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Aysegul Bayramoglu
Department of Nutrition and Dietetics, Faculty of Health Sciences, Kutahya Health Sciences University, Kutahya 43100, Turkey, atbayramoglu@gmail.com

Gokhan Bayramoglu
Department of Occupational Health and Safety, Faculty of Health Sciences, Artvin Coruh University, Artvin 08000, Turkey, gbayramoglu@artvin.edu.tr

Halil Ibrahım Guler
Department of Molecular Biology and Genetics, Faculty of Science, Karadeniz Technical University, Trabzon 61080, Turkey, hiboguler@gmail.com

Nezaket Coban
Department of Molecular Biology and Genetics, Institute of Science, Artvin Coruh University, Artvin 08000, Turkey, nezocoban@gmail.com

Mustafa Çagatay Korkmaz
Department of Measurement and Evaluation, Faculty of Education, Artvin Coruh University, Artvin 08000, Turkey, mcagatay@artvin.edu.tr

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Association of Methylenetetrahydrofolate Reductase rs1801133 Genetic Variants with Type 2 Diabetes Mellitus and Diabetic Nephropathy

Aysegul Bayramoglu[1*], Gokhan Bayramoglu[2], Halil Ibrahım Guler[3], Nezaket Coban[4], Mustafa Çagatay Korkmaz[5]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Kutahya Health Sciences University, Kutahya 43100, Turkey
[2]Department of Occupational Health and Safety, Faculty of Health Sciences, Artvin Coruh University, Artvin 08000, Turkey
[3]Department of Molecular Biology and Genetics, Faculty of Science, Karadeniz Technical University, Trabzon 61080, Turkey
[4]Department of Molecular Biology and Genetics, Institute of Science, Artvin Coruh University, Artvin 08000, Turkey
[5]Department of Measurement and Evaluation, Faculty of Education, Artvin Coruh University, Artvin 08000, Turkey

Abstract

Background: Type 2 diabetes mellitus (T2DM) is a complex metabolic disease with a genetic predisposition. Methylenetetrahydrofolatereductase (MTHFR) gene is one of the candidate genes associated with T2DM and diabetic nephropathy (DN). This research was carried out to determine the frequency of the C677T polymorphism (rs1801133) of the MTHFR gene and examine the role of rs1801133 polymorphism in T2DM and DN development.

Methods: DNA was obtained from peripheral blood samples (273 samples) using a DNA isolation kit. MTHFR rs1801133 polymorphism was determined using polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and electrophoresis. PCR products were cut by restriction enzyme HiNF I and analyzed by 2% agarose gel electrophoresis. The results were statistically analyzed.

Results: Although MTHFR rs1801133 genotype frequencies showed statistically significant differences between the control and T2DM patient groups (p = 0.001), no statistically significant difference was found between individuals with and without DN.

Conclusions: MTHFR gene rs1801133 polymorphism is related to T2DM but not to DN. CT and TT genotypes can be accepted as genetic markers.

Keywords: diabetic nephropathy, genetic variation, methylenetetrahydrofolate reductase, restriction fragment length polymorphism, type 2 diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is associated with environmental and genetic factors and is characterized by chronic hyperglycemia due to the absence of insulin secretion, decreased insulin effect, or unresponsiveness of insulin receptors.[1–4] This illness severely affects patients' quality of life and imposes a huge economic burden on national health and economy.[5] DNA methylation is associated with the development and progression of T2DM.[6,7] Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that functions in homocysteine remethylation cycle and in DNA methylation and converts homocysteine to methionine.[6–8] Methionine then combines with ATP to form S-adenosyl methionine, the primary methyl donor for DNA methylation.[9]

***Corresponding author:**
Aysegul Bayramoglu
Kutahya Health Sciences University, Faculty of Health Sciences, Department of Nutrition and Dietetics, Kutahya, Turkey
E-mail: atbayramoglu@gmail.com

T2DM causes macrovascular and microvascular complications, one of which is diabetic nephropathy (DN).[1] DN is a diabetic kidney disease and the primary cause of chronic kidney disease. Genetic and environmental factors contribute to the development and progression of DN.[10] Genetic studies provide useful and valuable information about potential targets for the pathobiology and treatment of DN.[11] Poor glycemic control is the most important cause of DN,[9] and high plasma homocysteine levels are associated with insulin resistance and DN. MTHFR is regulated by homocysteine metabolism.[9,12,13]

The human MTHFR gene is situated on 1p36.3 chromosome and encodes the MTHFR enzyme consisting of 656 amino acids.[14–16] MTHFR C677T polymorphism (rs1801133) is a C-to-T transition at base pair 677 that represents point mutation and decreased MTHFR activity.[12,14–18] Decreased MTHFR activity leads to increased plasma homocysteine level, which is associated with DN.[14]

The effects of MTHFR gene polymorphism on diabetes and DN have been investigated. Although studies

revealed that MTHFR rs1801133 polymorphism is a risk factor for DN and T2DM, the results are inconsistent.[11]

Therefore, this study aimed to reveal the frequency of MTHFR rs1801133 polymorphism in subjects with T2DM and DN and determine whether this frequency is related to both illnesses.

METHODS

Participants

Peripheral blood samples were drawn from 143 subjects (82 subjects without DN [DN^-] and 61 subjects with DN [DN^+]) who applied to the Internal Medicine Department of Artvin State Hospital, Turkey. The control group was selected from volunteers who came for routine health screening and did not have a family history of T2DM (130 volunteers). T2DM diagnosis was made by qualified clinicians on the basis of fasting blood glucose level ≥ 7.0 mmol/L, microalbuminuria (creatinine < 1.2 mg/dl, albuminuria; 30–300 mg/day), and HbA1c level of 6.5% for two consecutive routine screenings. This research was approved by the Local Ethics Committee of Karadeniz Technical University, Turkey (No: 2018/169). In accordance with the principles of the Declaration of Helsinki, informed consent was acquired from all subjects prior to study enrollment.

Genotyping

Genomic DNA was isolated using a DNA isolation kit (EZ-10 Spin Colon Blood Genomic DNA Minipreps Kit, Biotechnology Department Bio Basic Inc., Markham, Ontario, Canada). As shown in Table 1, the DNA samples were amplified in Bio-Rad Thermal Cycler (T100TM, Foster City, CA, USA) using allele-specific primers (Integrated DNA Technologies Inc., Leuven, Belgium) and PCR conditions. The addition of five units of HiNF I restriction enzyme (New England Biolabs, Ontario, Canada) at 37 °C overnight cleaved the 198 bp DNA fragment into 175 and 23 bp fragments. The digested products were separated by 2% agarose gel electrophoresis and viewed using a CCD camera. The findings were analyzed with gel analysis software (LabWorks, Cambridge, UK). For C677T mutation, the genotypes were identified as CC (198 bp), CT (198, 175, and 23 bp), and TT (175 and 23 bp).

TABLE 1. Primers and PCR conditions for MTHFR gene rs1801133 polymorphism

PRIMERS			
Sense: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' Antisense: 5'-AGGACGGTGCGGTGAGAGTG-3'			
PCR CONDITION			
Cycle	Number of Cycles	Tempature (+)	Time
Initial Denaturation	1	94 °C	3 min.
Denaturation		94 °C	60 s
Annealing	35	61 °C	60 s
Extension		72 °C	60 s
Final extension	1	72 °C	5 min.
Hold		4 °C	-

Statistical analysis

Statistical analysis was conducted using the SPSS v.19 Package program. Two groups with continuous and quantitative data were compared using two independent samples t-test and Mann–Whitney U test. Normality was examined using Shapiro–Wilk test. P-values below 0.05 were statistically significant. The strength of the correlation between MTHFR polymorphism and DN risk was measured using pooled OR and corresponding 95% CI. In the control and DN patient groups, the distribution of MTHFR genotypes and allele groups was determined by applying Pearson's Chi-square test. Clinical variables related to MTHFR genotypes were evaluated by two independent samples t-test.

RESULTS

Statistically significant differences in body mass index (BMI) ($p < 0.001$), glucose ($p < 0.001$), systolic blood pressure ($p < 0.001$), diastolic blood pressure ($p < 0.001$), HbA1c ($p < 0.001$), creatinine ($p = 0.002$), HDL ($p = 0.015$), total cholesterol ($p = 0.014$), and triglycerides ($p < 0.001$) were found between the patients and control group. No statistically significant differences in age ($p = 0.114$), gender ($p = 0.253$), and LDL ($p = 0.327$) were observed between the control group and patients with T2DM and DN (Table 2).

The genotype distributions and allele frequencies of the MTHFR C677T gene in the control, T2DM, and DN patient groups are shown in Table 3. The frequency of C677T genotype was CC 98.5%, CT 1.5%, and TT 0% in the controls and CC 86%, CT 13.3%, and TT 0.7% in the patients. The frequency of C677T genotype was CC 83.6%, CT 14.8%, and TT 1.6% in patients with DN and 87.8%, CT 12.2%, and TT 0% in patients without DN. The genotype distributions and allele frequencies were statistically significantly different between the control group and T2DM patient group ($p = 0.001$ and $p < 0.001$, respectively). No statistically significant differences in genotype distributions and allele frequencies were detected between T2DM patients with and without DN ($p = 0.627$ and $p = 0.493$, respectively).

Various models of gene inheritance were evaluated to determine a predisposition to increased risk or protection against T2DM and DN (Table 4, Table 5). According to the inheritance model, the CT-TT genotype was significantly associated with T2DM (OR: 10.40, 95% CI = 2.38–45.46, $p < 0.001$).

The distribution of some clinical parameters according to genotypes is presented in Table 6. No statistically significant difference in cholesterol, HDL, triglyceride, creatinine, and BMI was detected for the CT genotype. However, these parameters differed statistically for the CC genotype. Furthermore, a statistically significant difference in HbA_1c was detected for the CT genotype (p = 0.011). For LDL, a statistically significant difference was determined across all genotypes (CC, p = 0.165 and CT, p = 0.757).

TABLE 2. Demographic and biochemical characteristics of patients with T2DM, DN, and control

Parameters	T2DM and DN Patients (N = 143) mean ± SD	Control (N = 130) mean ± SD	*p
Age (year)	60.3 ± 12.9	57.5 ± 16.2	0.114*
BMI (kg/m^2)	31.4 ± 5.6	26.8 ± 5.1	< 0.001**
Fasting plasma glucose (mg/dL)	167.3 ± 81.6	93.6 ± 11.2	< 0.001**
Systolic blood pressure (mmHg)	136.5 ± 21.8	120.6 ± 10.9	< 0.001**
Diastolic blood pressure (mmHg)	80.5 ± 13.3	70.5 ± 8.5	< 0.001**
HbA_1c (%)	6.9 ± 1.3	6.8 ± 6.4	< 0.001**
Serum creatinine (mg/dl)	1.1 ± 1.3	0.8 ± 0.3	0.002**
Total Cholesterol (mg/dl)	200.1 ± 57.7	180.1 ± 39.9	0.014**
HDL (mg/dl)	43.9 ± 13.2	48.1 ± 19.6	0.015**
LDL (mg/dl)	120.5 ± 43.7	112.2 ± 37.1	0.327**
Triglycerides(mg/dl)	184.6 ± 134.4	137.9 ± 73.8	< 0.001**

BMI: Body Mass index, * Student t Test, **Mann–Whitney U test

TABLE 3. Genotypic and allelic frequencies of MTHFR polymorphism (rs180133 T/C) in T2DM, DN patients, and control subjects

MTHFR Genotype	Control (N = 130) N (%)	T2DM (N = 143) N (%)	p*	OR (95%CI)	DN- (N = 82) N (%)	DN+ (N = 61) N (%)	p*	OR (95%CI)
CC	128 (98.5)	123 (86.0)		Reference	72 (87.8)	51 (83.6)	0.627	Reference
CT	2 (1.5)	19 (13.3)	0.001	9.88 (2.25–43.33)	10 (12.2)	9 (14.8)	0.237	1.27 (0.48–3.34)
TT	0 (0)	1 (0.7)			0 (0)	1 (1.6)		**
C	258 (99.2)	265 (92.7)		Reference	154 (88.5)	111 (91.0)		Reference
T	2 (0.8)	21 (7.3)	< 0.001	10.22 (2.37–44.04)	20 (11.5)	11 (9.0)	0.493	0.76 (0.35–1.65)

CI: confidence interval; OR: odds ratio
*Pearson Chi-square test
**Owing to the lack of T allele, evaluation could not be made

TABLE 4. Analysis of the association between T2DM/control and MTHFR rs180133 polymorphism in different models of inheritance

Inheritance Model	Genotype	Control (N = 130) N (%)	T2DM (N = 143) N (%)	OR (95% CI)	p*
Dominant	CC	128 (98.5)	123 (86)	Reference	
	CT-TT	2 (1.5)	20 (14)	10.40 (2.38–45.46)	< 0.001
Recessive	TT	0 (0)	1 (0.7)	**	
	CC-CT	130 (100)	142 (99.3)		

95% CI: 95% confidence interval
*Pearson Chi-square Test
**Owing to the lack of T allele, evaluation could not be made

TABLE 5. Analysis of the association of DN^+/DN^- and MTHFR rs180133 polymorphism in different models of inheritance

Inheritance Model	Genotype	DN^- (N = 82) N (%)	DN^+ (N = 61) N (%)	OR (95% CI)	*p**
Dominant	CC	72 (87.8)	51 (83.6)	Reference	0.474
	CT-TT	10 (12.2)	10 (16.4)	1.41 (0.54–3.63)	
Recessive	TT	0 (0)	1 (1.6)	**	
	CC-CT	82 (88.5)	60 (98.4)		

95% CI: 95% confidence interval
*Pearson Chi-square test
**Owing to the lack of T allele, evaluation could not be made

TABLE 6. Distribution of MTHFR gene rs1801133 genotypes according to some clinical parameters of controls and patients

Parameters	Group	N	CC Genotype	N	CT Genotype
Cholesterol (mg/dl)	Control	128	179.7 ± 40.2	2	203.0 ± 0.7
	T2DM	123	197.6 ± 55.9	19	211.1 ± 66.7
*p**			0.004		0.868
HDL	Control	128	48.3 ± 19.7	2	38.9 ± 0.07
	T2DM	123	43.8 ± 12.9	19	44.1 ± 15.1
*p**			0.040		0.639
LDL	Control	128	111.8 ± 37.2	2	139.0 ± 0.7
	T2DM	123	118.8 ± 42.3	19	127.5 ± 50.5
*p**			0.165		0.757
Triglyceride (mg/dl)	Control	128	138.1 ± 74.0	2	129.0 ± 0.7
	T2DM	123	182.3 ± 132.2	19	197.4 ± 154.6
*p**			0.001		0.548
HbA1c	Control	128	6.9 ± 6.5	2	4.7 ± 0.007
	T2DM	123	6.9 ± 1.4	19	7.1 ± 1.2
*p**			0.940		0.011
Creatinine (mg/dl)	Control	128	0.8 ± 0.3	2	1.1 ± 0.0
	T2DM	123	1.1 ± 1.3	19	1.1 ± 0.8
*p**			0.008		0.971
BMI (kg/m^2)	Control	128	26.7 ± 4.9	2	38.1 ± 0.003
	T2DM	123	31.8 ± 5.7	19	30.8 ± 5.1
*p**			< 0.001		0.067

*Independent two samples T Test

DISCUSSION

Hyperglycemia and insulin resistance are associated with enhanced DNA methylation.[6,19] DNA methylation has been established in T2DM, and MTHFR is one of the important enzymes involved in DNA methylation.[6] MTHFR is an important enzyme involved in homocysteine metabolism. High homocysteine levels have been found in patients with T2DM and DN, one of the microcomplications of diabetes. Given the role of MTHFR in DNA methylation and homocysteine metabolism, the effects of MTHFR gene polymorphism in diabetes and DN have been investigated; however, the results are contradictory.[20] Therefore, our study assessed the correlation between MTHFR rs1801133 polymorphism in patients with T2DM and DN from a Turkish population.

Our research revealed statistically significant difference in BMI, glucose, systolic blood pressure, diastolic blood pressure, HbA1c, creatinine, HDL, total cholesterol, and triglycerides between the patient and control groups. However, no statistically significant differences in age, gender, and LDL were observed between the control group and patients.

Similar to our findings, other studies reported the lack of difference in terms of gender and age.[6,9,21,22] Fekih-Mrissa *et al*. and Ma *et al*. reported that patients with diabetes had high levels of glucose, creatinine serum concentrations, HDL, LDL, HbA1c, total cholesterol, triglycerides, and BMI.[21,22]

In our research, the genotype distributions and allele frequencies of the MTHFR gene differed significantly between the patients with T2DM and the control group. Nevertheless, no statistical difference was found among the patients with DN.

Mtiraoui *et al*.[23] showed an association between MTHFR C677T mutation and hyperhomocysteinemia and DN. El-Baz *et al*.[10] inferred that ACE and MTHFR gene polymorphisms might be considered as genetic risk factors for DN in patients with T2DM. Rahimi *et al*.[24] revealed that MTHFR 677T and MTHFR 1298 C alleles increase the sensitivity to the onset and progression of DN in Iranians with T2DM. Cui *et al*.[25] determined that MTHFR C677T polymorphism might constitute a risk factor for DN in the Chinese population. Another study inferred a correlation between MTHFR rs1801133 polymorphism and increased plasma homocysteine levels, which might constitute a genetic risk factor for DN in Chinese patients with T2DM.[26]

In the evaluation of gene inheritance models to control increased risk or susceptibility to protection against T2DM and DN, the CT-TT genotype was significantly associated with T2DM.

Similar to our findings, Poodineh *et al*.[6] conducted an evaluation of codominant, recessive models and indicated that the rs1801133 polymorphism is significantly linked to T2DM susceptibility in their population. In contrast to our results, Pirozzi *et al*.[27] determined that the CT-TT genotype is not significantly associated with T2DM.

In their meta-analysis, Yang *et al*.[28] stated that MTHFR rs1801133 polymorphism is associated with the risk of DN and the MTHFR 677T variant contributes to an increase in DN in Caucasians with T2DM. In their 2019 study, Ma *et al*.[11] stated that the T allele of rs1801133 might constitute a risk factor for DN in Chinese males with T2DM and synergy might occur between MTHFR rs1801133 and smoking in relation to susceptibility to DN. Another study in Asian population reported that that the development of DN is associated with MTHFR rs1801133 polymorphism, especially in early T2DM.[29] In the study of the C677T and A1298C polymorphisms of the MTHFR gene in a southern Indian population, Ramanathan[30] indicated that DN is associated with these polymorphisms and also provided evidence that the rs1801133 polymorphism is associated with the progression of chronic kidney disease in DN. Another report showed that the MTHFR C677T T allele or TT genotype might be an important genetic molecular marker to identify the risk of DN in subjects with T2DM and to assist with developing appropriate disease prevention and management strategies.[5] In summary, MTHFR rs1801133 variants may affect the risk of DN, and additional research is warranted on gene–gene and gene–environment interactions.[31]

In our study, no statistically significant difference in cholesterol, HDL, triglyceride, creatinine, and BMI for the CT genotype were found. However, these parameters statistically differed for the CC genotype. Furthermore, a statistically significant difference in HbA1c for the CT genotype (p = 0.011). For LDL, statistically significant difference was observed across all genotypes. In contrast to our results, Santana Bezerra *et al*.[9] determined that these parameters were not statistically significantly different between patients and control subjects.

CONCLUSIONS

Any new finding that may be an early diagnostic marker is critical for diagnosis, follow-up, and treatment of patients. Identifying the most important polymorphism variants in different populations and writing haplotype maps for different societies may be the key for disease development and treatment in each population. Investigating T2DM, which is known to have a genetic predisposition, in molecular detail is important for early diagnosis and may provide an auxiliary parameter for doctors. Our findings showed that MTHFR rs1801133 polymorphism is not associated with DN but is related to T2DM. It was concluded that the MTHFR rs1801133 polymorphism CT and TT genotypes may be a genetic biomarker for T2DM progression and development in Turkish population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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