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Methylenetetrahydrofolate reductase gene polymorphisms, lipid profiles, and basic renal functional markers as risk for myocardial infarction: a case-control study and haplotype analysis

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Abstract: Myocardial infarction (MI) is a serious cardiovascular disease and the primary cause of mortality, with a complex etiopathology. Identifying the genetic basis of myocardial infarction (MI) is essential for developing personalized medical treatments. This study examined the possible association between polymorphisms in the methylenetetrahydrofolate reductase (*MTHFR*) gene and MI. In the study, 120 patients with MI and 120 age-and-sex-matched controls were genotyped for C677T and A1298C *MTHFR* polymorphisms by the allele-specific or amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). In the case of the C677T polymorphism, the T/T and C/T genotypes were associated with a significantly increased risk of MI under the dominant genetic model (odds ratio (OR)=2.060; P=0.006). Although there was no significant association between the A1298C variant and MI, this polymorphism was linked to a higher level of creatinine in MI patients (P<0.002). A similar association was observed for the C677T polymorphism (P=0.003). An A-T haplotype represented an increased risk for MI (OR=1.630; P=0.014), whereas the A-C haplotype had a protective role (R=0.517; P=0.002). These findings indicate that C677T *MTHFR* polymorphism is strongly associated with and increased risk of MI, making it a potential genetic risk factor and a possible predictor of MI.

Keywords: myocardial infarction; methylenetetrahydrofolate reductase gene polymorphisms; myocardial infarction risk factors; haplotype analysis; renal functional markers

Abbreviations: myocardial infarction (MI); methylenetetrahydrofolate reductase (MTHFR); cardiovascular disease (CVD); single nucleotide polymorphism (SNP); amplification refractory mutation system- polymerase chain reaction (ARMS-PCR); high-density lipoprotein cholesterol (HDL-c); low-density lipoprotein cholesterol (LDL-c); coronary artery disease (CAD); acute coronary syndrome (ACS)

INTRODUCTION

Myocardial infarction (MI) is the leading cause of adult mortality in developed and developing countries [1,2]. The global prevalence of MI is 3.8% in people <60 and around 9.5% in those >60 [3]. Men of all ages suffer from MI more often than women [2]. At the root of MI development is atherosclerosis, a gradual, inflammatory process characterized by the buildup of atherosclerotic plaque. At critical moments, this plaque erodes and causes a complete narrowing of the artery, leading to myocardial necrosis [4].

Considering that MI is a complex cardiovascular disorder with multifactorial and polygenic etiology, recognition of the risks is essential for a more precise understanding and management of the disease [5]. Widely studied traditional risk factors such as obesity, diabetes, hyperlipidemia, hypertension, low physical activity, smoking, etc. are associated with the occurrence of cardiovascular incidents [6-9].

The genetic background of MI remains largely unknown, but there is growing evidence that genetic predisposition could play a crucial role in cardiovascular disease (CVD) development, with the heritability of MI estimated at 50-60% [10]. Thus, numerous studies have shown that a positive family history is one of the most important risk factors for heart disease [11]. Genetic variations that influence key processes such as blood pressure regulation, coagulation, and metabolic pathways related to the pathogenesis of atherosclerosis, such as homocysteine (Hcy) metabolism, may serve as potential genetic risk factors, increasing susceptibility to MI [12-14]. As early as 1969, McCully noted that Hcy can be toxic to the vascular wall [15]. High Hcy levels can contribute to MI onset through several pathways. Hcy stimulates inflammation by promoting the transcription of factors in neural tissue and raising the concentration of cytokines [16]. Furthermore, Hcy accumulation inside the cells reduces DNA repair and promotes apoptosis [14]. Increased plasma Hcy levels are associated with the development of atherosclerosis and the deterioration of the vascular endothelium [16]. Elevated Hcy levels (greater than 15 μmol/L) may result from genetic variations affecting enzymes involved in Hcy metabolism [12,15]. The enzyme methylenetetrahydrofolate reductase (MTHFR) plays a pivotal role in this process, catalyzing the irreversible conversion of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which is the major circulating form of folic acid required for the endogenous transformation of Hcy to methionine [17]. The *MTHFR* gene, which encodes for the corresponding enzyme, is located at the terminal part of the short arm of chromosome 1; it consists of 11 exons and is 2.2 kb long [18]. *MTHFR* is a highly polymorphic gene and has at least 247 single nucleotide variants [19]. The two most common and important single nucleotide polymorphisms (SNPs) in the *MTHFR* gene are C677T (rs1801133), and A1298C (rs1801131) [20]. Both variants can potentially impair MTHFR enzyme activity, which may lead to the accumulation of Hcy and raise the risk of CVD developing [21]. The C-to-T substitution at the 677th nucleotide position, which results in a thermolabile MTHFR variant (alanine to valine), leads to a 50-70% reduction in enzyme activity and is theoretically linked to severe hyperhomocysteinemia, whereas the A-to-C change at the 1298th position (glutamate to alanine) may reduce enzyme activity by up to 17% [22,23]. Considering that conventional risk factors cannot explain the complete etiology of MI, we assumed that the *MTHFR* variants could have significant implications for the etiopathology of MI. There is a discrepancy between the results of studies and meta-analyses that examined the association of *MTHFR* polymorphisms and risk for developing MI. The two most recent meta-analyses presented opposing results; one pointed out a notable association between *MTHFR* polymorphisms, C677T and A1298C, and MI [24]. Conversely, other meta-analyses showed that neither the *MTHFR* C677T nor A1298C variants confer risk for MI [25]. In the study of Mallhi et al. [19], *MTHFR* C677T polymorphism was classified as a genetic risk factor for the development of MI in the Pakistani population. Previous studies, however, did not find a significant association of *MTHFR* C677T polymorphism with MI in a test group compared to control group [5,26].

In our study, we investigated for the first time the association between C677T and A1298C MTHFR polymorphisms and MI in the Montenegrin population, analyzing the frequencies and the relationship of various haplotypes with MI risk. Additionally, our aim was to contribute to the knowledge about the role of *MTHFR* SNPs as a MI risk in conjunction with traditional risk factors, including cholesterol levels and basic renal function markers such as urea and creatinine.

MATERIALS AND METHODS

Ethics statement

The present study was approved by the Ethical Committee of Clinical Center of Montenegro (approval number: 03/01-9928/1) and conducted according to the principles of the Helsinki Declaration. A signed informed consent was obtained from all the subjects.

Study participants

This genetic association case-control study included 240 participants divided into two groups. The case group consisted of 120 patients from the Center for Cardiology, the Clinical Center of Montenegro, from September 2021 to January 2023, who were diagnosed with MI by a cardiology specialist. The control group included 120 healthy subjects or patients who visited the cardiology or other internal medicine units and

were medically confirmed free of cardiac disorders. The exclusion criteria were ages younger than 18 and older than 80, malignant disease, pregnancy, and chronic autoimmune disease.

Demographic data and biochemical parameters

Data about the patients' sex, age, and lifestyle choices were obtained from interviews with the patients or from their medical histories. Participants who drank at least one cup of coffee daily were defined as coffee drinkers, while alcohol consumers were subjects who drank strong liquor at least twice a week. Those who smoked 5 or more cigarettes were defined as "smokers". Biochemical parameters such as total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), serum urea, and creatinine were collected from the patients' medical history.

Blood samples collecting and genotyping

Peripheral blood samples were collected in EDTA tubes from all participants. Genomic DNA was extracted manually by a commercial extraction kit (QIAamp DNA Mini kit, Qiagen, Germany) according to the manufacturer's protocol. Quantification of DNA was carried out by the fluorometer Qubit 2.00 (Thermo Fisher Scientific, USA). For the genotyping of C677T and A1298C SNPs, the ARMS-PCR method was used, and fragments of interest were further amplified using the Attomol Quicktype PCR kit (Germany) with the addition of Taq DNA polymerase (Qiagen, Germany). After amplification in a Master Cycler Gradient PCR machine (initial denaturation for15 min at 95°C, 5 cycles for 1 min at 94 °C; 1 min at 63°C; 1 min at 72°C, 30 cycles for 30 s at 94°C; 30 s at 63°C; 30 s at 72°C, final elongation for 2 min at 72°C), the amplicons were separated on a 3% agarose gel by horizontal electrophoresis. The gel was stained with SYBR safe dye. The obtained results were visualized and examined on an UV transilluminator.

Statistical analysis

Descriptive information of categorical variables was reported as frequencies and analyzed with Pearson's χ^2-test and Fisher's exact test. Continuous variables were expressed as the mean±standard deviation and analyzed with the t-test or the Mann-Whitney U-test after testing the normality of data distribution with the Shapiro-Wilks test. To assess the representativeness of the participants, the distribution of rs1801131 and rs1801133 was tested for Hardy-Weinberg equilibrium using Pearson's χ^2-test. Differences between the study groups were analyzed using one-way ANOVA or the Kruskal-Wallis test after the homogeneity of variance was tested using Levene's test, and the normality of the data was tested using the Shapiro-Wilks test. After the Kruskal-Wallis test, Dunn's multiple comparison post-hoc test was used to determine specific pairwise differences between the study groups. Statistical analyses were performed in the R ver. 4.1.2 [27].

Tests implemented in PLINK ver. 1.07 [28] were used for genetic analyses. Pearson's χ^2-test was used to test allelic associations, whereas univariate logistic regression was applied to test genotypic associations according to the genotypic (2df), additive, dominant, and recessive models. The Akaike information criterion (AIC) was used to determine the best-fitting model, with the model with the lowest AIC indicated the best fit. Haplotype analyses were performed using Pearson's χ^2-test and logistic regression, while the probability of a possible haplotype was assessed using the E-M algorithm. To correct for multiple testing bias, a permutation test with $10e^6$ iterations was performed using the max(T) permutation procedure implemented in PLINK. A multivariate logistic regression analysis was performed to analyze the contributions of specific genetic variants, clinical parameters, and daily life activities to MI. The goodness of fit of the multivariate logistic regression was tested using the Hosmer-Lemeshow test. The odds ratio (OR) with a 95% confidence interval (CI) was used as a measure of the strength of an association. The significance level (P) was set at 0.05. The study power was calculated post-hoc after the best-fitting genetic model for the examined variants was determined. To calculate the power of the analysis in which the associations of C677T with MI was observed, factors such as sample size (N=240), the observed odds ratio (OR=2.060), and the minor allele frequency in the whole analyzed group (MI cases and controls) calculated by PLINK software (MAF=0.3396) were considered in addition to the best-fitting genetic model (dominant). The study power was calculated with the genpwr.calc function,

which is integrated into the genpwr package for power calculations in genetic association studies using R software ver. 4.1.2 [29,27].

RESULTS

Demographic characteristics of the study population

We analyzed 240 participants including 120 MI patients (34 women and 86 men) with a mean age of 65.45±7.46 years, and 120 control subjects (36 women and 84 men) with a mean age of 64.53±8.40. There were no significant differences in age and sex between MI patients and control subjects, nor in physical activity, alcohol consumption, or coffee drinking (all P>0.05). The proportion of smokers in MI patients was higher than in control subjects, with a statistical significance (P=0.037) The demographic characteristics, daily activities, and lifestyle habits of the studied populations are shown in Table 1.

Table 1. Demographic characteristics and daily activities of the study population

	Patients	Controls	P
Age (mean±SD)	65.45±7.46	64.53±8.40	0.282
Sex (n, %)			
Male (n, %)	86 (71.67)	84 (70.00)	0.887
Female (n, %)	34 (28.33)	36 (30.00)	
Physical activity (n, %)			
High (n, %)	54 (45.00)	61 (50.83)	0.438
Low (n, %)	66 (55.00)	59 (49.17)	
Cigarette consumers (n, %)	76 (63.33)	59 (49.17)	**0.037**
Alcohol consumers (n, %)	55 (45.83)	50 (41.67)	0.603
Coffee drinkers (n, %)	94 (78.33)	93 (77.50)	1

Table 2. Biochemical characteristics of the study population

	Patients	Controls	P
Total cholesterol (mmol/L)	5.22±1.05	4.96±0.91	0.070
Triglycerides (mmol/L)	1.90±1.19	1.67±0.74	0.280
HDL-c (mmol/L)	1.19±0.33	1.26±0.46	0.313
LDL-c (mmol/L)	2.87±1.06	2.69±0.97	0.204
Urea (mmol/L)	7.08±3.22	6.80±2.63	0.521
Creatinine (mmol/L)	101.57±49.90	83.55±32.53	**0.001**

HDL-c – high-density lipoprotein cholesterol, LDL-c – low-density lipoprotein cholesterol

Biochemical parameters of the populations studied

Analysis of the selected biochemical parameters, lipid status, and basic renal function markers urea and creatinine revealed that MI patients had significantly higher creatinine levels compared to control subjects (P=0.001). No significant differences were observed in the proportions of the other biochemical characteristics examined (all P>0.05). Biochemical parameter values are shown in Table 2.

MTHFR A1298C and C677T genotypes and allele distributions and association with MI

Patients and controls were in Hardy-Weinberg equilibrium for both A1298C and C677T *MTHFR* polymorphisms (P>0.05). Allele T of C677T was associated with an increased risk of MI, with an OR of 1.596 (P=0.015, 95% CI=1.090-2.336), and P=0.018 after $10e^6$ permutation correction (Table 3). Genotype analysis confirmed C677T association with MI according to the best-fitting dominant model of inheritance (P=0.006, OR=2.060, 95% CI=1.224-3.465, and P=0.007 after $10e^6$ permutation correction (Table 3). The estimated power of our study to detect an association of C677T in the dominant model was 90.40%. For A1298C, there was neither an allelic (P=0.766, OR=1.061, 95% CI=0.719-1.564) nor genotypic association of A1298C with MI. The results for all tested models are presented in Table 3, including exact P values, ORs, and CIs. Detailed information about allelic and genotype frequencies for both *MTHFR* polymorphisms is given in Supplementary Table S1.

Association of biochemical parameters with *MTHFR* A1298C and C677T SNPs in MI patients and controls

The data on biochemical parameters relating to the A1298C genotypes are shown in Table 4 and Supplementary Table S2. We observed differences between the study groups regarding A1298C genotypes in creatinine levels (P=0.002) (Table 4). The mean creatinine level in the A1298C A/C and A/A genotypes was significantly higher in the MI group compared to the control group (P=0.024, P=0.019, respectively) (Supplementary Table S2). We found no differences between the study groups regarding A1298C genotypes

Table 3. Allelic and genotype allele distribution for *MTHFR* A1298C and C677T in MI patients and controls and their association with the disease

	SNPs (alleles, gene)	
	A1298C	**C677T**
	(C/A, *MTHFR*)	**(T/C, *MTHFR*)**
Minor allele frequency		
Patients	0.312	0.392
Controls	0.300	0.287
Allelic association		
$\chi 2$	0.088	5.806
P[a]	0.766	0.015
OR (95% CI)	1.061 (0.719-1.564)	1.596 (1.090-2.336)
P[b]		**0.018**
Genotype frequencies	C/C,A/C,A/A	T/T,C/T,C/C
Patients	0.010/0.425/0.475	0.125/0.533/0.342
Controls	0.083/0.433/0.483	0.092/0.392/0.516
Genetic models		
General (2df)	C/C vs. A/C vs. A/A	T/T vs. C/T vs. C/C
t	0.1997	7.414
P[c]	0.905	0.024
P[b]		0.096
Additive (1df)	C/C > A/C > A/A	T/T > C/T > C/C
t	0.298	2,425
P[c]	0.765	0.015
OR (95% CI)	1.061 (0.718-1.568)	1.630 (1.098-2.420)
P[b]		**0.014**
AIC		**330.7**
Dominant (1df)	C/C + A/C *vs.* A/A	T/T + C/T *vs.* C/C
t	0.129	2,723
P[c]	0.897	0.006
OR (95% CI)	1.034 (0.623-1.716)	2.060 (1.224-3.465)
P[b]		**0.007**
AIC		**329.2**
GSP[d]		**90.40**
Recessive (1df)	C/C *vs.* A/C + A/A	T/T *vs.* C/T + C/C
t	0.447	0.828
P[c]	0.655	0.408
OR (95% CI)	1.222 (0.507-2.947)	1.416 (0.622-3.223)

Minor allele is underlined; χ^2, test statistics; [a]Pearson's χ^2 test; [b]$10e^6$ permutation test; df, degrees of freedom; t, test statistic; [c]Logistic regression analyses; OR, odds ratio; CI, confidence interval; [d]Genetic Statistical Power (post-hoc)

in serum levels of total cholesterol, triglycerides, HDL-c, LDL-c, and urea (all P>0.05) (Table 4).

The data on the biochemical parameters according to the C677T genotypes are shown in Table 5 and Supplementary S3. Differences in creatinine levels were observed between the study groups based on the C677T genotype (P=0.003). The mean creatinine level was found to be higher in the MI group with the C/T genotype compared to the control groups with the C/C, C/T and T/T genotypes (P=0.048, P=0.027, P=0.029, respectively) (Supplementary Table S3). No statistically significant differences were found between the C677T genotype groups in terms of total cholesterol, triglycerides, HDL-c, LDL-c, or urea levels (all P>0.05) (Table 5).

Genetic factors, clinical parameters, and daily activities associated with MI

Multivariable logistic regression analysis was performed to examine the association between *MTHFR* C677T genotypes, creatinine levels, cigarette consumption, and MI. The results are summarized in Table 6. The analysis showed that the C677T genotypes C/T and T/T were positively associated with MI and were independent predictors for MI (OR=3.032, 95% CI=1.490-6.171, P=0.002, and OR=4.879, 95% CI=1.588-14.996, P=0.006, respectively). Creatinine levels were associated with MI (OR=1.013, 95% CI=1.004-1.022, P=0.002). In addition, cigarette consumption was found to be associated with MI, suggesting that cigarette non-consumption had a protective effect on MI (OR=0.539, 95% CI=0.305-0.951, P=0.033). Overall, the model demonstrated a good fit to the

Table 4. Biochemical parameters for MI patients and controls according to the *MTHFR* A1298C (rs 1801131) genotype

Parameters	***MTHFR* A1298C (rs1801131) genotype N**						
	Patients			**Controls**			**P**
	C/C (12)	**A/C (51)**	**A/A (57)**	**C/C (10)**	**A/C (52)**	**A/A (58)**	
Total cholesterol (means ±SD)	5.43±1.098	5.21±1.059	5.19±1.051	5.05±0.761	4.99±0.982	4.91±0.874	0.377[a]
Triglycerides (means ±SD)	1.47±0.346	2.12±1.560	1.79±0.850	1.67±0.729	1.60±0.651	1.74±0.820	0.738[b]
HDL-c (means ±SD)	1.11±0.240	1.19±0.305	1.21±0.366	1.20±0.520	1.27±0.462	1.26±0.460	0.936[b]
LDL-c (means ±SD)	3.13±1.280	2.88±1.010	2.82±1.080	3.02±0.876	2.76±1.020	2.58±0.930	0.557[b]
Urea (means ±SD	8.99±6.340	6.80±2.800	6.94±2.520	7.63±3.800	6.94±2.520	6.53±2.510	0.595[b]
Creatinine (means ±SD)	91.9±21.60	99.3±49.40	106.0±54.60	104.0±46.20	82.7±36.70	80.7±24.10	**0.002[b]**

HDL-c – high-density lipoprotein cholesterol, LDL-c low-density lipoprotein cholesterol; [a]One-way ANOVA test; [b]Kruskal-Wallis test

Table 5. Biochemical parameters for MI patients and controls according to the *MTHFR* C677T (rs 1801133) genotype

Parameters	*MTHFR* C677T (rs1801133) genotype N						
	Patients			Controls			P
	T/T (15)	C/T (64)	C/C (44)	T/T (11)	C/T (47)	C/C (62)	
Total cholesterol (means ±SD)	5.28±1.130	5.20±0.970	5.23±1.170	5.31±0.622	4.82±0.908	5.00±0.942	0-239[a]
Triglycerides (means ±SD)	1.54±0.665	1.95±1.170	1.94±1.360	1.79±0.585	1.77±0.896	1.57±0.623	0.442[b]
HDL-c (means ±SD)	1.28±0.311	1.21±0.353	1.14±0.329	1.26±0.577	1.27±0.451	1.25±0.456	0.824[b]
LDL-c (means ±SD)	2.82±0.850	2.83±1.020	2.96±1.210	2.64±0.892	2.60±1.000	2.77±0.957	0.940[b]
Urea (means ±SD	5.96±1.970	7.07±2.840	7.52±4.020	6.89±2.090	6.71±2.640	6.86±2.750	0.767[b]
Creatinine (means ±SD)	89.9±24.20	107.0±54.20	97.8±49.70	72.8±13.50	83.5±35.10	85.5±32.90	**0.003[b]**

HDL-c – high-density lipoprotein cholesterol, LDL-c low-density lipoprotein cholesterol; [a]One-way ANOVA test; [b]Kruskal-Wallis test

Table 6. Genetic factors, clinical parameters, and daily activities associated with MI

	Multivariant Logistic (N=240)		
	B	OR (95% CI)	P
Intercept	-4.428	0.012 (0.002-0.065)	**<0.001**
C677T C/T	1.109	3.032 (1.490-6.171)	**0.002**
C677T T/T	1.585	4.879 (1.588-14.996)	**0.006**
Level of creatinine	0.013	1.013 (1.004-1.022)	**0.002**
Cigarette non-consumers	-0.618	0.539 (0.305-0.951)	**0.033**

OR – odds ratio; CI – confidence interval; Multivariate logistic regression

data, as indicated by the Hosmer-Lemeshow goodness-of-fit test (P=0.437) and a Nagelkerke R-squared value of 0.178, suggesting that 17.8% of the variance in MI could be explained by the predictors included in the model (Table 6).

Associations of *MTHFR* A1298C-C677T haplotypes among the MI patients and control groups

MTHFR A1298C and C677T were in linkage disequilibrium (LD) (D'=1.0, R^2=0.3, estimated using PLINK, ver. 1.07). Three common A1298C-C677T haplotypes with frequencies ≥5% were identified (A-T, C-C, and A-C). Their frequencies in the MI patients' group and the control group are shown in Table 7. Pearson's χ^2 test and logistic regression showed a statistically significant difference in the frequency distribution of haplotypes between patients and controls (global score, P=0.014 and P=0.007, respectively; $P_{corrected}$=0.005). Among individual haplotypes, the *MTHFR* A1298C and C677T A-T haplotype was associated with a higher risk of MI (OR=1.630, $P_{corrected}$=0.014), whereas individuals with the A1298C-C677T haplotype A-C had a lower risk of MI (OR= 0.517, $P_{corrected}$=0.002).

DISCUSSION

There are no recent studies on the association of *MTHFR* polymorphisms with MI in Caucasians. In the present study, we gained insight into the association of *MTHFR* gene variations with MI risk in the Montenegrin population, especially in the context of some traditional risk factors as well as parameters of lipid status and renal functional markers. Most of the MI patients were males (71.67%), and both MI subjects and controls were matched for sex and age. The analysis of lifestyle characteristics showed that smoking was a statistically significant risk factor in the group with MI compared to controls. Previous research emphasized the existence of a clear relationship between active

Table 7. Haplotype frequencies of *MTHFR* haploblock A1298C-C677T in MI patients and controls, and their association with MI

Haplotypes	Patients	Controls	χ2	Df	P[a]	t	P[b]	OR	P[c]
Global score	/	/	8.507	2	0.014	9.940	0.007	/	**0.005**
A-T	0.392	0.287	5.806	1	0.016	5.880	0.015	1.630	**0.014**
C-C	0.312	0.300	0.088	1	0.766	0.089	0.765	1.060	0.823
A-C	0.296	0.412	7.141	1	0.007	8.690	0.003	0.517	**0.002**

Among individual haplotypes, A-T increased the risk of MI and A-C decreased risk of MI. Significant P values are shown in bold.
[a]Pearson's χ2-test; [b]Logistic regression analyses; [c]10e6 permutation test

and passive smoking and the incidence of MI in both sexes. In addition to active smoking as a known risk factor for MI, the Tromsø Study showed that exposure to passive smoking for over 20 years significantly raises the risk of MI in women by 40% [30]. We also observed statistically significant elevated values of creatinine in the group with MI compared to the controls. Serum creatinine levels could represent a potential biomarker for the risk of developing CVD [31], as even slight changes in serum creatinine levels seem to lead to an increased likelihood of developing coronary artery disease (CAD) and MI [32]. Patients with CAD, a condition that precedes MI, exhibit elevated serum creatinine levels in males [33]. Consequently, individuals with elevated creatinine levels may be at a higher risk of developing MI.

Genotypic testing for the *MTHFR* polymorphisms C677T and A1298C showed that there is a significant relationship between the C677T SNP and MI. The dominant genetic model demonstrated a two-fold higher risk for developing disease. Our findings are consistent with a very recent study that indicated a strong association between the C/T and T/T genotypes and the risk of developing stroke in the Egyptian population [34]. Additionally, data obtained in previous research showed that the *MTHFR* T/T genotype was more frequent in patients with MI and was a genetic risk factor for MI in the Japanese male population [35]. A study examining the Eastern Turkish population pointed out that the frequency of the T/T genotype is significantly higher in patients with MI than in controls [36].

After examining the Chinese population, Hou et al. [5] noted that there were no links between *MTHFR* C677T polymorphism and MI. The discrepancy between the results may be attributed to potential differences in genetic background and the lower frequency of the examined polymorphism. In addition to the association between *MTHFR* C677T and MI based on the best-fitting dominant genetic model, our study also highlights the link between the C677T polymorphism and slightly elevated creatinine levels in MI patients compared to controls. Serum creatinine has often been overlooked as a marker for CVD, despite the fact that mildly elevated serum creatinine levels were suggested as an indicator of increased CVD mortality risk even 20 years ago [37]. An earlier study conducted on the Montenegrin population with type 2 diabetes mellitus emphasized the importance of elevated creatinine levels as an independent predictor for CVD [38]. Therefore, carriers of the *MTHFR* C677T T allele, with even slightly elevated creatinine levels, may be at greater risk of developing MI.

Given that the A1298C *MTHFR* polymorphism is located in the regulatory part of the enzyme, as opposed to C677T, which affects the catalytic domain of the MTHFR enzyme, the impact of reduced enzyme function in the A1298C is less pronounced [39]. There were inconsistent results on the effect of the A1298C SNP on increasing Hcy levels and its role as a risk factor for MI. Therefore, some studies have indicated that the A1298C MTHFR polymorphism is associated with elevated Hcy levels [40] and an increased risk of fatal myocardial infarction MI in women, with no similar association found in men. [41]. Our respondent group consisted mostly of men. Nasiri et al. [42] and Fuadi et al. [43] did not find an association between A1298C and the frequency of acute coronary syndrome (ACS). The results of our study did not show a positive association between the A1298C *MTHFR* polymorphism and an increased risk of MI. We observed a significant association between the A/A and A/C genotypes of the *MTHFR* A1298C polymorphism and higher creatinine levels in MI patients compared to controls. However, to better understand the potential role of the MTHFR A1298C polymorphism as a risk factor for MI, additional research is needed.

Haplotype analysis can be of great importance in assessing the synergistic effect of several genetic markers for disease occurrence. Our results show that the A-T haplotype is more frequent in MI patients than in the control group, representing a 1.63-fold greater risk of MI, reinforcing the association we observed between the *MTHFR* C677T genotype (C/T, T/T) and MI. Conversely, we found that the A-C haplotype might have a protective role as patients with this haplotype exhibit a lower risk of developing MI. This suggests that the presence of the allele T in this haplotype is significant enough to contributed to an increased risk of MI. Al-Mahroos et al. [44] emphasized a higher frequency of the A-C haplotype block in the control group compared to patients with CVD and thrombosis, identifying this haplotype as a protective factor against the risk of ischemic heart disease. Our study

is the first to highlight the importance of the A-T haplotype as a potential risk factor for CVD and MI among Caucasian Montenegrins. There are, however, limitations to our study. The sample size was not large enough and analyzing larger cohorts would be necessary to enhance the data's statistical. Additionally, Hcy levels were not tested.

CONCLUSIONS

The present study indicated a positive association between the C677T *MTHFR* polymorphism and MI in the Montenegrin population. Elevated creatinine levels in combination with *MTHFR* C677T and A1298C variants may increase the risk of MI. Our results highlight the importance of impaired folate metabolism and the role of *MTHFR* polymorphisms in MI onset. However, additional studies need to translate these findings into clinical practice, aiming for a more personalized approach to MI treatment and the reduction of mortality and morbidity.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: A dataset is provided after the data were anonymized and de-identified to protect participants' privacy. The data underlying the reported findings have been provided as a raw dataset available here:
https://www.serbiosoc.org.rs/NewUploads/Uploads/Perovic%20et%20al.%20Raw%20Dataset.pdf

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SUPPLEMENTARY MATERAL

Supplementary Table S1. Allelic and genotype frequency of *MTHFR* A1298C and C677T SNPs in both MI patients and control study cohorts

Groups	A1298C SNP						C677T SNP					
	Allele		Genotype			P	Allele		Genotype			P
	C	A	C/C	A/C	A/A		T	C	T/T	C/T	C/C	
Patients	75	165	12	51	57	0.904	94	146	15	64	41	**0.023**
Control	72	168	10	52	58		69	171	11	47	62	

The P value was calculated using Pearson's χ^2 test to compare the genotype distribution in two study groups

Supplementary Table S2. Post hoc comparisons of the level of creatinine between *MTHFR* A1298C genotypes determined by the Kruskal-Wallis test

Comparison	Z[a]	P[a]
Patients – Controls (genotype, level of creatinine (means ±SD)		
A/A (106.00±54.60) – A/A (80.7±24.10)	2.891	**0.019**
A/C (99.3±49.40) – A/A (80.7±24.10)	2.535	**0.042**
C/C (91.9±21.60) – A/A (80.7±24.10)	1.734	0.178
A/A (106.00±54.60) – A/C (82.7±36.70)	3.302	**0.014**
A/C (99.3±49.40) – A/C (82.7±36.70)	2.946	**0.024**
C/C (91.9±21.60) – A/C (82.7±36.70)	2.010	0.133
A/A (106.00±54.60) – C/C (104.0±46.20)	0.046	1.000
A/C (99.3±49.40) – C/C (104.0±46.20)	-0.106	1.000
C/C (91.9±21.60) – C/C (104.0±46.20)	0.062	1.000

[a]Dunn's multiple comparison test (post hoc)

Supplementary Table S3. Post hoc comparisons of level of creatinine between *MTHFR* C677T genotypes determined by the Kruskal-Wallis test

Comparison	Z[a]	P[a]
Patients – Controls, (genotype, level of creatinine (means±SD)		
C/C (97.8±49.7) – C/C (85.5±32.9)	2.014	0.110
C/T (107.0±54.2) – C/C (85.5±32.9)	2.949	**0.048**
T/T (89.9±24.2) – C/C (85.5±32.9)	1.337	0.302
C/C (97.8±49.7) – C/T (83.5±35.1)	2.060	0.118
C/T (107.0±54.2) – C/T (83.5±35.1)	2.917	**0.027**
T/T (89.9±24.2) – C/T (83.5±35.1)	1.414	0.295
C/C (97.8±49.7) – T/T (72.8±13.5)	2.303	0.080
C/T (107.0±54.2) – T/T (72.8±13.5)	2.764	**0.029**
T/T (89.9±24.2) – T/T (72.8±13.5)	1.918	0.118

[a]Dunn's multiple comparison test (post-hoc)