International Journal of Medical Science and Clinical Invention 10(2): 6553-6560, 2023
DOI:10.18535/ijmsci/v10i2.03
https://valleyinternational.net/index.php/ijmsci

Research Article,

Detection of Methylenetetrahydrofolate Redactase Gene Polymorphisms among Sudanese Patients with Type 2

Alaa Marghani Suliman[1], Tyseer A. M. Abdalrahman[3], Ruaa Mohammed Babeker[3], Enas Alzain Ahmed Mohamme[3], Maye M. Merghani[4], Nihad Elsadig Babiker[1,2,3*]

[1]University of Medical Science and Technology
[2]Darfur University College, Sudan
[3]National Center of Neurological Sciences, Sudan
[4]Nahda college, Sudan
Email Address: **nihadelsadig @yahoo.com**

Abstract

background: Type 2 diabetes mellitus (T2DM) is a major public health problem that not only affects individual life quality, but also increases social economic burden. The pointed of this research to detect MTHFR gene polymorphisms in Sudanese patients with type 2 diabetes mellitus.

Materials and Methods: This was case control study conducted at the NCNS Khartoum, Sudan during the period of August to December, 2022. From each participant 3 ml of venous blood was collected in sterile containers with Ethylene Diamine Tetra-acetic Acid (EDTA). Genomic DNA was isolated from peripheral blood leukocytes by the DNA extraction kits. The MTHFR gene was amplified using conventional PCR. PCR products were sent for sequencing to Macro gene Europe Laboratory.

Results: MTHFR gene was detected in 94% of the patients and 64% of controls. Sanger sequence revealed tow substitution polymorphisms C > T and A > C. Mutation taster software confirmed the presence of the polymorphisms, and emphasized the tow polymorphisms are single Base Exchange polymorphism and changed the amino acid sequences.

Conclusion: In the Sudanese patients with T2DM tow single base exchange polymorphisms were detected in the MTHFR gene (C>T ID rs 1801133 and A>C), according to the analysis the two polymorphisms change the amino acid sequence and affected in the normal gene function this increased the possibility of early disease complications.

Keywords: T2DM, MTHFR gene, Sanger sequence, polymorphisms, PCR.

Introduction:

Diabetes mellitus (DM) encompasses a heterogeneous group of disorders characterized by hyperglycemia associated with multiple disorders including metabolic, cellular, and blood disturbances leading to vascular complications [(1)]. Type 2 diabetes mellitus (T2DM) is a major public health problem that not only affects individual life quality, but also increases social economic burden [(2).] The number of people suffering from type 2 (T2) DM has been increasing due to the aging population, urbanization, and low physical activity. According to the International Diabetes Federation (IDF) estimate of 2013, 382 million (8.3%) adults had diabetes worldwide. The number has been increasing by twofold over the past 20 years, and 80% of the people with diabetes particularly live in low- and middle-income countries. [(3)] Several hematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), and the coagulation factors are shown to be directly associated with DM.[4,5]

Methylenetetrahydrofolate reductase (MTHFR) is a folate- metabolizing enzyme that participates in folic acid circulation and DNA synthesis MTHFR catalyzes the irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Dysfunction or low activity of MTHFR may decrease the level of methyl pool; consequently, it inhibits the

successful deoxynucleoside synthesis and intracellular methylation reactions [6,7].

The human gene *MTHFR* (OMIM number: 607093) is located on chromosome 1p36.3. Of all the identified SNPs, C677T (Ala222Val, rs1801133 C>T) is one of the most investigated genetic variations. The C677T polymorphism is a C to T transition at base pair 677, which results in the amino acid transition from Ala to Val. Such amino acid transition significantly decreases the activity of MTHFR [8] In Sudan, no published data were found on this subject and none involving the proposed panel of genetic variants. This study was design to detect the possible present of MTHFR gene polymorphisms in Sudanese patients with Type 2 diabetes mellitus (T2DM)

Material and methods:

A Descriptive Analytical case-control hospital-based study conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period august to December, 202**2.** All patients attending the medicine unit at Ibrahim Malik teaching hospital and diagnosed with Type 2 diabetes mellitus (T2DM) during the aforementioned period were included. In addition to that, apparently healthy participants were selected as control group. From each participant 3 ml of venous blood was withdrawn with minimal stasis from the ante-cubital vein using a dry sterile disposable syringe and needle. Blood samples were dispensed into sterile containers with Ethylene Diamine Tetra-acetic Acid (EDTA). They were labeled with subject's age, sex and identification number and stored at -20°C for molecular analysis. Ethical clearance for this study was obtained from ethical review committee, Faculty of Medical laboratory, Ibrahim Malik Teaching hospital and the participants was fully informed about the advantages and disadvantages before participation in the research (verbal informed consent).

The DNA was extracted by G-DEX IIB Genomic DNA extraction Kit (for blood). Primers were designed by using Prime3 software. The forward primer for MTHFR (C677T) was designed as "5-GGTCAGAAGCATATCAGTCATGAG -3"and reverse as "5-CTGGGAAGAACTCAGCGAACTCAG -3" with product size of 494bp fragment.

Method for polymerase chain reaction PCR:

14 ul double distilled water was placed in PCR tube, then 4 ul of master mix, 1 ul of forward primer, 1 ul of reverse primer and 2 ul of DNA sample was added then vortex. The PCR tube containing this mixture was place in commercial thermal cycler (Swift^{TM}MaxPro SWT-MXP-BLC-4)at following condition: Denaturation temperature 94^{o}C for 30 secs, annealing temperature at 61^{o}C for 30 sec and extension temperature at 72^{o}C for 30 secs, the final elongation was adjusted for 5 minutes at 72 °C. PCR reaction was set at 35 cycles.

The PCR amplification product was separated on agarose gel. Finally the. PCR products were sent for sequencing to Macro gene Europe Laboratory.

SPSS version 23 statistical software (SPSS Inc., USA) was used for statistical analysis. The sequencing results were analyzed using different bioinformatics soft-wares and tools. The obtained sequences, aligned using BioEdit-ClustalW software with a normal sequence from Gen Bank (National Center of Biotechnology Information, accession number NC_000001.11) were examined for the presence of polymorphisms.

Results:

A total of 100 participants were enrolled in this study, 50 were selected as cases and 50 were selected as control group. In the case group; 68% were male and 32% were female, the most affected age group between 41-60 years (70%), most of them are from Khartoum state (94%). In addition to the diabetes mellitus; 22% had a hypertension and 74% did not have history of any other chronic disease. The disease duration; 54% more than ten years and 46% less than ten years. (Table 1) (Figure 1)

Table (1): Distribution of sociodemographic data

		Frequency	Percent
Age	20-30yr	7	14.0
	31-40yr	8	16.0
	41-60yr	35	70.0
	Total	50	100.0

		Frequency	Percent
Age	20-30yr	7	14.0
	31-40yr	8	16.0
	41-60yr	35	70.0
Occupation	Khartoum	47	94.0
	Bahri	1	2.0
	Omdurman	2	4.0
	Total	50	100.0
Duration of disease	≤ 10 years	27	54.0
	> 10 years	23	46.0
	Total	50	100.0

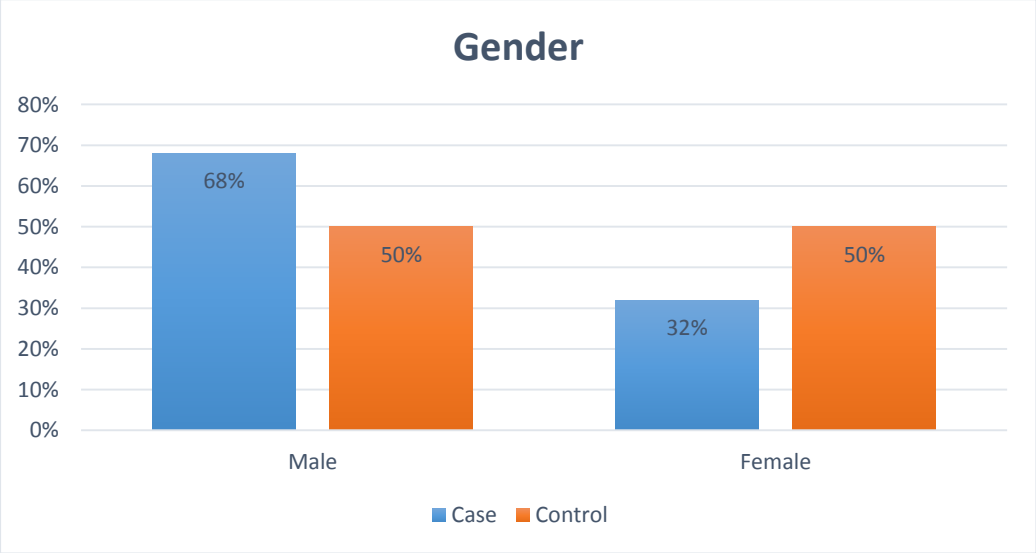

Figure (1): Distribution of gender of case and control

4.2 Molecular studies

In the present study 494 bp of MTHFR gene was detected in the gel electrophoresis after PCR (Figure 2). For the PCR results; MTHFR gene was detected in 94% of the patients and 64% of controls (figure 3). Chi-Square test was used to compared the PCR results between the case and control; it showed significant differences with (p value =0.00) (table 2)

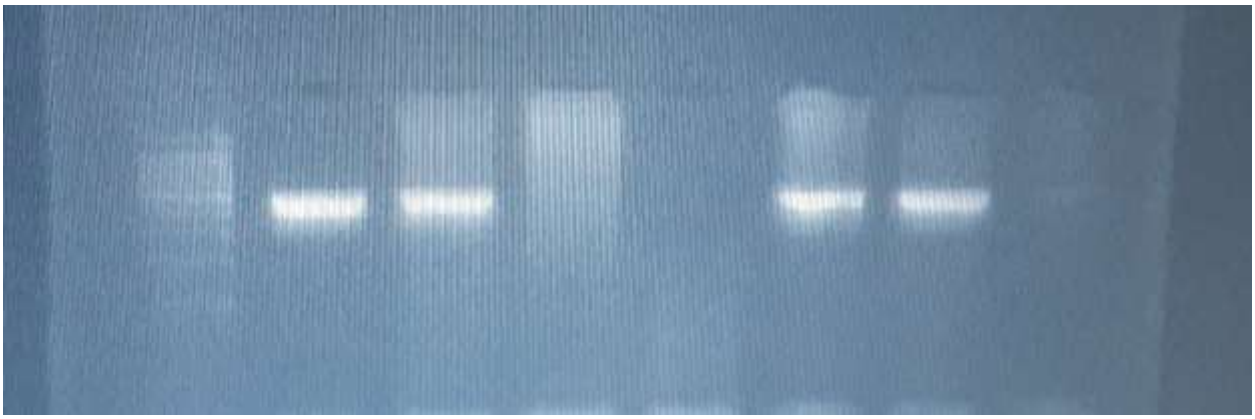

Figure (2): 494 bp of factor of MTHR allele detected with gel electrophoresis

Table (2): Chi-Square test for the PCR results

Study population	PCR results		P. value
	Positive	Negative	
Case	47 (94%)	3 (6%)	0.000*
Control	32 (64%)	18 (36%)	
Total	79 (100.0%)	21 (100.0%)	

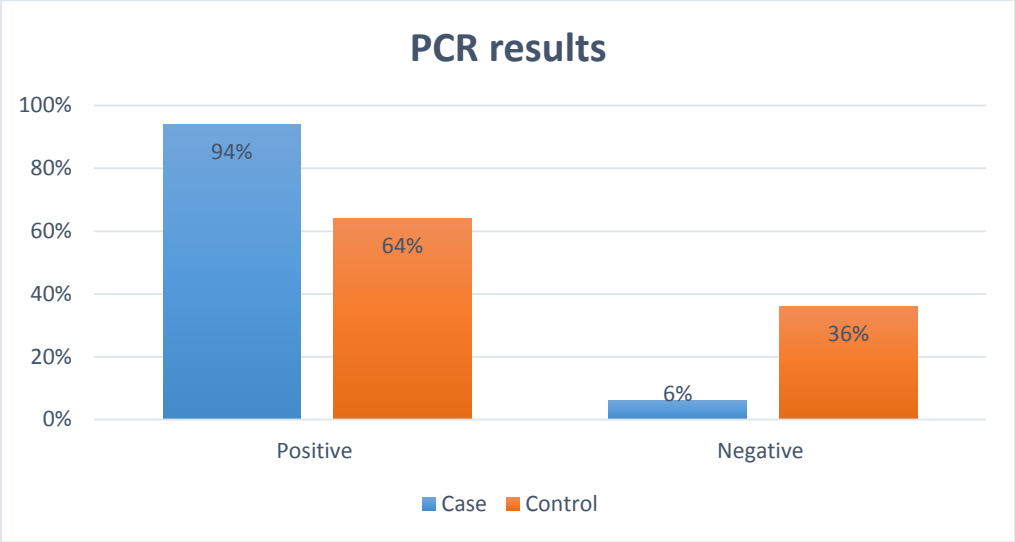

Figure (3): Distribution of PCR result in case and control

Sequencing results:

Different bioinformatics soft-wares and tools were used to analyze the sequencing results. The obtained sequences aligned using BioEdit-ClustalW software with a normal sequence from GenBank gene (accession number NC_000001.11in NCBI).

When the cases were compared with the normal reference two single Base Exchange were found C to T and A to C. While when the controls were compared with normal reference, no any single base exchange was found among the all control groups (figure 4)

Mutation taster was used to confirm the mutation which revealed; C>T single Base Exchange polymorphism was predicted, amino acid sequence was changed, and splice site also was changed, alteration location was at chromosome 1, alteration type was single base exchange, cDNA changes position was 794, reference ID (rs 1801133), and A>C also single Base Exchange polymorphism, amino acid sequence was changed, alteration location was at chromosome 1, cDNA changes position was 865. (figure 5,6)

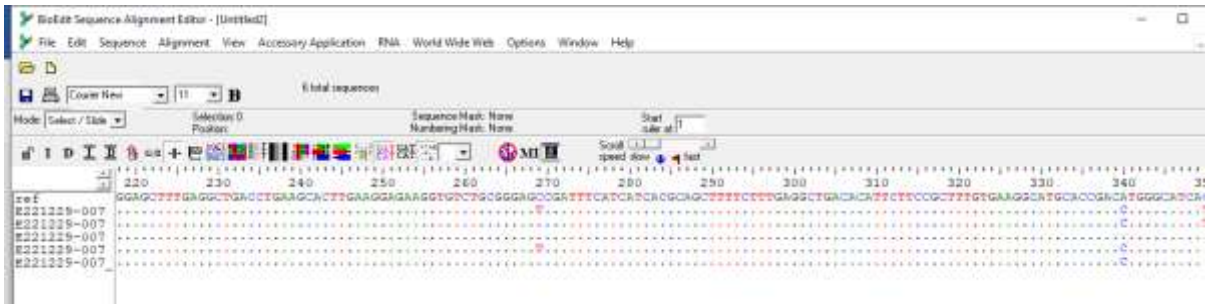

Figure (4): Multiple sequence alignment using Bio-Edit clustal W for cases group with reference gene sequence of MTHFR gene.

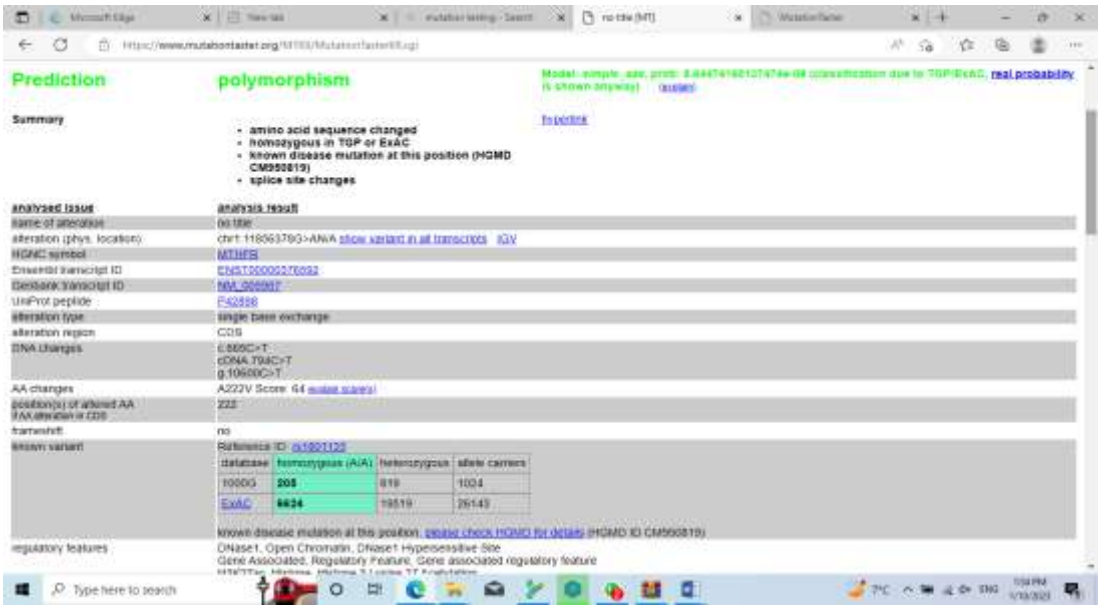

Figure (5): mutation taster application result of C>T singles Base Exchange

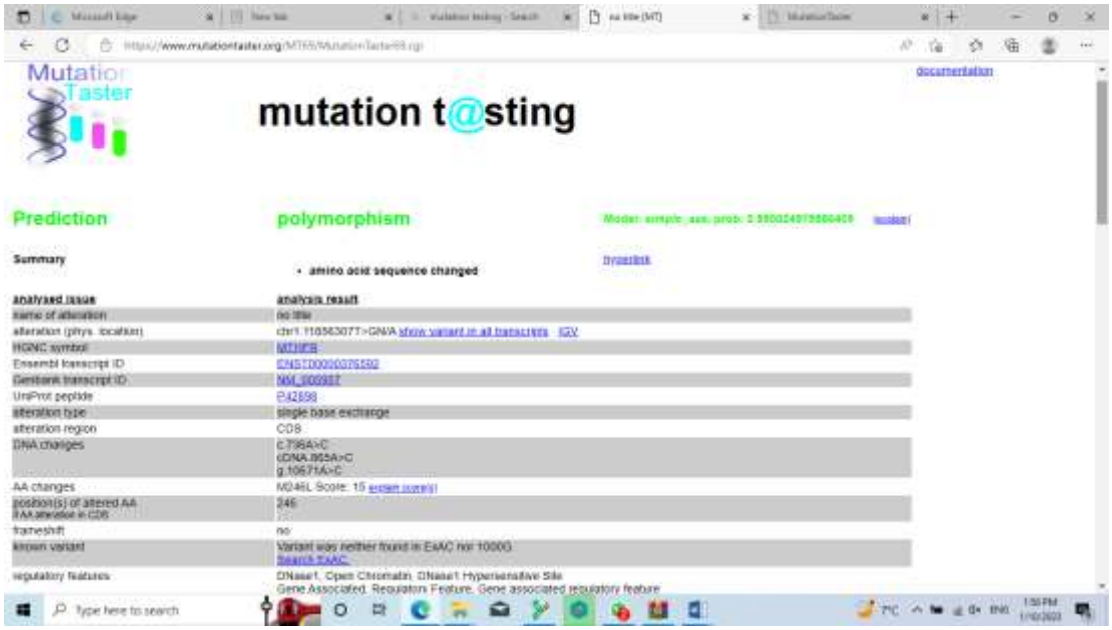

Figure (6): mutation taster application result of A>C singles Base Exchange

Discussion:

Type 2 diabetes mellitus (T2DM) is a major public health problem that not only affects individual life quality, but also increases social economic burden. Evidences suggest that T2DM is a complex disease caused by the combinations of environmental and genetic risk factors.[9] This was descriptive analytical case-control hospital-based study conducted at the research laboratory of the national center of neurological sciences to detect MTHFR gene polymorphisms in Sudanese patients with Type 2 diabetes mellitus (T2DM), the results revealed that; In the case group; 68% were male and 32% were female, the most affected age group between 41-60 years (70%). In addition to the diabetes mellitus; only 22% had a history of hypertension, and 54% had a disease duration more than ten years.

The results of this study is consist with Canadian study which reported; the prevalence of diabetes was over 19% in men over 50 years of age by 2005 in a population-based study in Ontario, Canada, the corresponding prevalence for women was just under 16%. Similarly, a recent Korean study reporting data from 2005 showed type 2 diabetes prevalence in above 30 year olds to be

around 7.9% in women but 10.2% in men with the biggest differences in prevalence's apparent in the 40–59-year-old brackets, where, remarkably, male diabetes prevalence was around double that in females. [10]

Also Alexandra Kautzky-Willer et al study mention; T2DM is more frequently diagnosed at lower age and body mass index in men; however, the most prominent risk factor, which is obesity, is more common in women. [11]

In present study only 22% had a history of hypertension although 54% had disease duration more than ten years and 46% less than ten years.

Patients with diabetes can have elevated blood pressure and 40–60% of diabetes cases exhibit high blood pressure. Hypertension is a well-known complication of diabetes mellitus and diabetes is a well-known complication of hypertension. [12]

For the PCR results; MTHFR gene was detected in 94% of the patients and 64% of controls when compared the PCR results between the case and control. the sanger sequencing results found two single Base Exchange C to T and A to C in the case group.

Mutation taster was used to confirm the mutation which revealed; C>T single Base Exchange polymorphism was predicted, amino acid sequence was changed, and splice site also was changed, alteration location was at chromosome 1, cDNA changes position was 794, reference ID (rs 1801133).

Meng et al point out that single nucleotide polymorphism involves a transition of C to T which in turn results in an alanine to valine substitution at codon 222 (Ala222Val) The mutation of MTHFR C677T (rs 1,801,133) is located in the catalytic domain of the enzyme, which results in a thermolabile enzyme with a decrease in enzyme activity of 50%. The MTHFR C677T mutation with decreased enzyme activity significantly lowers the serum folate level and consequently increases the blood level of homocysteine. The resulting hyperhomocysteinemia is one of the indicators of impaired methylation capacity and could lead to a number of pathological processes[13]

Previous studies have shown that the elevated level of homocysteine is linked with T2DM complications such as nephropathy, cardiovascular disease and stroke. In addition, several studies have reported an association of MTHFR gene polymorphism with T2DM and its complications such as, retinopathy, neuropathy, ischemic heart disease, nephropathy and retinopathy in different populations. [9]

In a study conducted in China in 2014, Wang et al. found that C677T in the MTHFR may influence the risk of T2DM and they detected a significant relationship between MTHFR C677T polymorphism and T2DM in the Chinese population.[14]

Khalid et al. Observed that there was a significant relationship between MTHFR C677T polymorphism and T2DM in Arab population. Also Meng et al revealed MTHFR C677T polymorphism was significantly associated with T2DM in Asian populations. [9,15]

In the other hand in a case–control study conducted in the population of Brazilian with T2DM no correlation was found between the MTHFR C677T in the development of T2DM. Also Nithya et al suggest that the MTHFR C677T gene polymorphism is not considered as a risk factor for the development of T2DM and its vascular complications in the studied population. Therefore, the inter individual variability with respect to geographical background and lifestyle factors could play a role in the pathogenesis of T2DM with vascular complications. [16, 17]

In this study the second polymorphism was A>C also mutation taster confirmed this was a single Base Exchange polymorphism and the amino acid sequence was changed.

Also A to C polymorphism in the *MTHFR* gene encodes for a glutamate to alanine substitution and leads to a decrease in enzyme activity. Combined heterozygosity for the C677T/A1298C polymorphisms in some studies is associated with higher homocysteine concentrations and decreased plasma folate. [18]

Najiba et al found the Prevalence of the 2 heterozygous polymorphisms of the thermolabile MTHFR gene (CT and AC) was encountered more commonly in patients with diabetes mellitus. Patients who carry the A1298C mutation are at risk for at least 1 complication of DM. Double heterozygous mutants were at the greatest risk for retinopathy and for suffering at least 1 complication. [19]

while another study done in United Arab Emirates to detect MTHFR C677T and A1298C polymorphisms in DMT2 patients and revealed that; MTHFR gene polymorphisms are not related

to T2DM in the Emirati population. However, these polymorphisms can be used as risk markers for CVA, nephropathy, high LDL cholesterol and triglycerides in T2DM patients and allow timely treatment. [18]

Finally, the relationship between T2DM susceptibility and MTHFR C677T genotype has been largely investigated but contradictory conclusions still remain

Conclusion:

In the Sudanese patients with T2DM tow single base exchange polymorphisms were detected in the MTHFR gene (C>T ID rs 1801133 and A>C), according to the analysis the two polymorphisms change the amino acid sequence and affected in the normal gene function this increased the possibility of early disease complications. Further well designed, large-scale, and in depth studies are warranted to check the relationship between the T2DM and the MTHFR polymorphisms

Reference:

[1] Elalamy I,Chakroun T, Gerotziafas GT, et al. Circulating platelet-leukocyte aggregates: a marker of microvascular injury in diabetic patients. *Thromb Res*. 2008;121(6):843–848.

[2] DeFronzo, R. A., Ferrannini, E., Groop, L., Henry, R. R., Herman, W. H., Holst, J. J., … Weiss, R. (2015). Type 2 diabetes mellitus. *Nature Reviews Disease Primers*, *1*, 15019.

[3] Rizvi A, Sanders MB. Assessment and monitoring of glycemic control in primary diabetes care: monitoring techniques, record keeping, tests of average glycaemia, and point of care evaluation. *J Am Acad Nurse Pract*. 2006;18:11–21

[4] Mirza S, Hossain M, Mathews C, et al. Type 2-diabetes is associated with elevated levels of TNF-alpha, IL-6 and adiponectin and low levels of leptin in a population of Mexican American: a cross-sectional study. *Cytokine*. 2012;57(1):136–142.

[5] Gkrania-Klotsas E, Ye Z, Cooper AJ, et al. Differential white blood cell count and type 2 diabetes: systematic review and meta-analysis of cross-sectional and prospective studies. *PLoS ONE*. 2010;5(10):e13405.

[6] Friso, S., Choi, S.-W., Girelli, D., Mason, J. B., Dolnikowski, G. G., Bagley, P. J., Selhub, J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proceedingsof the National Academy of Sciences of the United States of America* 2002; 99(8), 5606–5611. https ://doi.org/10.1073/pnas.06206 6299.

[7] Niclot, S.,Pruvot, Q., Besson, C., Savoy, D., Macintyre, E., Salles, G. Baudry-Bluteau, D. (2006). Implication of the folate-methionine metabolism pathways in susceptibility to follicular lymphomas. *Blood*,*108*(1), 278–285. https ://doi.org/10.1182/blood-2005-04-1567.

[8] 8. Liew, S. C., & Gupta, E. D. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: Epidemiology, metabolism and the associated diseases. *European Journal of Medical Genetics* 2015; *58*(1), 1–10.

[9] 9. Yanzi Meng, Xiaoling Liu, Kai Ma4, Lili Zhang, Mao Lu6, Minsu Zhao, Min-Xin Guan8, Guijun Qin. Association of MTHFR C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility. Mol Genet Genomic Med. 2019;7:e1020.

[10] 10. Naveed Sattar. Gender aspects in type 2 diabetes mellitus and cardiometabolic risk. Best Practice & Research Clinical Endocrinology & Metabolism 2013; 27(4): 501-507

[11] Alexandra Kautzky-Willer, Jürgen Harreiter, Giovanni Pacini, Sex and Gender Differences in Risk, Pathophysiology and Complications of Type 2 Diabetes Mellitus, *Endocrine Reviews*, Volume 37, Issue 3, 1 June 2016, Pages 278–316, https://doi.org/10.1210/er.2015-1137

[12] Ohishi, M. Hypertension with diabetes mellitus: physiology and pathology. *Hypertens Res* 2018; **41**: 389–393. https://doi.org/10.1038/s41440-018-0034-4

[13] MengY, LiuX, MaK, et al. Association of MTHFR C677T polymorphism and type 2 diabetes mellitus (T2DM) susceptibility. Mol Genet Genomic Med. 2019;7:e1020. https://doi.org/10.1002/ mgg3.102

[14] Wang, H., Hu, C., Xiao, S. H., & Wan, B. (2014). Association of tagging SNPs in the MTHFR gene with risk of type 2 diabetes mellitus and serum homocysteine levels in a Chinese population. Disease Markers, 2014, 725731. https://doi.org/10.1155/2014/725731

[15] Al-Rubeaan, K., Siddiqui, K., Saeb, A. T., Nazir, N., Al-Naqeb, D., & Al-Qasim, S. (2013). ACE I/D and MTHFR C677T polymorphisms are significantly associated with type 2 diabetes in Arab ethnicity: A meta-analysis. Gene, 520(2), 166–177. https://doi.org/10.1016/j. gene.2013.02.0

[16] Pirozzi, F. F., Belini Junior, E., Okumura, J. V., Salvarani, M., Bonini- Domingos, C. R., & Ruiz, M. A. (2018). The relationship between of ACE I/D and the MTHFR C677T polymorphisms in the pathophysiology of type 2 diabetes mellitus in a population of Brazilian obese patients. Archives of Endocrinology and Metabolism, 62(1), 21–26. https://doi.org/10.20945/2359-3997000000005

[17] Nithya K, Isabel W, Angeline T, Priscilla AS, Shakila H, Asirvatham AJ. MTHFR C677T gene polymorphism in Type 2 diabetes mellitus patients with and without vascular complications: A case-control study. Meta Gene. 2017 Dec 1;14:79-84.

[18] El Hajj Chehadeh SW, Jelinek HF, Al Mahmeed WA, Tay GK, Odama UO, Elghazali GE, Al Safar HS. Relationship between MTHFR C677T and A1298C gene polymorphisms and complications of type 2 diabetes mellitus in an Emirati population. Meta Gene. 2016 Apr 17;9:70-5. doi: 10.1016/j.mgene.2016.04.002. PMID: 27222819; PMCID: PMC4856855.

[19] Najiba Fekih-Mrissa, Meriem Mrad, Hazard Ibrahim, Imen Akremi, Aicha Sayeh, Amel Jaidane, Haroun Ouertani, Borni Zidi, Nasreddine Gritli. Methylenetetrahydrofolate Reductase (MTHFR) (C677T and A1298C) Polymorphisms and Vascular Complications in Patients with Type 2 Diabetes. Canadian Journal of Diabetes 2017; 41(4): 366-371