Erciyes Med J 2019; 41(3): 250–4 • DOI: 10.14744/etd.2019.39259
ORIGINAL ARTICLE – OPEN ACCESS

Investigation of the *Methylenetetrahydrofolate Reductase* Gene C677T and A1298G Polymorphism in Chronic Periodontitis

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ABSTRACT

Objective: This case control study examined the frequency and distribution of the C677T and A1298C single-nucleotide polymorphisms of the *methylenetetrahydrofolate reductase* (*MTHFR*) gene in Turkish patients with chronic periodontitis.

Materials and Methods: This study included 89 subjects: 49 patients with chronic periodontitis and 40 healthy persons. Medical and periodontal histories were taken, and PD, CAL, PI, and BOP were recorded as the clinical parameters. The blood samples were taken from the subjects, and their DNA was obtained. The *MTHFR* gene's single nucleotide polymorphisms *677C→T* and *1298A→C* genotyping were analyzed using the RFLP assay, then PCR.

Results: There was a significant difference between the groups except for age when the clinical findings. Single-nucleotide polymorphisms in the *MTHFR* gene C677T and 1298C regions were not found to be significantly different between the groups.

Conclusion: The study revealed no association between the *MTHFR* polymorphism and the risk of chronic periodontitis; however, increasing the number of patients is useful when performing further studies. This present study is important because it is, to the best of our knowledge, the first study to research the interrelation between the *MTHFR* gene and chronic periodontitis.

Keywords: *Methylenetetrahydrofolate reductase* (*MTHFR*), polymorphisms, periodontitis

Cite this article as:
Kocaman G, Orbak R. Investigation of the Methylenetetrahydrofolate Reductase Gene C677T and A1298G Polymorphism in Chronic Periodontitis. Erciyes Med J 2019; 41(3): 250-4.

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Submitted
19.02.2019

Accepted
07.05.2019

Available Online Date
19.08.2019

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INTRODUCTION

Periodontitis is a multifactorial disease that starts with microbial dental plaque accumulating in the gingival region. In periodontal tissues, inflammation caused by pathogenic bacteria leads to the destruction of periodontal supportive tissues. Periodontal destruction is considered to occur as a result of the reaction between the bacterial challenge and the host response (1). The host response is affected by environmental and genetic factors. During the disease activity, cytokines are mediated by accelerating the periodontal destruction. Studies on the DNA methylation of different cytokine genes explaining the activity of periodontal disease will supply important data on the pathogenesis of periodontal disease (1, 2).

DNA methylation is an epigenetic mechanism that regulates the transcription of many genes (3). Studies have shown that the *methylene tetrahydrofolatreductase* (*MTHFR*) enzyme on this metabolic pathway showed that it plays a key role. As a consequence of a reduced *MTHFR* activity, homocysteine is accumulated, methionine remethylation cannot be achieved, and ultimately DNA methylation is missing. DNA hypomethylation in DNA's making and repair disorder (4, 5). In the *MTHFR* deficiency, a disorder in the formation of methionine from homocysteine occurs and exposes the organism to both the reduction of methionine (and S-adenosylmethionine) and the toxic effects of homocysteine deposition (6).

The *MTHFR* gene takes part in the 1p36.3 chromosome and the C677T and A1298C single nucleotide polymorphisms identified in this gene. A low enzyme activity has been reported to be associated with these polymorphisms. As a consequence of the reduction in the *MTHFR* activity, the *5-methyl THF* level decreases, and the plasma *homocysteine* (*Hcy*) level increases with the levels of *5,10-methylene THF* (1, 3–7). Some of the *MTHFR* gene mutations cause deficiency of the its enzyme, and deficiency is very common in the general population. In *MTHFR* deficiency, especially the occurrence of arterial diseases has been suggested to be a risk factor (8), and clinical symptoms have been widely distributed, such as peripheral neuropathy, growth retardation, hypotonia, stroke, and thrombosis (8–11).

In severe *MTHFR* deficiency, hyperhomocysteinemia and homocysteinuria occur (12). Hyperhomocysteinemia is a risk factor for coronary heart, peripheral, vascular, and cerebrovascular disease (10–14). Recent studies reported an increased plasma *Hcy* levels in patients with chronic periodontitis (15). The aim of this study was to investigate the effects of the *MTHFR* gene C677T and A1298C gene polymorphisms on chronic periodontitis predisposition and disease prognosis.

MATERIALS and METHODS

Subjects and Sample Collection

The study was approved by the Ethics Committee of Atatürk University, and it was held on February 18, 2009, with the file number 2009.1.1/8. MTHFR gene polymorphisms were examined among chronic periodontitis patients and periodontally healthy individuals. A total of 49 patients and 40 healthy controls participated in the study.

The subjects were diagnosed with chronic periodontitis that included patients aged >35 years, a periodontal attachment loss greater than 5mm at teeth, with the probing depth greater than 6 mm, and there were lesions in each quadrant affecting more than two teeth. In the 6 months prior to the study, those who used any medications such as antibiotics, anti-inflammatory agents, and immunosuppressants and who suffered from systemic diseases were not included in the study. Written consent was obtained from all patients. Upon a periodontal examination, plaque index (PI), bleeding on probing (BOP) clinical attachment loss (CAL), and probing depth (PD) values of the subjects in both groups were recorded. For DNA isolation, peripheral blood samples were taken from the brachial artery of each patient.

DNA Isolation and Polymerase Chain Reaction

For genomic DNA isolation, 3 ml of blood was obtained from the subjects. Total genomic DNA was extracted using a Vivantis GF-1 Blood DNA Extraction User Guid (Vivantis Tech.) according to the manufacturer instructions. In vitro amplification DNA fragments used the PCR technique. The amplification of fragment 198 bp of MTHFR 677 was used for the sequence for 5'-CATCCCTATTGGCAGGTTAC-3' and reverse 5'-GACGGTGCGGTGAGA GTG- 3' primers. At the amplification of fragment, 163 bp of MTHFR 1298 was used sequence for 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' and reverse 5'-CACTTTGTGACCATT CCGGTTTG-3' primers as well. To obtain the PCR products, a reaction mix was prepared according to the instructions in a 0.5 ml tube. For the PCR mix, of a total volume of 25 µL that included 200 ng/ml DNA, 6 µl PCR Master Mix (Promega), and 0.5 µl 10 pmol of both primers was prepared. The PCR cycling was set to 30 cycles in 5 minutes at 94°C, then for 1 minute at 94°C, then for 1 minute at 57°C, and 15 seconds at 72°C, and then finally for a 10-minute extension at 72°C. The amplified product was exanimated in 3% agarose gel with electrophoresis under a UV transilluminator (Fig. 1).

Agarose Gel Electrophoresis

DNA fragments ranging from 100 to 50000 nucleotides were effectively separated by the agarose matrix. By altering the agarose concentration, DNA fragments can be separated in different sizes. We also used ethidium as a fluorescent dye. Bromide was for staining DNA fragments. The agarose gel electrophoresis technique can detect even fragments smaller than 5ng of DNA.

Restriction Fragment Length Polymorphism (RFLP)

For the digestion of the amplified PCR products, the restriction enzyme Hinf I for the region 677 and Mbo II for the 1298 region were used. For the Hinf I and Mbo II restriction digestions, mixtures of 2 µl buffer, 1,5 µl (10 U) of Hinf 1, 4 µl water, and 4 µl of PCR product were prepared, and then they were incubated at 37°C overnight.

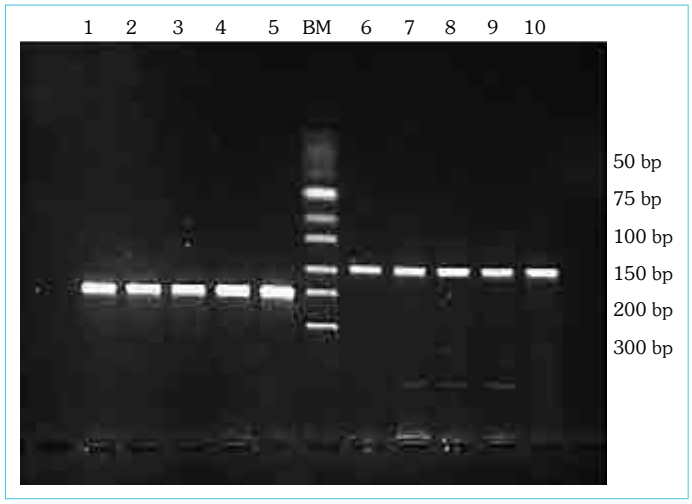

Figure 1. *MTHFR* gene PCR products

BM, weigh marker, 1-2-3-4-5 Lines show the PCR product for *MTHFR* 677 at 198 bp, and 6-7-8-9-10 Lines for *MTHFR* 1298 at 163 bp

Figure 2. RFLP analysis with *hinf I of MTHFR* 677

BM, weigh marker; Lanes 1-3-4, heterozygous type; Lanes 2, homozygous type; Lanes 5-6, wild type

Analysis of the MTHFR Codon 677 Genotype

There was only one band for the Wild type (677CC) of 677 polymorphism at 198 bp. Three bands were observed as heterozygous (677 CT) at 198,175 and 23 bp, and two bands were observed as homozygous (677 TT) at 175 and 23 bp (Fig. 2).

Analysis of MTHFR Codon 1298 Genotype

The Mbo II restriction enzyme was applied to digest the PCR product for analysis 1298 polymorphism. Only fragment of 56 bp in Wild type (1298 AA) was observed among the bands of 56, 30or31, 28, and 18 bp. The fragments of 84 bp, 56 in heterozygous (1298 AC) were observed among the bands of 84, 56, 30or31, 28, and 18 bp; and 84 bp fragments of homozygous 1298 (1298 CC) polymorphism were observed among 84, 30/31, and 18 bp (Fig. 3).

Statistical Analysis

The normality of dependent variables P–P plot and Kol-

Figure 3. RFLP analysis with *Mbo II of MTHFR* 1298

BM, molecular weight marker; Lanes 1-2, wild type; Lanes 3-4, heterozygous type; Lanes 5-6, homozygous type

mogrov–Smirnov test were tested. The gene polymorphisms were assessed with the count of direct gene. The difference between groups, the mean age, and clinical periodontal parameters that are not normally distributed were evaluated using the Mann–Whitney U test. The combined frequencies and the difference between the groups were analyzed using a two-proportional t test. The influence of the chronic periodontitis risk was calculated using the odds ratio and 95% confidence intervals. A p-value <0.05 was considered to be statistically significant.

RESULTS

In the analysis performed using clinical parameters, there was a significant difference between the plaque index (PI), bleeding on probing (BOP), clinical attachment loss (CAL), and probing depth (PD) parameters in the patient group compared to the control group (Table 1). No significant difference was found between the groups in the age parameter.

Genotyping

The genotype and allele frequencies of the *MTHFR* C677T and A1298C polymorphisms are shown in Table 2.

The genotyping distribution of the C677T and A1298C mutations was found to be chronic were periodontitis groups wild type at 69.4%, 677CT (heterozygous) at 24.5%, and 677TT (homozygous) at 6.1%, and for the control groups, they were 677CC at 70%, 677CT at 17.5%, and 677TT at 12.5%.

Table 1. Demographic and clinical Features of the studied subjects

	Controls (n=40)		Patients (n=49)		p
	n	%	n	%	
Age Mean (SD)	45.7	12.1	39.4	15.1	0.72
Ethnic origin	Turkish mixed population				
PD (SD) mm	1.8	0.4	3.5	0.65	**<0.001**
CAL (SD) mm	0.7	0.93	3.4	0.79	**<0.001**
BOP (SD)	0.6	0.49	1.7	0.50	**<0.001**
PI (SD)	1.01	0.62	2.1	0.61	**<0.001**

SD: Standard deviation; PD: Probing depth; CAL: Clinical attachment loss; BOP: Bleeding on probing; PI: Dental plaque on probing

Table 2. Distribution of the MTHFR C677T and MTHFR A1298C allele and genotype frequencies in chronic periodontitis patients and controls

Gene position	Alleles/Genotypes	Patients		Controls		p	Odds ratio (95% CI)
		n	%	n	%		
MTHFR 677	CC	34	69.4	28	70	0.368	1.51 (0.61 to 3.76)
	CT	12	24.5	10	17.5	0.432	1.48 (0.56 to 3.89)
	TT	3	6.1	2	12.5	0.523	1.81 (0.29 to 11.4)
	C	46	82	38	83	0.852	0.93 (0.45 to 1.94)
	T	15	18	12	17	0.852	1.07 (0.52 to 2.22)
MTHFR 1298	AA	18	36.7	14	35	0.578	1.29 (0.53 to 3.12)
	AC	27	55.1	21	52.5	0.502	1.49 (0.64 to 3.44)
	CC	4	8.2	5	12.5	0.771	0.62 (0.16 to 2.49)
	A	45	64	35	61	0.912	0.97 (0.55 to 1.71)
	C	31	36	26	39	0.912	0.97 (0.55 to 1.71)

CI: Confidence interval

Table 3. Frequencies of combined MTHFR C677T and MTHFR A1298C allelic variants in the chronic periodontitis patient and control groups

	Patients (n=49)						Controls (n=40)					
	MTHFR 677++		MTHFR 677+-		MTHFR 677--		MTHFR 677++		MTHFR 677+-		MTHFR 677--	
	n	%	n	%	n	%	n	%	n	%	n	%
MTHFR 1298++	12	24.5	4	8.2	2	4.1	10	25	3	7.5	1	2.5
MTHFR 1298+-	18	36.7	7	14.3	2	4.1	15	37.5	4	10	2	5
MTHFR 1298--	2	4.1	1	2	1	2	3	7.5	1	2.5	1	2.5

MTHFR: Methylene tetrahydrofolate reductase

The genotype distribution of the A1298C polymorphism found in chronic periodontitis groups was 1298AA at 36.7%, 1298AC at 55.1%, 1298CC at 8.2%, and in the control group, it was 1298AA at 36.7%, 1298AC at 55.1%, and 1298CC at 8.2%. In the control group, it was found to be 1298AA at 35%, 1298AC at 52.5%, and 1298CC at 12.5%. A significant difference in the genotype and prevalence were not found between patients and controls.

Both groups' *MTHFR* C677T and A1298C polymorphisms frequencies of allelic variants are presented in Table 3. In the combined frequencies, the difference between groups was not found between patients and controls.

DISCUSSION

Periodontitis is a chronic inflammation characterized by the bone loss in dental supportive tissues. Although oral bacterial infection is the main factor of periodontitis, genetic and environmental factors are known to be play a role in its progression and severity. Various studies have been conducted to identify genetic factors and the genes affecting periodontal diseases (16). Polymorphisms of genes involved in pathways important for cell metabolism take place in critical positions. In this case, the protein function and enzyme activity that the gene encodes can be significantly influenced by these changes. The deterioration of the function of proteins critical for the cell metabolism leads to various diseases or it increases the risk for some diseases. *MTHFR* is one of the polymorphic genes that can be given as an example.

The *MTHFR* polymorphisms are important risk factors for the development of cancer, vascular diseases, and diabetes (17–19). A single gene polymorphism in the *MTHFR* gene is known to reduce the enzyme activity encoded from the gene. In the studies, a decrease in the *MTHFR* enzyme was found to be 35% in the presence of heterozygous genotype (677 CT) and 70% in the presence of homozygous genotype (677 TT) compared to the normal genotype 677CC (20). The homozygosis in the A1298C polymorphism is more effective than the heterozigosis polymorphism in causing a reduction in the enzyme activity (7).

In the literature, different results of the *MTHFR* polymorphism have been reported in different populations and in different disease groups (21–23). In our study, it was found to be 12.5% of TT homozygotes of HC with chronic periodontitis patient in Turkish population. The ethnic and geographic variation of the C677T polymorphism is quite high. The TT rate is 1% in black population in America, sub-Saharan Africa, and South America, while in American Hispanics, Amerindian populations, Colombia, and Brazil, the rate is over 20%. The TT genotype frequency in the white society in Europe tends to increase from north to south. For North America and Australia, the rate is 8%–20%. The TT rate in Japan is 12% (7–23, 24). The results of this study are in compliance with the frequency reported in white Turkish population. In our study, the 1298 CC polymorphism homozygote ratio was found to be 12.5% in the healthy control group. In the literature, the CC prevalence for the *MTHFR* A1298C polymorphism in white-skinned individuals from North America ranges from 7% to 12%. The 1298CC frequency range is 4%–12% in most of the studies that come from Europe (10, 24).

With the *MTHFR* gene in the C677T polymorphism, the *MTHFR* enzyme activity decreased significantly, and homocysteine levels increased compared to 677CC genotypes in the 677CT or 677TT genotypes. It has been described as a risk factor in diseases such as cardiovascular diseases, neural tube defects, stroke, Down syndrome, breast and endometrial cancer, and diabetic nephropathy (8–11, 20–23). In our study, the 677CT ratios were found to be higher in the chronic periodontitis group compared to 677CC genotypes. This suggests that the *MTHFR* 677CT genotype may be considered to be a risk factor for chronic periodontitis. However, new studies are needed to be conducted on bigger populations.

Genetic polymorphisms may also be protective for a disease. In a study, a decreased *MTHFR* activity in lymphocytes of individuals with the 677 TT genotypes was reported to cause an increase in the concentration of *5,10-methylenetetrahydrofolate* required for the synthesis of thymine. This reduces DNA damage by inhibiting the formation of uracil by cytosine deamination. It has been suggested that the 677T allele increases the risk of acute leukemia, and collateral and lung cancers by establishing a higher *5,10 methylenetetrahydrofolate* level for the synthesis of thymine and purine in individuals with sufficient folate intake (25, 26). In our study, a TT genotype rate was found to be higher in the control group than the chronic periodontitis group, and this polymorphism can be interpreted as increasing the protection against chronic periodontitis.

The *MTHFR* enzyme activity is reduced by 40%–50% of the enzyme activity when both alleles are normal homozygous when A1298C and C677T mutations are double heterozygous. This enzyme activity is lower than the enzyme activity of individuals with the C677T heterozygote mutation. It has been suggested that

there is a significant increase in neural tube defects in individuals with the *MTHFR* 677T/1298C heterozygote status (27). In this study, double heterozygosity was higher in the chronic periodontitis group, and it was thought that it could be a risk factor for chronic periodontitis. The present study investigated the *MTHFR* 677 and 1298 polymorphisms, and the 677 TT and 1298 CC genotypes were found to increase in the HP group. The homozygous genotypes suggested that decrease the risk of CP. In addition, the *MTHFR* gene 677 and 1298 polymorphism, double heterozygotes (677 CT/1298 AC), was thought to be the induction in the risk of CP.

In conclusion, this study was the first to describe the relationship between the *MTHFR* gene C677T and A1298C polymorphisms and CP. This hypothesis could indicate a different genetic background between CP and *MTHFR*. The effects of the *MTHFR* gene polymorphism on periodontitis should be examined in further studies.

Ethics Committee Approval: The study was approved by the Ethics Committee of Atatürk University, and it was held on February 18, 2009, with the file number 2009.1.1/8.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – GK, RO; Design – GK, RO; Supervision – RO; Data Collection and/or Processing – GK; Analysis and/or Interpretation – GK, RO; Literature Search – GK; Writing – GK; Critical Reviews – RO.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: This study was supported by the Scientific Research Projects Coordinator of Atatürk University with the BAP project number 2009/325, and it was produced from a PhD thesis.

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