

«Tempori parce»

**ASSOCIATION OF MATRIX GLA-PROTEIN GENE ALLELIC
POLYMORPHISMS WITH ACUTE CORONARY SYNDROME IN THE
UKRAINIAN POPULATION**

2012

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Introduction

Based on the figures of Ukrainian statistics, like in majority of countries all over the world, cardio-vascular diseases take the first place in sickness and mortality rate in population. Among them, there are arteriosclerosis and its complications (myocardial infarction, aortic aneurysm, insult, thromboembolism etc.) [1]. WHO research shows that traditional approaches in the treatment of these and other common multifactorial diseases are ineffective and lead to substantial economic costs [2]. The problem is low efficiency of therapeutic and preventive measures related to the absence of etiological orientation due to insufficient understanding of the fundamental mechanisms that form the overwhelming majority of multifactorial diseases. Recently, research efforts of our foreign scientists have focused on the causes and mechanisms of these diseases. It was proved that compound coordinated action of genetic factors and environment are fundamental for forming these diseases [3].

With the development of molecular genetic technologies there have been ample opportunities for studying the genetic components of diseases. Today, a significant amount of accumulated data on the participation of various polymorphic genes has predispositioned the formation of multifactorial pathology [4].

However, inspite of the success of the global scientific community in understanding the human genome to have a relatively small number of genes, all of which are only partially explained in some of the links in the pathogenesis of myocardial infarction. Relative to the Ukrainian population such data are inconsiderable and disputable.

Considering that MGP probably brings effects on artery calcification development which is considered to be one of the main signs of degeneration process, it can be assumed that the genetic polymorphism of the promoter region may effect on the level of gene expression, MGP, and the variability of the 4th exon - the qualitative characteristics of the protein. Published data on this subject are ambiguous and contradictory [5 - 10].

OBJECTIVE:

To study distribution allelic variants of *MGP* T⁻¹³⁸→C (rs1800802) and G⁻⁷→A (rs1800801) promoter, Thr₈₃→Ala exon 4 (rs4236) polymorphisms in patients with acute coronary syndrome in the Ukrainian population.

RESEARCH OBJECTIVES:

- 1 To study distribution of allelic variants of *MGP* (SNP) polymorphisms in healthy patients - representatives of the Ukrainian population
2. To research the frequency of allelic polymorphisms of *MGP* in patients with acute coronary syndrome.
3. Perform a statistic analysis of the connection of different variants of genotype with the development of the acute coronary syndrome.

METHODS:

1. DNA extraction.
2. Polymerase chain reaction and restriction fragment length polymorphism (RFLP)
3. Electrophoresis of DNA amplified fragment
4. Statistical analysis was made with SPSS 17.0.

THE PRACTICAL SIGNIFICANCE OF THE STUDY:

The obtained results can be used as a basis for identifying people who are predisposed to the development of the SCS with intention of prevention of the diseases and prevent development of complications.

LITERATURE REVIEW

MGP Biochemistry

Human MGP molecule (molecular mass 10 kDa) consists of 84 aminoacids residues, 5 of them are γ - carboxyglutamic acids (Gla) (Fig. 1). Five forms of MGP were detached from several bones of rats which contained 79 and 83 residues while 5 and 1 residues are missing at the C terminus [11,12,13].

Thou in contrast to all known K- dependent proteins, MGP doesn't have propeptide form [13].

Although MGP contains a high percentage of hydrophilic residues, it is exceptionally water-insoluble (solubility <10 pg/ml), that is why its transport by plasma can be realized only in compound action with soluble proteins.

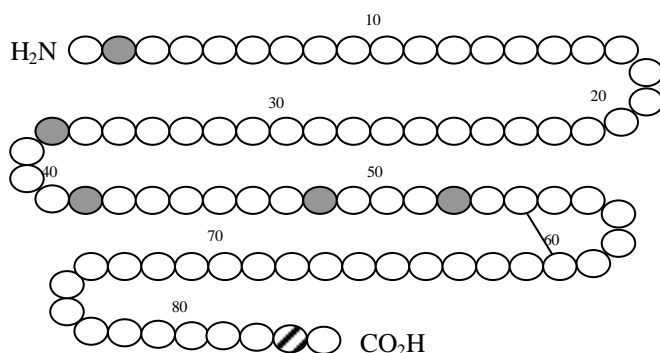


Figure 1. Scheme of the peptide chain of the molecule MGP.

Dark circles - glutamic acid residues, which have γ -carboxylation. In position 83 there may be threonine or alanine (polymorphism Tht83Ala)

The MGP molecule has been synthesized, cDNA structure predicts an 84-residue mature protein and a 19-residue transmembrane signal peptide and also contains 3 functional areas from N terminus: (1) transmembrane signal peptide, (2) putative recognition site for γ - carboxylase, (3) Gla- containing domain [13].

MGP formed in the cells undergoes post-translational modification, which consists of the carboxylation of five residues of glutamic acid (Glu) to form γ - carboxyglutamic acids (Gla). This reaction is catalyzed by the enzyme γ - glutamyl carboxylase (GGCX) and is connected with the oxidation of the reduced form of vitamin K (hydroquinone) into the 2,3-epoxide of vitamin K (Fig. 2). Thus, oxidation

of vitamin K can not occur without of Glu-residues MGP molecule carboxylation. In turn enough vitamin K for carboxylation of MGP reaction depends on the feedback of its reduction which is implemented by means of vitamin K- epoxide reducing complex (VKOR).

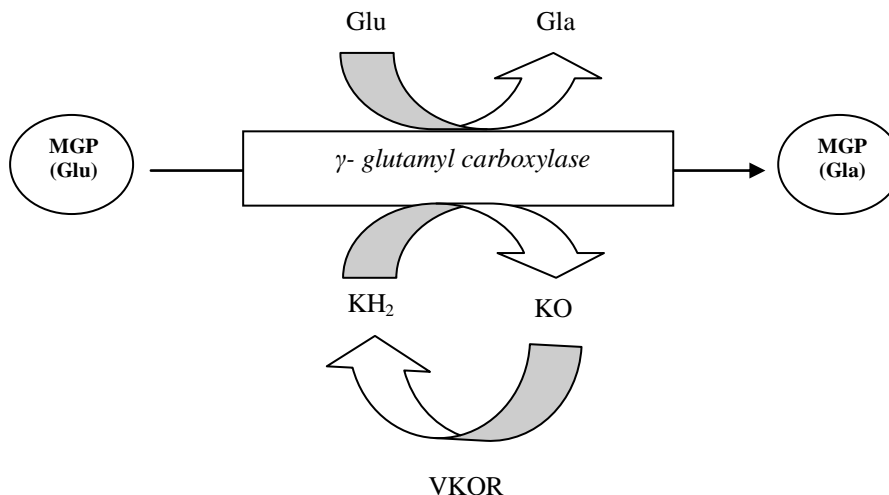


Figure 2. γ -carboxylation of MGP and vitamin K cycle
 KH₂ - reduced form of vitamin K (hydroquinone), KO - oxidized form of vitamin K (epoxide); VKOR - Vitamin K epoxide reductase

In addition to γ - carboxylation, MGP can undergo different posttranslation modifications, among them (1) specific proteolytic decomposition in C terminal molecule area [14,15], and (2) three serine residues phosphorylation in N terminal area [16].

Following the above reactions, MGP accumulates in the structures of the Golgi apparatus and then it is secreted in the extracellular space where its function is realized.

MGP gene

MGP gene in humans is represented by one copy of which is contained in the short arm of chromosome 12 (12p12.3-13.1) [13]. It is encoded with 84 amino acid residues of the mature protein and 19 residues of transmembrane signal peptides. The length of the gene has 3900 nucleotides and consists of four exons separated by three large intermediate sequences (introns) which account for more than 80% of the total

length of the gene [13] (Fig. 3). Each of the three functional regions of the protein has (1) transmembrane signal peptide (2) recognition site γ -carboxylase (3) the domain containing the residues of Gla – is encoded by separate exons of the MGP gene (exons 1,3,4). Exon 2 encodes a part of a protein, that consists of 11 amino acid residues (α -helical domain) and lies between the transmembrane and signal peptide recognition site γ -carboxylase. The function of MGP site is not known yet.

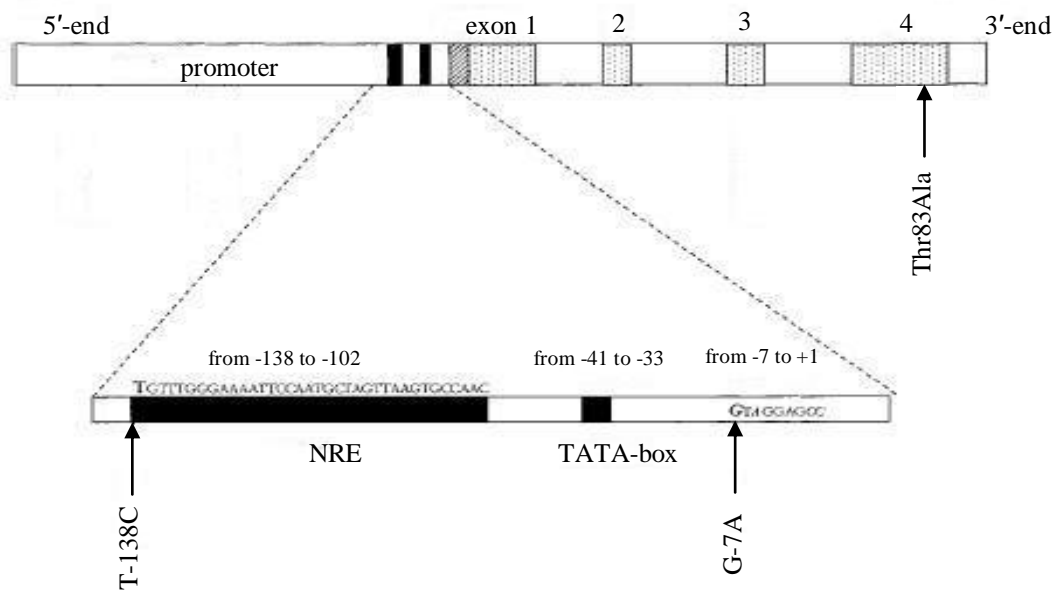


Figure 3. The structure of the gene and localization of MGP single nucleotide polymorphisms, three of his.
NRE – negative-responsive element

Such a 4-exon organization is typical for the osteocalcin gene (BGP). It differs substantially from the 2-exon organization of genes that encodes for the corresponding sections in other well-known vitamin K-dependent proteins.

Analysis of the promoter of the gene MGP showed that along with the typical TATA and CAT-boxes, it contains the control sequences (putative regulatory sequences) homologous to the previously identified elements responsible for the action of hormones and transcription factors (hormone and transcription factor responsive elements). In particular, it identifies two areas of the promoter containing potential binding sites for retinoic acid receptor and vitamin D [13].

MGP gene polymorphism

Today, more than 120 types of single nucleotide polymorphism (SNP) in the gene for human MGP are described. Among them, three types of polymorphism are the most explored, because of their association with various diseases: (1) T-138C (rs 1800802), (2) G-7A (rs 1800801), and (3) Ala83Thr (rs 4236) (Fig. 3).

Polymorphism T-138C which is a promoter as part of the gene has a site that forms complexes with nuclear proteins and takes their regulatory impact; G-7A is localized in the initial segment of the promoter from which starts the process of transcription; Thr83Ala - in the fourth exon encodes for Gla-domain. The last variant SNP determine the replacement of threonine into the alanine in the penultimate 83 molecules of MGP.

The question of how different types of MGP gene polymorphism affect its expression and the ability to perceive a variety of regulatory influences is now the focus of researchers. As one of the methods of approach to the solution, the incorporation of genetic constructs into the cultured cells containing the "normal" and "pathological" promoter variants of MGP and luciferase gene (luciferase test) is used.

The first kind of a research was fulfilled by Herrmann et al. [5]. The authors showed that the G-7A polymorphism does not affect the promoter activity of the gene MGP, meanwhile promoter activity with a minor allele of -138C (pathological variant), when compared with -138T (normal variant), was less than 20% in the MMC of rat vessels and 50% in cultured human fibroblasts.

Quite different results were obtained in the *Farzaneh et al* research. [6]. The authors found that the promoter polymorphism G-7A and T-138C significantly alter the transcriptional activity of a MGP gene in MMC of rats vessels in vitro. Thus, the promoter variant with minor allele -7A revealed activity in 1.5-times higher than -7G, and -138C variant was 4 times as active as -138T.

Analysis of the MGP gene promoter showed that the T-138C polymorphism regard to the site which is critical for the processes of transcription in vascular MMC. Here, in the position between -142 and -136 is an element that can bind the

activation protein-1 (AP-1). It is ascertained, that the T-138C polymorphism changes the binding site of the promoter with AP-1 complex. Alternative promoter allele-138T links good complexes AP-1, composed of *c-Jun*, *JunB*, *Fra-1* and *Fra-2* and activated phorbol compounds, while the ability to bind the AP-1 and subsequent activation of a promoter with -138C allele is very low [6].

The above data confirmed the work of *Kobayashi et al.* [7] which found that the promoter variant-138T in contrast to the-138C can form complexes with nuclear proteins (AP-1). However, what concerns us with the activity of these variants are the Japanese researchers who came to quite different than *Farzaneh et al.* conclusions: the introduction of MGP gene promoter in cultured cancer cells of human mammary gland have promoter activity -138T allele that was significantly higher than with the allele -138C.

Thus, the contradictory data about the effect of different types of MGP gene polymorphism on its transcription activity indicate the complexity of the problem and require further research in this direction.

MGP and calcification of the vascular wall

The presence of Gla-containing proteins in the vascular wall was first shown by *J.B. Lian et al.* [37] who extracted the Gla amino acid from alkaline hydrolysates of calcified atheromatous plaques of human aorta. There was no Gla in the hydrolysates of uninjured vessels and in calcified atherosclerotic plaques which gave grounds for the finding of a close connection between the Gla-containing proteins and processes of ectopic calcification.

R. L. Levy et al. [38] with EDTA-extraction isolated protein fraction containing Gla from atherosclerotic arteries. Low level of this fraction of protein was typical for the fatty strips and plaque, meanwhile in the calcified plaques its rate was considerable. The authors assumed that they had discovered a unique Gla-protein called atherocalcinom (molecular mass 80 kDa ,19 Gla-residues for 1000 amino acids). Subsequently, later the authors reported that atherocalcin was an artifact caused by contamination of blood vessels with serum proteins [39].

After the discovery of MGP, it has been shown that Gla-containing proteins are represented by this protein in the walls of blood vessels [40]. In the arterial wall the MGP is synthesized in MMC of media and intima, in areas of atherosclerotic affected areas and macrophages [41]. Using monoclonal antibodies it was shown that in normal artery wall of human MGP is associated with MMC and elastic membranes in the media and with the extracellular matrix in the adventitia [42]. It was found that MGP is related to different types of arterial calcification.

MGP and atherosclerosis. Calcification of atheromatous plaques is one of the process that completes the development of degenerative changes in the intima [43-45]. The research of accumulation and the expression of MGP in the human plaques showed that the protein molecules have a close spatial connection with the places of hydroxyapatite deposits: they were found on the border with the calcification of the cells [41,42]. However, MGP gene expression (corresponding to the formation of mRNA) in the MMC of atheromatous plaques was lower than with normal MMC constitutively expressing this protein. At the same time in plaques MMC began synthesis proteins related to the processes of osteo/chondrogenesis (osteocalcin, bone sialoprotein, alkaline phosphatase) and normally can not be synthesized in the arterial wall. These data gave reason to assume that the mineralization patterns of the vascular wall may be the result of an imbalance between procalcitonin (osteo / chondrogenesis) and anticalcitonin genic factors. MGP belongs to this group. [41].

MGP and Mönkenberg's arteriosclerosis. The close spatial connection between the MGP and cell calcification was found in the arteries of amputated extremities of diabetic patients. The deposition of calcium salts in the tunica media of arteries (arteriosclerosis Mönkenberga) was accompanied, as in case of atherosclerosis, with a decrease of MGP gene expression in MMC vessels [41,42]. Against the background of these changes MMC began to express the osteogenic proteins (see above). For Mönkenberg's arteriosclerosis the close relationship of MGP sites and elastic membranes, in places of vascular calcification disappears but in humans and in rats a significant amount of MGP were found in the extracellular matrix at the boundary of media with centre of mineralization [42].

MGP and calcification of MMC of blood vessels in vitro. When vascular MMC is cultured, it loses its contractile phenotype and gets the features of the modified MMC (migration, proliferation, synthesis of connective tissue components), characteristics of MMC atherosclerotic plaques. Later, vascular MMC form multi-cellular units in vitro which are spontaneous calcificated after 30 days. Since the first signs of this process MGP gene expression and some of osteogenic protein (osteocalcin, bone sialoproteins) increases in MMC [34,46,47]. On the other hand, there is a data that in the simulation of vascular MMC calcification, MGP expression in these cells is reduced [48]. It returns to its original level if the process of mineralization is inhibited with bisphosphonates.

Thus, based on the fact that the expression of MGP in calcification process may decrease as well as rise, it has been suggested two possible versions of events. The first is that the factors that inhibit MGP gene expression, may promote vascular mineralization. Second - in the case of initiation of calcification mechanisms it may increase the expression of adaptive proteins that limit this process. It is considered, that MGP is one of such protein. [46].

In addition to influence on the development of cardiovascular diseases, MGP allelic polymorphism may be related to some other illnesses and pathological processes such as kidney stones [49], osteoporosis [50,51], tooth loss [52] and plumbum intoxication [53, 54].

MATERIALS AND METHODS

Into the study were taken 115 patients (70.0% males and 30.0% females) with ACS 40 to 83 years old (mean age 58.5 ± 0.7 years) hospitalized to the reanimation and intensive therapy department of National Scientific Center "M.D. Strazhesko Institute of Cardiology", National Medical Academy of Science, Ukraine. Unstable angina pectoris (UAP) and acute myocardial infarction (MI) as a final diagnosis were established in 41 % and 59 % of patients, respectively. In 23 patients MI with no peak Q in electrocardiogram (ECG) was diagnosed while in 45 patients with MI peak Q was present in ECG. Diagnosis of acute MI and UAP were established on the basis of clinical, electrocardiography and biochemical examinations according to the recommendations of WHO experts and also according to recommendations of European and American cardiologic societies [55-57]. Patients with hereditary and innate diseases, severe metabolic pathologies including a severe form of diabetes mellitus, marked renal and liver failures, deficiencies of the haemostatic system, oncology and systemic pathologies, chronic heart failure of IIB-III stage, true cardiogenic shock were not included to the studied group. The control group consisted of 110 clinically healthy individuals with the absence of cardio-vascular pathologies, as confirmed by anamnesis, ECG examination, and measurement of arterial pressure and biochemical data. The control group and a group of patients did not differ by age and the ratio of persons of both sexes ($P > 0,05$ for the χ^2 -test).

Blood sampling was performed under sterile conditions into 2.7 ml monovettes containing EDTA potassium salt as an anticoagulant (Sarstedt, Germany), samples were frozen and stored at -20°C . DNA for genotyping was extracted from the venous blood using Isogene kits (Russia) according to manufacturer's protocol.

MGP T⁻¹³⁸→C polymorphism (rs1800802) was determined using PCR with subsequent analysis of restriction fragments length. For this aim a certain site of the *MGP* gene promoter was amplified using a pair of specific primers (Table 1).

PCR was performed for 33 cycles in a 25 μl volume containing 50-100 ng of DNA, 5 μl 5X PCR-buffer, 1.5 mM magnesium sulfate, 200 μM of each dNTP, 20 pM of each primer and 0.5U of *Taq* DNA polymerase ("Fermentas", Lithuania). PCR

was carried out in a thermocycler GeneAmp PCR System 2700 ("Applied Biosystems", USA). 6 μ l of the PCR products (142 bp) were subjected to digestion with 3U *Bse*MI ("Fermentas", Lithuania) and incubated at 37°C for 18 hours. Presence of thymine at the -138 position of promoter prevented restriction, and in the case of substitution for cytosine *Bse*MI cleaves the amplified fragment of the promoter into two fragments – 118 and 24 bp (Fig. 4 A).

MGP Promoter G⁻⁷→A Polymorphism (rs1800801). PCR was performed using primers (Table 1) for 33 cycles in a 25 μ l volume containing 50-100 ng of DNA, 5 μ l 5X PCR-buffer, 1.5 mM magnesium sulfate, 200 μ M of each dNTP, 20 pM of each primer and 0.5U of *Taq* DNA polymerase ("Fermentas", Lithuania). 6 μ l of the PCR product (500 bp) were subjected to digestion with 2U *Nco*I ("Fermentas", Lithuania) and incubated at 37°C for 18 hours. In the presence of guanine at the -7 position of promoter, *Nco*I restriction enzyme produces two fragments of 240 and 260 bp in length. Substitution of guanine for adenine prevents restriction and the amplified fragment of the promoter (500 bp) can not be cleaved (Fig. 4 B).

MGP Exon 4 Thr₈₃→Ala Polymorphism (rs4236). PCR was performed using primers (Table 1) for 33 cycles in a 25 μ l volume containing 50-100 ng of DNA, 5 μ l 5X PCR-buffer, 1.5 mM magnesium sulfate, 200 μ M of each dNTP, 20 pM of each primer and 0.5U of *Taq* DNA polymerase ("Fermentas", Lithuania). 6 μ l of the PCR product (173 bp) were subjected to digestion with 3U *Eco*477 ("Fermentas", Lithuania) and incubated at 37°C for 18 hours. Presence of adenine at the 3748 position of exon 4 prevented digestion, and in the case of substitution for thymine *Eco*477 cleaves the amplified fragment of exon 4 into two fragments – 127 and 46 bp (Fig. 4 C).

Table 1.

Details of PCR and RFLP for polymorphism analysis.

Polymorphisms	Primers	Annealing temperature (time)	PCR product size (bp)	Restriction enzymes	Fragments after restriction
T ⁻¹³⁸ →C (rs1800802)	(F) 5`-AAGCATACGATGGCCAAAACCTTCTGCA-3` (R) 5`-GAACTAGCATTGGAACCTTTCCCAACC-3`	57°C (1 min)	142 bp	<i>BseNI</i>	118 and 24 bp
G ⁻⁷ →A (rs1800801)	(F) 5`-CTAGTTCAGTGCCAACCCTTCCCCACC-3` (R) 5`-TAGCAGCAGTAGGGAGAGAGGCTCCCA-3`	64,5°C (45 s)	500 bp	<i>NcoI</i>	240 and 260 bp
Thr ₈₃ →Ala (rs4236)	(F) 5`-TCAATAGGGAAGCCTGTGATG-3`. (R) 5`-AGGGGGATACAAAATCAGGTG -3`	64,5°C (45 s)	173 bp	<i>Eco477</i>	127 and 46 bp

The RFLP results were analyzed by electrophoresis on an ethidium bromide-stained 2.5% agarose gel and detected by UV transillumination ("Biocom", Russia).

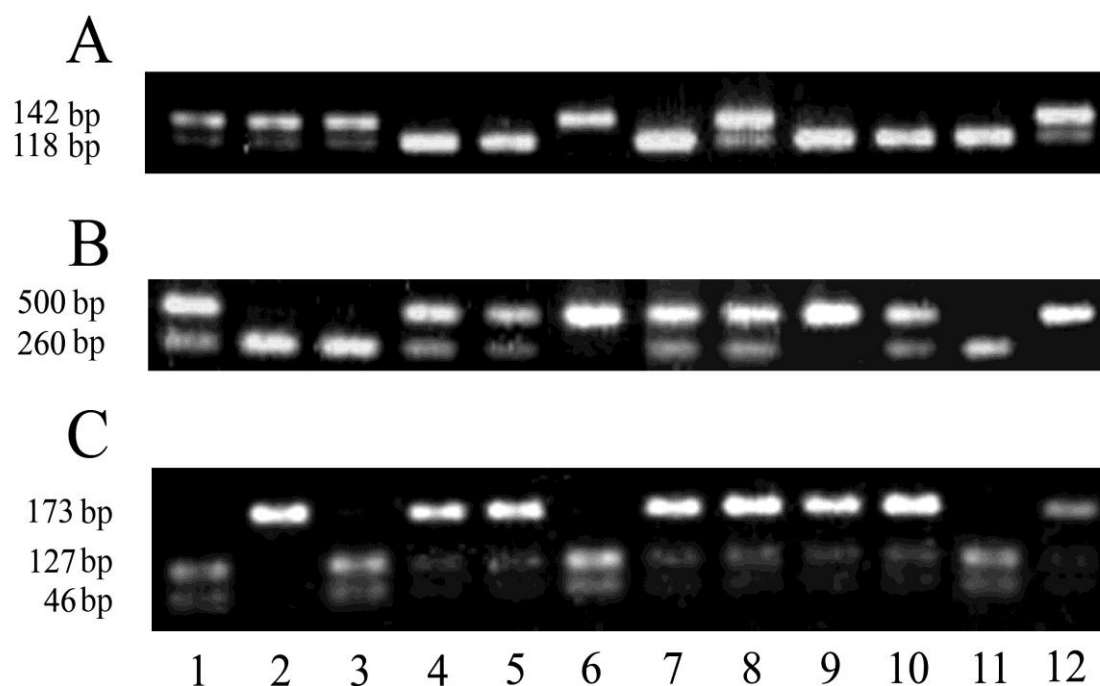


Fig. 4. Results of electrophoresis of restriction fragments of amplification products of *MGP* polymorphisms.

A – T⁻¹³⁸→C: lines 4, 5, 7, 9-11 - T/T genotype, 1-3, 8, 12 – T/C genotype, 6 – C/C genotype;
 B - G⁻⁷→A: lines 2, 3, 11 - G/G genotype, 1, 4, 5, 7, 8, 10 - G/A genotype, 6, 9, 12 - A/A genotype;
 C - Thr₈₃→Ala: lines 1, 3, 6, 11 - Thr/Thr genotype, 4, 5, 7-10, 12 - Thr/Ala genotype, 2 - Ala/Ala genotype.

Results and Discussion

Clinical characteristics of studied groups of 115 patients with ACS and 140 healthy humans are summarized in table 2. No differences between the groups were noted with respect to gender, age and glucose concentration. The prevalence of atherogenic risk factors (including cigarette smoking, overweight, hypertension, total cholesterol) was significantly higher in the patient group.

Table 2.

Clinical parameters of acute coronary syndrome (ACS) and control subjects
(mean and \pm SE)

Parameter	Acute Coronary Syndrome (n=115)	Healthy Subjects (n=140)
Sex, male/female	93/22	78/32
Age, years	58.7 \pm 0.5	54.0 \pm 0.8
Current smokers, %	36.5	25.7
BMI, kg/m ²	28.9 \pm 0.41	23.8 \pm 1.20 *
SBP, mm Hg	145.8 \pm 2.6	131.6 \pm 8.3 *
DBP, mm Hg	88.4 \pm 1.5	72.8 \pm 9.7 *
T-Cho, mmol/L	6.3 \pm 0.13	5.6 \pm 0.25 *
HDL-Cho, mmol/L	1.3 \pm 0.05	1.4 \pm 0.10
Glucose, mmol/L	5.8 \pm 0.6	4.7 \pm 0.7 *

BMI – body mass index, SBP – systolic blood pressure, DBP - diastolic blood pressure, T-Cho – total cholesterol, HDL-Cho – cholesterol of high density lipoproteins. * - p < .05

Association between Manifestations of ACS and Polymorphisms of MGP Gene. Results of genotyping of the patients by 3 sites of *MGP* are summarized in table 3.

Table 3.

Comparison of genotype for several polymorphisms of *MGP* gene in patients with acute coronary syndrome (ACS) and control subjects

Genotype	Promoter T ⁻¹³⁸ →C		Promoter G ⁻⁷ →A		Exon 4 Thr ₈₃ →Ala	
	Control (n=139)	ACS (n=107)	Control (n=140)	ACS (n=114)	Control (n=128)	ACS (n=115)
AA, n (%)	75 (54.0)	64 (59.8)	71 (50.7)	48 (42.1)	58 (45.3)	49 (42.6)
Aa, n (%)	57 (41.0)	35 (32.7)	63 (45.0)	52 (45.6)	55 (43.0)	50 (43.5)
aa, n (%)	7 (5.0)	8 (7.5)	6 (4.3)	14 (12.3)	15 (11.7)	16 (13.9)

Values are n (%). A – major allele, a – minor allele

As it can be seen, distribution of major allele homozygous, heterozygotes and minor allele homozygotes while analyzing G⁻⁷→A polymorphism of the

promoter was 42.1%, 45.6%, 12.3% correspondingly (in the control group 50.7%, 45.0%, 4.3%). Analysis of T⁻¹³⁸→C promoter polymorphism has shown that the respective figures were 59.8%, 32.7%, 7.5% (in control group – 54.0%, 41.0%, 5.0%) while at the assay of Thr₈₃→Ala polymorphism (exon 4) these were 42.6%, 43.5%, 13.9% (in control group – 45.3%, 43.0%, 11.7%). In the group of healthy individuals a minor allele variant A/A (G⁻⁷→A polymorphism) was observed in 2.9-folds more rare than in ACS. Using logistic regression analysis (table 4) it was estimated that A/A genotype (G⁻⁷→A polymorphism) was significantly (p=.02) associated with ACS (OR=4.302; 95% CI, 1.262 - 14.673) (Fig. 5)

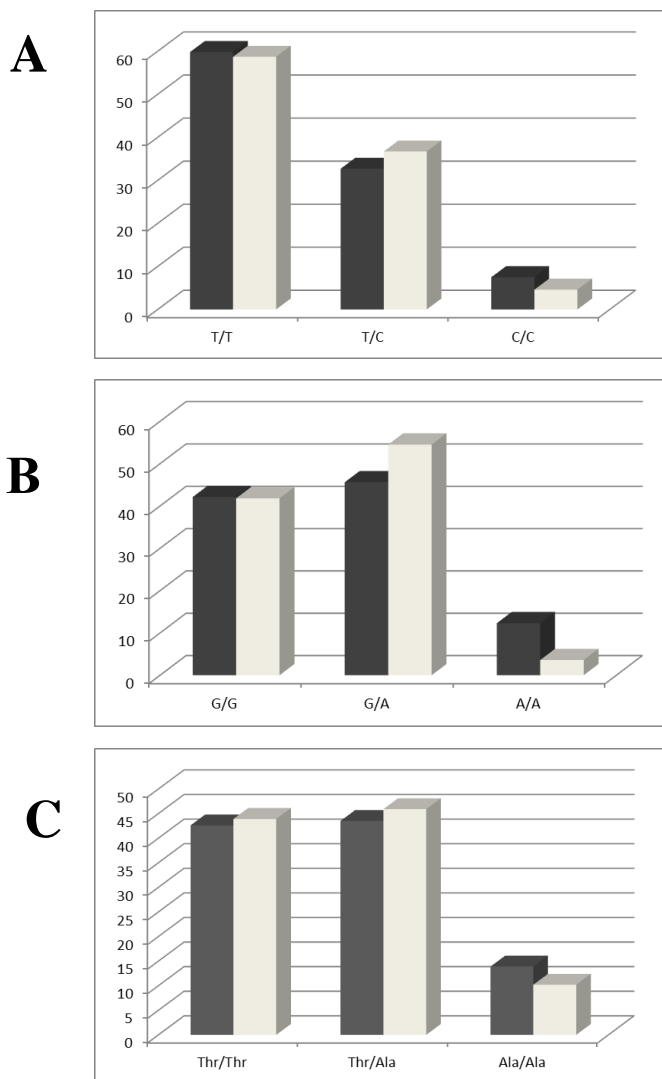


Fig. 5. Determination of the frequency of different genotypes with MGP gene allelic polymorphism in the genes of healthy individuals (light columns) and patients with acute coronary syndrome (dark columns): A - T-138 → C polymorphism, B - G-7 → A polymorphism; C - Thr83 → Ala polymorphism

Table 4.

Results of logistic regression analysis

SNP	Genotype	Coefficient of regression	SE	Wald statistics	P - value	OR	95% CI for	95% CI for
							OR lower	OR upper
Promoter	T/C	-.185	.287	.416	.519	.831	.474	1.458
T ⁻¹³⁸ →C	C/C	.502	.584	.738	.390	1.651	.526	5.189
Promoter	G/A	.431	.351	1.502	.220	1.538	.773	3.064
G ⁻⁷ →A	A/A	1.459	.626	5.434	.020	4.302	1.262	14.673
Exon 4	Thr/Ala	-.247	.358	.479	.489	.781	.387	1.574
Thr ₈₃ →Ala	Ala/Ala	-.402	.509	.623	.430	.669	.247	1.814

Homozygotes by major allele were considered as a reference group.

SE – standard error, OR – odds ratio, CI – confidential interval

In the present study we have explored associations between genetic variation in *MGP* gene and ACS risk. Analyzing *MGP* SNPs we have found that G⁻⁷→A promoter polymorphism is associated with ACS in male but not in female individuals. As to other two studied polymorphisms (T⁻¹³⁸→C, Thr₈₃→Ala), we have not found any relations (using logistic regression) between them and ACS in the Ukrainian population.

Herrman et al. have analyzed *MGP* polymorphism in the ECTIM Study which included myocardial infarction patients and control subjects from Northern Ireland and France, and the AXA study which consisted of health volunteers from France [5]. In the ECTIM Study, the genotype distributions did not differ between patients with myocardial infarction and control subjects. Only in a group of low-risk subjects were the Ala₈₃ and A⁻⁷ alleles are more frequent in case than in control subjects. In the AXA Study, none of the *MGP* polymorphisms was related to calcification or atherosclerosis of the carotid artery. On the other hand, the A⁻⁷ and Ala₈₃ alleles were associated with femoral calcification in the presence of atherosclerotic plaques. Most probably, the difference in findings between the carotid and femoral arteries might be explained by the significantly lower frequency of atherosclerotic/calcified plaques in the carotid than in the femoral arteries. According to these observations, the G⁻⁷→A or Thr₈₃→Ala polymorphism could influence the calcification process affecting atherosclerotic plaques, and it might contribute to the risk of myocardial infarction in low-risk individuals. The authors considered that it would be necessary to verify these results in other studies before any definitive conclusion can be drawn, especially since the associations were observed in subgroups of patients and not in the whole population.

In another study, associations between *MGP* SNPs and coronary artery calcification (CAC) in older men and women of European descent from Massachusetts (USA) were examined [58-60]. Various methods of analysis all revealed that in men, homozygous carriers of the minor allele of G⁻⁷→A, T⁻¹³⁸→C and Thr₈₃→Ala polymorphisms were associated with a decreased quantity of CAC,

relative to major allele carriers. This association was not found in women. In addition, genetic variation in *MGP* was shown to associate with serum MGP concentrations, but there were no association between serum MGP and CAC.

In the Coronary Artery Risk Development in Young Adults (CARDIA) Study, a population-based investigation of cardiovascular disease in younger African-American and non-Hispanic white participants, T⁻¹³⁸→C polymorphism of the *MGP* gene was analyzed for association with presence or absence of CAC [9]. This SNP was also studied in autopsy samples for an association with several measures of atherosclerotic calcification [61]. However, no association with T⁻¹³⁸→C polymorphism and measures of vascular calcification was found in either of these studies.

Italian scientists defined the distribution of two *MGP* polymorphisms (G⁻⁷→A, T⁻¹³⁸→C) in patients with chronic kidney disease (CKD) and age- and sex-matched healthy controls [18]. It was shown that the frequency of the minor A allele (G⁻⁷→A polymorphism) and the major T allele (T⁻¹³⁸→C polymorphism) was significantly higher in the CKD group versus controls. A/A and T/T homozygotes were associated with cardiovascular events in CKD patients. It was concluded that altered *MGP* gene polymorphism may be a negative prognostic factor for the progression to end-stage renal disease and for cardiovascular events in patients with CKD.

Thus, findings presented here are inconsistent. In some studies, *MGP* polymorphisms were shown to associate with arterial calcification and myocardial infarction [5, 10, 25], in others, no association between *MGP* SNPs and cardiovascular events was found [7, 9]. Moreover, in those studies, in which such association was still revealed, relation the same type of *MGP* polymorphism to arterial calcification was different. For example, in AXA Study the minor alleles -7A and 83Ala were associated with increased femoral artery calcification [5], while in Crosier et al. study [10] the same alleles were linked to decreased level of coronary artery calcification.

In vitro studies results do not clarify the question. In one investigation [15], analysis of *MGP* promoter activity revealed that the -138C allele reduced promoter

activity by 20% in rat vascular smooth muscle cells (VSMC) and by up to 50% in a human fibroblast cell line. Moreover, it was demonstrated that a nuclear protein specifically binds in the region covering the T⁻¹³⁸→C polymorphic site and that binding is enhanced in the presence of the T allele. Thus, the difference in promoter activity might be explained by differential binding of a nuclear protein that is important in MGP transcription. As regards the G⁻⁷→A polymorphism, the findings did not indicate that this SNP may be functional in vitro.

At the same time in another study [6], the influence of G⁻⁷→A and T⁻¹³⁸→C polymorphisms on gene expression was examined by using reporter gene constructs transiently transfected into VSMCs. It was demonstrated independent impact of both common polymorphisms on transcriptional activity of the MGP gene. The -7A variant had 1.5-fold higher activity than the -7G variant, whereas the -138C variant had 4-fold higher activity than the -138T variant. On the other hand, it was shown that the -138T allelic variant binds AP-1 complexes and is induced following phorbol 12-myristate 13-acetate treatment, while the -138C variant is refractive to this compound, confirming that AP-1 factors preferentially bind to the -138T variant. It was suggested that the -138C variant provides protection against tissue calcification in VSMC by resulting in higher levels of MGP transcription. Equally, the responsiveness of the -138T site to extracellular stimuli mediated via AP-1 may result in altered susceptibility to calcification. It is easy to see that these results are in the disparity with above mentioned findings [5].

In conclusion, we have defined significant association between the G⁻⁷→A promoter polymorphism of *MGP* gene and ACS (unstable angina pectoris + acute myocardial infarction) in Ukrainian population. The observed association suggest possible involvement of this polymorphism in coronary artery disease; however, these results must be substantiated in other studies focusing on appropriate end points.

Conclusion

1. Distribution of major allele homozygotes, heterozygotes and minor allele homozygotes in control group while analyzing T⁻¹³⁸→C promoter polymorphism 58,7%, 36,7%, 4,6 while analyzing G⁻⁷→A polymorphism of the promoter was - 41,8%, 54,5%, 3,6% , while at the assay of Thr₈₃→Ala polymorphism (exon 4) these were – 43,9%, 45,9%, 10,2%.

2. Distribution of major allele homozygotes, heterozygotes and minor allele homozygotes in group with acute coronary syndrome: while analyzing T⁻¹³⁸→C MGP promoter polymorphism were 59,8%, 32,7%, 7,5%, while analyzing G⁻⁷→A promoter polymorphism were - 42,1%, 45,6%, 12,3% , while at the assay of Thr₈₃→Ala polymorphism (exon 4) these were– 42,6%, 43,5%, 13,9%.

3. It was estimated that A/A genotype (G⁻⁷→A polymorphism) was significantly (p=.02) associated with acute coronary syndrome in Ukrainian population.

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