MTHFR C677T Gene Polymorphism and Association with Disorders

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Abstract: - The Methylenetetrahydrofolate reductase (MTHFR) is a general and important enzyme in human cells, which is responsible for the metabolism reactions of homocysteine and folate. The genetic material for MTHFR enzyme synthesis is situated on 1 chromosome p arm in the 1p36.3 position. A lot of single nucleotide mutations have been identified in this mentioned locus, but among them well-studied is the C677T gene mutation. The C677T/MTHFR polymorphisms impact MTHFR enzyme activity, leading to alterations in methionine and folate metabolism, homocysteine levels, and in most cases subsequent effects on DNA methylation. This literature review compiles information about the MTHFR C677T polymorphism and explores its potential association with various complex, multifactorial disorders, such as cancer, cardiovascular complications, neurological conditions, and diabetes mellitus, among others. The review synthesizes findings from diverse global populations, providing valuable insights for master's and doctorate students, as well as researchers specializing in this field.

Key-Words: - MTHFR C677T Gene, polymorphism, multifactorial disorders, complication, Methylenetetrahydrofolate reductase, Single nucleotide polymorphisms.

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1 Introduction

methylenetetrahydrofolate to 5-methyltetrahydrofolate, a key co-factor responsible for adding methyl to homocysteine and after it is converted into methionine. The genomic location of the MTHFR gene is located on the 1st autosomal chromosome, situated at the terminal part of the short arm (1p36.6), [1], and this genomic region is vital for controlling the folate metabolism, a fundamental process in cell metabolism involving the methylation of the vital macromolecules, such as DNA, RNA, and as well proteins. This genetic alteration results in the conversion of valine with alanine at genetic codon 222, representing a very general mutation that adversely affects the function of the enzyme. MTHFR enzyme genetic makeup involves two alleles. Presently, there are more than 20 gene mutations linked to this enzyme, particularly single nucleotide polymorphisms (SNPs) emphasis on the in-depth examination of the C677T- rs1801133 and A1298C - rs1801131, [2]. The C677T allele has been well studied from the scientific point of view and it is established that it has high clinical significance too. The A1298C variant is milder and less clinically significant.

In our current literature review, we will focus on exploring the relationship of C677T SNP with various types of complexes, and multifactorial disorders, such as diabetes mellitus, cardiovascular complications, cancer, and others (Table 1, Appendix). The C677T MTHFR is intricately linked with a range of diseases, including vascular disorders, cancers, neurological conditions, diabetes, psoriasis, and more [3], [4], [5], [6]. The prevalence and distribution of the C677T polymorphism exhibit geographical and ethnic variations, contributing to its complex epidemiology, [7].

Homozygous mutations demonstrate increased homocysteine levels, whereas individuals with heterozygous (C/T) mutations exhibit slightly raised homocysteine levels compared to controls, [8]. Increased levels of homocysteine, referred to as

hyperhomocysteinemia, are recognized as a developing susceptibility to several cardiovascular diseases.

2 Function of Methylenetetrahydrofolate Reductase (MTHFR) and Its Implications

The Methylenetetrahydrofolate reductase has a crucial role in methionine and folate metabolism, impacting the metabolism of the cell's DNA and ensuring the maintenance of appropriate homocysteine levels in the body, [9]. C677T/MTHFR gene mutations lead to decreased MTHFR enzyme activity, with individuals carrying the heterozygous genotype (C/T) having 70% of standard enzyme activity, and those with the T/T homozygous genotype having just 30%, [10]. This reduction interrupts the conversion of homocysteine to methionine, leading to decreased serum folate levels, increased homocysteine levels, and DNA hypomethylation.

Homocysteine assumes a pivotal role in the impairment of endothelial cells, expediting the initiation and advancement of atherosclerosis. It contributes to the development of unstable plaques via mechanisms involving endoplasmic reticulum stress, oxidative stress, inflammatory factors, and as well immune responses. Furthermore, homocysteine triggers injury to endothelial cells, initiates lipid oxidation, as well accelerates the progression of the atherosclerosis process, [11].

MTHFR is involved in the methylation processes of DNA, RNA, purine, and thymidine syntheses by acting as a methyl donor. These processes contribute to the development of various vascular diseases, [12]. The C677T/MTHFR polymorphisms impact MTHFR enzyme activity, leading to alterations in methionine and folate metabolism, elevated homocysteine levels, and subsequent effects on DNA methylation. The role of homocysteine in endothelial damage and atherosclerosis, coupled with the influence of MTHFR in methylation processes, underscores the complex interplay contributing to the development of carotid atherosclerosis (CAS).

3 The C677T/MTHFR Polymorphism Association with Cardiovascular Disease

In [12], is studied 730 participants (516 males, 214 females) of Han ethnicity from Chongqing, China. In individuals with carotid atherosclerosis, the occurrence of heterozygotes (C/T) and homozygotes (T/T) was notably high, and the rates of C/C homozygotes and the C allele were markedly low ($P < 0.05$) compared to the healthy controls. The disease occurrence risk was higher for C/T heterozygotes and T/T homozygotes (OR = 4.06, $P < 0.001$ and OR = 3.14, $P < 0.001$), in comparison to the C/C genotype. The mutation in the MTHFR gene poses a risk factor for carotid atherosclerosis in the Chinese population.

In a comprehensive study involving 3,247 participants aged 30 to 89 years (1,693 women, 1,554 men), it was revealed that the T/T homozygous poses a risk for carotid stenosis occurrence within the Japanese population, [13]. Another study corroborate these findings, demonstrating that the incidence of a T allele in the MTHFR gene significantly increases the risk of intima-media thickness (IMT) thickening, thus suggesting a link between C677T/MTHFR and carotid atherosclerosis, [14].

However, the overall conclusions drawn from various studies on this topic remain argumentative. For instance, some authors showed that the MTHFR gene's C/T polymorphism contributes to an elevated homocysteine level. Surprisingly, they found no significant correlation between this mutation and carotid artery stenosis, [15].

The author's group studies within the Georgian population hold particular relevance for us as it involves individuals from the same region as ours—specifically, the Adjara region and Batumi city. The study enrolled 101 patients with arterial thrombosis, with a predominant male representation at 71.3%. Notably, 83% of these patients had myocardial infarction, and 13% had experienced ischemic stroke. A comparison of the incidence between the cases and controls revealed a significantly elevated occurrence in the patient group ($P < 0.05$). Specifically, the incidence of the C677T polymorphism was 21.2% in the control group, while the cases exhibited an incidence of 33.2%. The considerable prevalence of MTHFR C677T among individuals with arterial thrombosis proposes a potential association, indicating an elevated risk of arterial thrombosis in the mentioned population. This association is further supported by the statistically significant p-value, [16].

Additionally, the study investigated in the Brazilian adolescent population, the relationship between the C677T polymorphisms and cardiovascular risk factors. Among 115 adolescents, a prevalence of hyper-homocysteinemia (19.1%) and alterations in lipid profile were observed. Specifically, the MTHFR C677T exhibited elevated levels of plasma vitamin B6 and oxidized low-density lipoprotein compared to the homozygous (CC) genotype suggesting potential associations between this genetic variation and certain biochemical markers related to cardiovascular health, [17].

The inconsistencies in these conclusions may be attributed to factors such as small sample sizes, diverse racial backgrounds, and insufficient modifications of confounding variables like smoking, drinking, sleep quality, and dietary habits. Therefore, it is suggested that the MTHFR variant C677T may act as a relatively independent risk factor for CAS or indirectly contribute to atherosclerosis by elevating plasma homocysteine levels. The varying outcomes across studies highlight the need for further research with larger and more diverse populations, precisely addressing confounding factors, to gain a more conclusive understanding of the link between MTHFR gene polymorphisms and cardiovascular complication risk.

4 The C677T/MTHFR Polymorphism Association with Diabetes Mellitus

The link between MTHFR gene polymorphism and individuals with diabetes mellitus type 2 has been studied in various types of research. A study investigating MTHFR gene polymorphism and diabetic complications (retinopathy and nephropathy) among Japanese individuals with type 2 diabetes found that incidence was higher of 677T/677T individuals with retinopathy than those with the other genotypes. There was a notable difference in genotype distribution, with frequencies of 677C/677C at 41.9%, 677C/677T at 31.1%, and 677T/677T at 61.5% ($P < 0.05$). The retinopathy occurrence of 677T/677T homozygotes was clinically significant. The presence of the 677T/677T homozygous genotype is associated with a higher likelihood of experiencing diabetic retinopathy, particularly a specific type known as non-proliferative retinopathy, in individuals with hyperglycemia. Conversely, 677T/677T homozygous failed to show any impact on susceptibility to diabetic nephropathy, even in subjects with hyperglycemia, [18].

MTHFR 677 TT genotype was found to be related to both predisposition to Type 2 DM and its complications, including diabetic retinopathy, diabetic polyneuropathy, and ischemic heart disease among the Egyptian population. In a research involving 203 T2DM patients and 311 controls, results showed that patients with MTHFR 677TT genotype have higher susceptibility to diabetes. Also indicated that this mutation confer susceptibility to diabetic retinopathy, diabetic polyneuropathy, and ischemic heart disease. The study also found the potential role of MTHFR 1298C in the incidence of diabetic retinopathy. ACE DD genotype exhibited a strong clinical correlation to diabetic polynephropathy with a p value <0.001, [19].

Another meta-analysis demonstrated a strong association between Diabetic Nephropathy (DN) in the Caucasian population with T2DM. MTHFR 677T genotype was found to be an important factor in increased risk of DN. Additionally; p-values were statistically significant for the results. In conclusion, the Caucasian population has increased susceptibility to DN in individuals with MTHFR 677T SNP, [20].

Another result showed that C677T mutation might be a risk factor for nephropathy but not for diabetes mellitus disease incidence in the Chinese population. Meta-analysis from 12 studies conducted in the Chinese population showed that C677T polymorphism appears to show a higher likelihood of DN in individuals with DM.

On the other hand, when examining the association of the same polymorphism with T2DM, no significant link was found, the p-value was 0.70, which is higher than the conventional significance threshold of 0.05, suggesting that the association between the C677T gene polymorphism and diabetes mellitus was not statistically significant, [21].

5 The C677T/MTHFR Polymorphism Association with Neurological Conditions

MTHFR gene T allele may play a crucial role as a key risk factor in the development of various neurological conditions. In a comprehensive meta-analysis, the research focused on exploring the possible link between C/T gene polymorphism, and the occurrence of hemorrhagic stroke (HS). The analysis involved data from fifteen case-control studies from various populations such as Chinese, Turkish, and Dutch (2034 individuals with

hemorrhagic stroke and 4485 control subjects). The results consistently indicated a significant correlation between the C/T genetic polymorphism and an elevated risk of HS across various genetic models, such as dominant, codominant, and recessive, [22].

Potential genetic associations between MTHFR polymorphisms and specific neurological conditions such as migraine with aura, subacute combined degeneration (SCD), and autism, particularly in certain populations. When investigating the association with migraine, studies showed no overall significant difference between migraineurs and controls. However, in the migraine with aura subgroup, the TT allele showed an elevated susceptibility compared to the CC genotype, [23]. A noteworthy difference in genotype distribution was observed between SCD patients and controls. SCD patients displayed yet again an increased incidence of the T allele. Results identified the TT and vitamin B12 deficiency as a potential risk factor for SCD, [24]. Contrary, autism and the MTHFR C677T polymorphism gene also showed a noteworthy association in the Chinese Han population, particularly in the Northern Han subgroup, [25].

6 The C677T/MTHFR Polymorphism Association with Cancer

The different types of cancers are associated with polymorphisms of MTHFR. Numerous studies have contributed valuable insights into the intricate relationship between MTHFR gene polymorphism and cancer risk, underscoring the importance of population-specific factors and cancer types in influencing outcomes. Breast cancer has high prevalence characteristics among females and based on WHO statistics is one of the major causes of female mortality. Some scientific literature shows the potential relationship between MTHFR gene C677T and also A1298C single nucleotide polymorphisms with breast carcinogenesis. The study conducted by authors aimed to explore the relationship between the MTHFR gene C677T polymorphism and breast cancer in North Indian women. Through genotyping, the researchers observed noteworthy differences in the incidences of the C/T genotype and the T allele amongst individuals with breast cancer and those without. These findings suggest a potential relation between above-mentioned polymorphisms of the MTHFR gene with an elevated genetic susceptibility to breast cancer in this specific population, [26].

In contrast, a separate population study focused on breast cancer in Mali did not reveal any statistical correlation between C/T and susceptibility to breast cancer. The genotypic analysis in the Malian population did not show any clinically significant differences in the distribution of C677T genotypes between individuals with breast cancer and those without, [27].

Individuals carrying certain variants of the C677T polymorphism may have an altered susceptibility to developing esophageal cancer compared to those with different genotypes. When comparing the T allele to the C allele and combining individuals with TT and CT genotypes versus those with the CC genotype, the study observed significant associations with esophageal cancer risk, [28].

The C677T polymorphism in the MTHFR gene is more strongly linked to leukemias and lymphomas than the A1298G polymorphism, as suggested by studies with significant findings. However, some research has not observed similar connections, [29]. There were some scientific publications about investigation the impact of gene variations (C677T and A1298C) on chronic lymphocytic leukemia (CLL) risk. Their study, involving 832 CLL patients and 886 healthy individuals, found that these gene variations did not significantly increase the chances of developing CLL. In simpler terms, having these specific genetic differences doesn't appear to significantly contribute to inheriting a risk for CLL, [30].

Another meta-analysis studied if MTHFR C677T is linked to the incidence of stomach cancer. After looking at information from 28 studies with over 5,700 cases and 8,500 controls, results showed a clear connection between this mutation and the risk of stomach cancer overall. However, when studied for different ethnic groups, the connection was strong for Asians but not for Caucasians. In simpler terms, this means the MTHFR C677T gene might slightly raise the risk of stomach cancer in Asians, but it doesn't seem to do the same in Caucasians, [31].

Similar studies demonstrating differences in cancer incidence were found for prostate cancer. Researchers looked into the connection between C677T and A1298C MTHFR genes and the risk of prostate cancer. They analyzed data from 21 studies involving over 21,000 participants. The overall findings showed no significant link between these gene variations and prostate cancer risk. However, when studied for specific groups, results showed that the C677T CT gene variation might increase prostate cancer risk in East Asians. Also, the

A1298C CC gene variation was linked to a slightly lower risk in Europeans but a higher risk in Asians. The study suggests that these gene-cancer associations may differ in various populations, and more large-scale studies are needed to confirm these findings, [32].

7 Other Possible Associations

There are numerous scientific papers that show other possible associations of different types of disorders with MTHFR C677T gene polymorphism. Among them is a potential link with venous thromboembolism (VTE). The mentioned disorder is a complex multifactorial disease, in which genetic factors, especially several types of single nucleotide mutations plays a crucial role. In the XXI century many studies have been reported about a possible link between MTHFR gene polymorphism and the possible risk of VTE, but unfortunately they give us alternative conclusions. Some of them suggested that MTHFR C/T polymorphism is related to the susceptibility to VTE, because the C/T genotype is a significant risk factor for VTE, but some of the published papers do not show any possible correlation. There are very important review papers, where 32 different studies are analyzed. There mentioned studies included patients as Asian as Caucasian population. 15 scientific papers were for the Asian population, the majority of them belonged to the Chinese population, and 17 papers were for the Caucasian (South Korea, China, Russia, Macedonia, Netherlands, America, Greece, Spain, Egypt, Canada, Australia, Tunisia, Turkey, Mexico). The publication year is from 1999 to 2019. The total sample size contained 8223 patients and 10,859 controls. The study suggested that C/T polymorphism may increase susceptibility to VTE in the Asians, but not in the Caucasians, [33].

The Mexican population has highlighted an elevated susceptibility of venous thromboembolism (VTE) and thromboembolism in individuals with C677T homozygous mutations (23%) compared to those with heterozygous C677T-A1298C mutations (16%), [34].Another research aimed to assess the prevalence of methylenetetrahydrofolate reductase variants in pulmonary hypertension patients and investigate whether homozygous or compound heterozygous variants correlate with the severity of the disease. Medical records of 105 pulmonary hypertension patients were retrospectively analyzed. The rate of the minor alleles 677C > T and 1298A > C was found to be 0.352 and 0.295, respectively. Patients with these variants exhibited a markedly elevated ratio of pulmonary to systemic vascular resistance during the initial heart catheterization. Moreover, a higher proportion of patients with homozygous or compound heterozygous variants had moderate to severe disease compared to those without. The study suggests that these variants could potentially influence disease progression or severity, [35].

The authors suggested that MTHFR polymorphisms could act as potential risk factors for adverse pregnancy outcomes. As the frequency of MTHFR gene polymorphisms increases, there are higher chances of pregnancy losses, particularly with the C677T polymorphism compared to A1298C. Noteworthy were significant variations observed in both perinatal complications and occurrences of early pregnancy losses when the study participants were categorized into different groups based on their MTHFR gene polymorphisms, [36]. In another research, when investigating the link between the MTHFR C677T genotype and various pregnancy-related factors it was observed that pregnant women with the T allele and those with the T/T genotype were linked to severe intervillous fibrin and combined with decidual thrombosis. In contrast, CC genotype women exhibited a protective effect against the above-mentioned pathologic variants. These findings suggest a potential genetic influence on specific pregnancy-related pathologies, [37].

When assessing the link between homocysteine levels, assessed through the MTHFR C677T gene variation, and the severity of COVID-19, the results showed a significant association between the presence of the MTHFR 677T allele, especially in Latinos, and higher rates of COVID-19 incidence and mortality compared to other ethnic groups. Statistical analysis suggests a strong link between the C677T gene variation and coronavirus-related deaths (there was found to be a strong relationship of 85% between the presence of the C677T gene variation and death caused by coronavirus (p value= 0.03)), [38].

The authors identified a potential link between COVID-19 and hypercoagulability in individuals who have mutations in the MTHFR gene. Among the COVID-19-infected patients, there was a noteworthy incidence of MTHFR gene mutations, affecting 30.3% of individuals. Additionally, outcomes such as patient recovery (P value = 0.025), and the severity of lung involvement, through CT (P value = 0.009). Mortality and the presence of radiological findings of thrombosis were statistically significant. The findings imply that the presence of MTHFR gene mutations may contribute to a tendency for blood clot formation in individuals

infected with COVID-19, raising concerns about the potential impact on the risk of thrombotic events in the Egyptian population, [39].

Interestingly, this association is noted even in cases where there is no elevation in homocysteine levels, a marker often associated with increased clotting risk. An interesting case series on three young females, previously healthy and aged 15-17, who experienced spontaneous thromboembolic diseases such as DVT and pulmonary emboli shortly after contracting COVID-19. Despite being in the normal range for homocysteine levels, these individuals had either homozygous or heterozygous mutations in the MTHFR gene. Suggesting an interesting yet complex interplay of genetic and environmental factors contributing to thromboembolic events following COVID-19 infection, [40].

8 Conclusion

A comprehensive review of numerous scientific studies has established a potential link between the MTHFR C677T gene and various types of somatic and infectious diseases. This connection is particularly emphasized when examining the MTHFR C677T polymorphism and its potential links to complex disorders with multiple contributing factors. These disorders include cancers, cardiovascular complications, neurological disorders, and among others. Notably, the most significant associations are observed in cardiovascular disorders, especially cases involving venous thrombosis and diabetes mellitus. The identification of an individual's homozygous or heterozygous state for this gene is crucial for effective disease treatment. Such information enhances treatment efficacy and aids in preventing further complications. The limitation of the study is that there are also some studies which do not show any significant association between the MTHFR C677T gene and disorders.

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Abbreviations

MTHFR- Methylenetetrahydrofolate reductase
SNPs- Single Nucleotide Polymorphisms
IMT- Intima-Media Thickness
OR- Odds Ratio
CAS- Carotid Atherosclerosis
T2DM- Type 2 Diabetes Mellitus
ACE- Angiotensin converting Enzyme
DN- Diabetic Nephropathy
HS- Hemorrhagic Stroke
SCD- Subacute Combined Degeneration
CLL- Chronic lymphocytic leukemia
VTE- Venous Thromboembolism
COVID-19- Coronavirus disease 2019
DVT- Deep Venous Thrombosis
pHWE- P values for Hardy–Weinberg equilibrium test

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- Aleena Parveen Shaikh – write the section 1 and 8
- Kristine Makharadze - write the section 2 and 3
- Marina Nagervadze - write the section 4 and 6
- Marina Koridze was supervising the writing process
- Rusudan Khukhunaishvili – write the section 7
- Salome Glonti – write the section 5.

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The authors have no conflicts of interest to declare that are relevant to the content of this article.

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APPENDIX

Table 1. MTHFR C677T Gene polymorphism and association with disorders

Type of disease	Number of studying participants	Ethnicity	SNP	Potential association	References
Breast cancer	19,260 BC cases and 23,364 controls	Asian and Caucasian	C677T	Strong trend toward the risk for BC in the TT and CT genotypes. Increased risk in Asian and Caucasian populations	[41]
Carotid atherosclerosis	730 participants (516 males, 214 females)	China	C677T	The risk of disease occurrence was higher for individuals with C/T heterozygotes and T/T homozygotes	[12]
Carotid stenosis	3,247 participants aged 30 to 89 years (1,693 women, 1,554 men),	Japanese population	C677T	MTHFR homozygous T/T poses a risk for carotid stenosis	[13]
Diabetic Nephropathy (DN) with T2DM	10 case-control studies 1590 individuals (DN) and 1555 T2DM without DN.	Caucasian population	C677T	The presence of the MTHFR 677 T variant was found to be a factor contributing to an increased risk of DN within this particular population.	[20]
Venous thromboembolism (VTE)	The total sample size is nearly 20,000, containing 8223 patients and 10,859 controls	Caucasian and Asian	C677T	MTHFR C677T polymorphism may increase susceptibility to VTE in the Asians, but not in the Caucasian	[33]
T2DM	190 type 2 diabetic patients	Japanese population	C677T	Incidence was higher of 677T/677T homozygous individuals with retinopathy than those with the other genotypes.	[18]
COVID-19	33 patients with COVID-19 and 13 healthy controls	Egyptian population	C677T	COVID-19 patients with MTHFR gene mutations (30.3%) show significant links to hypercoagulability, impacting recovery, lung involvement, mortality, and thrombosis.	[39]
Pulmonary hypertension	105 patients	USA	677C > T, 1298A > C	Homozygous or compound heterozygous variants are associated with a higher proportion of moderate to severe disease.	[35]
Hemorrhagic stroke (HS)	6519 individuals (2034 with hemorrhagic stroke and 4485 control subjects)	Various populations including Chinese, Turkish, and Dutch	C677T	Significant associations between MTHFR gene C677T polymorphism and an elevated risk of hemorrhagic stroke. The associations were observed across various genetic models.	[22]
Prostate cancer	Over 21,000 participants from 21 studies	Various populations, including East Asians and Europeans	C677T, A1298C	No significant link. However, subgroup analyses indicated that the C677T CT gene variation might increase prostate cancer risk in East Asians. Additionally, the A1298C CC gene variation was associated with a slightly lower risk in Europeans but a higher risk in Asians.	[32]