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ORIGINAL ARTICLE

Association of *MMP1* and *MMP3* haplotypes with myocardial infarction and echocardiographic parameters of the left ventricle

Tamara Djuric[1] | **Jovana Kuveljic**[1] | **Ana Djordjevic**[1] | **Milica Dekleva**[2,3] | **Goran Stankovic**[3,4] | **Aleksandra Stankovic**[1] | **Maja Zivkovic**[1]

[1]Laboratory for Radiobiology and Molecular Genetics, "Vinca" Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

[2]Department of Cardiology, University Clinical Center "Zvezdara", Belgrade, Serbia

[3]Faculty of Medicine, University of Belgrade, Belgrade, Serbia

[4]Cardiology Clinic, Clinical Center of Serbia, Belgrade, Serbia

Correspondence
Tamara Djuric, Department of Radiobiology and Molecular Genetics, "Vinca"
Institute of Nuclear Sciences – National Institute of the Republic of Serbia, University of
Belgrade, P.O. Box 522, Belgrade 11001, Serbia.
Email: tamariska@vin.bg.ac.rs

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Abstract

Background: Myocardial infarction (MI) leads to ischemia and afterward to left ventricular (LV) remodeling. Matrix metalloproteinase−1 (MMP1) and −3 (MMP3) belong to the family of endopeptidases and together they can dissolve most of the components of the extracellular matrix. *MMP1* and *MMP3* variants have been investigated solely in association with ischemic heart disease and LV dysfunction, but not in haplotype. The aims of this study were to investigate the association of haplotypes inferred from *MMP1* rs1799750 (−1607 1G/2G; NC_000011.9:g.102670497del) and *MMP3* rs35068180 (−1612 5A/6A; NC_000011.9:g.102715952dup) with MI and their effect on the change in echocardiographic parameters of LV structure and function in patients within 6 months after MI.

Methods: The study included 325 patients with the first MI and 283 healthy controls. Gene variants were detected by PCR-RFLP method. Parameters of LV structure and function were assessed by conventional 2D echocardiography, 3–5 days and 6 months after the first MI, on a subgroup of 160 patients. Haplotype analysis was performed with Thesias software.

Results: Haplotypes 2G-5A and 1G-6A were significantly and independently associated with MI compared with the reference haplotype 2G-6A (adjusted, $p = 0.009$ and $p = 0.026$, respectively). After Bonferroni correction for multiple testing, *MMP1* and *MMP3* haplotypes lost their association with the change in LV long diameter and stroke volume within 6 months after MI.

Conclusion: *MMP1* and *MMP3* haplotypes are strongly associated with MI. Further studies are needed to validate this result and to examine their association with echocardiographic parameters of LV structure and function after MI.

KEYWORDS

haplotypes, LV remodeling, MMP1, MMP3, myocardial infarction

1 | INTRODUCTION

Myocardial infarction (MI), underlined by atherosclerosis, is provoked by plaque rupture in most of the cases (Davies, 1995). Consequential ischemia leads to myocardial damage and afterwards to left ventricular (LV) remodeling. Essential part of remodeling is degradation of extracellular matrix (ECM) because it provides the entry of inflammatory cells, proliferation and differentiation of interstitial cells and the overall structure for scar formation (Spinale, 2007).

Matrix metalloproteinase 1 and 3 (MMP1 and MMP3) both belong to the family of endopeptidases and together they can dissolve most of the components of the ECM. MMP1 is a collagenase that cleaves particularly collagen type III, which is, along with collagen type I, the main component of myocardial ECM (Spinale, 2007). These fibers account for the strength of the plaque fibrous cap and provide its structural support. Collagen degradation makes the cap thinner and the plaque vulnerable (Sukhova et al., 1999), hence more prone to rupture. MMP3, stromelisyn-1, can degrade various components of the ECM and activate other MMPs, as well as its own proenzyme. It is shown that the *MMP1* gene (OMIM: 120353) with the insertion variation 1G/2G, that adds one guanine nucleotide in the promoter region creating an Ets binding site, has a higher expression (Kanamori et al., 1999; Rutter et al., 1998). 2G allele is correlated with higher mRNA and protein levels compared with the 1G allele (Cao et al., 2010; Galis et al., 1995) and has been associated with a predisposition to ischemic heart disease but also with better survival of heart failure (Velho et al., 2011). In the promoter of *MMP3* gene (OMIM: 185250) variation 5A/6A adds one adenine nucleotide (Ye et al., 1995) and has been shown to decrease transcription in vitro (Ye et al., 1996) and under in vivo conditions (Zhu et al., 2006) compared with the 5A allele. Furthermore, it has been shown that NFkB p50 and p65 subunits interact with the *MMP3* promoter in macrophages of the atherosclerotic plaque, with greater binding to the 5A allele than to the 6A allele. Reporter gene assays in transiently transfected macrophages showed that the 5A allele had greater transcriptional activity than the 6A allele (Souslova et al., 2010). 5A/5A genotype has been associated with LV systolic and diastolic dysfunction (Abd El-Aziz & Mohamed, 2016) as well as with CAD (Beton et al., 2016) and with increased risk of MI (Beyzade et al., 2003).

MMP1 variant −1607 1G/2G (rs1799750) and *MMP3* variant −1612 5A/6A (rs35068180) are insertion/deletion variants mapped in the promoters of their respective genes and according to the LDlink (Machiela & Chanock, 2015) rs35068180 is not available on genotyping chips, while *MMP1* rs1799750 is available on few Affimetrix and Illumina genotyping chips that are used in genotyping of large sample collections such as UK biobank. The two largest GWASs in the last decade, that were investigating MI as the phenotype of interest, did not find significant association of rs1799750 with MI (Deloukas et al., 2013; Hartiala et al., 2021) Nevertheless, both variants are mapped on chromosome 11q22.3, only 38 kb apart and are in moderate linkage disequilibrium ($r^2 = 0.27$, $D' = 0.54$) in CEU, according to Ensembl database (Howe et al., 2021) making them a worthy candidates for the haplotype analysis. Up to date, their haplotypes have been investigated in association with MI only in Japanese (Nojiri et al., 2003), and it is well known that Japanese population has significantly different *MMP3* 5A and 6A allele frequencies compared with CEU (Howe et al., 2021). In regard to other CVD phenotypes, their haplotypes have been analyzed only in association with stroke (Huang et al., 2017), and with CAD together with *MMP12* variants (Dalepiane et al., 2007). In Serbian population, which has not been covered by the published GWASs, these two variants have not been analyzed in association with MI.

So, the aim of this study was to investigate the association of haplotypes inferred from *MMP1* variant −1607 1G/2G (rs1799750; NC_000011.9:g.102670497del) and *MMP3* variant −1612 5A/6A (rs35068180; NC_000011.9:g.102715952dup) with MI in CAD patients with the first MI. In addition, we have analyzed their possible effect on change in echocardiographic parameters of LV structure and function on a subgroup of prospectively followed CAD patients within 6 months after the first MI.

2 | MATERIAL AND METHODS

2.1 | Study population

The study had included 680 subjects, 325 patients that survived the first MI and 283 healthy controls, all of which were unrelated Caucasians of European descent from Serbia. The samples were collected from consecutively admitted patients due to the first MI in the Coronary Care Unit in the Department of Cardiology, University Clinical Center "Zvezdara", Belgrade, Serbia ($n = 160$) and at the Cardiology Clinic, Clinical Center of Serbia, Belgrade, Serbia ($n = 165$). The 160 patients from the University Clinical Center "Zvezdara", Belgrade, Serbia, were prospectively followed up 6 months after the first MI in the same clinic. MI was diagnosed according to the World Health Organization criteria. Common inclusion criteria for the patients from both clinics were ischemic MI and stenosis >50% in at least one coronary artery assessed by angiography, which was performed in accordance with standard local practice and existing clinical practice guidelines for all the patients. Exclusion

criteria were previous MI, tumors, chronic inflammatory diseases, autoimmune disease, or renal failure. Additional exclusion criteria for the patients that were prospectively followed were as follows: age over 70 years, history of any other heart disease, significant rhythm disturbances, previous pacemaker or cardioverter-defibrillator implantation. For those patients parameters of LV structure and function, measured by conventional 2D echocardiography, were evaluated at admission and 6 months after the first MI. Demographic characteristics, co-morbidities, risk factors (hypertension, diabetes mellitus, hypercholesterolemia, cigarette smoking, and family history of cardiovascular disease) were recorded at admission. All standard biochemical analyses were performed at admission, during hospital treatment and on the day of discharge.

Control samples were collected from the individuals undergoing annual medical check-up at Occupational Medical Center, Vinča Institute of Nuclear Sciences – National Institute of the Republic of Serbia, Belgrade, Serbia. All of them underwent clinical, ultrasound, and ECG examination and those with no evidence of cerebrovascular or cardiovascular diseases, chronic inflammatory diseases, diabetes mellitus, or renal failure were included in the study.

Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, a diastolic blood pressure ≥ 90 mmHg, or current treatment with antihypertensive drugs. Subjects with a fasting glucose level of ≥7.0 mmol/ L, or taking insulin or oral hypoglycemic drugs were characterized to have DMT II.

The study was approved by the Ethics Committee of the participating medical centers and each participant gave their written informed consent to participate in the study.

2.2 | Echocardiography

For the patients that were followed up 6 months after the first MI, 2D echocardiography examinations were performed 3–5 days of admission and repeated after 6 months. Doppler-echocardiographic data were obtained using commercially available, second harmonic imaging system Toshiba XG/Artida (Toshiba Medical Systems, Japan). All echocardiographic measurements were obtained according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging (Lang et al., 2015; Schiller et al., 1989).

Using M mode images, LV end-diastolic and end-systolic diameters were assessed; LV end-diastolic and end-systolic volumes and ejection fraction were measured using the modified biplane Simpson's method from the apical four- and two-chamber views (Schiller et al., 1989).

Myocardial tissue deformation (strain) was assessed during systole by speckle tracking technique using Toshiba 2D Tissue Tracking system. End-systole was defined as an aortic valve closure in the apical long-axis view. Global longitudinal strains were calculated from three conventional apical imaging planes. Global circumferential strains were measured from basal and apical short-axis imaging planes, whereas global radial strain was obtained from short-axis view at the papillary muscle level (Geyer et al., 2010).

2.3 | Genetic analysis

Peripheral blood samples for genomic DNA isolation were collected within 3–5 days after MI. Genomic DNA was extracted from whole blood samples collected with EDTA by standardized BloodPrep® DNA Chemistry isolation kit (Applied Biosystems, Forester City, CA, US) on the ABI PRISM™ 6100 Nucleic Acid PrepStation (Applied Biosystems, Forester City, CA, US) or purified by the proteinase K/phenol extraction method. The *MMP1* rs1799750 (NC_000011.9:g.102670497del; GRCh38.p13 chr 11) and *MMP3* rs35068180 (NC_000011.9:g.102715952dup; GRCh38.p13 chr 11) were genotyped by polymerase chain reaction (PCR) on ABI 9700 (Applied Biosystems, USA) and restriction fragment length polymorphism analysis. The PCR for *MMP1* was performed by the following conditions: denaturing cycle at 95°C for 5 min followed by 35 cycles at 95°C for 30 s, annealing temperature at 54°C for 45 s and 72°C for 45 s with the final step at 72°C for 5 min, using primers 5′-TTC ACC CTC TAA TAT GAA GAG CC-3′ as forward and 5′-TCT TGG ATT GAT TTG AGA TAA GTC AGA TC-3′ as reverse. The PCR product was digested by Bgl II restriction enzyme (MBI Fermentas, Vilnius, Lithuania) (Djuric et al., 2012). Primers for *MMP3* were 5′-GAT TAC AGA CAT GGG TCA C-3′ as forward and 5′-TTT CAA TCA GGA CAA GAC GAA GTT T-3′ as reverse. The PCR conditions were the same, except for the annealing temperature at 53°C for 30 s. The PCR product was digested by Pdm I restriction enzyme (MBI Fermentas, Vilnius, Lithuania) (Djurić et al., 2005).

Approximately 10% of the samples were randomly selected and genotyped a second time by another investigator. Results in the repeated genotyping were 100% concordant with the results of the original genotyping.

2.4 | Statistical methods

Allele and genotype frequency distribution of the analyzed variants and deviation from Hardy–Weinberg equilibrium were estimated by chi-square (χ^2) test. Mean of normally

distributed continuous variables between two groups were compared by unpaired Student's *t*-test. For variables with significantly skewed distribution, comparisons were made by nonparametric Mann–Whitney U-test. Values of continuous variables were expressed as mean ± standard deviation (SD) and *p* value <0.05 were considered statistically significant for the main demographic and biochemical parameters. Association of *MMP1* and *MMP3* variants solely with MI was assessed by logistic regression analysis and has been shown as crude and adjusted odds ratio (OR) and its 95% confidence interval (CI). The OR's were adjusted for age, gender, body mass index (BMI), hypertensive status, smoking status, total cholestrol (TC), high-density lipoprotein cholesterol (HDLC), triglyceride (TG). The statistical analyses were performed using the software package Statistica Version 8.

The frequencies of *MMP1* and *MMP3* haplotypes as well as their association with the first MI or echocardiographic parameters of LV structure and function were performed by the Thesisas software (www.genecanvas.org). The program performs haplotype-based association analysis in unrelated individuals. This program is based on the maximum likelihood model and is linked to the stochastic-EM (Expectation–Maximization) algorithm (Tregouet et al., 2004). Thesias allows the simultaneous estimation of haplotype frequencies and of their associated effects on the phenotype of interest. For haplotype-phenotype association it uses the likelihood ratio test. The 95% confidence interval of the estimate is provided as well as the *p*-value associated the χ^2 test testing the nullity of this estimate. The Thesisas software was also used to estimate the LD parameters within the studied groups. We had the study power of 80% for the observed association of haplotypes with MI (number of cases $n = 325$, prevalence of heart attack in Serbia 2.5% (Jovic et al., 2016), observed disease haplotype frequencies, control to case ratio at the significance level of 0.05), which was calculated using the Power for Genetic Association Analyses (PGA) tool (Menashe et al., 2008).

p-values <0.05 were considered statistically significant, except when analyzing (a) solely *MMP1* and *MMP3* variants in the same sample groups in association with MI where Bonferroni correction for multiple testing was performed and *p* value <0.025 was considered statistically significant and (b) association of *MMP1* and *MMP3* haplotypes with 12 echocardiographic parameters of LV structure and function where $p < 0.004$ was considered statistically significant when Bonferroni correction for multiple testing was performed.

3 | RESULTS

Main characteristics of controls and patients with the first MI are shown in Table 1. MI patients were older, had a

TABLE 1 Main characteristics of controls and MI patients

Variable	Controls $n = 283$	MI patients $n = 325$	*p* value
BMI, kg/m^2	24.2 ± 3.6	27.3 ± 8.4	<0.001
Age, year	40.8 ± 14.8	57.9 ± 10.8	<0.001
TC, mmol/L	5.9 ± 1.4	5.5 ± 1.1	<0.05
TG, mmol/L	1.6 ± 1.3	1.8 ± 1.1	<0.001[#]
HDLC, mmol/L	1.3 ± 0.4	1.1 ± 0.4	<0.001
LDLC, mmol/L	3.7 ± 1.1	3.6 ± 1.0	ns
Gender M, %	50.9	75.1	<0.001[*]
Hypertension, %	11.7	58.9	<0.001[*]
Smokers, %	51.9	79.9	<0.001[*]
DMT II, %	0.0	29.7	N/A
Multivessel disease, %	0.0	54.3	N/A
STEMI, %	0.0	88.3	N/A

Note: Values are mean ± SD for BMI, Age, TC, TG, HDLC, LDLC, DMT II; *p* – mean of normally distributed continuous variables were compared by unpaired Student's *t*-test; *p* values <0.05 were considered statistically significant.

Abbreviations: BMI, body mass index; DMT II, diabetes mellitus type 2; HDLC, high density lipoproteins cholesterol; LDLC, low density lipoproteins cholesterol; N/A, not applicable; ns, non significant; TC, total cholesterol; TG, triglycerides.

[#]Mann–Whitney *U* test was used to compare values between controls and MI patients for continuous variables that had skewed distribution.

*Chi-square test was used for categorical variables.

TABLE 2 Haplotype association of the *MMP1* 1G/2G (rs1799750) and *MMP3* 5A/6A (rs35068180) variants with the first MI

Haplotype	Controls (frequency) $n = 283$	MI patients (frequency) $n = 325$	Crude OR (95% CI)	p	Adjusted OR (95% CI)	p
2G-6A	0.390249	0.306542	Ref. haplotype		Ref. haplotype	
2G-5A	0.095228	0.178895	2.26 (1.40–3.64)	0.0008	2.46 (1.25–4.85)	0.009
1G-6A	0.192739	0.235529	1.50 (1.04–2.17)	0.03	1.87 (1.08–3.24)	0.026
1G-5A	0.089612	0.114940	1.07 (0.79–1.45)	0.67	0.88 (0.58–1.35)	0.56

Note: Adjusted OR – OR was adjusted for age, gender, BMI, hypertensive status, smoking status, total cholesterol, high-density cholesterol, triglycerides.
Abbreviations: CI, confidence interval; MI, myocardial infarction; OR, odds ratio.

greater BMI and TG, lower TC, HDLC, and higher percentage of hypertensives, smokers and males.

The genotype and allele frequency distribution of the investigated genetic variants were in Hardy–Weinberg equilibrium ($p > 0.05$). There was no significant association of investigatedvariants, when analyzed individually, with the first MI. The *MMP1* rs1799750 and *MMP3* rs35068180 genotype and allele frequency distribution are presented in Table S1. The allele frequency in Serbian population for *MMP1* is exact match with gnomAD database allele frequency for non–Finnish European populations (0.47), but for *MMP3* there are no data in gnomAD. According to Ensembl the overall *MMP3* MAF in Europe is 0.45 and is similar to 0.41 in our population. The frequencies of the haplotypes inferred from *MMP1* rs1799750 and *MMP3* rs35068180 in the control group and group of patients with the first MI are presented in Table 2. Compared with the reference haplotype 2G-6A, haplotype 2G-5A was significantly and independently associated with MI (adjusted OR = 2.46, 95% CI 1.25–4.85, $p = 0.009$) as well as haplotype 1G-6A (adjusted OR = 1.87, 95% CI 1.08–3.24, $p = 0.026$). The OR's were adjusted for age, gender, BMI, hypertensive status, smoking status, TC, HDLC, TG. The study power for these associations was >80%. The LD between investigated variants in study group (combined controls and patients) was $D' = 0.34$, $r^2 = 0.11$.

We were analyzing the possible association of *MMP1* and *MMP3* haplotypes with a change in echocardiographic parameters of LV structure and function within 6 months (Δ values) in patients that were followed up for 6 months after the first MI. Main clinical characteristics for this subgroup of patients are given in Table S2. Compared with the reference haplotype 1G-6A, haplotype 2G-6A was associated with a change in LV long diameter, a parameter of LV structure (Table 3). Compared with the same reference haplotype 1G-6A, haplotypes 2G-6A and 1G-5A were associated with a change in stroke volume, a parameter of LV function (Table 4). After Bonferroni correction for multiple testing these associations lost their significance.

4 | DISCUSSION

The main finding of the study was an independent association of *MMP1* and *MMP3* haplotypes with the first MI in Serbian patients. Both MMPs belong to the family of endopepdidases, and together they can dissolve most of the components of the ECM. MMP1 is a collagenase that degrades type I and III fibrillary collagens, which are the main components of myocardial ECM (Spinale, 2007). On the other hand, MMP3 can degrade various components of the ECM and also activate other MMPs, such as full activation of pro-MMP1 (Suzuki et al., 1990), as well as its own pro-enzyme.

The data regarding the associations of *MMP1* and *MMP3* with promotion of cardiovascular diseases or their endpoints are somewhat controversial. The meta analysis that incorporated data from the studies investigating *MMP3* and *MMP-9* variants up to the year 2006 has found a significant association of the 5A allele with acute MI (Abilleira et al., 2006). Later on, the study in Caucasians from Germany that has investigated four *MMP3* haplotype tagging variants and rs35068180 (*MMP3* 5A/6A) did not find the association with prior or acute MI when analyzing variants either separately or in haplotype (Koch et al., 2010). Moreover, the same authors conducted a meta-analysis of *MMP3* 5A/6A with atherosclerotic coronary disease in patients with various cardiovascular subphenotypes (MI, coronary heart disease, CAD, or the acute coronary syndrome) and did not find the association neither in Caucasian, nor in East Asian populations (Koch et al., 2010). Nevertheless, on the protein level, the elevated baseline MMP3 levels in plasma of the patients that underwent cardiography were independently associated with 5-year-risk of AMI in men (Cavusoglu et al., 2016), and with fatal and nonfatal cardiovascular outcomes compared with stable CAD patients (Guizani et al., 2019). Considering the *MMP1* and coronary artery disease the data are scarce and also controversial. Similar to MMP3, MMP1 baseline plasma levels were an independent predictor of all-cause mortality at 5 years follow

TABLE 3 Association of *MMP1* and *MMP3* haplotypes with a change in echocardiographic parameters of LV structure within 6 months after the first MI

Echocardiographic parameter	Mean (95% CI)	*p* value
Δ LV end-diastolic diameter (mm)		
1G6A	0.11 [−1.78–2.00]	Ref
2G6A	2.44 [0.26–4.62]	0.168
1G5A	1.08 [−1.88–4.02]	0.647
2G5A	0.42 [−1.94–2.78]	0.829
Δ LV end-systolic diameter (mm)		
1G6A	0.60 [−1.82–3.02]	Ref
2G6A	0.32 [−3.56–4.22]	0.919
1G5A	−1.30 [−5.14–2.52]	0.490
2G5A	−0.30 [−3.12–2.50]	0.596
Δ LV end-diastolic volume (ml)		
1G6A	6.32[−6.66–19.30]	Ref
2G6A	2.58 [−12.40–17.56]	0.764
1G5A	−5.92 [−25.98–14.12]	0.416
2G5A	3.02 [−10.52–16.58]	0.726
Δ LV end-sistolic volume (ml)		
1G6A	6.22[−6.84–19.26]	Ref
2G6A	2.74 [−12.28–17.78]	0.782
1G5A	−5.66 [−25.80–14.46]	0.432
2G5A	2.92 [−10.66–16.50]	0.726
Δ LV long diameter (mm)		
1G6A	1.98 [−0.62–4.60]	Ref
2G6A	−2.56 [−5.64–0.48]	0.038
1G5A	−4.40 [−10.12–1.32]	0.065
2G5A	4.04 [0.46–7.60]	0.434

Note: By the Bonferroni correction for multiple testing *p* values <0.004 were considered statistically significant.

up in CAD patients (Cavusoglu et al., 2015). There was no significant association with combined endpoints (re-infarction, stroke, acute decompensated heart failure) in Caucasian patients with STEMI MI during follow-up (Pavkova Goldbergova et al., 2017), or with MI in Iranian population (Ghaderian et al., 2010). In Spanish population, *MMP1* 1G/2G, analyzed solely, was not associated with the first acute MI in male patients before 55 years of age, while in haplotype with other two *MMP1* promoter variants 2G allele was associated with significant risk for MI (Román-García et al., 2009). In Brazilian patients with systolic heart failure the 2G allele carriers were related to a higher prevalence of ischemic etiology and better heart failure–related prognosis (Velho et al., 2011).

In this study, when analyzed solely neither *MMP3* rs3506818 nor *MMP1* rs1799750 were significantly associated with MI. Only in haplotype analysis 2G-5A and 1G-6A haplotypes were significantly and independently associated with MI compared with the reference haplotype 2G-6A, set by Thesias software. It was shown that the *MMP1* gene with the insertion variation (2G allele), that adds one guanine nucleotide in the promoter region, creates the binding site for ETS family of transcription factors (Rutter et al., 1998), and is correlated with higher mRNA and protein levels compared with the 1G allele (Cao et al., 2010; Galis et al., 1995), which could lead to higher ECM degradation. Variation 5A/6A in the promoter of the *MMP3* gene adds one adenine nucleotide (Ye et al., 1995) and has been shown to decrease transcription in vitro (Ye et al., 1996) and under in vivo conditions (Zhu et al., 2006) compared with the 5A allele, leading to the accumulation of the ECM and progression of atherosclerosis. *MMP3* mRNA and protein levels has been found to be higher in individuals who are homozygous for the 5A allele than in those who are homozygous for the 6A allele, while heterozygous individuals had intermediate levels of MMP3 expression in arterial tissues (Medley et al., 2003). Moreover, it has been shown that NFkB, as a key regulator of inflammation, interact with the *MMP3* gene promoter. This transcription factor binds more readily to the

TABLE 4 Association of *MMP1* and *MMP3* haplotypes with a change in echocardiographic parameters of LV function within 6 months after the first MI

Echocardiographic parameter	Mean (95% CI)	*p* value
Δ LV short diameter (mm)		
1G6A	−0.18 [−4.12–3.76]	Ref
2G6A	1.16 [−5.34–7.64]	0.749
1G5A	1.16 [−4.14–6.40]	0.698
2G5A	1.18 [−3.34–5.70]	0.697
Δ LV ejection fraction (%)		
1G6A	1.78 [−0.90–4.46]	Ref
2G6A	2.48 [1.24–6.20]	0.786
1G5A	2.28 [2.42–6.98]	0.879
2G5A	−0.70 [−4.28–2.88]	0.281
Δ Stroke volume (ml)		
1G6A	−1.64 [−9.02–5.74]	Ref
2G6A	14.64 [4.32–24.94]	0.015
1G5A	14.76 [2.48–27.06]	0.048
2G5A	0.62 [−8.64–9.88]	0.712
Δ Apical circumferential strain (%)		
1G6A	−0.26 [−3.06–2.54]	Ref
2G6A	1.37 [−2.16–4.90]	0.515
1G5A	5.93 [0.90–10.96]	0.059
2G5A	1.08 [−1.62–3.80]	0.539
Δ Basal circumferential strain (%)		
1G6A	0.55 [−1.42–2.52]	Ref
2G6A	2.41 [−0.54–5.34]	0.366
1G5A	2.18 [−2.14–6.50]	0.557
2G5A	1.41 [−1.86–4.68]	0.631
Δ Global longitudinal strain (%)		
1G6A	−0.49 [−1.96–0.98]	Ref
2G6A	−0.47 [−3.30–2.36]	0.991
1G5A	−0.42 [−2.60–1.76]	0.964
2G5A	−0.84 [−3.00–1.32]	0.794
Δ Global radial strain (%)		
1G6A	3.08 [−1.04–7.18]	Ref
2G6A	1.81 [−4.66–8.28]	0.772
1G5A	0.85 [−5.80–7.50]	0.647
2G5A	1.24 [−4.20–6.68]	0.566

Note: By the Bonferroni correction for multiple testing *p* values <0.004 were considered statistically significant.

5A allele than the 6A allele and colocalize with MMP3 in macrophages and smooth muscle cells in atherosclerotic plaques (Souslova et al., 2010). So, the 6A allele so far was associated with the progression of atherosclerosis (Djurić et al., 2008; Hirashiki et al., 2003), while the 5A allele, was associated with MI (Liu et al., 2006). In light of our results, it seems that the haplotype made of 2G and 5A alleles, which are both associated with higher expression and proteolytic activity of MMP1 and MMP3, are bearing the true risk for MI. Excessive ECM degradation makes atherosclerotic plaques more vulnerable and prone to sudden rupture, which is the main cause of MI worldwide (Davies, 1995). The observed, weaker association of the haplotype inferred from 1G and 6A alleles with MI, could be due to the lower promoter activity of both *MMPs* and accumulation of the ECM, leading to plaque development and overall progression of atherosclerosis in coronary arteries. It is of note, that all of MI patients investigated in

this study had an ischemic coronary artery disease (more than 50% stenosis) and had STEMI MI as a consequence. Moreover, 54% of them had multivessel disease, which means that majority of the patients had advanced atherosclerosis prior to MI.

After MI, a highly regulating process of cardiac repair/remodeling follows the necrotic loss of cardiomyocites. With regard to their function in ECM turnover, MMP1 and MMP3 have also been analyzed in a context of left ventricle remodeling. It has been shown that accumulation of collagen, or decreased colagenolytic effect, has been correlated with LV dysfunction (Mukherjee & Sen, 1990). Ongoing cells loss with collagen replacement after MI may contribute to deterioration in cardiac geometry and function (Fomovsky et al., 2010). So, we have performed analysis of change in echocardiographic parameters of LV structure and function (LV diameters, volumes, strains, stroke volume and ejection fraction) within 6 months after the MI. We have shown association of haplotypes 2G-6A and 1G-5A with decrease of the LV long diameter and 2G-6A with increase of stroke volume compared with the 1G-6A reference haplotype. Although, this association was not significant after Bonferoni correction for multiple testing, it seems that haplotypes containing combination of alleles that affect *MMP1* and *MMP3* expression in opposite direction have better prognosis for LV diameters and stroke volume, compared with the reference haplotype 1G-6A. Still, a recent study has shown association of the *MMP3* 5A/5A genotype with LV systolic and diastolic dysfunction in 112 males with AMI, after 6 months of follow-up compared with 6A/6A (Abd El-Aziz & Mohamed, 2016). This discrepancy in the results, beyond the fact that we have analyzed the haplotypes of two *MMP* gene variations, rather than single one, opens the discussion about the dual role that collagen could have at LV remodeling. On the one hand, collagen accumulation may partly result in a stiff LV with dominant diastolic LV dysfunction, but on the other hand, an insufficient collagen deposition may lead to LV thinning and dilation with dominant systolic LV failure (Frangogiannis, 2019). Still, much more research is needed, on a larger sample size, and including other *MMPs* associated with cardiovascular diseases as well as their tissue inhibitors (*TIMPs*) to deepen the knowledge and unravel the complex networks of MMPs and ECM remodeling in humans.

This study has certain limitations that need to be addressed. The number of patients that have been followed up has been rather small to elucidate the true association of the investigated variants with echocardiographic parameters of LV structure and function 6 months after MI. Even though these variants have been recognized as functional ones, analysis of their effect on the protein level in this study would give additional value in analysis of their involvement in MI and subsequent remodeling process. MMP1 and MMP3 cover great deal of ECM remodeling in the heart and blood vessels, still there are other MMPs and TIMPs that could be included in the research of ischemic heart disease and its end points. Nevertheless, haplotype analysis gives a more accurate estimation of the possible associations of genetic variants with phenotype of interest than investigation of single variants.

In conclusion, we report strong and independent association of *MMP1* and *MMP3* haplotypes with the first MI in patients from Serbia. Further research should be focused on greater sample size and multiple *MMPs* and *TIMPs* variants. These markers could be promising targets for preventive screening and risk assessments of MI.

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AUTHOR CONTRIBUTIONS

Study Design: Tamara Djuric. Data Collection: Milica Dekleva, Goran Stankovic, Ana Djordjevic. Statistical Analysis: Jovana Kuveljic, Tamara Djuric. Data Interpretation: Tamara Djuric, Jovana Kuveljic, Ana Djordjevic. Manuscript Preparation: Tamara Djuric, Jovana Kuveljic, Maja Zivkovic. Literature Search: Jovana Kuveljic, Tamara Djuric. Funds Collection: Maja Zivkovic, Aleksandra Stankovic.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Tamara Djuric https://orcid.org/0000-0002-9857-9828
Ana Djordjevic https://orcid.org/0000-0002-0316-8461

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