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## **Association between of polymorphism rs1801133 in MTHFR gene and diabetes mellitus type II for Iraqi patients**

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**Abstract**---Diabetes mellitus (DM) is a clinical syndrome caused by metabolic problems. Recently, researchers have recently concentrated their efforts on identifying additional risk variables, like as homocysteine (Hcy), a non-independent vascular risk factor linked to insulin resistance in type 2 diabetes mellitus (T2DM). Aimed our current study to association between of polymorphism rs1801133 in MTHFR gene and diabetes mellitus type II for Iraqi patients. Samples of blood were collected from Iraqi with T2DM (50 patenits) from auditors to specialized laboratories and consulting clinics in Baquba District / Diyala Governorate, Iraq, as well as 21 healthy participants, and their ages ranged (35-54 years). Genotyping was performed by PCR/SNP (specific primers), risk score for T2DM was determined by Hardy-Weinberg equilibrium (HWE). (HWE) was analyzed in T2DM patients and healthy participants, and it was discovered that the MTHFR (rs1801133) genotypes were in agreement with the equilibrium, with no clear differences ( $p > 1.020 - 0.298$ ) between the observed and expected genotype frequencies. When rs1801133 genotype and allele frequencies were compared in T2DM patients and healthy participants, it was shown that there were no significant differences in these frequencies. In addition, the GG genotype of rs1801133 scored high in patients and healthy participants and was considered a preventive fraction (RR = 0.89). While, AA and GA

genotypes were considered the etiological fraction and associated with T2DM (RR = 1.23, 1.04). Even according to allele analysis G allele may be preventive, while A allele could be etiological for disease. Conclusions. The results indicated that AA, GA genotype and A allele is a risk factor with T2DM for rs1801133 and might have a role in the etiopathogenic mechanism in Iraqi patients with T2DM. However, more researchs with bigger sample sizes are required to confirm our findings.

**Keywords**---rs1801133, MTHFR, T2DM, hyperhomocysteinemia.

## **Introduction**

Diabetes mellitus (DM) is a clinical syndrome caused by metabolic problems such as hyperglycemia and problems with glucose, protein, and lipid metabolism. The destruction of insulin-secreting beta cells in the pancreas causes diabetes, which results in an absolute or relative shortage of insulin production [1,2]. According to the World Health Organization (WHO), Diabetes is anticipated to be the seventh greatest cause of death by 2030, and the current global diabetes population of 387 million is expected to rise to 592 million by 2035 [3]. Non-Insulin Dependent Diabetes (NIDDM) is the most frequent chronic ailment in adults, in the next 20 years, the number of diabetics is expected to climb from 386.7 million to 591 million. [4]. Furthermore, researchers have recently concentrated their efforts on identifying additional risk variables, like as homocysteine (Hcy), a non-independent vascular risk factor linked to insulin resistance in type 2 diabetes mellitus (T2DM) [5]. Recent research indicates that high homocysteine levels are a risk factor for diabetes and coronary heart disease, with risk of high Hcy levels being many times higher than the risk of other pathological disorders including high blood pressure, high cholesterol, and smoking [6].

Homocysteine (a natural, non-essential amino acid containing sulfur) plays a major role in destroying the arteries of the body if it increases more than it does by smoking, obesity, or cholesterol itself, and this is due to a deficiency in cystathionine b-synthase (CBS) or methyl tetrahydrofolate reductase enzymes (MTHFR) or cofactors (vitamin) in the metabolism of homocysteine [7]. Methylenetetrahydrofolate reductase (MTHFR) is one of the most important enzymes in homocysteine metabolism [8]. It is the primary enzyme involved in the metabolism of folic acid and homocysteine, and it has recently attracted attention as a biologically significant molecule. As the enzyme works to convert methylenetetrahydrofolate to methyltetrahydrofolate, which is required to convert Hcy to methionine, and in absence, deficiency or decreased of activity of enzyme. Remethylation of Hcy to methionine will not occur, resulting in an increased Hcy level in the blood [9].

Specific polymorphisms within the coding sequences of these enzymes have recently been shown to impact the metabolic pathways controlled by the enzymes [8]. The most frequent polymorphism of the MTHFR gene is the occurrence of a mutation of MTHFR C677T (rs1801133) in the catalytic domain of the enzyme, resulting in a 50% decrease in enzyme activity, and thus this decrease in enzyme activity accompanied by a significant decrease in folic acid levels and thus an

increase in homocysteine levels in the blood, which is one of the indicators impaired of methylation capacity can result in a variety of illnesses [10]. As a result, single nucleotide polymorphisms (SNPs) linked to the risk and progression of diabetes and its primary chronic problems in diabetic individuals impact the activity and function of the MTHFR enzyme [11]. Therefore, our current study came to investigate association between of polymorphism rs1801133 in MTHFR gene and diabetes mellitus type II for Iraqi patients.

## **Methods and Materials**

### **Participants**

A total of 50 T2DM individuals were enrolled in the research, (from auditors to specialized laboratories and consulting clinics in Baquba District / Diyala Governorate, Iraq) who aged over 35 years old, and 20 healthy control subjects. during October 2020 – September 2021 for diagnosis and treatment. Blood samples were kept in tubes EDTA and the samples were saved frozen at 20-25C until used for each isolate to extract DNA. Add to that, each participant gave their informed consent after they were informed of the study and given a special questionnaire to collect information.

### **Preparation PCR mixture and PCR conditions**

Using Primer3plus software designed the forward and reverse primers for rs1801133, and Macrogen/Korea provided them. The forward primer sequence 5'GAACTCAGCGAACTCAGCAC' and the revers: 5'CCCTATTGGCAGGTTACCCC3'. Polymerase chain reactions were performed using thermocycler device (USA). The PCR products were successfully amplified employing a reaction mixture consisting of 1,5 µL of every primer, 12.5µL of Go Tag Green Master Mix/ Promega, 3 µL of patient's DNA and 6.5 µL of D.W with final volume 25 µL. The final thermocycling program's amplification conditions were as follows: initial denaturation was a single cycle at 95 °C for 5 minutes, followed by 35 cycles of denaturation at 95 °C for 30s, annealing at 58 °C for 30s, and extensions at 72 °C for 30s, followed by a final cycle extension at 72 °C for 10 minutes. The PCR product has a size of 300bp.

### **Statistical analysis**

Alleles and genotype frequencies were analyzed using chi-square tests, while Hardy-Weinberg Equation was used to determine the populations studied were in genetic equilibrium from site <https://wpcalc.com/en/equilibrium-hardy-weinberg>. The calculation of the differences in genotypical and allelic frequency between studied groups was based on WINPEPI Computer program (version 11.63), to calculate Fisher's exact test, calculate the relative risk (RR), Preventive or Etiological fraction (PF or EF) with a confidence interval of 95% (CI). A statistically significant difference was considered with a P value of less than 0.05 [12].

## Results

Three genotypes (GG, GA, and AA) were identified, as well as two alleles (G and A) for the rs1801133 (G/A; Chromosome1). The genotypes in T2DM of Iraqi patients and healthy participants were found to be in agreement with the equilibrium, with no significant differences ( $p>1.020-0.298$ ) between the observed and expected genotype frequencies (Table 1).

## Discussion

According to results of this study, the MTHFR gene - rs1801133 resulted in the emergence of three genotypes (GG, GA, and AA) that correspond to two alleles (Table 1),(Figure1). (G and A). These genetic patterns were found that consistent with the Hardy–Weinberg equation (HWE) in Table 2 .

Table 1  
Numbers and percentage frequencies (observed and expected) of MTHFR gene (rs1801133) genotypes and their (HWE) in T2DM of Iraqi patients and healthy participants

Groups			MTHFR gene rs1801133 - Genotypes or alleles					H-WX2 P ≤
			GG	GA	AA	G	A	
T2DM (No. =50)	Observed	No.	26	18	6	70	30	<b>1.020</b>
		%	52	36	12	70	30	
	Expected	No.	24.5	21	4.5	Not estimated		
		%	49	42	9			
Controls (No. =20)	Observed	No.	11	7	2	29	11	<b>0.298</b>
		%	55	35	10	72.5	27.5	
	Expected	No.	10.51	7.98	1.51	Not Estimated		
		%	52.55	39.9	7.55			

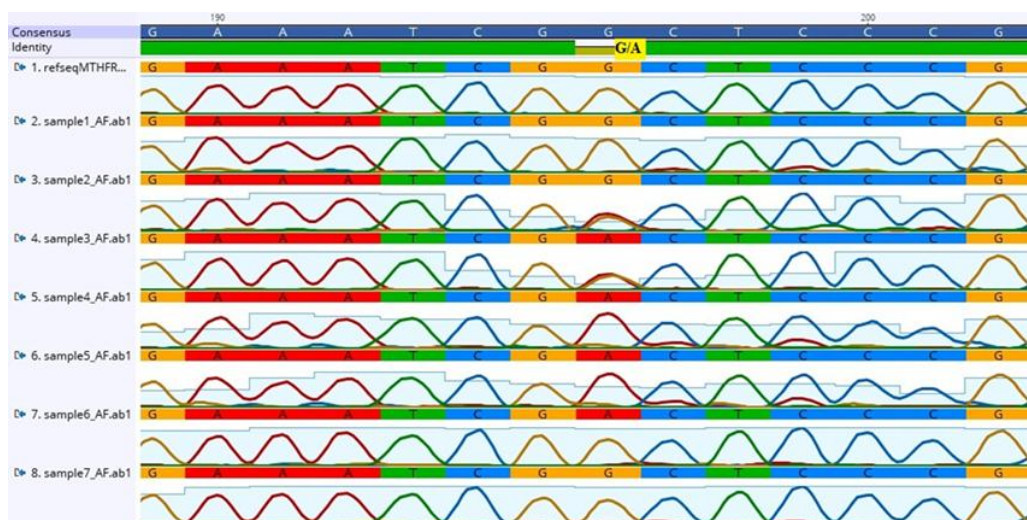


Figure 1. Shows a comparison of the nitrogenous base alignment of a part of the MTHFR gene between T2DM patients and individuals controls, showing SNP of rs1801133

Using Fisher's exact test, the rs1801133 polymorphism analysis showed that frequency of all genotypes was non-significantly higher in the control group than in the patients group. The genotype AA and A allele recorded lower ratio in both the patients (12 vs 30%) and control groups (10 vs 27.5% respectively) with Probability Fisher (0.840 vs 0.760), making it the least frequent genotype in the Iraqi population. On the other hand, the frequency of GG genotype and G allele (55 vs 72.5% respectively) was significantly elevated in control compared to the patients group (52 vs 70% respectively) with Probability Fisher (0.898 vs 0.760). Depending on the values of RR (1.23, 1.04), both AA, GA genotypes and A allele (1.13) were considered the etiological fraction and associated with T2DM. While, GG genotype with values of RR (0.89). And G allele (0.89) were considered the Preventive fraction and no associated with T2DM.

Table 2

Statistical analysis of association between genotypes and alleles of MTHFR gene (rs1801133) for T2DM versus Controls

Type of comparison	Statistical Evaluation			Fisher's Exact Probability	95% Confidence Intervals
	rs1801133 Genotype or Allele	Relative Risk (RR)	Preventive or Etiological fraction (PF or EF)		
T2DM versus Controls	GG	0.89	%11.4	0.898	0.30 - 2.56
	GA	1.04	%4.3	0.892	0.35 - 3.25
	AA	1.23	%18.5	0.840	0.23 - 9.53
	G	0.89	%11.5	0.760	0.38 - 1.99
	A	1.13	%11.5	0.760	0.50 - 2.63

The results of our current study were agreement with results of a previous study by Zidan et al. (2019) on evaluating role of the polymorphism C677T (rs1801133) of MTHFR gene in patients with T2DM and susceptibility to diabetic nephropathy, as the results showed an increased prevalence of C677T polymorphism for CT and TT genotypes and for the T allele, significantly and statistically significant in diabetic patients compared to control ( $P < 0.001$ ). It was also indicated by a statistically significant difference in the TT and CT genotypes and the T allele in T2DM with nephropathy compared with T2DM without nephropathy group ( $P < 0.01, 0.05$ ) [13]. The polymorphism C677T of gene MTHFR is characterized by an alanine/valine substitution at site 226 of the protein in the N-terminal catalytic domain of the MTHFR gene specifically in fourth exon of a cytosine/thymine base substitution at site 677 [14]. Moreover, study of Zhong et al. (2013) reinforced that this mutation reduces enzyme activity, with individuals with CT and TT genotypes having 65,35% less activity, respectively, compared to those with wild-type CC genotype [15]. Mutations in the MTHFR gene affect the specific activity of the enzyme in addition to increasing the concentrations of folate, which leads to an increase in the levels of homocysteine in the blood, and this would cause platelet aggregation and eventually the occurrence of endothelial vascular damage in patients with diabetes [14].

In another recent study conducted on the Iranian community on the relationship of MTHFR polymorphisms (rs1801133, rs1801131) with the risk of developing T2DM in southeastern Iran, the results of the study concluded that the MTHFR polymorphisms rs1801131 A/C and rs1801133 T/C may be Genetic biomarkers of T2DM development and progression in the Iranian community sample, the study also indicated that the C allele is a potential risk factor for T2DM for the rs1801133 polymorphism and this did not agree with the results of our current study [16]. Recently, recent studies have shown that both genetic and non-genetic variables influence Hcy levels. Genetic factors ensure the occurrence of genetic defects in the MHTFR gene, which leads to high levels of Hcy [17, 18]. Whereas, non-genetic factors include abnormalities in folic acid, vitamin B6 and vitamin B12 in the diet that also lead to increased levels of Hcy [19]. In conclusion, results indicate that AA, GA genotype and a allele is a risk factor with T2DM for rs1801133 and might have a role in the etiopathogenic mechanism in Iraqi patients with T2DM. Furthermore, this is the first publication to describe the genotype distribution of the MHTFR gene rs1801133 genetic polymorphism in Iraqi patients with T2DM and to show related between MHTFR gene rs1801133 genotypes and T2DM disease.

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### **Ethical approval**

The local ethical committee at Council College of Education for Pure Sciences/ the University of Diyala approved the project.

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### **Conflicts of interest**

None

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