosset et al., [17] and Van der Put et al., [23] as the target genes were amplified by PCR followed by restriction digestion with the endonuclease.

For MTHFR C677T polymorphism Primer sequences were as follows: The forward primer is 5TGAAGGAGAAGG TGTCTGCGGGA-3' and reverse primer 5'AGGACG GTGCG GTGAGAGTG-3'. PCR was carried out in a 25- μL reaction volume containing 100 ng of genomic DNA, 0.4 $\mu\text{mol/L}$ of each primer, 0.2 mmol/L of dNTPs, 2 mmol/L of MgCl_2 in 10% PCR buffer and 1 unit of *Taq* polymerase (Promega, UK). PCR conditions were optimized for an initial 2 min denaturation at 93°C followed by 35 cycles of denaturation at 93°C for 1 min, annealing at 58°C for 1 min, extension at 72°C for 1 min with final extension at 72°C for 10 min. 12.5 μL of the PCR products were digested with 5 units of *Hinf*I (Promega, UK) overnight at 37°C . RFLP product was separated on 2% agarose gel and visualized by ethidium bromide staining. Wild type (677CC) produced a single band at 198 bp. Heterozygotes (677CT) produced 198-, 175-, and 23-bp fragments. Homozygous mutants (677TT) produced 175- and 23-bp fragments (Fig. 1).

For MTHFR A1298C polymorphism, Primer sequences were: Forward primer 5'-CTT TGG GGA GCT GAA GGA CTA CTA C-3' and the reverse primer 5'-CAC TTTGTGAC-CATTCCGTTG-3'. 10 mM Tris·HCl, 50 mM of KCl, 1 mg/ml of gelatin, 3.0 mM of MgCl_2 , 200 μM each of dNTP, and 1.25 units of DNA *Taq* polymerase (Sigma). PCR conditions were optimized for an initial 2-min denaturation cycle at 92°C followed by 35 cycles of denaturation at 92°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for

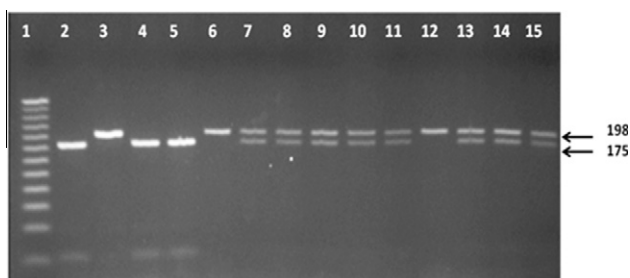


Figure 1 Agarose Gel electrophoresis for MTHFR C677T after digestion with *Hinf*I. Lane 1: 25 bp DNA marker, lanes 2, 4 and 5 (TT) genotype with two fragments 175 and 23 (faint). Lanes 3, 6 and 12 (CC) genotype with one fragment 198 bp. Lanes 7–11, and 13–15 (CT) genotype with three fragments 198, 175 and 23 bp (not seen).

30 s followed by a 7-min final extension at 72°C . 12.5 μL of PCR product was digested with 2.5 μL of *Mbo*II buffer and 2.5 units of *Mbo*II restriction enzyme (Promega, UK). RFLP products were separated on 2% agarose gel and visualized by ethidium bromide staining. Wild types (1298AA) produced five fragments of 56, 31, 30, 28, and 18 bp, heterozygotes (1298AC) produced six fragments of 84, 56, 31, 30, 28, and 18 bp, and the homozygous mutants (1298CC) produced four fragments of 84, 31, 30, and 18 bp. The major visible bands were those of 84 and 56 bp (Fig. 2).

2.2.4. Statistical analysis

Data were analyzed using SPSS Win statistical package version 15. Numerical data were expressed as mean, standard deviation and range. Qualitative data were expressed as frequency and percentage. AGT M235T and ACE I/D genotype frequencies were estimated by gene counting, and the differences between the studied groups were evaluated by Pearson's χ^2 test and ANOVA test. Allele and genotype frequencies were determined by allele counting, and the odds ratio (OR) and 95% confidence interval (CI) were also determined. Concordance of genotype frequencies with Hardy–Weinberg equilibrium was tested by χ^2 test.

3. Results

Clinical and laboratory data of studied groups are summarized in Table 1. Compared to healthy controls, individuals who developed diabetes had significantly higher mean values of fasting glucose, HbA1c and triglyceride levels.

Comparing the frequency of different genotypes among patients and controls (Table 2) revealed that: Evaluation of MTHFR C677T gene mutations (C/T or T/T compared to the wild type C/C) revealed that homozygous mutation of MTHFR C677T gene conferred increased T2DM risk (OR = 3.5, 95% CI = 1.06–11.57, $p = 0.032$). Statistically significant difference in C677T/T allele was found between T2DM and control cases with 2.2 risk for occurrence of T2DM (OR:2.189, 95% CI:1.17–4.10, $p = 0.013$). Individuals homozygous for MTHFR A1298C gene mutation (C/C homotype) had an increased risk of T2DM (OR = 2.2, 95% CI = 0.7–6.88, $p = 0.168$). This risk increase was also observed with A/C heterotype (OR = 2.5, 95% CI = 1.19–5.39, $p = 0.015$). A1298C C allele carry 2.9 fold risk for

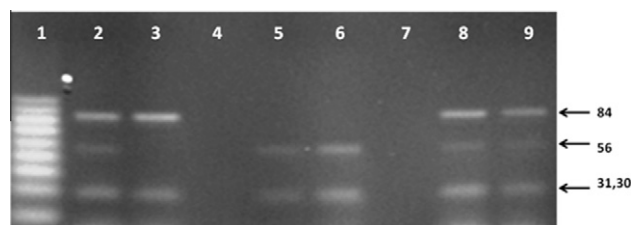


Figure 2 Agarose gel electrophoresis for A1298C polymorphism after digestion with *Mbo*II. Lane 1:10 bp DNA marker, lanes 2, 8 and 9 (AC) genotype which gave six fragments (84, 56, 31, 30, 28 and 18 bp) lanes 5 and 6 (AA) genotype which gave five bands (56, 31, 30, 28 and 18 bp) lane 3 (CC) genotype which gave four bands (84, 31, 30 and 18). The major visible bands were those of 84 bp and 56 bp.

Table 1 Clinical and laboratory data of study participants—T2DM versus control.

Characteristics	T2DM (n = 60)	Controls (n = 104)	P Value
<i>Gender</i>			
Male (%)	52 (86.67%)	93(89.42%)	0.385
Females (%)	8 (13.33%)	11 (10.58%)	
Age (year)	46.53 ± 6.53	44.76 ± 7.21	0.141
Duration of diabetes	7.8 ± 2.1 years	—	
Systolic blood pressure	136.91 ± 18.02	132.57 ± 13.52	0.191
Diastolic blood pressure	82.55 ± 6.59	82.02 ± 6.75	0.656
BMI (kg/m ²)	28.83 ± 3.38	27.27 ± 3.54	0.246
<i>Biochemical profile</i>			
Fasting blood sugar (mg/dl)	163.62 ± 61.45	98.53 ± 9.88	<0.001*
HB A1C (%)	6.89 ± 0.89	4.88 ± 0.65	<0.001*
Cholesterol (mg/dl)	197.52 ± 40.50	180.76 ± 47.91	0.317
Triglycerides (mg/dl)	194.26 ± 91.68	119.06 ± 66.61	0.013*
HDL cholesterol (mg/dl)	39.41 ± 8.64	41.87 ± 15.09	0.307
LDL cholesterol (mg/dl)	131.03 ± 41.15	115.77 ± 42.16	0.977
Urea (mg/dl)	21.85 ± 9.08	20.52 ± 9.43	0.972
Creatinine (mg/dl)	0.91 ± 0.15	0.95 ± 0.17	0.229

* Significant *p*-value < 0.05.**Table 2** The frequency of MTHFR C677T and A1298C genotypes in T2DM patients (n = 60) and controls (n = 60).

Item	T2DM patients no (%)	Controls no (%)	OR	95% CI	<i>p</i> -Value
<i>Genotypes</i>					
<i>MTHFR 677</i>					
677 C/C	37 (61.67)	45 (75)	0.536	0.25–1.17	0.116
677 C/T	11(18.33)	11(18.33)	1.00	0.398–2.58	1.02*
677 T/T	12(20)	4 (6.67)	3.5	1.06–11.57	0.032*
T allele	35(29.26)	17(15.8)	2.189	1.17–4.10	0.013*
<i>MTHFR 1298</i>					
1298 A/A	20 (33.33)	38 (63.33)	0.29	0.137–0.61	0.001
1298 A/C	30(50)	17(28.33)	2.53	1.19–5.39	0.015*
1298 C/C	10 (16.67)	5 (8.33)	2.2	0.7–6.88	0.168
C allele	50(41.66)	27(22.5)	2.87	1.62–5.07	<0.001*
<i>Combined genotypes</i>					
677 CC/1298AA	16(26.67)	28(46.67)	0.42	0.19–0.89	0.023*
677 CC/1298 AC	1(1.67)	7(11.67)	0.19	0.015–1.08	0.028*
677 CC/1298 CC	3(5)	3(5)	1.00	0.19–5.65	0.728
677 CT/1298 AA	18(30)	13(21.67)	2.03	0.88–4.73	0.096
677 CT/1298 AC	6(10)	3 (5)	2.83	0.76–19.26	0.298
677 CT/1298 CC	6(10)	1(1.67)	6.56	0.76–56.22	0.041*
677 TT/ 1298 AA	3(5%)	4(6.67%)	0.73	0.16–3.44	0.697
677 TT/ 1298 AC	4(6.67%)	1(1.67%)	4.21	0.46–38.86	0.171
677 TT/ 1298 CC	3(5%)	0(0%)	ND	ND	ND

* Significant *p*-value < 0.05.

development of T2DM (OR = 2.87, 95% CI = 1.62–5.07, *p* < 0.001).

Dual mutant genotype TT/CC was detected in 5% of cases, while it was absent in controls. Pronounced risk for T2DM was evident in C677T/CT-A1298C/CC combined form (OR = 6.56, 95% CI = 0.76–56.2, *p* 0.041).

Furthermore, statistical comparison between T2DM patients with wild type alleles and those with mutant alleles of MTHFR C677T (CT and TT) or A1298C (AC and CC) genes revealed no statistically significant differences between the two

patient groups, as regards their gender, glucose/lipid indexes, kidney function or blood pressure (Table 3).

Both groups were tested for the Hardy–Weinberg equilibrium (HWE) at the C677T locus. The frequencies of CC, CT and TT genotypes in T2DM patients were 61.67%, 18.33% and 20% respectively, and in control subjects were 75%, 18.33% and 6.67% respectively. Marked deviation from HWE was detected in both T2DM group ($p^2 = 0.5017$, $2pq = 0.4413$ and $q^2 = 0.085$) and control group ($p^2 = 0.708$, $2pq = 0.266$ and $q^2 = 0.025$). Regarding

Table 3 Association between MTHFR genetic polymorphisms versus clinical and biochemical parameters in T2DM patients.

	C677T genotype			A1298C genotype		
	C/C (<i>n</i> = 37)	C/T and T/T (<i>n</i> = 23)	<i>p</i> Value	A/A (<i>n</i> = 20)	A/C and C/C (<i>n</i> = 40)	<i>p</i> Value
SBP	139.14 ± 15.86	133.0 ± 21.47	0.289	137.87 ± 18.68	136.11 ± 17.96	0.795
DBP	83.76 ± 5.24	80.42	0.249	83.67 ± 5.22	81.61 ± 7.58	0.287
BMI (kg/m ²)	27.11 ± 3.27	27.53 ± 3.88	0.294	26.80 ± 4.33	26.57 ± 2.30	0.176
FBS (mg/dl)	164.25 ± 60.54	158.67 ± 83.09	0.599	180.46 ± 61.06	148.0 ± 59.71	0.558
HB A1C (%)	6.95 ± 0.97	6.77 ± 0.70	0.115	6.97 ± 1.00	6.83 ± 0.79	0.374
Chol (mg/dl)	201.84 ± 38.95	187.87 ± 45.05	0.593	202.21 ± 38.14	192.95 ± 43.83	0.756
TG(mg/dl)	169.62 ± 97.28	163.26 ± 80.61	0.708	183.38 ± 110.47	153.74 ± 70.03	0.057
HDL (mg/dl)	39.43 ± 8.72	39.63 ± 9.13	0.884	41.52 ± 8.66	37.73 ± 8.62	0.432
LDL (mg/dl)	134.80 ± 39.41	116.63 ± 39.72	0.850	126.83 ± 40.73	130.61 ± 40.13	0.648
Urea (mg/dl)	19.81 ± 8.35	25.79 ± 9.43	0.746	20.74 ± 8.91	22.82 ± 9.32	0.669
Creatinine (mg/dl)	0.91 ± 0.13	0.92 ± 0.17	0.414	0.89 ± 0.15	0.93 ± 0.15	0.449

A1298C locus, when both groups were tested for the HWE the frequencies of AA, AC and CC genotypes of the A1298C gene in T2DM patients were 33.33%, 50% and 16.67% respectively, and in control subjects were 63.33%, 28.33% and 8.33% respectively. No deviation from HWE was detected in T2DM group ($p^2 = 0.340$, $2pq = 0.486$ and $q^2 = 0.173$) or in control group ($p^2 = 0.601$, $2pq = 0.348$ and $q^2 = 0.037$).

4. Discussion

Type 2 diabetes is a complex disorder accounting for about 90–95% of all diabetes syndromes. Despite numerous reports suggesting a substantial genetic contribution to the susceptibility of type 2 diabetes, no major susceptibility genes have been identified so far [24]. The role of MTHFR C677T and A1298C polymorphisms has been widely studied across the world in different populations, but the results are controversial. A closer association between the MTHFR C677T polymorphism and T2DM than for the MTHFR A1298C polymorphism have been reported and explained by the fact that C677T polymorphism decreases the enzyme activity more than does A1298C polymorphism: 70% versus 30% respectively [18]. Indeed, C677T polymorphism is located in the exon 4 coding for the N-terminal catalytic domain of MTHFR enzyme, whereas A1298C polymorphism is located in the exon 7 coding for the C-terminal regulatory domain [25].

Our results revealed significant difference in the distribution of MTHFR C677T genotypes ($p < 0.001$) and mutant T allele ($p = 0.015$) between diabetic patients and control subjects. MTHFR C677T/TT homotype was found to be significantly higher in T2DM patients compared to controls, and conferred an almost 3.5-fold increased risk for T2DM (OR: 3.5, 95% CI: 1.06–11.57, p -value: 0.032) in accordance with Movva et al. [26] who found fourfold risk for developing T2DM in Indian population (OR: 4.0423; 95% CI: 1.8753, 8.7133). Other studies also have confirmed the association of this polymorphism with diabetic complications as nephropathy in a Polish population [27] and coronary heart disease in a Chinese population [28] as well as Egyptian population [29]. However, in some previous case-control studies, MTHFR C677T polymorphism was not associated with T2DM in different populations: Taiwan [30], Tunisia [31,32], the Czech Republic [33], Turkey [34], China [35], Germany [36], and Brazil [37]. The reason for this discrepancy in the literature may reflect a potential gene-environmental interaction.

C677T/TT genotype was more frequent in T2DM patients than in the healthy controls (20% versus 6.7%). Of interest to note is that the Brazilian population holds the lowest frequency reported for MTHFR C677T/TT genotype (9%) [37] and the highest (19.1%) is in Chinese population [28]. Hence, it can be concluded that ethnicity is one of the most important factors that play a role in C677T gene polymorphism and susceptibility to T2DM. The C677T polymorphism of the MTHFR gene has been reported to cause reduced enzyme activity and impaired homocysteine/folate metabolism, leading to moderate hyperhomocysteinemia [38]. However, no literature data directly associate the MTHFR-linked homocysteine and folate metabolism with T2DM. This hyperhomocysteinemia may damage the vascular endothelium, which is responsible for vasopressant effects and may cause a status of elevated blood pressure [39].

In the current study regarding MTHFR A1298C gene polymorphism, a significant association with T2DM ($p < 0.001$) was evident. The frequency of 1298 CC genotype was higher in the patients compared to controls (16.67% versus 8.33% respectively) in accordance with previous results in Taiwanese [30] and Moroccan populations [40]. Calculation of the risk estimate revealed that 1298CC homozygous and AC heterozygous genotype were associated with 2.2 and 2.5 times risk for T2DM respectively.

Few studies have reported the association between MTHFR A1298C polymorphism and T2DM. In a Polish population, the frequency of the A1298C genotypes did not differ among patients with different stages of diabetic nephropathy [27]. However, this polymorphism was reported to be associated with the risk for development of neural tube defects [23] and cardiovascular diseases [41].

Our results revealed no significant relation between lipid/glucose metabolic indexes with MTHFR genotypes among diabetic patients. Thus we can assume that MTHFR polymorphisms which may play some roles in the pathogenesis and complications in Caucasians T2DM patients are unlikely to be applied in Egyptian patients. Lack of association between the MTHFR genotypes with diabetic related indexes in our study could be attributed to ethnic variations in MTHFR genotypes, as a given population may have elements in its genetic reservoir that are protective against certain disease despite the high prevalence of disease-susceptible alleles [30]. Additionally, linkage disequilibrium of the mutant allele with a nearby non causative polymorphism may underlie the findings. Therefore, the possibility of linkage disequilibrium

of the variant alleles with a nearby non-causative polymorphism in our population cannot be ruled out.

Significant difference between diabetic and control groups for those was found to be heterozygous for 677CT and homozygous for the mutant 1298CC (OR: 6.56, 95% CI: 0.76–56.22, *p*-value: 0.041). Double heterozygosity 677 CT/1298 AC revealed 2.8 risk for T2DM (OR:2.83, 95% CI 0.76–19.26, *p*-value:0.298) confirming previous results of Van der Put et al., [23] who reported that 677C→T and 1298A→C polymorphisms can act synergistically, given that heterozygosity for both polymorphisms causes lower MTHFR enzymatic activity than heterozygosity alone for either of them and a trend to higher or significantly higher plasma total homocysteine levels. Also, double heterozygous patients had a higher risk for diabetic nephropathy than those with the 677CT genotype [42]. On the contrary, Friedman et al. [43] found that combined heterozygosity of A1298C mutation with other MTHFR mutations including C677T has been associated with decreased total plasma homocysteine levels.

MTHFR 677 CC/ 1298 AA combined genotype was significantly higher in the controls compared to patients with decreased risk of T2DM, thus it may have a protective role against the susceptibility to T2DM. No statistically significant difference could be encountered between the patients and the controls regarding the frequency of the combined genotypes; 677 CC/ 1298 CC, 677 CT/ 1298 AA, 677 TT/1298 AA and 677 TT/1298 AC. The combined genotypes MTHFR 677 TT/ 1298 CC were not detected in the controls, so the statistical difference between the two groups and the risk estimates for these combined genotypes were not determined. This could be an evidence of increased T2DM risk of this combined genotype. In conclusion: our data suggest that the MTHFR C677T and A1298C polymorphism are risk factor for T2DM in Egyptian patients. Our data also suggest that the two gene polymorphisms may act synergistically to increase the risk of diabetes. Furthermore, it should be noted that the size of the studied population was relatively small and therefore, large-scale prospective studies are needed to confirm these findings.

Conflict of interest

No author has declared conflict of interest.

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