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Original Research Article

Distribution of the methylenetetrahydrofolate reductase A1298C polymorphism among patients undergoing endovascular treatment for lower-extremity arterial disease

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ABSTRACT

Background: The methylenetetrahydrofolate reductase (MTHFR) A1298C polymorphism reduces enzyme activity, leading to impaired homocysteine (Hcy) metabolism and moderate hyperhomocysteinemia. Elevated Hcy promotes endothelial dysfunction and accelerates atherosclerosis, increasing the risk of cardiovascular disease. Peripheral arterial disease may progress to critical limb ischemia, often requiring endovascular revascularization such as angioplasty with stenting.
Methods: This retrospective study consisted of 69 patients divided into 58 males and 11 females. The inclusion criteria for this study consist of patients diagnosed with chronic arterial insufficiency according to the Fontaine classification, patients with informed consent, patients who underwent lower-extremity revascularization, patients with surgical indication of atherosclerotic lesions and the exclusion criteria included patients without arterial chronic sufficiency, patients without obtained informed consent, patients with more than one intervention.
Results: Out 69 patients, 16 patients were found with hemodynamically significant atherosclerotic lesions in the aortofermoral segment, 37 in the femorotibial segment and 16 in both segments. The distribution of MTHFR A1298C polymorphisms with AA genotype was 37 patients (53.6%) with AC genotype was 21 patients (30.4%) and with CC genotype was 11 patients (16.0%).
Conclusions: This study revealed the most predominant revascularization procedure was angioplasty combined with stenting, accounting for 55.1% of all interventions. The most prevalent allele of the MTHFR A1298C genetic polymorphism was normal AA genotype in patients with lower-extremity arterial disease who underwent isolated endovascular or hybrid interventions on the main arteries were detected in 37 (53.6%) patients.

Keywords: Hyperhomocysteinemia, Angioplasty, Stenting, MTHFR A1298 polymorphism, Genotype

INTRODUCTION

Lower-extremity arterial disease (LEAD) is a chronic systemic vascular condition resulted by atherosclerosis and leads to lower limb ischemia by narrowing of arteries in the leg, often asymptomatic in early stages of this disease. This condition affects over 200 million people globally. The prevalence of LEAD is over 40 adults is 9.7%, and it is significantly influenced by risk factors such as smoking, diabetes, hypertension, physical inactivity and high cholesterol levels.[1,2] Thus, treatment includes aggressive risk factors control and lifestyle modifications, additionally with endovascular therapies consisting of balloon angioplasty, stenting and drug eluded balloons in order to establish revascularization.[3]

The MTHFR gene is responsible for producing MTHFR enzyme, which is necessary for processing folate (B_9) and methylating Hcy into methionine. The role of MTFHR gene is significant in B_9 cycle in regulation of B_9 and methionine metabolic pathways, which is crucial for DNA methylation and stability shown in Figure 1. Therefore, A1298C polymorphism declines activity of MTHFR enzyme, resulting in elevated Hcy and decreased 5-MTHF, thereby leading to inclined levels of blood lead levels resulting in elevated oxidative stress, especially in homozygous carriers (A1298CC).[4,5] MTHFR gene polymorphism is relatively frequent genetic variation in MTHFR gene, resulting from substitution of adenine to cytosine at nucleotide 1298, which replaces glutamic acid to alanine.[6] Clinical significance of this mutation is associated with thrombosis and declined cardiovascular health resulting from high Hcy levels.[7] Furthermore, this polymorphism is often investigated as risk factor for frequent miscarriage, schizophrenia and elevated susceptibility of rheumatoid arthritis.[8]

In addition, studies show the combined effects of C677T and A1298C, compound heterozygosity by 677CT/1298AC significantly reduced the MTHFR activity by 50-60%.[9,10] However, MTHFR polymorphism studies in LEAD patient revealed an important knowledge gap, which was a result of overshadowing from the C667T variant. Even though the A1298C declines the enzyme activity, research is currently inconclusive for the independent vascular risk suggesting smaller and insignificant role of A1298C in comparison to C667T variant.[11] Studies also fail to isolate the clinical effect of A1298C alone, since most explored the combined effects of C667T/A1298C. Following flowchart depicts progression of MTFHR A1298C polymorphism to LEAD shown in Figure 2.

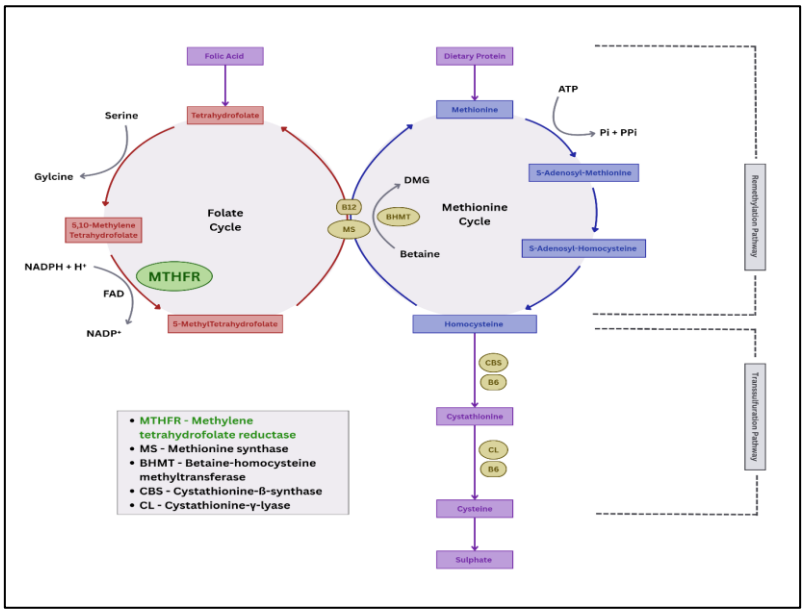

Figure 1: Metabolic pathways of folate cycle and methionine influenced by MTHFR.

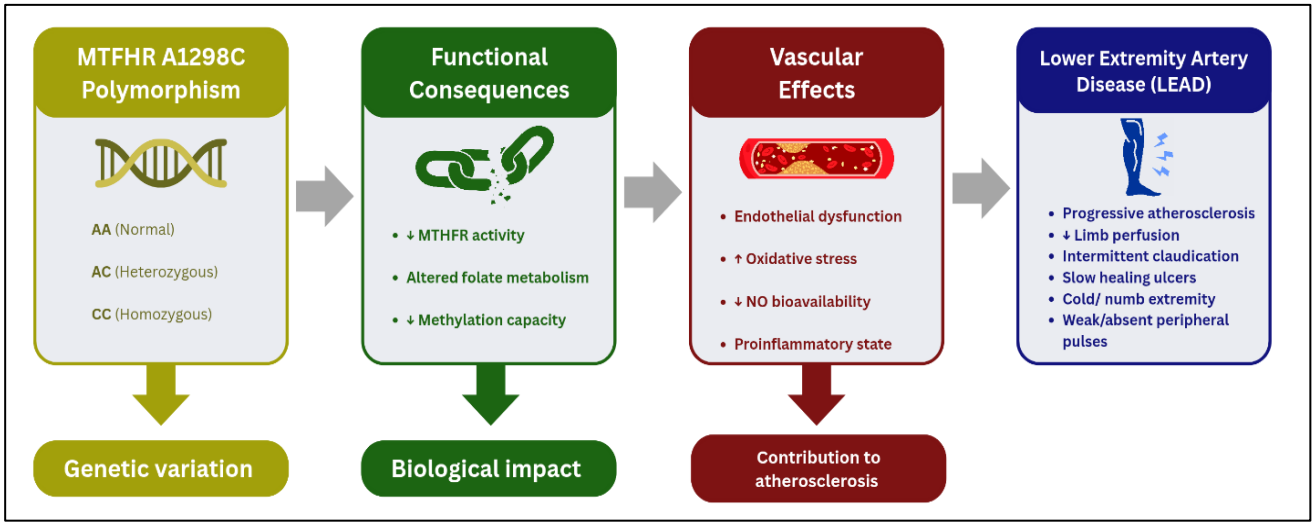

Figure 2: Progression of MTHFR A1298C polymorphism to LEAD.

Furthermore, understanding of genetic factors in LEAD is significant for early detection, risk assessment of lower limb ischemia and develop individualized therapies to improve clinical outcomes and reduce risk of amputation.

Thus, this study aims to elaborate the distribution and effect of A1298C polymorphism in LEAD patients undergoing endovascular interventions.

METHODS

This retrospective study was conducted in the Surgical department of Grodno University Clinical Hospital during the period of June 2021 to December 2022, which consisted of 69 patients divided into 58 males and 11 females. The inclusion criteria for this study consist of patients diagnosed with chronic arterial insufficiency according to the Fontaine classification, patients with informed consent, patients who underwent lower-extremity revascularization, patients with surgical indication of atherosclerotic lesions and the exclusion criteria included patients without arterial chronic sufficiency, patients without obtained informed consent, patients with more than one intervention.

The patients were divided into 3 groups according to the genotypes such as AA, AC and AC. Blood samples were collected from all participants following a strict compliance to ensure sample integrity. Genomic DNA was extracted using validated commercial kits optimized for high purity and yield, suitable for downstream genotyping applications.

The MTHFRA1298C polymorphism was genotyped with the polymerase chain reaction–based methods specific to this variant, such as PCR-RFLP (restriction fragment length polymorphism) or PCR specific to alleles, modified to the lab conditions in Belarus. These techniques were implemented for their reliability and reproducibility in distinguishing single nucleotide polymorphisms within the MTHFR gene.

The quality control measures consisted of duplicate genotyping subset of samples, inclusion of positive and negative controls in each assay batch, and verification of genotype distributions according to Hardy-Weinberg equilibrium. These procedures enabled the accuracy and consistency of the genetic data received in this study conducted in Belarus.

The statical modelling was conducted using the STATISTICA 12.0 software package with a primary check for normal distribution using a distribution histogram. The quantitative data, the distribution of which was not normal, were given as a median, 25% and 75% quartiles. Since most of the quantitative features did not tally with the normal distribution law, non-parametric methods were utilized for comparison. The Mann-Whitney test was used to assess differences in quantitative traits among two independent groups. At a significance level of p less than 0.05, it was considered that the studied indicator in the compared groups had statistically significant differences.

The clinical assessment of the patients was conducted upon evaluation of severity and symptoms of LEAD, which were categorized according to the Fontaine classification into stage I: asymptomatic, stage IIa: mild intermittent claudication (walking distance until pain begins >200 m), stage IIb: moderate to severe intermittent claudication (walking distance until pain begins <200 m), stage III: ischemic rest pain and stage IV: necrosis, ulceration, or gangrene of the affected leg. The cardiovascular risks such as diabetes mellitus, arterial hypertension, coronary heart disease were documented during the initial assessment of each patient for the all genotypes examined.

The following endovascular procedures was implemented to treat LEAD such as balloon angioplasty**,** balloon angioplasty with stent implantation, balloon angioplasty with stenting and femoropopliteal bypass, balloon angioplasty with stenting of the external iliac artery and superficial femoral artery, hybrid procedure: balloon angioplasty with stenting and common femoral artery endarterectomy, balloon angioplasty with stenting and endarterectomy, hybrid procedure: balloon angioplasty with stenting and femorotibial bypass.

The study was performed according to Good Clinical Practice standards and the principles of the Declaration of Helsinki. The informed consent was collected from all participants before the inclusion in the study.

RESULTS

Distribution of MTHFR A1298C genotypes and Allele frequencies

The genotype distribution among the 69 patients consisted of 37 (53.6%) with the AA genotype, 21 (30.4%) with AC, and 11 (16.0%) with CC. The allele frequency analysis showed the A allele accounted for 68.8% (n=95) and the C allele for 31.2% (n=43) in study population. Distribution of MTHFR A1298C genotypes and Allele frequencies are shown in Table 1 and 2.

Table 1: Distribution of MTHFR A1298C genotypes.

Genotype	N	Percentage (%)
AA	37	53.6
AC	21	30.4
CC	11	16.0
Total	69	100

Table 2: Distribution of Allele frequencies.

Allele	N	Percentage (%)
A	95	68.8
C	43	31.2

Baseline clinical characteristics by genotype

There were no statistically significant differences were observed in age across genotypes (AA-median 63 years [59; 69], AC-62 years [60; 65], CC-68 years [61.5; 73], p=0.34). Sex distribution showed pattern toward statistical significance (p=0.05), with males predominating in all groups but especially in AC (95.2%) and CC (90.8%) genotypes. Prevalence of DM and AH did not vary significantly between genotypes (DM, p=0.15; AH consistent across groups). CHD significantly more frequent in AA (94.6%) and CC (100%) genotypes compared to AC (71.4%) (p=0.01). Encephalopathy rates and the stage of chronic arterial insufficiency were comparable among genotypes (Table 3).

Laboratory parameters by genotype

Median Hcy levels were higher in the CC genotype group (21.7 [16.3; 23.7] mmol/l) compared to AA (15 [13.4; 19.1] mmol/l) and AC (15.7 [12.2; 21.8] mmol/l), despite this difference was statistically not significant (p=0.14).

Other measured thiol-related parameters, including cysteine, cysteinylglycine, glutamylcysteine, and glutathione, showed no significant variation among genotypes (all p>0.15) (Table 4).

Angiographic characteristics

The lesion localization was statically not significant by genotype. Aorto-iliac-femoral involvement was noted in 21.6% of AA, 28.6% of AC, and 18.2% of CC patients (p=0.76). Femoro-popliteal-tibial involvement was the most predominant among all genotypes (AA: 54%, AC: 57.1%, CC: 45.4%, p=0.82).

Combined involvement of both regions was observed in 24.3% of AA, 14.3% of AC, and 36.4% of CC patients (p=0.36) (Table 5).

Table 3: Baseline clinical characteristics by genotype.

Parameters	AA	AC	CC	P value
Age (in years)	63 [59; 69]	62 [60; 65]	68 [61.5; 73]	0.34
Gender	Male-28 (75.7%) Female-9 (24.3%)	M-20 (95.2%) F-1 (4.8%)	M-10 (90.9%) F-1 (9.1)	0.05
DM	12 (32.4%)	9 (42.9%)	1 (9.1%)	0.2
AH	2 [2; 2]	2 [2; 2]	2 [2; 2]	
CHD	35 (94.6%)	15 (71.4%)	11 (100%)	0.01
Encephalopathy	8 (21.6)	7 (33.3%)	3 (27.4%)	0.6
Stage of chronic arterial insufficiency	3 [2; 4]	3 [2; 4]	3 [2; 4]	0.753
Stage 2	13 (35.1%)	7 (33.3%)	5 (45.5%)	0.852
Stage 3	6 (16.2%)	5 (23.8%)	3 (27.3%)	0.706
Stage 4	18 (48.6%)	9 (42.8%)	3 (27.3%)	0.640
Localization of plaques				
1-Aorto-iliac-femoral position	8 (22.9%)	6 (28.6%)	2 (18.2%)	0.64
2-Femoro-popliteal-tibial position	18 (51.4%)	12 (57.1%)	5 (45.5%)	
3-Both	9 (25.7%)	3 (14.3%)	4 (36.4%)	

Table 4: Laboratory parameters by genotype.

Parameters (mmol/l)	AA	AC	CC	P value
Hcy	15 [13.4; 19.1]	15.7 [12.2; 21.8]	21.7 [16.3; 23.7]	0.14
Cysteine (Cys)	409.7 [330.97; 487.5]	349.5 [282.2; 446.2]	475.4 [318.0; 538.8]	0.15
Cysteinylglycine	31.8 [26.2; 38.1]	28.5 [25.9; 38.5]	34.3 [27.3; 41.5]	0.57
Glutamylcysteine (gGluCys)	9.1 [6.2; 11.4]	6.7 [6.3; 8.7]	9.1 [6.1; 12.4]	0.34
Glutathione (GSH)	3.7 [2.8; 4.3]	3.7 [3.0; 4.5]	4 [2.7; 4.5]	0.93

Table 5: Angiographic characteristics.

Parameters	AA	AC	CC	P value
Aorto-iliac-femoral position	8 (21.6%)	6 (28.6%)	2 (18.2%)	0.76
Femoro-popliteal-tibial position	20 (54%)	12 (57.1%)	5 (45.4)	0.82
Both	9 (24.3%)	3 (14.3%)	4 (36.4%)	0.36

Procedural characteristics

The procedural types were categorized into balloon angioplasty, angioplasty with stenting, and the hybrid procedures. Balloon angioplasty alone was performed in 24.3% of AA, 33.3% of AC, and 9.1% of CC patients (p=0.32). Angioplasty combined with stenting was the

most frequent intervention (AA: 54%, AC: 57.1%, CC: 54.5%, p=0.67). Hybrid procedures, including combinations of angioplasty, stenting, bypass, and endarterectomy, were more frequent in the CC group (36.4%) in comparison to AA (21.6%) and AC (9.6%), though this difference was not statistically significant (p=0.16). The procedural characteristics are shown in Table 6.

Table 6: Procedural characteristics.

Parameters	AA	AC	CC	P value
Balloon angioplasty	9 (24.3%)	7 (33.3%)	1 (9.1%)	0.32
Angioplasty+ stenting	20 (54%)	12 (57.1%)	6 (54.5%)	0.67
Hybrid procedure	8 (21.6%)	2 (9.6%)	4 (36.4%)	0.16

DISCUSSION

This study investigated the distribution of MTHFR A1298C polymorphism and its correlation with clinical, laboratory, angiographic and procedural characteristics in patients with lower extremity arterial disease (LEAD). The AA genotype was the most frequent (53.6%), followed by AC (30.4%) and CC (16.0%) respectively. The allele frequencies showed a prevalence of the A allele (68.8%) over the C allele (31.2%). There were no significant differences observed in age or most baseline clinical characteristics among the genotypes, except for CHD, which was significantly more predominant in AA and CC genotypes in comparison to AC. The Hcy levels, although significantly elevated in the CC group, did not reveal any statistical significance. Angiographic lesion localization and procedural interventions were comparable between the genotypes, with a minor pattern with regard to hybrid procedures in the CC group.

These results correspond with the global findings indicating that MTHFR polymorphisms, mainly A1298C, may influence cardiovascular disease risk through effects on Hcy metabolism, in spite with variable clinical impact. The noticed association among CC and AA genotypes with higher CHD prevalence explored a potential connection between this polymorphism and atherosclerotic strain in LEAD patients. In addition, the elevated Hcy in CC carriers, despite not being statistically significant in this study, is compatible with the recommended mechanism of impaired methylation and endothelial dysfunction contributing to LEAD progression.

The genotype distribution and correlated clinical characteristics elaborated the potential application of MTHFR A1298C genotyping in risk stratification for LEAD patients. Thus, identifying patients with AA or CC genotypes, who appear more susceptible to concomitant CHD, leading to more targeted observation and aggressive control of cardiovascular risk factors. Although, Hcy differences were not statistically significant, observing and managing increased Hcy in CC genotype carriers could be evaluated as part of an individualized medical approach. The procedural characteristics of this study was insignificant by genotype, suggesting that current interventional approach remain relevant across genetic subgroups. However, the pattern of hybrid procedures in CC patients is prominent, thus may require further investigation to optimize protocol development.

The comparisons with similar studies reveal an adherence of elevated Hcy levels among CC carriers, despite the lack of statistical significant data in our study could be attributed to sample size limitation.[12] Furthermore, there are several investigations indicating significant Hcy elevation in CC genotype individuals, approving this hypothesis of impaired methylation and endothelial dysfunction resulting the progression of LEAD.[13] Our findings on angiographic and procedural characteristics, showed no significant genotype-based differences, aligning with the reports of genetic variation without markedly influence for interventional outcomes, thus current treatment protocols remain generally relevant.

However, some contrasting studies have indicated differing genotype distributions and variable clinical associations, particularly due to ethnic, demographic, or methodological differences. Some studies revealed no significant correlation among MTHFR A1298C polymorphism and cardiovascular risk factors or disease severity, highlighting the difficulty and diversity of genetic influences on LEAD.[14] These differences underscore the requirement for larger, ethnically diverse cohorts and longitudinal designs to validate the polymorphism's clinical impact and to coordinate contrasting evidence.

In conclusion, this study contributes to the currently developing body of evidence supporting the clinical significance of MTHFR A1298C genotyping in LEAD, while also identifying the need to analyze the findings within global and population-specific data.

Limitations

The limitations of this study consisted of relatively small sample size, which may decline sensitivity to detect significant differences, particularly in Hcy levels and clinical outcomes. This cross-sectional design limits causal analysis between genotype and disease characteristics.

CONCLUSION

The study revealed that MTHFR A1298C polymorphism in LEAD patients reflects global genotype patterns, with AA genotype and A allele most predominant. AA and CC genotypes are associated to higher coronary heart disease prevalence, suggesting elevated atherosclerotic risk. Increased Hcy levels in CC carriers were statistically not significant, mainly due to sample size, thus assisting the role of impaired methylation in LEAD progression. No significant genotype differences were observed in angiographic or procedural outcomes, though CC patients showed an association towards more complex hybrid interventions.

Recommendations

The future studies should concentrate on larger, longitudinal cohorts to validate these correlations and examine the physiologic pathways connecting MTHFR polymorphisms to LEAD progression and treatment outcomes.

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