RELATIONSHIP OF POLYMORPHISMS OF MTR GENE rs1805087, MTRR GENE rs1801394 TOHOMOCYSTEIN CONCENTRATION ANDCOVID-19 SEVERITY IN UZBEK PATIENTS.

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Abstract.

One of the most severe complications of COVID-19 caused by SARS-CoV-2 infection is hypercoagulopathy-induced ischemic damage to vital organs, which causes many cases of disability and death in patients infected with COVID-19. Therefore, by thoroughly studying the factors that cause hypercoagulopathy, selecting patients who are prone to the development of this pathological process, and carrying out special proliferative and therapeutic procedures for them, it is possible to reduce the above-mentioned indicators of morbidity and mortality. One of the factors causing hypercoagulopathy is the polymorphisms of the MTR and MTRR genes, which are thrombophilic genes, and their study in Uzbek patients infected with COVID-19 helps to understand the correlation of different alleles of these genes with the amount of homocysteine in these studied patients, as well as their significancecan give in the pathogenesis of COVID-19.

Keywords. MTR, MTRR, rs1805087, rs1801394 , wild allele, minor allele folate cycle, hyperhomocysteinemia, endothelial dysfunction.

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Introduction. The coronavirus infection (COVID-19) is a new infectious disease with severe complications that is rapidly spreading around the world. In December 2019, an epidemic outbreak of an unknown infection was observed in Wuhan, and the development of pneumonia in a large number of patients caused an emergency in the Chinese health system [6]. The Chinese Center for Disease Control and Prevention examined a swab prepared from patients' throat swabs and confirmed that the case was caused by a new type of beta-coronavirus [5]. The new virus was called SARS-CoV-2 (severe acute respiratory syndrome coronavirus) [7]. Studies have shown that SARS-CoV-2 binds to ACE2 in the vascular endothelium and, as a result of its cytopathic effect, causes massive apoptosis and necrosis of endothelial cells, as well as endothelial dysfunction induced by the cytokine storm in the pathogenesis of COVID-19 - hypercoagulopathy, thrombophilia and thrombosis in vessels, causes an increase in blood vessel permeability, microcirculation disorders. These changes cause endothelial dysfunction and the development of local or

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systemic vasculitis [8,9]. In particular, one of the most dangerous complications of COVID-19 is venous thromboembolism (VTE). Long-term immobilization, dehydration, acute inflammatory process, comorbidities such as hypertension, diabetes, obesity, and cardiovascular disease increase the risk of VTE in patients with COVID-19 [19]. VTE occurs as deep vein thrombosis (DVT) and pulmonary artery thromboembolism (PAT), while ATE occurs as myocardial infarction, ischemic stroke, peripheral artery thrombosis, and embolism[20].

Therefore, by thoroughly studying disorders related to hypercoagulopathy, we can identify patients who are likely to develop hyperthrombosis and thereby reduce the likelihood of such severe complications by implementing specific prophylactic and therapeutic measures. One condition that may increase the likelihood of hypercoagulopathy is a folate cycle disorder.

Folic acid enters the body through the alimentary tract in the form of polyglutamate and is broken down by the enzyme glutamate carboxypeptidase II (GCPII) into monoglutamates for absorption through SLC19A1 and PCFT transporters in enterocytes. Inside the cell, folic acid is re-deconjugated for storage. Folic acids stored in the form of folate polyglutamate within the cell must be converted back to dihydrofolate (DHF) to enter

[1, 2, 3, 4] (see Figure 1).

the folate cycle. Then, dihydrofolate is reduced back to tetrahydrofolate (THF) (using NADPH with the enzyme dehydrofolate reductase), tetrahydrofolate is transformed into 5,10-methylenetetrahydrofolate (5,10-methyleneTHF) by the enzyme Serine-hydroxymethyltransferase. And finally, 5, 10-methyleneTHF is converted into the active form - 5-methyltetrahydrofolate by the enzyme MTHFR (methylenetetrahydrofolate reductase). The next steps in the metabolism of folate and homocysteine continue with the formation of methionine from 5-methyltetrahydrofolate, giving homocysteine its methyl group (-CH3). As a result, 5-methyltetrahydrofolate itself becomes tetrahydrofolate. This process is controlled by the enzyme MTR (methionine synthase), and B12 plays a role as a cofactor in this case. But after a certain time, MTR becomes inactivated as a result of oxidation of B12. In this case, the enzyme MTRR (methionine synthase reductase) returns V12 to an active state. Methionine formed from homocysteine is converted to S-adenosylmethionine (SAM) by the enzyme methionine adenosyltransferase. SAM is important in the organism as a universal methyl group donor, in the synthesis of nucleotides, epigenome production and its maintenance, synthesis of neurotransmitters, phospholipids, hormones

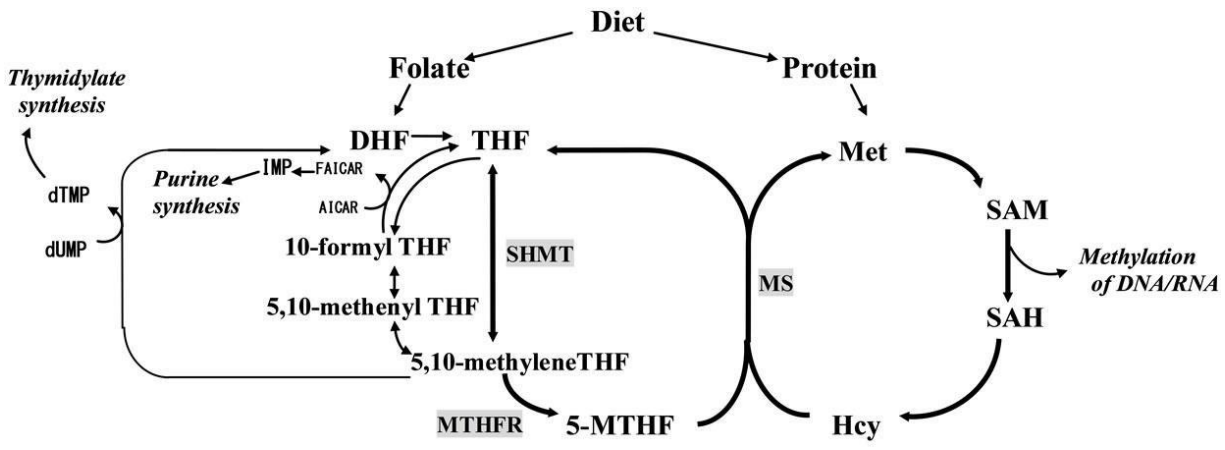

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Figure 1. The folate and methionine cycle. DHF = dihydrofolate; THF = tetrahydrofolate; 5-MTHF = 5-methyltetrahydrofolate; 5,10-methyleneTHF = 5,10-methylenetetrahydrofolate; 5,10-Methenyl THF = 5,10-Methenyltetrahydrofolate; 10-formyl THF = 10-formyltetrahydrofolate; Hcy = homocysteine; Met = methionine; SAM = s-adenosyl methionine; SAH = s-adenosyl homocysteine; dUMP = deoxyuridine monophosphate; dTMP = deoxythymidine monophosphate; AICAR = 5-amino-4-imidazole carboxamide ribonucleotide; FAICAR = 5-formamidoimidazole-4-carboxamide ribonucleotide; IMP = inosine monophosphate; SHMT = serine hydroxymethyltransferase; MTHFR = methylenetetrahydrofolate reductase; MS = methionine synthase [5] .

Deficiency of folic acid and group B vitamins, developed due to paresis or digestive disorders in the body, including a defect in folate metabolism genes, leads to an increase in homocysteine in the blood, and as a result of disruption of the cell epigenome and DNA replication, especially epithelial regeneration and hematopoiesis [15, 16] can cause a decrease in the synthesis of neurotransmitters [17], neurodegenerative disorders due to the accumulation of toxic substances in the brain, neuropsychological disorders due to disruption of the synthesis of neurotransmitters in the brain, and cardiovascular diseases [18, 25]. At the same time, as a result of the violation of the folate cycle, homocysteine remethylation is disturbed, as a result of which homocysteine can accumulate in the blood in high concentrations.

It is known that homocysteine in high concentration damages the endothelial layer of blood vessels and activates the coagulation process. Endothelium is a barrier between the vascular wall and circulating blood and produces vasoactive substances, mediators and their inhibitors. Through these biologically active substances, the endothelium is of primary importance in controlling vascular tone . Nitrous oxide is one of these substances. It is constantly produced by the endothelium and has several protective functions, including vasodilation, inhibition of smooth muscle proliferation , and reduction of platelet and other blood cell aggregation. At the same time, nitric oxide reacts with homocysteine and "neutralizes" it. However, hyperhomocysteinemia or neutralization does not occur. Similarly, homocysteine induces the formation of oxygen radicals. The mechanism for this is that hyperhomocysteine inhibits the activity of some antioxidants, particularly glutathione peroxidase, superoxide dismutase, and heme oxygenase-1. In addition, hyperhomocysteine increases the activity of NADF-oxidases, as a result of which many free oxidative radicals are generated. This leads to a decrease in the activity of endothelial lipid synthetase. As a result, the synthesis of nitric oxide, which is an important factor of vasodilatation and endothelial protection, decreases in hyperhomocysteinemia [19, 20].

Result of increased oxidant stress and endothelial dysfunction due to increased homocysteine, rostacyclin, which is considered a vasoactive substance, reduces the amount of PGI_2 and increases the amount ofthrombocyte A_2. Both are synthesized from arachidonic acid under the action of cyclooxygenase. Thromboxane enhances thrombosis formation by increasing platelet aggregation, causing strong narrowing of blood vessels. Prostacyclin is mainly synthesized in the endothelium of blood vessels, unlike thromboxane, it relaxes vascular muscles, reduces platelet aggregation, and stimulates fibrinolysis [20].

Studies have shown that homocysteine activates elastase, and as a result of elastin degradation, the endothelium becomes fragile, which leads to the deposition of calcium, cholesterol, and lipids that deform the vascular wall. At the same time, homocysteine reduces the production of endothelin-1. Endothelin-1 is a protein composed of 21 amino acids synthesized by vascular endothelium. It

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increases the proliferation of smooth muscle cells by binding to special transmembrane receptors and has a strong vasoconstrictor effect. Normally, endothelial cells resist the adhesion of blood cells to the vessel surface, have antithrombotic and fibrinolytic properties. Endothelial injury as a result of hyperhomocysteinemia increases platelet aggregation [21].

According to the literature, homocysteine disrupts the activity of tissue plasminogen activator, causes the aggregation of lipoprotein and fibrin, as a result of which fibrinolysis decreases. At the same time, homocysteine in high concentration disrupts the activity of antithrombin III and protein, which are natural anticoagulants, alters the normal antithrombotic properties of the endothelium, which activates blood clotting factors V, X and XII [21].

In addition, homocysteine not only alters endothelial cells, but also impairs their regeneration by causing hypomethylation in these cells. Similarly, as a result of folate cycle deficiency, the production of HDL (high-density lipoprotein) from hepatocytes decreases, which increases the risk of atherosclerosis [22].

Material and methods. In the scientific study, 80 patients were examined and they formed the main group. Based on the clinical symptoms of COVID-19 and the severity of the disease, the patients of the main group were divided into: Group I - 20 mild cases, Group II - 26 cases of moderate severity, and Group III - 34 cases of severe cases. When dividing the patients into groups in this order, we used the 8th revision of the manual of the Ministry of Health of the Republic of Uzbekistan entitled "TEMPORARY RECOMMENDATIONS ON THE TREATMENT OF PATIENTS WITH CORONAVIRUS INFECTION" (**https://ssv.uz/uploads/documentation/823b672dcd7e9a5f0fde55dba1cc18ab.pdf**). The purpose of dividing the groups in this order was to determine the relationship between the tested gene polymorphisms and the severity of the disease.

In a clinical study, thrombophilia genes were examined in the blood of 80 patients with coronavirus infection. In the detection of MTHFR gene A1298C (rs 1801131), C677T (rs 1801133) polymorphisms in the venous blood of patients, nucleotide sequencing was carried out using polymerase chain reaction in a DT-Lite 48 amplifier, using DNA-technology (Russia) reagents. Similarly, the amount of homocysteine in the plasma of all group patients and healthy subjects was checked (by the IFA method in the "Human" (Germany) reagent) and the correlation between their genotypes and the amount of homocysteine was checked.

Statistical processing of research results. The χ 2 criterion was used to evaluate genotypes meeting taking into account the Hardy-Weinberg equilibrium and to compare the level of distribution of genotypes and alleles. If the χ 2 criterion was used to confirm the presence of a predisposition to the studied pathology through the association of alleles and genotypes, the pathogenetic significance of alleles and genotypes in the studied disease was confirmed by the relative risk (RR) and extimolar ratio (OR) with a 95% confidence interval (95% CI:). A level of $p < 0.05$ was considered statistically significant. Statistical data processing was performed using Statistica 6.1 (StatSoft, USA).

Results. According to the results of the study conducted in the Uzbek population, the distribution frequency of alleles and genotypes of the MTR gene 2756 A>G (rs1805087) polymorphism was as shown in the table below (Table 1).

Table 1

Distribution frequency of alleles and genotypes of MTR gene in 2756 A>G (rs1805087) polymorphism

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G groups	Distribution of alleles				Distribution of genotypes					
	A		G		A / A		A / G		G / G	
	n	%	n	%	n	%	n	%	n	%
Main group, (n = 80)	136	85	24	15	59	73.75	18	22.5	3	3.75
First group, (n = 20)	39	97.5	1	2.5	19	95	1	5	0	0
Second group, (n = 26)	46	88.46	6	11.54	20	76.92	6	23.08	0	0
Third group, (n = 34)	51	75	17	25	20	58.82	11	32,35	3	8.82
Control group, (n = 20)	38	95	2	5	18	90	2	10	0	0

The results obtained on the distribution of genotypes of the MTR gene rs1805087 polymorphism were checked based on the Hardy-Weinberg law, and the results showed no significant deviation from the results obtained based on the given law (χ^2<3.84; R>0.05) (Table 2).

Table 2

Comparison of empirical - observed results with theoretical - expected results calculated by Hardy-Weinberg law of MTR gene rs1805087 polymorphism.

Main group					
Alleles	Distribution of alleles				
A	0.85				
G	0.15				
Genotypes	Distribution of genotypes		χ 2	p	df
	Observed	Expected			
A / A	0.7375	0.7225			
A/G	0.225	0.255			
G/G	0, 0 375	0.0225			
General	1	1	1.1	0.57	1

Control group					
Alleles	Distribution of alleles				
A	0.95				
G	0.05				
Genotypes	Distribution of genotypes		χ 2	p	df
	Observed	Expected			
A / A	0.90	0.905			
A / G	0.1	0.095			
G / G	0.0	0.0			
General	1	1	0.05	0.97	1

On the other hand, when the significance of the MTR gene 2756 A>G (rs1805087) polymorphism in different groups was examined, the presence of a positive relationship between the minor allele and

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the heterozygous genotype in the pathogenesis of Mild COVID-19 development was not confirmed. A, Gfrequency of alleles and A/A, A/G, G/G genotypes were as follows: 97.5%, 2.5% and 95%, 5%, 0%, respectively. When analyzing the differences in the frequency of distribution of alleles and genotypes, no reliable correlation was found between mild COVID-19 and different genotypes, in particular between heterozygous A/G and homozygous A/A genotypes - χ^2=0.4; R=0.6. (Table 3).

Table 3

Significance of different alleles and genotypes of MTR gene 2756 A>G (rs1805087) polymorphism in the development of mild COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	p	RR	95% CI	OR	95% CI
	First group		Control group							
	n	%	N	%						
A	39	97.5	38	95.0	0.3	p = 0.6	1.0	0.04 - 24.33	2.1	0.19 - 22.52
G	1	2.5	2	5.0	0.3	p = 0.6	1.0	0.19 - 4.97	0.5	0.04 - 5.34
A / A	19	95.0	18	90.0	0.4	p = 0.6	1.1	0.04 - 25.79	2.1	0.18 - 24.21
A / G	1	5.0	2	10.0	0.4	p = 0.6	0.5	0.02 - 12.22	0.5	0.04 - 5.43

Frequency of A, G allelesin moderately severe COVID-19and A/A, A/G, G/G genotypes were as follows: 88.46%, 11.54% and 76.92%, 23.08%, 0%, respectively. When analyzing the significance of the MTR gene 2756 A>G (rs1805087) polymorphism in moderate-severe COVID-19 disease, although wild-type homozygous A/A genotype was protective (OR=0.4; 95% CI: 0.07-1.98), heterozygous and genotype has an inducing effect (OR=2.7; 95% CI: 0.5-14.6), but these results were found to be statistically insignificant (χ^2=1.3; R=0.3) (4- table).

Table 4

Significance of different alleles and genotypes of the MTR gene 2756 A>G (rs1805087) polymorphism in the development of moderate-severe COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	P	RR	95% CI	OR	95% CI
	Second group		Control group							
	N	%	n	%						
A	46	88.5	38	95.0	1.2	p = 0.3	0.9	0.39 - 2.23	0.4	0.08 - 2.02
G	6	11.5	2	5.0	1.2	p = 0.3	1.1	0.1 - 11.8	2.5	0.49 - 12.43
A / A	20	76.9	18	90.0	1.3	p = 0.3	0.9	0.32 - 2.28	0.4	0.07 - 1.98
A / G	6	23.1	2	10.0	1.3	p = 0.3	2.3	0.86 - 6.16	2.7	0.5 - 14.46

On the other hand, in the development of severe form of COVID-19, A, Gfrequency of alleles and A/A, A/G, G/G genotypes was as follows: 75%, 25% and 58.82%, 32.35%, 8.82%, respectively. In patients of this group, when the importance of the alleles and genotypes of this gene polymorphism in the development of severe form of COVID-19 was analyzed , there was no statistically reliable correlation between the severe course of COVID-19 and the occurrence of the heterozygous A/G genotype: χ^2 =3.4; R=0.1; OR=4.3, 95% CI 0.92-20.12, protective significance of A/A homozygous genotype - wild-type form in the development of severe COVID-19 (OR=0.2; 95%CI: 0.04-0, 7) was found and the statistical reliability of this result was confirmed (χ 2=5.9; R=0.025) (Table 5).

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Table 5

The importance of different alleles and genotypes of MTR gene 2756 A>G (rs1805087) polymorphism in the development of severe form of COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	P	RR	95% CI	OR	95% CI
	Third group		Control group							
	N	%	n	%						
A	51	75.0	38	95.0	6.9	p=0.01	0.8	0.5 - 1.26	0.2	0.04 - 0.62
G	17	25.0	2	5.0	6.9	p=0.01	1.3	0.09 - 17.26	6.3	1.61 - 24.98
A / A	20	58.8	18	90.0	5.9	p=0.025	0.7	0.33 - 1.31	0.2	0.04 - 0.7
A / G	11	32.4	2	10.0	3.4	p = 0.1	3.2	1.61 - 6.51	4.3	0.92 - 20.12

Similarly, the study of the distribution frequency of alleles and genotypes of the second thrombophilic gene investigated by our study – MTRR gene 66 A>G (rs1801394) polymorphism showed that 2 (10%) patients in group 1 had heterozygous A/G genotype, 16 in group 2 A heterozygous A/G mutation was detected in (61.54%) patients, a heterozygous A/G mutation was detected in 9 (26.47%) patients in group 3, and a G/G homozygous wild genotype was detected in 23 (67.65%) patients (see Table 6 see).

Table 6

Distribution frequency of alleles and genotypes of MTRR gene 66 A>G (rs1801394) polymorphism

G groups	Distribution of alleles				Distribution of genotypes is average					
	A		G		A / A		A / G		G / G	
	n	%	n	%	n	%	n	%	n	%
Main group, (n = 80)	87	54.38	73	45.63	30	37.5	27	33.75	23	28.75
First group, (n = 20)	38	95	2	5	18	90	2	10	0	0
Second group, (n = 26)	36	69.23	16	30.77	10	38,46	16	61.54	0	0
Third group, (n = 34)	13	19,12	55	80,88	2	5.88	9	26,47	23	67.65
Control group, (n = 20)	36	90	4	10	17	85	2	10	1	5

MTRR A66G (rs1801394) polymorphism was tested based on the Hardy-Weinberg law. In the main group, the obtained results showed a deviation from the results calculated based on the Hardy-Weinberg law (χ^2 =8.1; R<0.05) (Table 7). The reason for the observed deviation is due to the fact that the group of patients treated with COVID-19 and having different severity levels of COVID-19 showed different results, and when the results obtained in subgroups were examined separately by the Hardy-Weinberg equation, no statistically significant deviation was observed (χ^2 <3.84; R>0.05). On the other hand, although there was a deviation in the indicators determined in the control group, it was found to be statistically insignificant (R>0.05).

Table 7

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Comparison of empirical-observed results with theoretical-expected results calculated by Hardy-Weinberg law of MTRR A66G (rs1801394) polymorphism.

Main group					
Alleles	Distribution of alleles				
A	0.544				
G	0.456				
Genotypes	Distribution of genotypes		χ 2	p	df
	Observed	Expected			
A / A	0.375	0 , 30			
A/G	0.3375	0.496			
G/G	0.2875	0.204			
General	1	1	8	0.016	1

Control group					
Alleles	Distribution of alleles				
A	0.90				
G	0.10				
Genotypes	Distribution of genotypes		χ 2	p	df
	Observed	Expected			
A / A	0.85	0.81			
A / G	0.10	0.18			
G / G	0.05	0.01			
General	1	1	3.9	0.14	1

MTRR gene 66 A>G (rs1801394) polymorphism in the development of mild form of COVID-19 was not confirmed. A, Gfrequency of alleles and A/A, A/G, G/G genotypes were as follows: 95%, 5% and 90%, 10%, 0%, respectively. The obtained results showed that it was almost no different from the following indicators in the control group: 90%, 10 and 85%, 10%, 5%. When analyzing the differences in the frequency of distribution of alleles and genotypes, a positive correlation was not revealed between mild COVID-19 and heterozygous A/G genotype: χ^2=0.6; R=0.99; OR=1.0, 95% CI 0 (Table 8).

Table 8

The importance of different alleles and genotypes of MTRR gene 66 A>G (rs1801394) polymorphism in the development of mild form of COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	P	RR	95% CI	OR	95% CI
	First group		Control group							
	n	%	N	%						
A	38	95.0	36	90.0	0.7	p = 0.4	1.1	0.11-10.11	2.1	0.38 - 11.85
G	2	5.0	4	10.0	0.7	p = 0.4	0.9	0.29 - 3.15	0.5	0.08 - 2.66
A / A	18	90.0	17	85.0	0.2	p = 0.7	1.1	0.12 - 9.52	1.6	0.24 - 10.58
A / G	2	10.0	2	10.0	0.0	p=0.99	1.0	0.13 - 7.57	1.0	0 - 0

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The frequency ofA, G allelesin moderate-severe COVID-19and A/A, A/G, G/G genotypes was as follows: 69.23%, 30.77% and 38.46%, 61.54%, 0%, respectively. However, the mutant homozygous G/G genotype was not detected in mild and moderate COVID-19. When analyzing the distribution frequency differences of MTRR gene 5p15.31 66 A>G (rs1801394) polymorphism of alleles and genotypes, it was found that there is a strong positive correlation between the occurrence of moderate COVID-19 and heterozygous A/G genotype, and the heterozygous genotype is positive in increasing the risk of moderate COVID-19. It was found that there is an effect (OR=14.4, 95% CI 3.3-62.78, χ^2 =12.6; R=0.01). On the other hand, homozygous normal A/A genotype has a statistically reliable, protective role in the development of a moderate form of COVID-19(OR=0.1, 95% CI 0.03-0.43, χ^2 =10.1; R=0 .01) (Table 9).

Table 9

Significance of different alleles and genotypes of the MTRR gene 66 A>G (rs1801394) polymorphism in the development of moderate-severe COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	P	RR	95% CI	OR	95% CI
	Second group		Control group							
	n	%	n	%						
A	36	69.2	36	90.0	5.7	p=0.025	0.8	0.41 - 1.44	0.3	0.08 - 0.78
G	16	30.8	4	10.0	5.7	p=0.025	1.3	0.22 - 7.68	4.0	1.29 - 12.44
A / A	10	38.5	17	85.0	10.1	p = 0.01	0.5	0.16 - 1.28	0.1	0.03 - 0.43
A / G	16	61.5	2	10.0	12.6	p = 0.01	6.2	2.21- 17.15	14.4	3.3 - 62.78

The frequency of A, G allelesin severe COVID-19and A/A, A/G, G/G genotypes was as follows: 19.12%, 80.88% and 5.88%, 26.47%, 67.65%, respectively. Interestingly, among all patients, the homozygous G/G mutant genotype was detected only in the group of patients with severe form of COVID-19(Table 10).

Table 10

Significance of different alleles and genotypes of the MTRR gene 66 A>G (rs1801394) polymorphism in the development of severe COVID-19.

Alleles and genotypes	Number of alleles and genotypes				χ 2	P	RR	95% CI	OR	95% CI
	Third group		Control group							
	N	%	n	%						
A	13	19.1	36	90.0	51.1	p = 0.01	0.2	0.08 - 0.53	0.0	0.01-0.07
G	55	80.9	4	10.0	51.1	p = 0.01	4.7	0.72- 30.96	38.1	14.03-103.34
A / A	2	5.9	17	85.0	34.6	p = 0.01	0.1	0.01 - 0.91	0.0 1	0 - 0.05
A / G	9	26.5	2	10.0	2.1	p = 0.2	2.6	1.27 - 5.54	3.2	0.66 - 15.85
G / G	23	67.6	1	5.0	20.0	p = 0.01	13.5	5.31 - 34.5	39.7	7.92 - 199.36

As shown in Table 10, when the differences in the distribution frequency of alleles and genotypes of the MTRR gene 66 A>G (rs1801394) polymorphism were analyzed, a strong correlation was found between the severe form of COVID-19 and the mutant homozygous G/G genotype (χ^2 =20.0; R =0.01) and it was confirmed that this genotype has an inducing significance in the development of severe form of COVID-19 (OR=39.7, 95% CI 7.92-199.36). On the other hand, the

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homozygous wild genotype - A/A was found to have a strong protective effect on the development of COVID-19 (OR=0.01, 95% CI 0.001-0.05, χ^2 =34.6; R=0.01). Similarly, heterozygous genotype A/G induction significance (OR=3.2, 95% CI 0.66-15.85) was not statistically significant (χ^2 =2.1; R=0.2) (Table 10).

Thus, during the research, we determined the homocysteine values of patients of different genotypes observed in the investigated polymorphisms of MTR and MTRR genes (Table 11). According to him, the results obtained in the first group were not statistically significantly different from the results observed in the control group (p>0.05). Similarly, there was no statistically significant difference (p>0.05) between the results of the wild-homozygous genotype patients in the second group (those infected with the moderate form of COVID-19) and the control group (p>0.05), while the results of the third group of patients were statistically different from the results of the control group. reliably prevailed 1.7 times (p<0.05). On the other hand, the homocysteine results observed in the patients of the second group with MTR gene 2756 A>G polymorphism heterozygous - A/G genotype and MTRR gene 66 A>G polymorphism heterozygous - A/G genotype were approximately 3 times higher than the results obtained in the control group tested for this polymorphism. ,2 times and 2.7 times higher (p<0.05), the indicators of the third group were about 3.43 and 2.2 times higher than the control group, respectively (p<0.05). On the other hand, when plasma homocysteine concentrations were compared in patients with different genotypes, the result of patients with heterozygous A/G genotype for the MTR gene A2756G polymorphism in the second and third groups was higher than the result of wild homozygous A/A genotype patients in these groups, 2 and 2, respectively. An excess of 01 times was detected. Similarly, in the second group, the result of heterozygous A/G genotype patients with MTRR gene A66G polymorphism was found to be approximately 2.2 times higher than that of wild homozygous A/A genotype patients, while in the third group, this indicator was confirmed to be 1.9 times higher. And finally, the concentration of homocysteine in patients with homozygous non-wild genotype for the MTR and MTRR genes in the third group was found to be 2.6 and 2.7 times higher than the amount of homocysteine in patients with homozygous wild genotype in this group , respectively (see Table 11). All reported comparisons were considered statistically significant (p<0.05).

Table 11

Results of homocysteine (µmol/l) in different genotypes of MTR gene 2756 A>G and MTRR 66 A>G polymorphisms

Groups	MTR 2756 A>G			MTRR 66 A>G		
	A/A	A/G	G/G	A/A	A/ G	G/G
First Group	5.2±0.4	-	-	5.1 ± 0.7 _	-	-
Second Group	7.9 ± 1.9	1 5 , 9 ±1.2 [ab]	-	5.8 ± 1.3	12.8±1.4 [ab]	-
Third Group	8, 7±1.14 [a]	1 7,5 ±1,1 [ab]	22.5 ± 3.2 [ab]	5,5 ±0,7	1 0 .7±1.2 [ab]	14.7± 1.31 [abc]

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Control Group	5 , 1 ±0.7	-	-	4.79 ± 1.1	5.76 ± 0.9	-

Instruction : a - statistical reliability compared to the control group - p <0.05; b - statistical reliability compared to the indicator shown by patients with wild homozygous genotype of the same polymorphism and the same group - p < 0.05, c - statistical reliability compared to the indicator shown by patients with the heterozygous genotype of the same polymorphism and the same group - p < 0.05. The presence of the "-" sign means that patients with the indicated genotype in this group were not found or were not found in sufficient numbers to draw conclusions about statistical reliability.

Discussion. MTR - methionine synthase, as mentioned above, plays an important role in the folate cycle, methionine synthesis and adequate maintenance of plasma homocysteine. The MTR gene is located in the 1q43 locus, and as a result of the rs1805087 polymorphism, the A nucleotide in the 2756 locus is changed to a G, the aspartate in the enzyme sequence changes to glycine, thereby changing the protein structure, which causes a decrease in enzyme activity [12, 13, 14]. As a result, the function of methionine synthesis decreases by transferring the methyl group from 5-methyltetrahydrofolate to homocysteine. This can lead to folate cycle disorders and hyperhomocysteinemia.

As shown in Table 11, according to the MTR gene A2756G polymorphism, patients carrying the mutant allele had significantly higher levels of homocysteine compared to patients with the wild-type homozygous genotype, reaching the level of hyperhomocysteinemia (>15 µmol/l). Our results confirmed that the MTR gene plays an important role in the folate cycle and homocysteine remethylation.

A similar result, that is, the existence of a positive association between the MTR gene A2756G polymorphism and hyperhomocysteinemia, was reported by Abdelila Laraki and colleagues [11] and Biselli J. And it was also determined through the research carried out by his colleagues.

Similarly, the enzyme methionine synthase reductase expressing the MTRR gene is important in maintaining the active state of cobalamin, a cofactor of the methionine synthase (MTR) enzyme. It is known that cobalamin is a very important intermediate that takes the methyl group from 5-methyltetrahydrofolate and transfers homocysteine from the remethylation process of homocysteine by methionine synthase. Therefore, free cob(I)alamine and methylated cob(III)alamine appear depending on their state in the cobolamine reaction. However, since cob(I)alamine has a strong tendency to oxidation, under the influence of oxidants, it can turn into an inactive state - cob(II)alamine. In this case, the activity of the MTR enzyme is also inactivated. In this case. and the methionine synthase reductase enzyme can convert inactive cob(II)alamin into active methylated cob(III)alamin by removing the methyl group from S-adenosylmethionine, and thus has an important role in maintaining homocysteine concentration in plasma [10, 11].

As shown in Table 11, although patients carrying the G allele of the MTRR gene 66 A>G polymorphism, in particular, patients with heterozygous A/G and homozygous G/G genotypes, the level of homocysteine was significantly superior to the result observed in patients with the normal wild-type homozygous genotype and controls. its amount did not reach the level of hyperhomocysteinemia (>15 µmol/l). Also, interestingly, in the subjects with the minor allele in the control group, homocysteine levels did not reliably differ from those in the wild-type homozygous group, but

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such a difference was observed in those infected with COVID-19 (Table 11). In our opinion, the reason for this is that the susceptibility to folate cycle deficiency, which occurs in individuals carrying the minor allele of the MTRR gene rs1801394 polymorphism, does not change as a result of which it is compensated under normal conditions. On the other hand, patients with COVID-19 and carrying the minor allele of the MTRR gene rs1801394 polymorphism are particularly susceptible to folate deficiency, and its relative reduction in absorption may lead to more significant and/or faster manifestations of folate cycle deficiency symptoms (e.g., increased homocysteine) in such patients. may be the cause. For what reasons can folic acid deficiency occur in the pathogenesis of COVID-19? According to the results of our meta-analysis, the reduction of vitamin D synthesis in the pathogenesis of COVID-19 leads to the disruption of PCFT transcription, which is involved in its control, and thereby causes a relative decrease in folic acid absorption [26, 27]. Or, another explanation is that poor dietary intake of patients may cause relatively low folic acid absorption [28, 29].

Also, according to the results of the investigated gene polymorphisms, the frequency of both mutant alleles of both genes in patients with moderate and severe form of COVID-19 was significantly different from that of the control group and patients with mild form of COVID-19. This is the MTR gene rs1805087 polymorphism and MTRRThe rs1801394 polymorphism indicates that patients with the minor allele are more likely to develop moderate and severe forms of COVID-19.

The reason for this, in our opinion, is due to its toxic effect on the endothelial system as a result of an increase in the amount of homocysteine, and therefore, the hyperproduction of cytokines in the pathogenesis of COVID-19 may have a cobormide effect on this process and cause a more severe inflammatory process in patients. Patients carrying the mutant allele may have a reduced activity of the enzyme methyltetrahydrofolate reductase, which may predispose them to moderate or severe disease in patients with COVID-19.

In conclusion, it can be said that polymorphisms of thrombophilia risk genes, in particular, alleles and genotypes of MTRR A66G (rs1801394) and MTR A2756G (rs 1805087) have not been confirmed to have protective-protective or inducing significance in the development of a mild level of the pathogenesis of COVID-19.

Heterozygous A/G genotype of MTRR gene A66G (rs1801394) polymorphism in moderate-severe COVID-19 was found to be reliably high, and it was found that these genotypes have an inducing effect on moderate-severe COVID-19, and the MTRR gene A66G polymorphism A/A genotypes were found to be more likely to cause moderate-severe COVID-19. It was found that there is a protective effect in the development of 19. On the other hand, no statistically significant correlation was found between the MTR 2756 A>G (rs1805087) gene polymorphism and the heterozygous genotype , but the A/A genotype was found to be statistically reliable in the development of the disease.

The heterozygous A/G genotype of MTRR gene A66G (rs1801394) polymorphism in severe COVID-19 was found to be reliably high and these genotypes have an inducing effect on moderate-severe COVID-19, and the MTRR gene A66G polymorphism A/A genotypes were found to have an inducing effect on moderate-severe COVID-19. It was found that it has a protective effect on the development of COVID-19. On the other hand, no statistically significant correlation was found between MTR 2756 gene A>G (rs1805087) polymorphism heterozygous genotype, but the A/A genotype was found to be statistically reliable in the development of the disease. Thus, the G/G genotype of the MTRR gene rs1801394 polymorphism was

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confirmed to be a statistically reliable risk factor for the development of a severe form of COVID-19.

References

1. Brandalize AP, Bandinelli E, Borba JB, Félix TM, Roisenberg I, Schüler-Faccini L. Polymorphisms in genes MTHFR, MTR and MTRR are not risk factors for cleft lip/palate in South Brazil. Braz J Med Biol Res. 2007 Jun;40(6):787-91. doi: 10.1590/s0100-879x2006005000112. PMID: 17581676.

2. Armando D'Angelo, Jacob Selhub; Homocysteine and Thrombotic Disease. Blood 1997; 90 (1): 1–11. doi: https://doi.org/10.1182/blood.V90.1.1.

3. VidmarGolja M, Šmid A, KarasKuželički N, Trontelj J, Geršak K, Mlinarič-Raščan I. Folate Insufficiency Due to MTHFR Deficiency Is Bypassed by 5-Methyltetrahydrofolate. J Clin Med. 2020 Sep 2;9(9):2836. doi: 10.3390/jcm9092836. PMID: 32887268; PMCID: PMC7564482.

4. Zheng Y, Cantley LC. Toward a better understanding of folate metabolism in health and disease. J Exp Med. 2019 Feb 4;216(2):253-266. doi: 10.1084/jem.20181965. Epub 2018 Dec 26. PMID: 30587505; PMCID: PMC6363433.

5. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan, China: the mystery and the miracle // J. Med. Virol.-2020.-№92(4).-B.401–402

6. Zhu N.etal., 'ANovelCoronavirusfromPatientswithPneumoniainChina, 2019', N. Engl. J. Med., vol. 382, no. 8, pp. 727–733, Feb. 2020, doi: 10.1056/NEJMoa2001017

7. Lu R., Zhao X., Li J. et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395(10224):565-574; doi: 10.1016/S0140-6736(20)30251-8

8. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-Mediated Inflammatory Responses: From Mechanisms to Potential Therapeutic Tools. Virol Sin. 2020;35(3):266-271. doi:10.1007/s12250-020-00207-4

9. Savla SR, Prabhavalkar KS, Bhatt LK. Cytokine storm associated coagulation complications in COVID-19 patients: Pathogenesis and Management. Expert Rev Anti Infect Ther. 2021;19(11):1397-1413. doi:10.1080/14787210.2021.1915129.

10. Ludwig ML, Matthews RG. Structure-based perspectives on B12-dependent enzymes. Annu Rev Biochem 1997; 66: 269-313.

11. Laraqui A, Allami A, Carrié A, Coiffard AS, Benkouka F, Benjouad A, Bendriss A, Kadiri N, Bennouar N, Benomar A, Guedira A, Raisonnier A, Fellati S, Srairi JE, Benomar M. Influence of methionine synthase (A2756G) and methionine synthase reductase (A66G) polymorphisms on plasma homocysteine levels and relation to risk of coronary artery disease. ActaCardiol. 2006 Feb;61(1):51-61. doi: 10.2143/AC.61.1.2005140. PMID: 16485733.

12. Chen J., Stampfer M.J., Ma J., Selhub J., Malinow M.R., Hennekens C.H.. et al. (2001) Influence of a methionine synthase (D919G) polymorphism on plasma homocysteine and folate levels and relation to risk of myocardial infarction. Atherosclerosis 154, 667–672 10.1016/S0021-9150(00)00469-X

13. Ma LM, Yang HP, Yang XW, Ruan LH. Methionine synthase A2756G polymorphism influences pediatric acute lymphoblastic leukemia risk: a meta-analysis. Biosci Rep. 2019 Jan 15;39(1):BSR20181770. doi: 10.1042/BSR20181770. PMID: 30559146; PMCID: PMC6331679.

14. Al Farra HY. Methionine synthase polymorphisms (MTR 2756 A>G and MTR 2758 C>G) frequencies and distribution in the Jordanian population and their correlation with neural tube defects in the population of the northern part of Jordan. Indian J Hum Genet. 2010 Sep;16(3):138-43. doi: 10.4103/0971-6866.73405. PMID: 21206701; PMCID: PMC3009424.

15. Hobbs C.A., Sherman S.L., Yi P., Hopkins S.E.,Torfs C.P., Hine R.J., Pogribna M., Rozen R., James S.J.Polymorphisms in Genes Involved in FolateMetabolismas Maternal Risk Factors for Down syndrome // Am. J.Hum.Genet.2000.Vol.67,№3.P.623–630.doi:10.1086/303055OhrvikVE.

16. Susan J. Duthie, Sabrina Narayanan, Gillian M. Brand, Lynn Pirie, George Grant, Impact of Folate Deficiency on DNA Stability, The Journal of Nutrition, Volume 132, Issue 8, August 2002, Pages 2444S–2449S, https://doi.org/10.1093/jn/132.8.2444S

17. Miller AL. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev. 2008 Sep;13(3):216-26. PMID: 18950248.

18. Kronenberg G, Harms C, Sobol RW, Cardozo-Pelaez F, Linhart H, Winter B, Balkaya M, Gertz K, Gay SB, Cox D, Eckart S, Ahmadi M, Juckel G, Kempermann G, Hellweg R, Sohr R, Hörtnagl H, Wilson SH, Jaenisch R, Endres M. Folate deficiency induces neurodegeneration and brain dysfunction in mice lacking uracil DNA glycosylase. J Neurosci. 2008 Jul 9;28(28):7219-30. doi: 10.1523/JNEUROSCI.0940-08.2008. PMID: 18614692; PMCID: PMC3844834.

19. Diane M. Ward, Opal S. et al. Altered sterol metabolism in budding yeast affects mitochondrial iron–sulfur (Fe-S) cluster synthesis Received for publication, January 5, 2018, and in revised form, May 11, 2018. DOI 10.1074/jbc.RA118.001781 Cox 2018 Ward et al. Published under exclusive license by The American Society for Biochemistry and Molecular Biology, Inc.

20. Shi S, Qin M, Shen B, et al. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China [published online ahead of print, 2020 Mar 25]. JAMACardiol. 2020;e200950. doi:10.1001/jamacardio.2020.0950.

21. Fetisova I.N. Polimorfizm genov folatnogosikla i bolezni cheloveka // Vestnik Ivanovskoy medisinskoyakademii.2006.T.11,№1-2.S.77–82

22. Dobroxotova Yu.E., Djobava E.M., AmintayevaL.A., Aliyeva A.N., Artizanova A.P., Bolkunova N.V. Endotelialnaya disfunksiya: gomosistein i oksid azota u beremennix grupp visokogo riska. Sovremenniye podxodi k terapii. Rol foliyevoy kisloti //Problem reproduksii. 2010.T.16,№6.S.98–103

23.Yakub M, Moti N, Parveen S, Chaudhry B, Azam I, Iqbal MP. Polymorphisms in MTHFR, MS and CBS genes and homocysteine levels in a Pakistani population. PLoS One. 2012;7(3):e33222. doi:10.1371/journal.pone.0033222. Epub 2012 Mar 21. PMID: 22470444; PMCID: PMC3310006.

24. Caccamo D, Gorgone G, Currò M, Parisi G, Di Iorio W, Menichetti C, Belcastro V, Parnetti L, Rossi A, Pisani F, Ientile R, Calabresi P. Effect of MTHFR polymorphisms on hyperhomocysteinemia in levodopa-treated Parkinsonian patients. Neuromolecular Med. 2007;9(3):249-54. doi: 10.1007/s12017-007-8006-x. PMID: 17914182.

25. Lucock M. Folic Acid: Nutritional Biochemistry, Molecular Biology, and Role in Disease Processes. Mol. Genet. Metab. 2000;71:121–138. doi: 10.1006/mgme.2000.3027.

26. Eloranta J.J., Zaïr Z.M., Hiller C., Häusler S., Stieger B., Kullak-Ublick G.A. Vitamin D3 and its nuclear receptor increase the expression and activity of the human proton-coupled folate transporter. Mol. Pharmacol. 2009;76:1062–1071. doi: 10.1124/mol.109.055392.

27. Gropper S.S., Smith J.L. Advanced Nutrition and Human Metabolism. 7th ed. Cengage Learning; Belmont, CA, USA: 2017.

28. Ikizler T.A. A Patient with CKD and Poor Nutritional Status. Clin. J. Am. Soc. Nephrol. 2013;8:2174–2182. doi: 10.2215/CJN.04630513.

29. Meisel E, Efros O, Bleier J, Beit Halevi T, Segal G, Rahav G, Leibowitz A, Grossman E. Folate Levels in Patients Hospitalized with Coronavirus Disease 2019. Nutrients. 2021 Mar 2;13(3):812. doi: 10.3390/nu13030812. PMID: 33801194; PMCID: PMC8001221.