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Prevalence of Polymorphisms of Genes Responsible for Coagulation System and Folate Metabolism and Their Predictive Value for Thrombosis Development in MINOCA Patients: Immediate and Long-Term Prognoses

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Abstract: (1) Background. One of the causes of myocardial infarction (MI) with nonobstructive coronary arteries (MINOCA) is thrombus formation in situ followed by lysis, resulting in a morphologically normal angiogram but with an underlying prothrombotic state that is potentially predisposed to recurrence. Recent studies have shown that a subset of MINOCA patients may have thrombophilic conditions at screening. Objective: To compare the prothrombotic trend in MINOCA patients with that of subjects with MI and obstructive coronary arteries (MIOCA) by testing for known congenital thrombophilias and markers of coagulation activation. (2) Materials and methods. Screening included congenital thrombophilias (factor V Leiden; assessment of protein C, protein S, and antithrombin III) and eight genes. Of these, four genes represented the folate pathway enzymes: MTHFR 677 C>T (rs1801133), MTHFR 1298 A>C (rs1801131), MTR 2756 A>G (rs1805087), and MTRR 66 A>G (rs1801394). The other four genes represented the blood coagulation system: F13 (163 G>T) rs5985, F1 (−455 G>A) rs1800790, GP IIb–IIIa (1565 T>C) rs5918, and PAI-I (−675 5G>4G) rs1799889. Additionally, we examined the levels of homocysteine and lipoprotein (LP) (a). (3) Results. Our study included 269 patients: 114 MINOCA patients and 155 MIOCA patients with lesions of one coronary artery. The frequencies of polymorphisms in the genes of the blood coagulation system and the folate pathway did not differ between the groups. The following genes were associated with in-hospital mortality in the MINOCA group: MTHFR 1298 A>C rs1801131 (OR 8.5; 95% CI 1.67–43.1) and F1 (−455 G>A) rs1800790 (OR 5.8; 95% CI 1.1–27.8). In the MIOCA group, the following genes were associated with in-hospital mortality: MTHFR 1298 A>C rs1801131 (OR 9.1; 95% CI 2.8–28.9), F1 (−455 G>A) rs1800790 (OR 11.4; 95% CI 3.6–35.9), GP IIb–IIIa (1565 T>C) rs5918 (OR 10.5; 95% CI 3.5–30.8), and PAI-I (−675 5G>4G) rs1799889 (OR 12.9; 95% CI 4.2–39.7). We evaluated long-term outcomes (case fatality rate, recurrent MI, and stroke) over a period of 12 months in both groups. The variables associated with these outcomes were laboratory parameters, such as protein C deficiency, hyperhomocysteinemia, and a content of LP (a) > 30 mg/dL. However, we did not reveal the prognostic value of polymorphisms of the studied genes representing the blood coagulation system and the folate pathway. (4) Conclusion. We established no statistically significant differences between the MINOCA and MIOCA groups in the prevalence of congenital thrombophilias and the prevalence of folate pathway enzyme genes and blood coagulation system genes. The MTHFR 1298 A>C (rs1801131) and F1 (−455 G>A) rs1800790 genes were associated with in-hospital mortality in both groups. More significant prognostic factors in both groups during the one-year period were protein C deficiency, hyperhomocysteinemia, and LP (a) > 30 mg/dL.

Keywords: MINOCA; factor V Leiden; gene polymorphisms; MTHFR 677 C>T (rs1801133); MTHFR 1298 A>C (rs1801131); MTR 2756 A>G (rs1805087); MTRR 66 A>G (rs1801394); F13 (163 G>T) rs5985; F1 (−455 G>A) rs1800790; GP IIb–IIIa (1565 T>C) rs5918; PAI-I (−675 5G>4G) rs1799889

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1. Introduction

Myocardial infarction (MI) with nonobstructive coronary arteries (MINOCA) is clinically identified by the general criteria for MI in the absence of coronary artery obstruction [1–3]. Multicenter registries demonstrated that MINOCA occurs in 1–13% of patients with MI [3,4]. A meta-analysis including 28 studies showed that compared to patients with MI and obstructive coronary arteries (MIOCA), patients with MINOCA were younger, more often female, and their prognosis was likely to be more favorable [3–6].

The etiology of MINOCA remains unclear to date. Several potential explanatory mechanisms have been proposed, such as vasospasm, spontaneous coronary dissection, microcirculatory dysfunction, Takotsubo cardiomyopathy, or myocarditis [1,7]. MINOCA was mentioned in recent guidelines for acute MI by the European Society of Cardiology, and it was emphasized that the underlying cause must be determined for each patient as it would affect subsequent treatment [1,6,8]. One of the causes of MINOCA is in situ thrombus formation followed by lysis, resulting in a morphologically normal angiogram but with an underlying prothrombotic state that is potentially predisposed to a recurrence [1,3,5,7].

A recent systematic review pointed out that 14% of patients with MINOCA may have thrombophilic conditions that can be detected by screening [4,7]. Congenital thrombophilias identified in patients with MINOCA include factor V Leiden (FVL), prothrombin gene mutation, and a deficiency in proteins C and S [9]. The presence of the Leiden mutation, an unfavorable polymorphism of the coagulation factor II gene, and a deficiency in antithrombin III and proteins C and S, predisposes the patient to the development of venous thrombosis [9–13]. However, the role of congenital thrombophilia in the development of arterial thrombosis has been little studied to date [10,14,15].

In the last two decades, many studies have been conducted to identify the prevalence of polymorphic variants of the candidate genes responsible for the development of arterial thrombosis [10,15–18]. However, their association with MI remain largely unexplored [14,19]. Small, highly selective studies of patients with MINOCA revealed an association between the carriage of polymorphisms of factor II and V genes and a deficiency in antithrombin III and proteins C and S, which implied a more important role of the hypercoagulable state in this cohort of patients [9,15,20,21].

Currently, the effects of four folate pathway enzyme genes and eight genes of the blood coagulation system are studied in association with the risk of thrombosis [3,20,22,23]. The former pool of genes includes methylenetetrahydrofolate reductase MTHFR 677 C>T (rs1801133), MTHFR 1298 A>C (rs1801131), methionine synthase MTR 2756 A>G (rs1805087), and methionine synthase reductase MTRR 66 A>G (rs1801394). The latter pool of genes encompasses F2 (20210 G>A) rs1799963, F5 (1691 G>A) rs6025, F7 (10976G>A) rs6046, F13 (163 G>T) rs5985, F1 (−455 G>A) rs1800790, GP Ia–IIa (807 C>T) rs1126643, GP IIb–IIIa (1565 T>C) rs5918, and PAI-I (−675 5G>4G) rs1799889.

Individual thrombophilic disorders differ in their prevalence in the general population and their impact on prothrombotic potential [13,17,19]. They range from a hundredfold increase in the risk of thrombosis in homozygous FVL to a negligible effect of protein S deficiency on the incidence rate of venous thrombosis [3,9,20,24,25]. To date, there are very few studies that have examined all known thrombophilias of established clinical significance in patients with MINOCA [1,3,4]. Several small studies have produced conflicting results [3,7]. In the largest meta-analysis involving eight available studies on 478 patients with MINOCA who underwent partial thrombophilia screening, abnormal results were detected in 14% of the study subjects and, as expected, the most common thrombophilia was FVL, which was characteristic for 12% of patients [4,5,7].

In light of the above findings, we hypothesized that the prevalence of thrombophilia is higher in MINOCA patients versus MIOCA patients. Therefore, the objective of our study was to compare the prothrombotic trend in MINOCA patients with that of MIOCA subjects by testing for known congenital and acquired thrombophilias and markers of coagulation activation.

2. Materials and Methods

2.1. Study Population

This study was part of a large, prospective cohort follow-up study of patients with acute MI who were treated at the research clinic of the Regional Clinical Hospital No. 1 in Krasnodar, Russia.

The study protocol complied with the ethical principles of the 1975 Declaration of Helsinki and was in accordance with the Ethical Guidelines for Epidemiological Research adopted in the Russian Federation. This study was approved by the Ethics Committee of the State Budgetary Public Health Institution, Ochapovsky Regional Clinical Hospital #1. (Protocol #4; date: 5 November 2018) and conducted according to international standards of good clinical practice.

The general patient database included those with coronary artery disease (CAD) who were hospitalized urgently with acute coronary syndromes (ACS) of all types and etiologies. This article includes an analysis of patients who were admitted for MI from November 2018 to November 2020 and met the following criteria:

(1) General diagnostic criteria for acute MI based on elevated troponin levels in combination with clinical criteria;
(2) A performed coronary angiography demonstrated nonobstructive (coronary stenosis < 50%) coronary arteries (MINOCA) or obstructive CAD (stenosis $\geq$ 50%) with isolated stenosis of one coronary artery and subsequent revascularization (MIOCA + 1 stent).

A cohort of patients with single-vessel CAD who were subjected to percutaneous coronary intervention (PCI) was selected as a comparison group, which was dictated by the greater comparability of the studied groups. Patients were excluded from this analysis if they were on long-term anticoagulant treatment or had non-cardiac causes of troponin elevation, such as heart failure or chronic lung and/or kidney disease. Thus, this study is a cohort observational study. At the first stage of the study, we compared the prevalence of various forms of hereditary thrombophilia polymorphism, folate cycle enzyme genes, and blood clotting system genes in two groups: MINOCA vs. MIOCA. Next, we studied their prognostic value for hospital mortality and long-term one-year outcomes (mortality, MI, and stroke) in each group separately.

The inclusion of the patients in the study did not affect management and treatment tactics. Intraoperative data were collected from the clinic's electronic database (JEMYS) in compliance with all legal principles. Written informed consent for treatment and diagnosis, as well as consent to the processing of personal data, was obtained from each patient during their hospitalization.

2.2. Laboratory Diagnostics

Laboratory screening included congenital thrombophilias (FVL; an evaluation of protein C, protein S, and antithrombin III), four genes of folate pathway enzymes, and four genes of the blood coagulation system. The former gene pool included MTHFR 677 C>T (rs1801133), MTHFR 1298 A>C (rs1801131), MTR 2756 A>G (rs1805087), and MTRR 66 A>G (rs1801394). The latter genes were F13 (163 G>T) rs5985, F1 (−455 G>A) rs1800790, GP IIb–IIIa (1565 T>C) rs5918, and PAI-I (−675 5G>4G) rs1799889.

A genetic analysis of factor V Leiden mutations (dbSNP: rs6025) was conducted using TaqMan genotyping assays. Free protein S levels were measured through an immunoturbidimetric assay. Protein C deficiency was diagnosed at 60% or less. Antithrombin III activity was assessed using an assay based on factor Xa inhibition, with antithrombin III deficiency diagnosed when the level was below 75% [12,20]. Plasma factor VIII activity was determined via coagulometric analysis, using deficient plasma (Siemens Healthcare Diagnostics), and levels of 150% or more were considered elevated. Total homocysteine (tHcy) was determined in fasting plasma via enzymatic analysis. Hyperhomocysteinemia was defined as tHcy $\geq$ 15 μmol/L. LP (a) was assessed in serum by ELISA, with a lower detection limit of 1.2 mg/dL. An LP (a) level > 30 mg/dL was considered elevated. The intra-assay and inter-assay CVs for all commercially available assays were <7%. The evaluation of

the genes representing the folate pathway enzymes and the blood coagulation system was carried out by polymerase chain reaction methods, using a reagent kit manufactured by DNA-Technology LLC.

2.3. Statistical Analysis

The database contained quantitative and categorical variables. Using the Shapiro–Wilk test, all quantitative data were examined for normal distribution. It was established that the data did not have a normal distribution. Therefore, we used nonparametric statistical methods. Continuous variables were analyzed using the Mann–Whitney U test. Fisher's exact test or the χ^2 test were used for categorical variables, as appropriate. The association between the in-hospital case fatality rate and any comorbidities was assessed using univariate and multivariate logistic regression analyses, and it was expressed as an odds ratio (OR), 95% confidence interval (CI), and *p*-value. Long-term outcomes (case fatality rate, MI, stroke, and hospital readmission) within 12 months were evaluated using univariate and multivariate Cox proportional hazards regression, and they were expressed as a hazard ratio (HR), 95% CI, and *p*-value. The quantitative data were presented as the median and interquartile range, Me (Q1; Q3). The categorical variables were presented as the absolute number and relative frequency, n (%). For statistical analyses, we used the STATISTICA 10 (Dell, Round Rock, TX, USA), Microsoft Office Excel, version version 2002 (16.0.12527.20278), and MedCalc (MedCalc Software Ltd., Ostend, Belgium) software packages.

3. Results

3.1. Patient Characteristics

Our study included 269 participants: 114 MINOCA patients and 155 MIOCA subjects with lesions of one coronary artery. Table 1 presents the clinical data of patients in both groups. Instrumental and laboratory data, in addition to the patients' medicamentous therapy, are presented in Tables 2 and S1.

Table 1. Clinical parameters of patients.

Parameters	MIOCA n = 155	MINOCA n = 114	*p*
Clinical data			
Age, years	54 (45;66)	48 (42;54)	<0.001 *
Male gender, %	50.3	38.6	0.056
BMI, kg/m^2	28.2 (26;31)	28.6 (25.8;31)	0.862
Prior MI, %	17.4	16.7	0.871
Family history of IHD, %	49	45.6	0.579
Prior stroke, %	3.2	7	0.153
Hypertension, %	72.3	73.7	0.795
Hypercholesterolemia, %	23.2	21.9	0.802
PAD, %	14.8	17.5	0.550
CHF NYHA class II–IV, %	61.3	57.9	0.575
Diabetes, %	11.6	11.4	0.957
Smoking, %	51.6	48.3	0.585
Hospitalization data			
Pain syndrome (or its equivalent), %	83.2	83.3	0.981
Classes of AHF	1 (1;3)	1 (1;1)	0.002 *
AF, %	16	1.7	<0.001 *
ST Elevates, %	28.4	33.3	0.384
LVEF, %	55 (45;55)	55 (52;55)	0.018 *
LA volume, mL^3	40 (37;42)	39 (37;41)	0.212
LVESD, mm	50 (47;54)	50 (47;54)	0.439

Table 1. *Cont.*

Parameters	MIOCA n = 155	MINOCA n = 114	*p*
Thrombolytic therapy, %	20	21.9	0.700
The effectiveness of thrombolysis, %	15.5	15.8	0.945
PCI, %	100	0	<0.001 *

BMI—body mass index; MI—acute myocardial infarction; IHD—ischemic heart disease; PAD—peripheral artery disease; CHF NYHA—chronic heart failure, New York Heart Association classification; AHF—acute heart failure; AF—atrial fibrillation; ST—ST segment on the ECG; LVEF—left ventricular ejection fraction; LA—left atrium; LVESD—left ventricular end systolic diameter; PCI—percutaneous coronary intervention; *—statistically significant differences.

Table 2. Laboratory data and hospital outcomes.

Parameters	MIOCA n = 155	MINOCA n = 114	*p*
Laboratory data			
MTHFR 677 C>T (rs1801133), %	14.8	14.9	0.987
MTHFR 1298 A>C (rs1801131), %	10.3	8.8	0.671
MTR 2756 A>G (rs1805087), %	20.7	22.8	0.670
MTRR 66 A>G (rs1801394), %	32.9	33.3	0.941
F13 (163 G>T) rs5985, %	23.9	21.1	0.586
F1 (−455 G>A) rs1800790, %	11	11.4	0.910
GP IIb–IIIa (1565 T>C) rs5918, %	22	24.6	0.613
PAI-I (−675 5G>4G) rs1799889, %	23.2	25.4	0.675
LDL, mmoL/L	2.4 (1.7;3.7)	2.7 (1.9;4)	0.159
LP (a), mg/dL	45 (9;106)	45 (11;108)	0.525
LP (a) > 30 mg/dL, %	58	64	0.322
CRP, mg/L	14 (10;32)	14 (10;32)	0.635
Factor V Leiden, %	25.8	19.3	0.211
Protein C deficiency, %	41.9	39.5	0.685
Protein S deficiency, %	43.2	40.4	0.637
Deficiency of antithrombin III, %	11.6	8.8	0.452
Factor VIII > 150%, %	12.9	10.5	0.553
Hyperhomocysteinemia, %	32.3	38.6	0.282
Hospital outcomes			
Hospital mortality, %	11.6	7	0.208
Bleeding, %	0.65	5.3	0.018 *
Stroke, %	5.8	2.6	0.213
Mechanical complications, %	1.3	7	0.014 *

LDL—low-density lipoprotein; LP—lipoprotein; CRP—C-reactive protein; *—statistically significant differences.

The tables show that the MINOCA group was younger: the median age in this group was 48 (42;54) years versus 54 (45;66) years in the MIOCA group ($p < 0.001$). In the MIOCA group, in the acute period, the acute heart failure (AHF) class (sensu the classification by T. Killip) prevailed; on the ECG, we detected atrial fibrillation more often (16% vs. 1.7%, $p < 0.001$) and left ventricular ejection fraction (LVEF) significantly less often ($p = 0.018$). In addition, patients from the two groups were statistically significantly different in terms of in-hospital non-fatal outcomes, such as bleeding ($p = 0.018$) and mechanical complications ($p = 0.014$), which were more common in the MINOCA group.

The frequency of polymorphisms in the genes of the blood coagulation system and the folate pathway did not differ between the groups. The prevalence of the MTHFR 677 C>T (rs1801133) and MTHFR 1298 A>C (rs1801131) genes in both groups was about 15% and 9%, respectively. MTRR 66 A>G rs1801394 was detected more often than the other genes studied, being present in over one-third of patients in both groups. These data are presented in Table 2.

3.2. Uni- and Multivariate Logistic Regression Analysis

A univariate analysis in the MINOCA group revealed various statistically significant independent variables associated with the in-hospital fatality rate: peripheral artery disease (PAD) (OR 10.1; 95% CI 2.2–46.8), pain syndrome (feedback) (OR 0.15; 95% CI 0.03–0.73), AHF class (OR 2.76; 95% CI 1.53–4.97), LVEF (OR 0.87; 95% CI 0.79–0.96), LP (a) concentration (OR 1.01; 95% CI 1.0–1.02), hyperhomocysteinemia (OR 13; 95% CI 1.54–110.2), and the following genes: MTHFR 1298 A>C (rs1801131) (OR 8.5; 95% CI 1.67–43.1) and F1 (−455 G>A) rs1800790 (OR 5.8; 95% CI 1.1–27.8). A multivariate logistic regression analysis yielded the following most powerful factors: PAD (OR 14.1; 95% CI 1.6–126.9) and AHF class (OR 2.96; 95% CI 1.25–7.0) (Table 3).

Table 3. Uni- and multivariate logistic regression analyses in patients with MINOCA for the outcome of hospital mortality.

Parameters	Univariate Logistic Regression Analysis OR (95% CI)	*p*	Multivariate Logistic Regression Analysis OR (95% CI)	*p*
Prior stroke	5.55 (0.92–33.6)	0.062	-	
PAD	10.1 (2.2–46.8)	0.003 *	14.1 (1.6–126.9)	0.018 *
Hypercholesterolemia	4.05 (0.94–17.5)	0.061	-	
Pain syndrome	0.15 (0.03–0.73)	0.023 *	0.16 (0.02–1.4)	0.095
Classes of AHF	2.76 (1.53–4.97)	<0.001 *	2.96 (1.25–7.0)	0.014 *
AF	15 (0.856–266)	0.095	-	
LVEF	0.87 (0.79–0.96)	0.006 *	-	
MTHFR 677 C>T (rs1801133)	-		-	
MTHFR 1298 A>C (rs1801131)	8.5 (1.67–43.1)	0.018 *	-	
MTR 2756 A>G (rs1805087)	-		-	
MTRR 66 A>G (rs1801394)	-		-	
F13 (163 G>T) rs5985	-		-	
F1 (−455 G>A) rs1800790	5.8 (1.1–27.8)	0.043 *	-	
GP IIb–IIIa (1565 T>C) rs5918	-		-	
PAI-I (−675 5G>4G) rs1799889	-		-	
LP (a)	1.01 (1.0–1.02)	0.034 *	-	
CRP	1.02 (0.99–1.05)	0.107	-	
Factor V Leiden	-		-	
Protein C deficiency	-		-	
Protein S deficiency	-		-	
Deficiency of antithrombin III	-		-	
Factor VIII > 150%	-		-	
Hyperhomocysteinemia	13 (1.54–110.2)	0.003 *	8.7(0.7–106)	0.089

OR—odds ratio; CI—confidence interval; AHF—acute heart failure; PAD—peripheral artery disease; AF—atrial fibrillation; LVEF—left ventricular ejection fraction; LP—lipoprotein; CRP—C-reactive protein; *—statistically significant differences.

A univariate analysis in the MIOCA group resulted in several significant independent variables associated with the in-hospital case fatality rate: PAD (OR 3.5; 95% CI 1.2–10.6), hypercholesterolemia (OR 3.1; 95% CI 1.13–8.6), family history of CAD (OR 4.2; 95% CI 1.3–13.5), AHF class (OR 2.26; 95% CI 1.49–3.4), LVEF (OR 0.85; 95% CI 0.79–0.9), LP (a) content (OR 1.01; 95% CI 1.0–1.03), C-reactive protein (CRP) (OR 1.04; 95% CI 1.02–1.07), hyperhomocysteinemia (OR 24.2; 95% CI 5.3–110.8), and the following genes: MTHFR 1298 A>C (rs1801131) (OR 9.1; 95% CI 2.8–28.9), F1 (−455 G>A) rs1800790 (OR 11.4; 95% CI 3.6–35.9), GP IIb–IIIa (1565 T>C) rs5918 (OR 10.5; 95% CI 3.5–30.8) and PAI-I (−675 5G>4G) rs1799889 (OR 12.9; 95% CI 4.2–39.7). A multivariate logistic regression analysis detected the following most powerful factors: PAD (OR 12.7; 95% CI 1.4–115), LVEF (OR 0.86; 95% CI 0.78–0.95) and the PAI-I (−675 5G>4G) rs1799889 gene (OR 922; 95% CI 12.2–69718) (Table 4).

Table 4. Uni- and multivariate logistic regression analyses in patients with MIOCA for the outcome of hospital mortality.

Parameters	Univariate Logistic Regression Analysis OR (95% CI)	*p*	Multivariate Logistic Regression Analysis OR (95% CI)	*p*
PAD	3.5 (1.2–10.6)	0.034 *	12.7 (1.4–115)	0.024 *
Hypercholesterolemia	3.1 (1.13–8.6)	0.033 *	-	
Family history of IHD	4.2 (1.3–13.5)	0.008 *	-	
Classes of AHF	2.26(1.49–3.4)	<0.001 *	-	
LVEF	0.85 (0.79–0.9)	<0.001 *	0.86 (0.78–0.95)	0.005 *
MTHFR 677 C>T (rs1801133)	-		-	
MTHFR 1298 A>C (rs1801131)	9.1 (2.8–28.9)	<0.001 *	-	
MTR 2756 A>G (rs1805087)	-		-	
MTRR 66 A>G (rs1801394)	-		-	
F13 (163 G>T) rs5985	-		-	
F1 (−455 G>A) rs1800790	11.4 (3.6–35.9)	<0.001 *	-	
GP IIb–IIIa (1565 T>C) rs5918	10.5 (3.5–30.8)	<0.001 *	-	
PAI-I (−675 5G>4G) rs1799889	12.9 (4.2–39.7)	<0.001 *	922 (12.2–69718)	0.002 *
LP (a)	1.01 (1.0–1.03)	<0.001 *	-	
CRP	1.04 (1.02–1.07)	<0.001 *	1.04 (0.99–1.1)	0.065
Factor V Leiden	-		-	
Protein C deficiency	-		-	
Protein S deficiency	-		-	
Deficiency of antithrombin III	-		-	
Factor VIII > 150%	-		-	
Hyperhomocysteinemia	24.2 (5.3–110.8)	<0.001 *	8.7 (0.69–109)	0.094

OR—odds ratio; CI—confidence interval; PAD— peripheral artery disease; IHD—ischemic heart disease; AHF—acute heart failure; LVEF—left ventricular ejection fraction; LP—lipoprotein; CRP—C-reactive protein; *—statistically significant differences.

3.3. Uni- and Multivariate Cox Proportional Hazards Regression

To assess long-term outcomes within 12 months of MI, we used Cox proportional hazards regression, which takes into account the time of the event. A univariate analysis in the MINOCA group demonstrated only protein C deficiency (HR 3.9; 95% CI 1.02–15) as a significant independent variable associated with the case fatality rate, whereas it established several variables associated with recurrent MI: LP (a) > 30 mg/dL (HR 8.7; 95% CI 1.2–66.2), protein C deficiency (HR 4.3; 95% CI 1.4–13.8), and hyperhomocysteinemia (HR 5.0; 95% CI 1.6–15.9). Multiple variables were associated with acute cerebrovascular disorders: the level of LP (a) > 30 mg/dL (HR 8.5; 95% CI 1.1–64.8), protein C deficiency (HR 2.9; 95% CI 1.0–8.7), and hyperhomocysteinemia (HR 3.5; 95% CI 1.2–11.4). A multivariate analysis yielded the following significant variables for recurrent MI and cerebrovascular disorders: protein C deficiency (HR 6.6, 95% CI 2.03–21.2; and HR 4.3, 95% CI 1.43–12.9, respectively) and hyperhomocysteinemia (HR 7.4, 95% CI 2.3–23.9; and HR 3.7, 95% CI 1.24–11.2, respectively) (Table 5).

A univariate analysis in the MIOCA group revealed several groups of significant independent factors. The variables associated with the case fatality rate were congestive heart failure (CHF) (HR 5.5; 95% CI 1.3–23.9), LP (a) level > 30 mg/dL (HR 4.5; 95% CI 1.3–15.5), and protein C deficiency (HR 4.6; 95% CI 1.5–14.1). The variables associated with recurrent MI included LP (a) > 30 mg/dL (HR 10.1; 95% CI 2.4–42.8), protein C deficiency (HR 9.6; 95% CI 2.8–32.4), and hyperhomocysteinemia (HR 3.8; 95% CI 1.7–8.8). The variables associated with acute cerebrovascular disorders were PAD (HR 4.2; 95% CI 1.3–13.9), arterial hypertension (feedback) (HR 0.11; 95% CI 0.03–0.42), and the concentration of LP (a) > 30 mg/dL (HR 4.6; 95% CI 1.01–20.8). In multivariate analysis, the following variables remained statistically significant in their association with the case fatality rate: CHF (HR 5.3; 95% CI 1.23–23.2) and protein C deficiency (HR 4.5; 95% CI 1.47–13.7). For recurrent MI, such variables were protein C deficiency (HR 9.5; 95% CI 2.8–31.9) and hy-

perhomocysteinemia (HR 3.7; 95% CI 1.6–8.6). For cerebral disorders, the multivariate analysis yielded the following variables with statistically significant associations: arterial hypertension (feedback) (HR 0.09; 95% CI 0.03–0.33) and the level of LP (a) > 30 mg/dL (HR 6.3; 95% CI 1.4–28.8) (Table 6).

Table 5. Uni- and multivariate Cox proportional hazards regression analyses in patients with MINOCA to assess long-term outcomes.

Parameters	Univariate Cox Proportional Hazards Regression HR (95% CI)	*p*	Multivariate Cox Proportional Hazards Regression HR (95% CI)	*p*
Mortality				
Protein C deficiency	3.9 (1.02–15)	0.036 *	-	
MI				
LP (a) > 30 mg/dL	8.7 (1.2–66.2)	0.005 *	-	
Protein C deficiency	4.3 (1.4–13.8)	0.008 *	6.6 (2.03–21.2)	0.002 *
Hyperhomocysteinemia	5.0 (1.6–15.9)	0.004 *	7.4 (2.3–23.9)	<0.001 *
Stroke				
LP (a) > 30 mg/dL	8.5 (1.1–64.8)	0.005 *	-	
Protein C deficiency	2.9 (1.0–8.7)	0.045 *	4.3 (1.43–12.9)	0.009 *
Hyperhomocysteinemia	3.5 (1.2–11.4)	0.021 *	3.7 (1.24–11.2)	0.019 *

HR—Hazard ratio; CI—confidence interval; LP—lipoprotein; *—statistically significant differences.

Table 6. Uni- and multivariate Cox proportional hazards regression analyses in patients with MIOCA to assess long-term outcomes.

Parameters	Univariate Cox Proportional Hazards Regression HR (95% CI)	*p*	Multivariate Cox ProportionalHazards Regression HR (95% CI)	*p*
Mortality				
CHF NYHA class II-IV	5.5 (1.3–23.9)	0.006 *	5.3 (1.23–23.2)	0.026 *
MTHFR 1298 A>C (rs1801131)	4.5 (1.3–15.5)	0.007 *	-	
F1 (−455 G>A) rs1800790	3.4 (0.96–11.6)	0.097	-	
LP (a) > 30 mg/dL	3.4 (0.96–11.6)	0.097	-	
Protein C deficiency	4.6 (1.5–14.1)	0.003 *	4.5 (1.47–13.7)	0.008 *
MI				
LP (a) > 30 mg/dL	10.1 (2.4–42.8)	<0.001 *	-	
Protein C deficiency	9.6 (2.8–32.4)	<0.001 *	9.5 (2.8–31.9)	<0.001 *
Hyperhomocysteinemia	3.8 (1.7–8.8)	0.002 *	3.7 (1.6–8.6)	0.002 *
Stroke				
PAD	4.2 (1.3–13.9)	0.035 *	-	
Hypertension	0.11 (0.03–0.42)	<0.001 *	0.09 (0.03–0.33)	<0.001 *
LP (a) > 30 mg/dL	4.6 (1.01–20.8)	0.023 *	6.3 (1.4–28.8)	0.017 *
Hyperhomocysteinemia	3.03 (0.9–9.4)	0.060	-	

HR—Hazard ratio; CI— confidence interval; CHF NYHA—chronic heart failure, New York Heart Association classification; LP—lipoprotein; PAD—peripheral artery disease; *—statistically significant differences.

4. Discussion

In addition to the main risk factors for CVD (age, gender, hypertension, diabetes mellitus, smoking, and dyslipidemia), genetic polymorphisms of coagulation genes and hereditary thrombophilia significantly increase the risk of MI [26]. Considering the multifactorial and multigenic occurrence of thrombotic events in addition to the classical factors of thrombosis, there may be a connection between polymorphisms and thrombotic events.

Congenital thrombophilia leading to an increased propensity for intravascular thrombosis has been postulated as one of the possible causes of MINOCA in previous studies and reviews [2,3,7]. According to the results of previous studies, the frequency of occurrence of genetic polymorphisms responsible for the development of thrombophilia varies depending on the ethnic composition of the population [27].

In this study, we did not discover support for our hypothesis that MINOCA patients patients have a significantly higher prevalence of thrombophilia than MIOCA patients since no statistically significant differences were established between the groups in these parameters (Table 2). In a previously conducted pilot study in patients with MINOCA, the carriage of genetic markers responsible for the development of thrombosis was revealed; however, this prevalence does not differ relative to patients with MIOCA [28]. This necessitates large-scale studies to evaluate this hypothesis. Additional variables examined in our study, including factor VIII concentration and the severity of homocysteinemia, also did not differ between the groups.

Our goal did not involve the identification of clinical differences between the two groups (MINOCA and MIOCA), since these were different groups in terms of the pathophysiology of MI. Logically, patients with obstructive CAD were older and more severe somatically at admission (Table 1); however, the groups did not differ in the long-term case fatality rate or the incidence of recurrent MI, cerebrovascular events, and hospitalization rates (Figures S1–S4). We examined the prevalence of polymorphisms of the genes responsible for the blood coagulation system and folate pathway in these two cohorts, in addition to their association with immediate and long-term case fatality rates, recurrent episodes of MI, and cerebrovascular events within 12 months after hospitalization. We did not find statistically significant differences in the prevalence of folate pathway enzyme genes and blood coagulation system genes between the MINOCA and MIOCA groups. In both groups, the prevalence of the MTHFR 677 C>T (rs1801133) and MTHFR 1298 A>C (rs1801131) genes was approximately 15% and 9%, respectively. The MTRR 66 A>G rs1801394 gene was found more often than other studied genes and was present in over one-third of patients in both groups. Based on the results of the univariate analysis, we found a statistically significant relationship of the following genes with in-hospital case fatality rate in the MINOCA group: MTHFR 1298 A>C (rs1801131) (OR 8.5; 95% CI 1.67–43.1, $p = 0.018$) and F1 (−455 G>A) rs1800790 (OR 5.8; 95% CI 1.1–27.8, $p = 0.043$). In the MIOCA group, the following genes were significantly associated: MTHFR 1298 A>C (rs1801131) (OR 9.1; 95% CI 2.8–28.9, $p < 0.001$), F1 (−455 G>A) rs1800790 (OR 11.4; 95% CI 3.6–35.9, $p < 0.001$), GP IIb-IIIa (1565 T>C) rs5918 (OR 10.5; 95% CI 3.5–30.8, $p < 0.001$), and PAI-I (−675 5G>4G) rs1799889 (OR 12.9; 95% CI 4.2–39.7, $p < 0.001$). The multivariate logistic regression analysis yielded a statistical significance for only one of them, and only in the MIOCA group: PAI-I (−675 5G>4G) rs1799889 (OR 922; 95% CI 12.2–69718, $p < 0.002$). The rest of the genes in the multifactorial model were offset by clinical factors (Tables 3 and 4).

The analysis of the long-term one-year results also demonstrated that only in the MIOCA group did the MTHFR 1298 A>C (rs1801131) and F1 (−455 G>A) rs1800790 genes have borderline statistical significance in relation to the case fatality rate (Table 6). The more powerful prognostic factors in both groups were protein C deficiency, hyperhomocysteinemia, and LP (a) > 30 mg/dL, both in the univariate analysis and in the multivariate Cox proportional hazards regression.

Homocysteine has been discussed for quite a long time as a risk factor for the development of acute coronary syndrome and stroke. Opinions are divided as to whether the elevated levels of homocysteine observed in patients with ACS and stroke are the cause or consequence of this acute event. Hyperhomocysteinemia is a confirmed risk factor for cardiovascular events associated with increased thrombogenicity, oxidative stress, and endothelial dysfunction [29,30]. Over 80 clinical and epidemiological trials established that hyperhomocysteinemia, in addition to conventional factors, is an independent risk factor for the development of cardiovascular diseases in patients with atherosclerotic lesions of the coronary arteries [29,31]. An increase in the homocysteine content in blood has a

damaging effect on the vascular endothelium and stimulates thrombus formation [30,31]. The incidence of hyperhomocysteinemia in the general population is 5–10% [29,32]. In the elderly, these figures are higher: 30–40% [30,32]. In patients with MI, the level of homocysteine is higher than the reference values in 75% of cases [30]. In addition to the genetic factor, the prevalence of hyperhomocysteinemia varies depending on the geographical, ethnic, and social status due to the lifestyle and dietary habits of the population [27].

In our study, the prognostic role of homocysteine was confirmed for in-hospital fatality rate and for the development of recurrent MI and acute cerebrovascular events in the long term. Higher homocysteine levels and a significant prevalence of methylenetetrahydrofolate reductase mutation were previously reported in younger patients with MINOCA [29,32].

One of the reasons for the increase in the concentration of homocysteine in the blood is a decrease in enzymatic activity due to hereditary enzyme defects, which are encoded by the genes MTHFR 677 C>T, MTHFR 1298 A>C, MTRR 66 A>G, and MTR 2756 A>G [11,23,31]. According to the results of some studies, an increase in the risk of developing venous and arterial thrombosis, in addition to the risk of CAD and MI, was found in patients with unfavorable polymorphisms of the rs1801133 and rs1801131 genes [11,23]. However, in other studies, such associations were not found [29,32]. According to the results of a meta-analysis of 80 studies, there is no convincing evidence for the association of a MTHFR polymorphism (677 C>T) and coronary heart disease in Europe, North America, and Australia [11].

Xiaobo Dong et al. presented a systematic review of the literature from the following databases: Embase, Pubmed, Web of Science, Cochrane Library, China National Knowledge Infrastructure, and the WanFang electronic database. This meta-analysis demonstrated a significant relationship between polymorphisms of the MTHFR A1298C gene and stroke risk within the allelic genetic model C (OR = 1.19; 95% CI = 1.07–1.32; $p = 0.001$), dominant genetic model (OR = 1.19; 95% CI = 1.06–1.33; $p = 0.004$) and recessive genetic model (OR = 1.43; 95% CI = 1.15–1.77; $p = 0.001$). When analyzing the subgroups, the authors revealed a correlation between the polymorphism of MTHFR A1298C and the susceptibility to ischemic stroke [33].

Data on these polymorphisms are contradictory, which does not currently allow for the formulation of recommendations for the primary and secondary medicamentous prevention of hyperhomocysteinemia [34]. Hence, the clarification of this issue requires further research. Deficiencies in vitamins B6 and B12 and folic acid in the diet can block one of the main pathways of homocysteine metabolism, which causes an increase in its level [31,32]. In this regard, studies on homocysteine-lowering vitamin therapy are ongoing [29,30]. The results of these studies are also contradictory, thereby requiring further investigation. In the future, it should be established whether folic acid supplementation in hyperhomocysteinemia in patients with MINOCA may be useful, since the available data for typical MI patients have demonstrated negative results, implying the uselessness of this routine marker assessment [30].

Deficiency in vitamins B6 and B12 and folic acid in consumed food can block one of the main pathways of homocysteine metabolism, which leads to hyperhomocysteinemia [31]. In this regard, studies for research into homocysteine-lowering vitamin therapy are being conducted. However, the research results are contradictory and require further study [29,35]. For patients with MINOCA, the prognostic value of introducing early preventive measures, including specific antithrombotic therapy and homocysteine-reducing vitamin therapy, has not been fully studied. [1,31].

According to the published sources, a deficiency in antithrombin III and proteins C and S imposes a significant risk of MI development [9]. Antithrombin III deficiency (both hereditary and acquired) can lead to heparin resistance at higher than usual doses of heparin required to achieve the target partial thromboplastin time or activated clotting time [10,24]. Direct inhibitors of thrombin and factor Xa do not require antithrombin III, and therefore antithrombin III deficiency should not affect their effectiveness [9,10,24].

Protein C, protein S, and antithrombin III deficiencies account for 14–25% of familial thrombotic disease cases (including systemic thrombosis), although most of these tend to be venous rather than arterial [9]. The deficiency manifests itself either in the quantity (quantitative deficiency) or in the molecular function (qualitative deficiency) of these coagulant proteins [9,24]. Protein C is a vitamin-K-dependent factor that is converted by thrombin into an active protease [9]. Once in its active form, it limits coagulation through the proteolysis of coagulation factors Va and VIIIa [13,19]. Protein S acts as a cofactor for activated protein C [9], while antithrombin III acts as an inhibitor of coagulation protease in the coagulation cascade with the exception of factors Va and VIIIa, which are regulated by proteins C and S [9,13,24]. In conjunction with each other, these factors help to maintain the delicate balance between vascular hemostasis and fibrinolysis [9]. In our study, the prognostically unfavorable role of protein C deficiency (unlike protein S) was observed in the long term for the development of overall case fatality rates and recurrent MI (Tables 5 and 6).

We also assessed the level of LP (a) as a cardiovascular risk factor. LP (a) is known to be genetically determined and is associated with premature atherosclerosis and thrombosis. The results of large genetic epidemiological studies provided strong evidence of associations between high concentrations of LP (a) and an increased risk of CAD, heart failure, and mortality [34,36–39]. Meta-analysis and subsequent publications of two large genetic epidemiological studies demonstrated the importance of LP (a) as one of the risk factors for cardiovascular events and a possible new target for treatment. [37,40–42]. Additionally, genetic epidemiological studies provided evidence of a causal relationship between high levels of LP (a) and atherosclerotic PAD at the level of 12–16% [37,40,41]. The level for the risk of venous thrombotic complications was 30% [43]. In some studies, MINOCA patients had a more favorable lipid profile than MIOCA subjects with a concomitantly similar LP (a) concentration [1,10,39,44]. In our study, LP (a) levels did not differ between groups. This could be due to the fact that the group with MIOCA included patients with single-vessel lesions rather than those with multifocal manifestations of atherosclerosis.

In regression models, the level of LP (a) demonstrated a statistically significant predictive value both in relation to early events (in-hospital case fatality rate) and concerning long-term outcomes, such as the development of one-year case fatality rates, recurrent MI, and acute cerebrovascular events (Tables 5 and 6). In light of the expected new medicines for antisense therapy and the emergence of new evidence confirming the importance of LP (a) in early atherogenesis, we propose to include LP (a) in the spectrum of the diagnostic panel in patients with MINOCA.

Other congenital conditions that may contribute to the thrombotic phenotype are elevated levels of factors VIII, IX, and XI and certain types of hereditary fibrinogen disorders [21,25,28]. Factor VIII level is partly genetically determined; however, it is strongly influenced by various clinical conditions (e.g., acute-phase proteins and venous thrombosis per se) [23]. In addition, the limit value for elevated factor VIII is the subject of debate [24]. Elevated levels of other coagulation factors are rarely considered as a cause of thrombophilia, and they are therefore not habitually investigated [13,19,39].

Limitations of the Study

We did not measure coagulation factors IX, X, XI, and XII and many other markers of thrombophilia since their clinical relevance in screening for this disorder is unclear, even though they are increasingly tested in many laboratories.

5. Conclusions

It was found that in patients with MINOCA, the prevalence of hereditary thrombophilia, folate cycle enzyme genes, and blood clotting system genes is the same as in patients with MIOCA.

The genes MTHFR 1298 A>C (rs1801131) and F1 (−455 G>A) rs1800790 were independent factors associated with hospital mortality in both groups. Genes GP IIb–IIIa

(1565 T>C) rs5918 and PAI-I (−675 5G>4G) rs1799889 were independent factors associated with hospital mortality in patients with MINOCA.

Significant long-term prognostic factors in both groups were protein C deficiency, hyperhomocysteinemia, and a content of LP (a) > 30 mg/dL. However, we did not reveal the predictive value of the polymorphisms of the studied genes representing the blood coagulation system and the folate pathway.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cardiogenetics13020006/s1, Table S1: Laboratory characteristics of patients and drug therapy; Figure S1: Kaplan–Meier survival and freedom from mortality; Figure S2: Kaplan–Meier survival and freedom from MI; Figure S3: Kaplan–Meier survival and freedom from acute cerebrovascular incident/stroke; Figure S4: Kaplan–Meier survival and freedom from hospitalization.

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