

**PLASMA HOMOCYSTEINE LEVEL AND C677T POLYMORPHISM IN MTHFR GENE IN PATIENTS WITH ACUTE CORONARY SYNDROME****Andrey V. Grek,  
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Cardiovascular diseases (CVD) of atherosclerotic origin and accompanying complications are a major cause of mortality in the world and Ukraine, in particular. Endothelial dysfunction is the key cause of atherosclerosis and atherothrombosis. One of the causes of endothelial dysfunction is hyperhomocysteinemia that may occur on the background of MTHFR (methylenetetrahydrofolate reductase) mutation.

Thus, the goal of the study was to investigate the interrelation between homocysteine (Hc) level and MTHFR polymorphism in patients with acute coronary syndrome (ACS).

161 patients with ischemic heart disease and ACS have been examined. The control group comprised 87 healthy individuals. Homocysteine level was the highest in the patients having ACS with ST-segment elevation and complicated course, and was 1.8 times higher than Hc level in the control group. The patients with the most severe ACS course comprised 27 % of homozygotes for the major allele C and 41 % of homozygotes for the minor allele T. Comparing the distribution of MTHFR gene C677T polymorphism in patients with ACS that were stratified by plasma Hc level, we observed a statistically significant association,  $P < 0.030$  by chi-square test. We confirmed that these patients had a high T/T genotype frequency of MTHFR C677T polymorphism. The obtained data proved the association of T/T genotype of MTHFR C677T polymorphism with increased Hc level as well as ACS severity.

**Key words:** MTHFR, acute coronary syndrome, cardiovascular diseases, homocysteine

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**Introduction**

Cardiovascular diseases (CVD) of atherosclerotic origin and accompanying complications are a major cause of mortality in the world and Ukraine, in particular [1-3]. In low- and middle-income countries more than 80% of mortality causes are accounted for by CVD. According to estimates provided by WHO, CVD remain a major cause of death; by 2030 the death rate due to CVD will increase to 23.3 million [1, 4]. Since traditional risk factors for CVD do not fully explain the prevalence

and incidence of these diseases, it is urgent to search for new risk factors of cardiovascular diseases, early detection of which would help to reduce the death rate from this disease.

Among the numerous modern theories of the pathogenesis of atherosclerosis, much attention is paid to the concept, which states atherosclerotic process as the inflammation that occurs in arterial wall due to solution of continuity of arterial endothelium [5]. However, a lot of scientific studies have defined later that the initiator of atherogenesis is endothelial dysfunction (an imbalance among mediators acting oppositely) rather than endothelial lesion [6-9].

There are many factors causing endothelial dysfunction, such as high concentrations of low density lipoproteins and modified low density lipoproteins, increasing of free radicals in blood, Chlamidia pneumonia infection [10-15]. In 1969 the homocysteine theory of atherogenesis was first suggested [16]. Homocysteine is an amino acid that is biosynthesized from methionine and is a homologue of the amino acid cysteine. Methionine is obtained from the diet as a part of protein; it is metabolized to give S-adenosylhomocysteine, which is converted into Hc in the process of hydrolysis. According to the homocysteine theory, Hc is one of the main causative factors for endothelial dysfunction [17-20].

The association between the increase of plasma Hc concentration and increased risk of cardiovascular diseases was conclusively established during Framingham Heart Study (1996). European Collaborative Study found out that hyperhomocysteinemia represents an independent modified CVD risk factor. The most reliable evidence of the association between CVD and Hc has been obtained in prospective cohort design studies: Physicians Health Study, British United Provident Study, Trombo Study, British Regional Heart Study [21-24]. The findings demonstrated that Hc is an independent risk indicator for the diseases of cardiovascular system, which is even more informative, than cholesterol. Hyperhomocysteinemia (HHc) unfavorably influences the mechanisms involved in vascular tone regulation, lipid metabolism and coagulation cascade [25, 26]. It has been published that Hc stimulates platelet aggregation, disrupts the function of tissue plasminogen activator, contributes to binding of lipoproteins to fibrin as well as inhibits the function of such natural anticoagulants as antithrombin III and

protein C. In addition, Hc stimulates some coagulation factors (i.e. V, X и XII) [27, 28].

Blood Hc concentration can rise due to many causes. Mostly, Hc concentration increase occurs due to the lack of vitamins. Low intake of folic acid and B6, B12 and B1 vitamins is especially harmful. The following factors are considered to increase Hc: smoking; alcohol abuse; excessive use of coffee, meat and cholesterol-containing products; diabetes mellitus and renal failure. It is known that HHc can be caused by hereditary diseases, attributable to the deficit of enzymes involved in Hc metabolism [32]. In particular, the increase of plasma Hc concentration occurs in the setting of methylenetetrahydrofolate reductase (MTHFR) mutation, which causes disorders in vitamin B12-dependent remethylation of Hc to methionine [29].

The most well-known MTHFR gene polymorphism is the one, in which cytosine nucleotide (C) at 677 location in exon 4 is replaced with thymidine (T); this leads to substitution valine amino-acid residue for alanine residue in the location of binding with flavin adenine dinucleotide cofactor. Due to the structural change in this region, enzyme dissociation with cofactor goes too quickly to perform its catalytic function properly and adequately. In 1999 L.B. Bailey and J.F Gregory demonstrated in vitro a 30% decrease of MTHFR activity in homozygotes for T-allele and 65% decrease – in heterozygotes as compared with normal level [30]. Moreover, it has been shown that TT-genotype initiates approximately a twofold increase of Hc level as compared with CC-individuals [31].

The goal of the study is to investigate the interrelation between Hc level and MTHFR polymorphism in patients with acute coronary syndrome (ACS).

## Materials and Methods

The study was approved by the Ethics Committee of Medical Institute of Sumy State University. Prior to the study, each patient gave written informed consent to participate in the study. 161 patients with ischemic heart disease have been examined. The control group comprised 87 healthy individuals. The examined patients were undergoing treatment at Sumy Regional Cardiology Dispensary of Sumy Regional Council Municipal Institution from January to July of 2012.

ACS was diagnosed on the basis of: anamnesis and complaints, clinical examination, blood pressure measurement, over-time 12-lead ECG, laboratory tests (complete blood count and clinical urinalysis, over-time creatine phosphokinase (3 times), creatine phosphokinase-MB, lactate dehydrogenase, lipid profile). All patients were treated according to the Order № 436 of Ministry of Healthcare of Ukraine, dated 03.07.2006. The patients were divided into 4 clinical groups: Group I consisted of 28 patients having ACS with ST-segment elevation and uncomplicated course; group II comprised 24 patients having ACS with ST-segment elevation and complicated course; group III consisted of 55 patients having ACS without ST-segment elevation and uncomplicated course; group IV – 54 patients with ACS without ST-segment elevation and complicated course.

Hc level study was performed at the clinical diagnostic laboratory of FLORIS Health Center (accreditation certificate № 001415 of 16.11.2009) by means of enzyme immunoassay analyzer «IMMULITE ONE, DPA» (USA), applying «IMMULITE 1000 Homocysteine» and «IMMULITE 1000 vitamin B12» reagents according to the manufacturer's instructions. Hc level evaluation was performed under the classification of DACH.Liga: Hc level <10 µmol/L is a safe level, 10-12 µmol/L is considered a border-line level, 12-30 µmol/L is equal to a moderate HHc [32].

C677T polymorphism in exon 4 of the MTHFR gene was determined by polymerase chain reaction with restriction fragment length polymorphism assay (PCR-RFLP) at the molecular-genetic research laboratory of Medical Institute of Sumy State University.

Statistical analysis was performed using SPSS-17 program. Thus the significance of differences was estimated by means of chi-square test. P-value <0.05 was considered significant.

## Results

According to the results of Hc content study, Hc level in patients with ACS was higher than that in the control group. The highest plasma Hc concentration was observed in the second group of patients and constituted  $16.6 \pm 0.31$  mcmol/L, which is 1.3 times higher than the indicators in the first group; 1.7 times higher than in the third group; 1.3 times higher than in the fourth group and 1.8 times higher than in the control group (Table 1).

Comparing the distribution of MTHFR gene C677T polymorphism in patients with ACS from different clinical groups, we observed a statistically significant association,  $P < 0.001$ , by chi-square test (Table 2). The group II patients with the most severe ACS course comprised 27 % of homozygotes for the major allele C, 32 % of heterozygotes and 41 % of homozygotes for the minor allele T. The patients of group I constituted 47.6%, 33.3% and 19.1%; the patients of group III constituted 52.7%, 43.6% and 3.7%; the fourth group constituted 65.4%, 28.8% and 5.8% for C/C, C/T and T/T, respectively. On the other hand, 50% of ACS patients with the minor allele T had ACS with ST-segment elevation and complicated course.

Comparing the distribution of MTHFR gene C677T polymorphism in patients with ACS depending on the concentration of Hc in plasma blood, we observed the absence of a statistically significant association,  $P > 0.05$ , by chi-square test. Among the patients with moderate HHc, the distribution of C/C, C/T and T/T genotypes were: 53.3%, 25%, 21.7%. Patients with safe and boundary levels of Hc, carrying distribution of genotypes for the following polymorphism: 55.6%, 33.3% and 11.1% and 42.9%, 42.9% and 14.3% respectively. Among patients homozygous for the minor allele T 76.4 % had a moderate HHc (Table 3).

**Table 1.** Homocysteine level in patients with acute coronary syndrome

	Control. mcmol/L	Group I. n=18	Group II. n=18	Group III. n=33	Group IV. n=23
Homocysteine. mcmol/L	9.1±0.48	12.7±0.14	16.6±0.31	9.9±0.56	13.2±0.26

**Table 2.** Genotype frequency of methylenetetrahydrofolatereductase gene C677T polymorphism in subjects with acute coronary syndrome from different clinical groups

Control groups	Frequency	MTHFR genotype		
		C/C	C/T	T/T
I	N	10	7	4
	%	12.7%	13.2%	22.2%
II	N	6	7	9
	%	7.6%	13.2%	50.0%
III	N	29	24	2
	%	36.7%	45.3%	11.1%
IV	N	34	15	3
	%	43.0%	28.3%	16.7%

**Table 3.** Genotype frequency of methylenetetrahydrofolatereductase gene C677T polymorphism in subjects with acute coronary syndrome and different plasma homocysteine levels

Homocysteine level groups	Frequency	MTHFR genotype		
		C/C	C/T	T/T
Safe level	n	10	6	2
	%	20.8%	22.2%	11.8%
Borderline level	n	6	6	2
	%	12.5%	22.2%	11.8%
Moderate hyperhomocysteinemia	n	32	15	13
	%	66.7%	55.6%	76.4%

## Discussion

Hence, the highest Hc level was observed in group II patients with complicated ACS and ST elevation. Hc level in group II patients was significantly higher as compared with that in groups I ( $p < 0.001$ ), III ( $p < 0.001$ ) and IV ( $p < 0.001$ ). We also noted that Hc level was significantly higher in the patients of group I, comparing with group III ( $p < 0.05$ ), but had no significant difference with the concentration Hc patients IV group ( $p > 0.05$ ), and significantly lower in the patients of group III, compared to the fourth group ( $p < 0.01$ ) (Table 1). These data demonstrate a relationship between the level Hs and the severity of clinical course of ACS. This are consonant with the results provided by

Nygaard O et al. (1997) and Oudi ME (2010), who demonstrated that Hc level in ACS patients with three-vessel disease was higher than in ACS patients with one- or two-vessel disease; the levels were  $23.7 \pm 10.37$ ,  $16.3 \pm 5.46$  and  $13.9 \pm 4.89$   $\mu\text{mol/L}$ , respectively [33, 34]. Studies of foreign authors also demonstrated that the increase in Hc, beginning with 10  $\mu\text{mol/L}$ , is associated with the increased risk of cardiovascular disease [32, 34, 35]. The results of our study show that ACS patients, compared to the control group, had a significantly higher Hc level, which was associated with the severity of clinical course.

A considerable association was observed between MTHFR gene C677T polymorphism and ACS course severity. This is confirmed by the

maximum frequency of detected minor allele homozygotes in patients with the most severe course of ACS. These results are congruent with other researches' findings that indicate an interrelation between C677T polymorphism and arterial thrombosis; the risk of the latter was higher in homozygotes for T-allele [36-38]. The data about association of 677T allele with ischemic attacks in our study can be explained, in our opinion, by the interrelationship between MTHFR gene C677T polymorphism and plasma Hc level. So, moderate HHs was found in 65.2 % of patients. The frequency of patients with genotype T/T by S677T polymorphism of MTHFR gene in 6.5 times were higher than the frequency of patients with genotype C/C and C/T. Established in our trial connection between MTHFR gene C677T polymorphism and increased level Hs comparing with patients C/C genotype is consonant with investigation's results of another scientists. Brattström et al. (1998), Martin Y.N. et al. (2006) and Xu H. et al. (2013) proved that T/T-genotype and associated increase of Hc level are the indicators of instability of atherosclerotic plaques [39-41]. Established connection between T/T-genotype, Hc level and ACS course severity is confirmation with these results.

## Conclusions

These results indicate that Hc increase is associated with the development of severe ACS.

The frequency of T/T allele of MTHFR gene C677T polymorphism was the highest in the patients of the second group, who had the most severe ACS, as compared to other groups.

We have confirmed the association of MTHFR gene C677T polymorphism and high homocysteinemia with ACS course severity.

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