Original Article

Methylenetetrahydrofolate Reductase Gene Polymorphisms In Egyptian Patients With Type 2 Diabetis Mellitus

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Abstract

Background: Type 2 diabetes mellitus (T2DM) is a major public health problem around the world. MTHFR gene, located on chromosome 1 (1p36.3), encodes for methylenetetrahydrofolate reductase enzyme. Two of the most investigated polymorphisms in the MTHFR gene are C677T(rs1801133) and A1298C (rs1801131). These polymorphisms 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.

Patients and Methods: A whole number of 102 individuals were selected, classified into two groups: group (1) were 51 healthy subjects, group (2) were 51 diabetic patients. MTHFR gene polymorphism (rs1801133) and(rs1801131) were genotyped with polymerase chain reaction, followed by enzymatic digestion with HinfI and MboII enzymes, respectively.

Results: C677T and A1298C genetic polymorphisms conveyed an increase in T2DM risk (OR = *5.98*, 95% CI = 1.*24–28.83*, p = 0.03 and OR = *1.97*, 95% CI = *0.61*–6.*36*, p = 0.00*5* 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 = 9.3, 95% CI = 1.12–77.38, p 0.04).

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, because the size of the examined population was very small, large-scale prospective investigations are required to validate these findings

Keywords: Type 2 diabetes mellitus, gene polymorphism, MTHFR gene, Risk factor

INTRODUCTION

DM is regarded as a global epidemic, with more than 400 million people suffering from the condition worldwide. (1) Despite the fact that diabetes is possibly treatable, it is nevertheless the 9th biggest cause of mortality, with a 90 percent rise in burden between 1990 and 2010. (2) The International Diabetes Federation (IDF) estimates that T2DM will reach 18.7 million people by 2025. (3) By 2045, the number of people living with diabetes is anticipated to rise to 578 million (10.2 percent) and 700 million (10.9 percent). (4) In Egypt, the prevalence of diabetes mellitus is estimated to be at 15.56 percent in people aged 20 to 79. (5)

Diabetes mellitus (DM) is a condition marked by high blood glucose levels. (DM) is a condition in which blood glucose levels are abnormally high. It becomes unmanageable over time, allowing other complex metabolic illnesses such diabetic neuropathy (DN), diabetic retinopathy, diabetic foot, and cardiovascular problems to develop. (6)

T2DM develops as a consequence of the interplay of genes and environmental variables. Despite the fact that several mutation s have been related to the risk of T2DM, no significant susceptibility genes have been discovered thus far.[7].

The 5,10Methylenetetrahydrofolate reductase (MTHFR) gene is located near the end of the short arm of chromosome 1. (1p36 .6).

This enzyme is essential for folate metabolism, which is an essential step for cell metabolism in the DNA, RNA, and protein methylation (8) .

MTHFR C677T and A1298C are 2 common genetic polymorphisms which lead to reduced MTHFR activity and increased homocysteine level (9).

The role of MTHFR C677T and A1298C polymorphisms has been widely studied across the world in variable populations, but the results are controversial. The association between MTHFR A1298C polymorphism and T2DM is reported to be closer than MTHFR C677T polymorphism with T2DM and explained by the fact that C677T polymorphism decreases the enzyme activity more than does A1298C polymorphism: 70% versus 30% respectively [10].

Indeed, the C677T polymorphism is found in exon 4, which codes for the MTHFR enzyme's N-terminal catalytic domain, whereas the A1298C polymorphism is found in exon 7, which codes for the C-terminal regulatory domain. [11].

Subjects and Methods

This study was done in Medical Biochemistry and Internal Medicine Departments, Faculty of Medicine, Zagazig University. This study included 102 subjects aged classified into two groups: group of 51 Egyptian T2DM patients **(mean age ± SD 44.92±3.20 years)**, group of 51 diabetic patients **age and sex matched healthy unrelated Egyptian subjects (mean age ± SD 46.07±5.36).**

Both patients and controls are enlisted from outpatient clinics of the Endocrinology Unit of Internal Medicine Department; Zagazig University hospital. According to the 2017 The American Diabetes Association (ADA) (12) standards for the diagnosis of diabetes ,patients were diagnosed T2DM if they have one of the next standards: (i) fasting plasma glucose (FPG) level of 126 mg/dL (7 mmol/L) or higher; fasting is defined as no caloric intake for at least 8 hours, (ii) a 2-hours plasma glucose level of 200 mg/dL (11.1 mmol/L) or higher during a 75-g oral glucose tolerance test (OGTT),or (iii) a random plasma glucose of 200 mg/dL (11.1 mmol/L) or more in a individuals with typical symptoms of hyperglycaemia (polyuria, polydipsia, polyphagia, weight loss) or hyperglycemic crisis or (iiii) haemoglobin A1c (HbA1c) level of 6.5% or more. (12). All patients were non-smoker, normotensive, non-obese, no history of coronary artery disease (CAD), liver, cancer, autoimmune disorders, and inflammatory diseases and type-1 DM.

The entire included groups were exposed to whole history taking, full clinical anthropometric dimensions. Estimation of body mass index (BMI) was done through dividing body weight in kilograms by (height in square meters) (13). Laboratory tests including fasting and 2 hours post prandial blood glucose levels, HbA1c%, lipid profile [serum total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c) and calculation of low-density lipoprotein cholesterol (LDL-c)]. Estimation of urea and creatinin level was done.All participants contracted an acquainted written agreement before registration in our research and the study design was permitted by the Ethical Committee of Faculty of Medicine, Zagazig University.

1- After an overnight fasting,6 ml of intravenous blood was taken from every individual by sterile vein-puncture and separated into three samples: The first sample was 2ml of blood were collected in an EDTA containing tubes and were divided into two aliquots; for colorimetric estimation of glycated haemoglobin as percent of total haemoglobin by Stanbio Laboratory, Boerne, Texas, USA (14) and the reamaining part was stored at -20 °C for total DNA and RNA extraction. The second sample was 1 ml blood into sodium fluoride tube, for measurement of FBG and 2 hours postprandial blood glucose via enzymatic colorimetric technique by Spinreact kit, Girona, Spain (15). The remaining blood was moved into a plain tube, allowed to clot at 37°C, centrifuged for 15 minutes at 4000 r.p.m. Total cholesterol and triglyceride levels were tested by routine enzymatic techniques (Spinreact, Girona, Spain) (16**), (**17). HDL cholesterol concentration was detected after precipitation of the apoB-containing lipoproteins (18). The LDL cholesterol level was calculated with the Friedewald formula (19). Urea and creatinin were estimated by using commercial kits from (Spinreact, Spain) (20**), (**21**).**

Detection of MTHFR C677T and A1298C genetic polymorphism

DNA Extraction:

Genomic DNA was extracted from white blood cell pellets by salting out extraction method [22] 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.

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

0.5–2.0 lg 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., [23] and Van der Put et al., [24] 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 5TGAAGGAGAAGGTGTCTGCGGGA-3
The reverse primer is 5AGGACGGTGCG GTGAGAGTG-3
PCR was carried out in a 25-uL reaction volume containing 100 ng of genomic DNA 0.4 lmol/L of each primer, 0.2 mmol/L of dNTPs, 2 mmol/L
of MgCl2 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 uL of the PCR products were digested with 5 units of Hinf1 (Promega, UK) overnight at 37 C.
RFLP product was separated on 2% agarose gel and visualized by ethidium bromide produced 198-, 175-, and 23- bp fragments. Homozygous mutants (677TT) produced 175- and 23-bp fragments. 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 TTTGTGACCATTCCGGTTTG-3. 10 mM TrisÆHCl, 50 mM of KCl, 1 mg/ml of gelatin, 3.0 mM of MgCl2, 200 lM 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 30 s followed by a 7-min final extension at 72 C. 12.5 lL of PCR product was digested with 2.5 ll of MboII buffer and 2.5 units of MboII restriction enzyme (Promega, UK). RFLP products was 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.

Statistical analysis

All data were collected, tabulated and statistically analyzed using SPSS 20.0 for windows (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as the mean ± SD & median (range), and qualitative data were expressed as absolute frequencies (number) & relative frequencies (percentage). Independent samples Student's t-test was used to compare between two groups of normally distributed variables while Mann Whitney U test was used for non- normally distributed variables. Kruskal Wallis test was used to compare between more than two groups of non- normally distributed variables. Percent of categorical variables were compared using Chi-square test or Fisher's exact test when appropriate. Spearman's rank correlation coefficient was calculated to assess relationship between various study variables, (+) sign indicate direct correlation & (-) sign indicate inverse correlation, also values near to 1 indicate strong correlation & values near 0 indicate weak correlation. All tests were two sided. p-value < 0.05 was considered statistically significant (S), p-value ≥ 0.05 was considered statistically insignificant (NS)

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 = 5.98, 95% CI = 1.24–28.83, p = 0.026). Statistically significant difference in C677T/T allele was found between T2DM and control cases with 2.8 risk for occurrence of T2DM (OR:2.837, 95% CI:1.35–5.97, p = 0.026). Individuals homozygous for MTHFR A1298C gene mutation (C/C homotype) had an increased risk of T2DM (OR = 1.97, 95% CI = 0.6–6.35, p = 0.255). This risk increase was also observed with A/C heterotype (OR = 2.5, 95% CI = 1.11– 5.79, p = 0.026). A1298C C allele carry 2.4-fold risk for development of T2DM (OR = 2.37, 95% CI =1.3 - 4.33, p < 0.001). Dual mutant genotype TT/CC was detected in 4% of cases, while it was absent in controls. Pronounced risk for T2DM was evident in C677T/CT-A1298C/CC combined form (OR = 9.30, 95% CI =1.12 to 77.4, p 0.039). 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 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 inT2DM patients were 64.70%, 15.68% and 19.6% respectively, and in control subjects were 80.4%, ,15.68% and 3.92% respectively. Marked deviation from HWE was detected in both T2DM group (p2 = 0.5263, 2pq = 0.3982 and q2 = 0.075) and control group (p2 = 0.778, 2pq = 0.207 and q2 = 0.0138).

Regarding A1298C locus, when both groups were tested for the HWE the frequencies of AA, AC and CC genotypes of the A1298C gene inT2DM patients were 33.33%, 49% and 17.64% respectively, and in control subjects were 62.74%, 27.45% and 9.8% respectively. No deviation from HWE was detected in T2DM group (p2 = 0.3346, 2pq = 0.487 and q2 = 0.177) or in control group (p2 = 0.584, 2pq = 0.359 and q2 = 0.055).

Table(1): Baseline characteristics of type-2 DM patients versus controls

Parameters	**Controls (*n* =51)**	**Type-2 DM patients (*n* =51)**	***P* value***
Age (years) Range Mean ± SD	 40– 53 46.07±5.36	 40 – 50 44.92±3.20	 0.19135
FBG (mg/dl) Range Mean ± SD	 75 –106 89.42≠7.51	 146 –205 165.72±14.25	 <0.001
PPBG (mg/dl) Range Mean ± SD	 129 – 141 135.12 ±4.11	 238 – 390 *297.02±39.49*	 <0.001
HbA_{1c} (%) Range Mean ± SD	 4 –5.1 4.45≠0.35	 6 – 8.4 7.28±0.64	 <0.001
Total cholesterol (mg/dl) Range Mean ± SD	 155 – 177 166.02±5.83	 165 –250 204.30±24.33	 <0.001
Triglycerides (mg/dl) Range Mean ± SD	 71–128 95.63≠14.54	 89 – 208 166.63±35.08	 <0.001
LDL-C (mg/dl) Range Mean ± SD	 62.6 –98.6 83.25± 8.91	 103–184 144.2±40.08	 <0.001
HDL-C (mg/dl) Range Mean ± SD	 42 – 58 51.47±4.86	 31 – 52 38.5±5.99	 <0.001
Homocysteine (mmol/L)	(4.12_14.82) 10.41±2.89	(12-20.86) 14.73±2.46	<0.001

Table (2): Genotype distributions and allelic frequencies of MTHFR A1298C (rs ******) polymorphism in type-2 DM patients(n=51) and controls(n=51)

MTHFR A1298C Polymorphism	Type-2 DM patients, *n* (%)	Controls, *n* (%)	Odds ratio (95% confidence interval)	p value*	P1 AA/AC	P2 AA/CC
Genotype						
AA	17 (33.33)	32 (62.74)	0.2969 (0.1317 - 0.6694)	0.0034	0.006	
AC	25 (49)	14 (27.45)	2.5412(1.1143 - 5.7953)	0.0266		0.047
CC	9 (17.64)	5 (9.8)	1.9714(0.6115 - 6.3553)	0.2557		
Alleles						
A	**59(57.84)**	**78(76.47)**	-----	-----		
C	43 (42.15)	24 (23.53)	2.3686(1.2959 - 4.3295)	0.005		

The chi-square statistic is 8.8373. The *p*-value is .012051. The result is significant at $p < .05$.

HWE
Patients (p2 = 0.3346,
2pq = 0.4876755 and q2 = 0.17766225)
Control (p2 = 0.58476609,
2pq = 0.359867 and q2 = 0.05536609)
(No Deviation)

Table (3): Genotype distributions and allelic frequencies of MTHFR C677T (rs ******) polymorphism in type-2 DM patients and controls

MTHFR C677T Polymorphism	Type-2 DM patients, *n* (%)	Controls, *n* (%)	Odds ratio (95% confidence interval)	p value*	P1 CC/CT	P2 CC/TT
Genotype						
CC	33 (64.70)	41 (80.4)	0.4472(0.1820- 1.0984)	0.0792		0.012
CT	8 (15.68)	8 (15.68)	1.0233 (0.3523 - 2.9722)	0.9663	0.69	
TT	10 (19.6)	2 (3.92)	5.9756(1.2384 - 28.8339)	0.0260		
Alleles						
C	**74(72.54)**	**90(88.23)**	------	---------		
T	28 (27.45)	12 (11.76)	2.8378(1.3500-5.9653)	0.006		

The chi-square statistic is 6.1982. The *p*-value is .04509. The result is significant at $p < .05$.

HWE
Patients(p2 = 0.52635025,
2pq = 0.3982995 and q2 = 0.07535025)
Control(p2 = 0.77862976,
2pq = 0.20754048 and q2 = 0.01382976)

Marked Deviation

Table 4: The frequency of combined genotypes MTHFR C677T and A1298C genotypes in T2DM patients (n=51) and control (n= 51)

Combined MTHFR genotypes	Type-2 DM patients, *n* (%)	Controls, *n* (%)	Odds ratio (95% confidence interval	p value*
667CC/1298AA	14(27.45)	26(51)	0.3638 (0.1595 - 0.8297)	0.016
667CC/1298AC	1(1.96)	8(15.7)	0.1075 (0.0129 - 0.89420	0.0391
667CC/1298CC	2(3.9)	2(3.9)	1.0000 (0.1354 to 7.3861)	1.0000
667CT/1298AA	14(27.45)	9(17.65)	1.7658 (0.6850 to 4.5514)	0.2392
667CT/1298AC	5(9.8)	2(3.9)	2.6630 (0.4921 to 14.4107)	0.2556
667CT/1298CC	8(15.68)	1(1.96)	9.3023 (1.1183 to 77.3815)	0.0391
667TT/1298AA	2(3.9)	2(3.9)	1.0000 (0.1354 to 7.3861)	1.0000
667TT/1298AC	3(5.88)	1(1.96)	3.1250 (0.3141 to 31.0948)	0.3310
667TT/1298CC	2(3.9)	0(0)	ND	ND

Table :Association between MTHFR A1298C genetic polymorphisms versus clinical and biochemical parameters in T2DM patients.

Parameters	Type-2 DM patients (*n* =51)			$P^{*¥}$ value
	AA (*n* =17)	AC (*n* =25)	CC (*n* =9)	
Total cholesterol (mg/dl)	208.29±24.42	200.2±17.69	207±20.75	0.42
Triglycerides (mg/dl)	167.06±25.14	150.48±20.92	148±26.02	0.05
LDL cholesterol (mg/dl)	139±26.62	133.96±23.58	129.33±24.64	0.62
HDL cholesterol (mg/dl)	38.53±2.32	37.76±2.01	37.22±2.17	0.30
FBG (mg/dl)	158±8.58	160.52±7.20	159.78±7.41	0.58
PPBG (mg/dl)	277.88±23.12	285.48±24.17	287.22±27.83	0.54
HBA1c (%)	6.95±0.46	7.09±0.38	6.84±0.46	0.27

Parameters	Type-2 DM patients (*n* =51)			$P^{*¥}$ value
	CC (*n* =17)	CT (*n* =25)	TT (*n* =9)	
Total cholesterol (mg/dl)	207.65±24.07	198.88±17.75	207.11±21.43	0.34
Triglycerides (mg/dl)	166.88±25.38	161.64±25.76	158.33±28.98	0.70
LDL cholesterol (mg/dl)	136±26.04	135.16±23.84	130.56±24.79	0.86
HDL cholesterol (mg/dl)	38.24±2.68	37.88±2.05	37.78±1.86	0.84
FBG (mg/dl)	157±10.11	159.12±7.26	160±7	0.61
PPBG (mg/dl)	276.59±23.80	286.6±25.39	288.22±28.96	0.39
HBA1c (%)	6.85±0.57	7.03±0.39	6. 66±0.54	0.13

Discussion

T2DM is a polygenic metabolic condition that is complex and multifactorial. Various environmental and genetic risk factors influence its etiology. (25).

The enzyme methylenetetrahydrofolate reductase (MTHFR) methylates homocysteine to generate methionine (26), and its dysfunction can lead to HHcy (27). Therefore, numerous studies have investigated whether reduced MTHFR activity is a risk factor for T2DM (28).

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 [10].

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 (29)

Between diabetic patients and control people, there was a significant difference in the distribution of MTHFR C677T genotypes (p = 0.045) and mutant T allele (p = 0.006). MTHFR C677T/TT homotype was found to be significantly higher in T2DM patients compared to controls, and was associated with a nearly 5.98 fold increased risk of T2DM (OR: 5.98, 95 percent CI: (1.24 - 28.83, p value: 0.026), in agreement with Movva et al. [30], who found a fourfold increased risk of T2DM in the Indian population (OR: 4.0423; 95% CI: 1.8753 - 8.7133).

Other investigations have linked this polymorphism to diabetes complications such diabetic nephropathy in Japanese people [31], Iranian people (32), and coronary heart disease in Chinese Han people [33] and Egyptian people [34]. Previous case-control investigations in Taiwani [35], Tunisia [36,37], the Czech Republic [38], Turkey [39], China [40], Germany [41], and Brazil [42] found no association between the MTHFR C677T polymorphism and T2DM. This discrepancy in the literature could be the result of a gene-environment interaction..

In 2013, Khalid et al. found a significant link between MTHFR C677T polymorphism and T2DM in the Arab population [43], and Zhong et al. conducted a metanalysis of the relationship between MTHFR C677T polymorphism and T2DM, concluding that there was no link between the two, regardless of the patient's ethnicity or the presence of serious DM-related complications [45].

T2DM patients had a higher rate of C677T/TT genotype than healthy controls (19.6 percent versus 3.9 percent). It's worth noting

that the Brazilian population has the lowest reported frequency of MTHFR C677T/TT genotype (9%) [45], while the Chinese population has the highest (19.1%). China is expected to have 380 million T2DM sufferers by 2025(46)

So, we can conclude that ethnicity is one of the most important factors that play a role in C677T gene polymorphism and susceptibility to T2DM. **Meng Y et al.,** provided strong evidence that MTHFR C677T was significantly associated with T2DM in Asians, but not in Caucasians or Africans. Thus, it is necessary to identify the role of C677T in different ethnicities.

The C677T polymorphism of the MTHFR gene has been reported to cause reduced enzyme activity and impaired homocysteine/folate metabolism, resulting in moderate hyperhomocysteinemia [47].

Errera et al. have demonstrated that the frequencies of MTHFR C and T allele were 68% and 32% in Brazilian T2DM cases (48). Similar results were obtained in our study where the C and T allele frequencies were 72.5% and 27.5%, respectively in T2DM cases. The frequency of MTHFR CC genotype was 36.71% in T2DM cases without dyslipidemia which is higher in comparison with Turkish T2DM cases (29%) (49) However, there is no evidence linking MTHFR-linked homocysteine and folate metabolism to T2DM in the literature. Hyperhomocysteinemia can affect the vascular endothelium, which is responsible for vasopressor effects, resulting in high blood pressure. [50].

In the current study regarding MTHFR A1298C gene polymorphism, a significant association with T2DM (p 0.005) was evident. The frequency of 1298 CC genotype was higher in the patients compared to controls (17.64%versus 9.8% respectively) in accordance with previous results in Taiwanese [51] and Moroccan populations [52]. Calculation of the risk estimate revealed that 1298CC homozygous and AC heterozygous genotype were associated with 1.97 and 2.5times riskfor T2DM respectively.

Elbaz et al. clearly recognised that the MTHFR A1298C polymorphism may be considered a genetic risk factor for diabetic neph ropathy in Egyptians with type 2 diabetes (53), but Chehadah et al. discovered that the MTHFR gene A1298C polymorphism is n ot associated to T2DM in the Emirati population.

ElHajj et al, has found that in T2DM patients, it can be utilized as a marker for CVA, nephropathy, elevated LDL cholesterol, and triglycerides (54)

Ay et al. also have found that MTHFR A1298C polymorphism may be regarded a genetic risk factor for diabetic nephropathy among individuals with type 2 diabetes in the Turkish community.

In diabetic patients, our findings demonstrated no significant relationship between lipid/ glucose metabolic indices and MTHFR g enotypes. As a result, we can infer that MTHFR polymorphisms, which may have a role in the development and consequences of T2DM in Caucasians, will not be relevant in Egyptian patients. The lack of association between MTHFR genotypes and diabetic r elated indexes in our study could be attributed to ethnic variations in MTHFR genotypes, as a population's genetic reservoir may contain elements that protect against disease despite the high prevalence of disease-susceptible alleles [45].

Significant difference between diabetic and control groups for those was found to be heterozygous for 677CT and homozygous for the mutant 1298CC (OR: 9.30, 95% CI: 1.12 - 77.3815), p-value: 0.039). Double heterozygozity 677 CT/1298 AC revealed 2.6risk for T2DM (OR:2.66, 95% CI 0.491 to 14.41, pvalue:0.256) confirming previous results of Van der Put et al., [47] who reported that 677C/T and 1298A/C polymorphisms can act synergistically, given that heterozygosity for both polymorphisms cause 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
[56].

Onthecontrary,**Friedman et al.** [57] discovered that coupled heterozygozity of the A1298C mutation with other MTHFR variants, particularly C677T, is linked with lower total plasma homocysteine levels.

MTHFR 677 CC/ 1298 AA combined genotype was significantly higher in controls compared to patients with a lower risk of T2 DM, suggesting that it may have a protective role in T2DM susceptibility.

There was no statistically significant difference in the frequency of the combination genotypes 677 CC/ 1298 CC, 677 CT/ 1298 AA, 677 TT/1298 AA, and 677 TT/1298 AC between patients and controls.

Because the combined genotypes MTHFR 677 TT/ 1298 CC were not found in the controls, the statistical difference between the two groups and risk estimations for these combined genotypes could not be determined. This might be evidence of an elevated T2 DM risk from this combination.

Conclusions and future direction

our findings indicate that the MTHFR C677T and A1298C polymorphisms are risk factors for T2DM in Egyptian individuals. Our findings also show that the two gene polymorphisms may work together to raise the risk of diabetes.

Furthermore, because the size of the examined population was very small, large-scale prospective investigations are required to validate these findings.

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