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MTHFR gene at rs A1298C polymorphism in type II diabetes among Iranian population

Negin Jafarian[1], Mohammad Hasan Sheikhha[2,3], Ali Akbar Samadani[4]

ABSTRACT

Introduction: Diabetes mellitus type II is a complex endocrine and metabolic disorder. Correspondingly, interference between multiple environmental, genetic and epigenetic factors cause a progressive and heterogeneous disorder with varying degrees of insulin resistance and dysfunction of islets. In this account, *MTHFR* gene (Methylene tetrahydrofolate reductase) is located on the chromosome 1 and its relationship with diabetes is unclear.

Methods: In a case-control study, blood samples of 150 patients with type II diabetes referred to the Central Laboratory in Yazd city, Iran was tested to determine the polymorphism A1298C of *MTHFR* gene using 4P-ARMS-PCR method as the control group 150 normal subjects without diabetes were compared.

Results: AA Genotype was reported % 66.4 in patients and 42% in the control group. AC genotype was % 66.30 in patients and % 33.9 in controls. Finally CC genotype was report % 66.64 inpatients and % 66.48in the control group. Our study showed that the prevalence of polymorphisms studied in patients had a significant difference with controlled group (p = 0.000). When checking diabetes complications, there was significant difference between these polymorphisms and neuropathy (p = 0.008).

Conclusion: *MTHFR* gene could be raised as one of the genes associated with susceptibility to type II diabetes. A1298C polymorphism of this gene can also be considered for the incidence of neuropathy.

Keywords: *MTHFR*, polymorphism A1298C, diabetes mellitus, neuropathy

INTRODUCTION

Diabetes is a chronic disorder in which a series of inadequate insulin production cannot keep glucose homeostasis normally. In this way, insulin insufficiency leads to decreased pancreatic islet beta cells or cause a resistance to insulin action that as a final result keeps a chronic increase in systemic glucose levels (1).

Diabetes types II include 90to 95 percent of diabetes and in majority of patients are diagnosed between 40 and 60 years (2). According to the World Health Organization (WHO) report, in Iran with more than four million people are suffering with the disease. The prevalence of diabetes in Yazd province is significantly higher compared to other provinces and it's about 14.2% (3).

Micro-vascular complications of diabetes are such as nephropathy, neuropathy and retinopathy, which leads to the common consequences associated with diabetes; include kidney failure, blindness, and amputation of lower limbs (4).

Diabetes is attributed to the interaction between environment and epigenetic factors (5). Although there is metabolic defects in type II diabetes, somewhat a variety of predisposing genes have been identified that interact with environmental factors during pregnancy, early childhood and later adulthood (6).

[1] *Department of Biology, Ashkzar Branch, Islamic Azad University, Ashkzar, Iran.*
[2] *Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[3] *Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*
[4] *Gastrointestinal and liver diseases research center (GLDRC), Guilan University of Medical Sciences, Rasht, Iran*

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Correspondence: *Mohammad Hasan Sheikhha[a,b]*
[a] Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
[b] Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
E-mail: sheikhha@yahoo.com

Correspondence: *Ali Akbar Samadani*
Gastrointestinal and liver diseases research center (GLDRC), Guilan University of Medical Sciences, Rasht, Iran
E-mail: a_a_hormoz@yahoo.com

Table 1: *Specific primers sequences for ARMS-PCR (Different alleles such as A, C and etc.)*

Polymorphism	Primer's Name	Primer sequences
A1298C	Common F	GAAGAAGTTTGCATGCTTGTGGTTG
	Common R	CAGGCAAGTCACCTGGGAGAGA
	Primer A	GGCAAAGAACGAGACTTCAAAGACACATT
	Primer C	GAGGAGCTGACCAGTGATGC

5, 10-Methylenetetrahydrofolate reductase enzyme encoded by the *MTHFR* gene and involved in turning of 5 and 10 methylene tetrahydrofolate to 5 methyl tetrahydrofolate; that the products are suitable substrate for conversion of homocysteine to methionine (7).

Plasma Homocysteine is a potentially toxic amino acid with negative pathological effects on the vascular endothelium, athreogenesis and coagulation factors five and eight, and led to increased levels of thrombin, platelet aggregation and resulting in venous thrombosis (8).

Mutations in the *MTHFR* gene results in a decrease in gene activity and thereby reduce the substrate for methionine synthase which is followed by increased levels of homocysteine. The same applies to induce platelet aggregation and ultimately, damage to vascular endothelial. *MTHFR* gene is located on chromosome number one (p36.31) and has 12 exons that encode a protein of 77 kDa (9).More than 40 point mutations and 9 common variants on *MTHFR* gene are known including polymorphism A1298C significantly creates a change on *MTHFR* enzyme activity.

A1298C polymorphism makes adenine to cytosine in nucleotide 1298 within exon 7 of *MTHFR* gene, which led to the displacement of glutamate by valine (p.Glu429Ala, rs1801131) (10).Since the gene *MTHFR* is regulating metabolism of homocysteine enzyme in the body, therefore it can have a role in diabetic nephropathy.Importantly, In this project, the performance of *MTHFR* gene polymorphism at rs A1298C in type II diabetes was investigated.

MATERIAL AND METHODS

In this study, polymorphisms A1298C of *MTHFR* gene in 150 persons without diabetes and 150 with type II diabetes were investigated. The patients had one complication of nephropathy, retinopathy or neuropathy and were referred to the Central Laboratory of Yazd, Iran.

Criteria for diagnosis of nephropathy associated with microalbuminuria (30 to 300 mg per 24 hours), which was confirmed by a physician.

The diagnosis of retinopathy was done with retina examination by a physician and observation of neovascularization (based on WHO criteria) and finally the examinations carried out by endocrinologist about neuropathy and numbness, according to standard indices.

After getting consent from patients a questionnaire about their diabetes was completed. Five cc of blood was taken from each participant in the tube containing EDTA. Genomic DNA was extracted with kit (GeneALL, ExgeneTM SV mini, 100p Korea). Single nucleotide polymorphism was tested by ARMS-PCR method using the primers metioned in **Table 1**. After using the software for data collection and analysis on SPSS19, the appropriate tests such as Chi-square, t-tests and ANOVA were used.

RESULTS

Determining the Genotype of the Polymorphism A1298C

After running the PCR product on 2% agarose gel a 593 bp bands is related to internal control (outer primers), a 361 bp is related to allele C, and a 281 bp for the allele A. Considering the number and length of the bands on gel, we realized the genotype and single nucleotide polymorphisms (**Figure 1**).

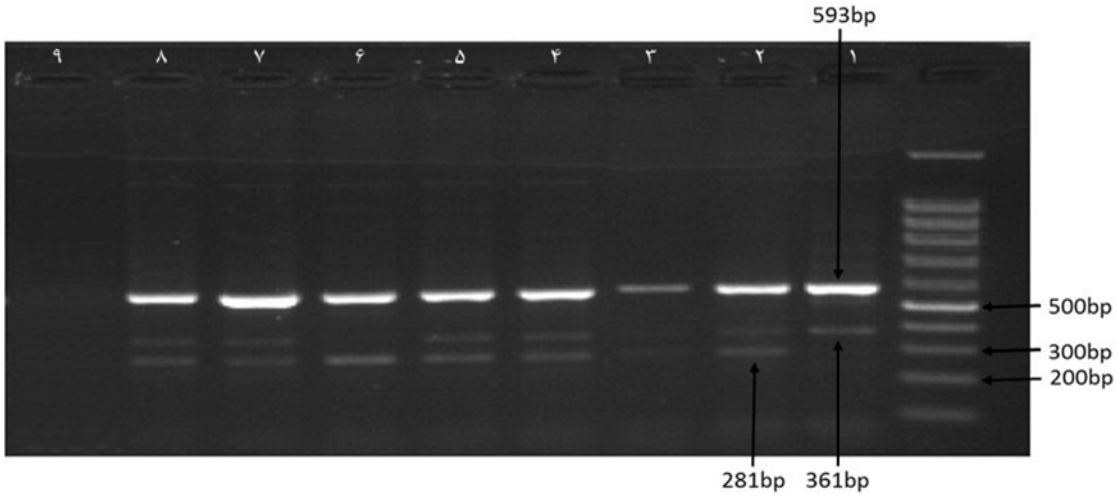

Figure 1: *Gel electrophoresis showing A1298C polymorphism genotypes*

Table 2: *The frequencies of genotypes and allelic polymorphism A1298C of MTHFR gene in two groups: case and control*

Genotypes	Controls		Diabetics		P-value
	Percent	Number	Percent	Number	
AA	42	63	4/7	7	<0.005
CC	9/3	14	30/7	46	
AC	48/7	73	64/6	97	
Total	100	150	100	150	
			Allele frequency		
A	66/4	199	37	111	<0.005
C	33/6	101	63	189	
Total	100	300	100	300	

Table 3: *Comparing the genotype and allele frequency Retinopathy in diabetic patient with Retinopathy and non-Retinopathy*

Genotypes	No complication of retinopathy		With complications of retinopathy		P-value
	Percent	Number	Percent	Number	
AA	5/31	5	3/58	2	
AC	58/52	55	75	42	0.123
CC	36/17	34	21/42	12	
Total	100	94	100	56	
			Allele frequency		
A	34/57	65	41/08	46	
C	65/43	123	58/92	66	0.000
Total	100	188	100	112	

In this way, different bands are classified as numbers 1, 2 and 3 including:

1) Individuals with healthy homozygous AA: 281bp and control band 593 bp
2) Heterozygous individuals AC: 281bp and 361 bp and 593 bp control band
3) Individuals with mutant homozygous CC: 361 bp band and 593bp control band

The samples were classified into two groups and each group was determined in genotype and allele frequencies. According to the genotype and allele frequencies of polymorphism A1298C in the two study groups, there was a significant relationship between genotypes in cases and controls. Prevalence of genotypic and allelic polymorphisms is shown in **Table 2**.

A1298C polymorphism in patients with diabetes mellitus was investigated in two groups of with and without diabetic complications. Notably, significant relationship was not observed with complication of retinopathy in diabetic patients (**Table 3**).

While, a significant relationship was observed with complication of nephropathy (**Table 4**).

Table 4: *Comparing the genotype and allele frequency retinopathy in diabetic patient with Nephropathy and non-Nephropathy*

Genotype	No complication of Nephropathy		With complications of Nephropathy		P-value
	Percent	Number	Percent	Number	
AA	4/28	5	6/07	2	
AC	63/25	74	69/69	23	0.634
CC	32/47	38	24/2	8	
Total	100	117	100	33	Total
			Allele frequency		
A	35/89	84	40/9	27	
C	64/11	150	59/10	39	
Total	100	234	100	66	

Table 5: *Comparing the genotype and allele frequency retinopathy in diabetic patient with Nephropathy and non-Nephropathy*

Genotype	No complication of Nephropathy		With complications of Nephropathy		P-value
	Percent	Number	Percent	Number	
AA	5/20	5	3/71	2	
AC	72/92	70	50	27	0.008
CC	21/88	21	46/29	25	
Total	100	96	100	54	
			Allele frequency		
A	41/6	80	28/70	31	
C	58/4	112	71/30	77	
Total	100	192	100	108	

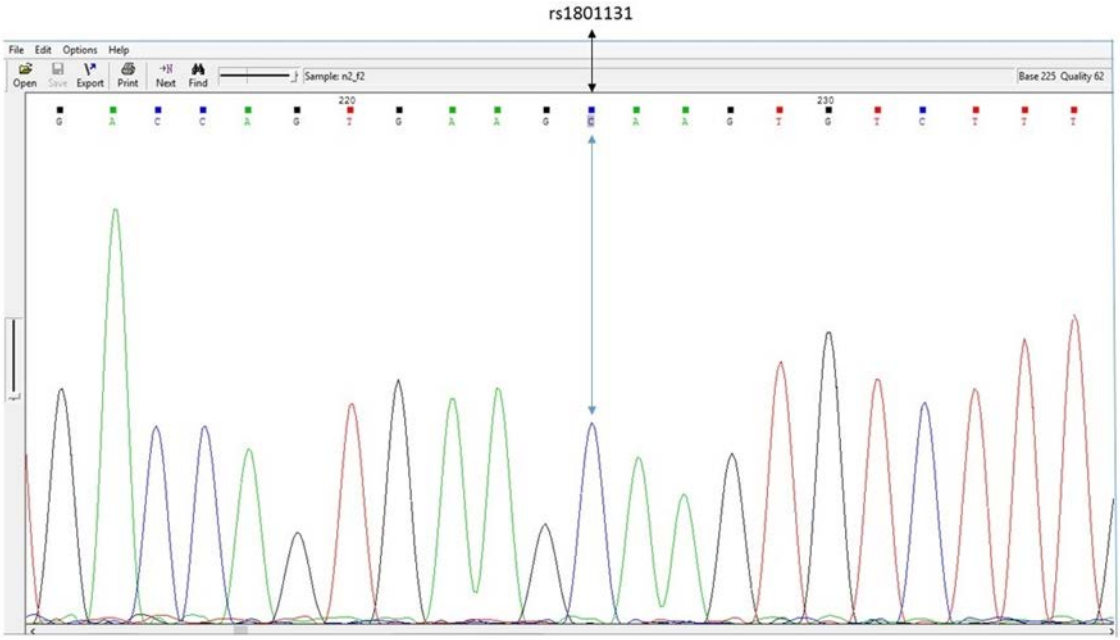

Figure 2: *The results of sequencing a homozygous mutant sample (CC) for cases*

A significant relationship was observed with complication of Neuropathy in diabetic patient (**Table 5**).

Among the 108 diabetic patients in the study groups that the HbA1C test was performed using ANOVA, statistical analysis significant correlation was observed between the polymorphism A1298C with HbA1C demographic characteristics.

To approve genotypes obtained from the process ARMS-PCR-4P as well as electrophoresis detection accuracy of the results, a number of samples were randomly selected and sequenced (**Figure 2**, **Figure 3**).

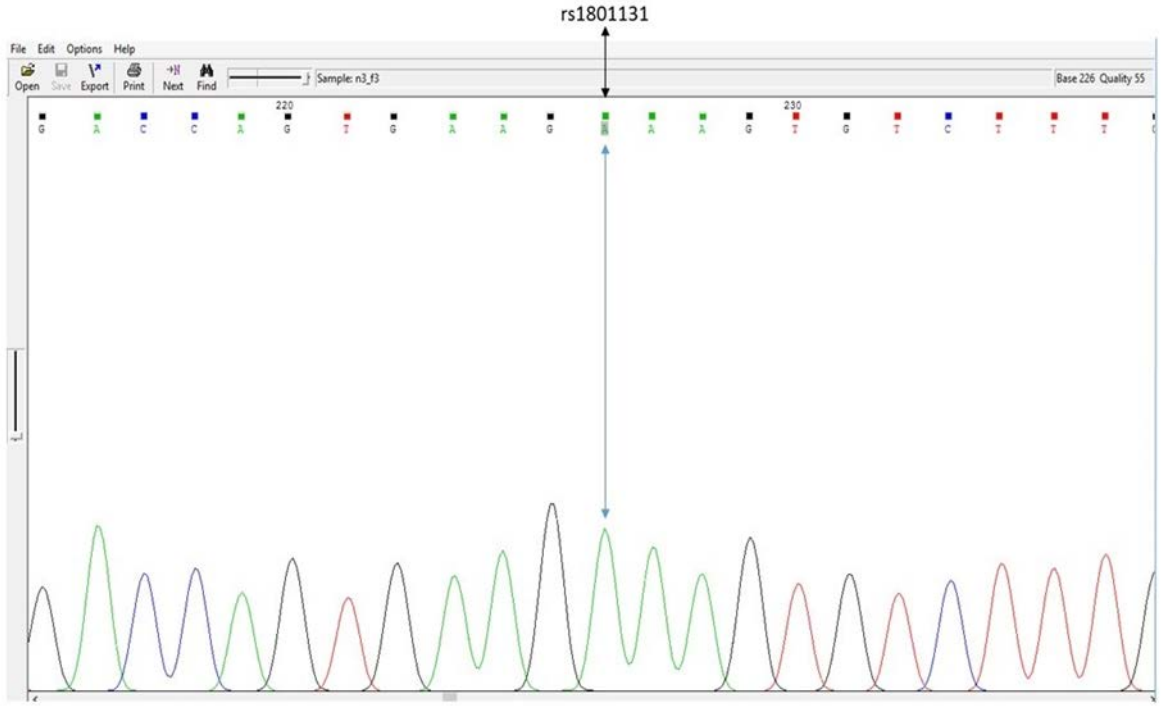

Figure 3: *The results of sequencing a sample of healthy homozygous (AA) for cases*

DISCUSSION

Diabetes mellitus type II is a complex metabolic and endocrine. Interaction between multiple genetic and environmental factors cause a progressive, heterogeneous disorder with varying degrees of insulin resistance and dysfunction of pancreatic beta cells and is associated with changes in biochemical, physiological and pathological liver (11). Unfortunately, diabetes mellitus type II is increasing and this increase is more significant in developing countries, including in our country. Patients with diabetes are more prone to the risk of complications such as vision disorders, cardiovascular attacks, amputation and kidney failure (11). 5 and 10 methylene tetrahydrofolate reductase enzyme is a key enzyme regulating the plasma homocysteine level codes by *MTHFR* gene. Polymorphism A1298C with a mutation in the *MTHFR* gene, affects the specific activity of the enzyme as well as increase the folate concentrations in serum homocysteine levels. The same applies to induce platelet aggregation and ultimately, vascular endothelial injury in diabetes (12, 13). A1298C polymorphism was investigated in people with diabetes. Polymorphism A1298C with the frequency of AA = 6.07%, AC = 69.69%, and CC = 24.2% in cases with complications of diabetic nephropathy compared to a lot of AA = 4.28%, AC = 63.25%, and CC = 32.47% in cases without complications of diabetic nephropathy showed no significant relationship P = 0.634. While polymorphism A1298C with frequencies of AA = 3.71%, AC = 50%, CC = 46.29% in cases with complications of diabetic nephropathy compared to a lot of AA = 5.20%, AC = 72.92%, CC = 21.88% in the groups, without the complications of diabetic nephropathy showed a significant relationship P = 0.008.

Several studies were consistent with our study results, such as AbdRaboh et al who studied the effect of A1298C and C677T polymorphisms in patients with type II diabetes mellitus in Egyptian people using RFLP techniques. According to the data of their study, polymorphism of the *MTHFR* gene increases the risk (OR: 2.2, 95% CI = 0.7-6.9, P = 0.004) in the type II diabetes (10). Rizk El-Baz and his colleagues also examined the effect of the polymorphism A1298C on diabetic nephropathy in 202 patients with type II diabetes, and 102 with diabetic nephropathy with technique RFLP-PCR. The results of their study showed that polymorphism A1298C causes an increased risk of macro-albuminuria in patients with diabetic nephropathy (P = 0.02) (14). Shuai Wu and colleagues in 2012 examined the effect of *MTHFR* gene and *ACE* in progression of diabetic nephropathy. The outcome of their meta-analysis study that was conducted in China and Pakistan represents a significant correlation (OR = 1.43: 95% CI: 1.08-1.90, P = 0.014) (15).

Serbulent Yigit and his colleagues in Turkey performed a study on 230 peripheral diabetic nephropathy and 282 healthy controls and they reported a significant difference between *MTHFR* gene in normal group (P = 0.002) and patients with peripheral diabetic nephropathy (P = 0.003) (16).

While some studies have different results of our study such as Boyi Yang and colleagues who, in China and the UK reviewed had study on 15094 patients with high blood pressure and hypertension in pregnancy and 21633 controls, and found that there was no significant associated between A1298C polymorphism and high blood pressure and hypertension in pregnancy (oR: 1.06, 95% CI = 0.90-1.26, P = 0.155) (17).In the case of different results, we can be pointed to possible causes difference in communities, such as race, number of patient study procedures and so on. Considerably, the quality and quantity of extracted nucleic acids is really effective in final results (18,19) and also a compatible and suitable primer designing is very significant and required in this way (20).

CONCLUSION

Diabetes mellitus type II is a complex and elaborated endocrine and metabolic disorder. In this way, interaction between multiple genetic and environmental factors cause a progressive, heterogeneous disorder with varying degrees of insulin resistance and pancreatic beta-cell dysfunction and is related with changes in biochemical, physiological and pathological liver. Meaningly, obesity and overweight are the main factors involved in insulin resistance and glucose intolerance.

Additively, when beta cells are no longer able to produce enough insulin to overcome insulin resistance, impaired glucose tolerance progresses to diabetes type II.

Conclusively, 5 and 10 methylene tetrahydrofolate reductase enzyme is a key enzyme regulating plasma homocysteine level that coded by the *MTHFR* gene. Polymorphism A1298C, by creating mutations in the *MTHFR* gene affects the specific activity of the enzyme as well as increase the folate concentrations in serum homocysteine levels. That would be induced platelet aggregation and ultimately, vascular endothelial damage in diabetes.

CONFLICT OF INTERESTS

The authors have no conflict of interest in this study.

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