BRITISH MEDICAL JOURNAL

British Medical Journal

Volume 3, No.1, January 2023

Internet address: http://ejournals.id/index.php/bmj
E-mail: info@ejournals.id
Published by British Medical Journal
Issued Bimonthly
3 knoll drive. London. N14 5LU United Kingdom
+44 7542 987055

Requirements for the authors.

The manuscript authors must provide reliable results of the work done, as well as anobjective judgment on the significance of the study. The data underlying the work shouldbe presented accurately, without errors. The work should contain enough details andbibliographic references for possible reproduction. False or knowingly erroneous statementsare perceived as unethical behavior and unacceptable.

Authors should make sure that the original work is submitted and, if other authors'works or claims are used, provide appropriate bibliographic references or citations. Plagiarismcan exist in many forms - from representing someone else's work as copyright to copying orparaphrasing significant parts of another's work without attribution, as well as claimingone's rights to the results of another's research. Plagiarism in all forms constitutes unethicalacts and is unacceptable. Responsibility for plagiarism is entirely on the shoulders of theauthors.

Significant errors in published works. If the author detects significant errors or inaccuraciesin the publication, the author must inform the editor of the journal or the publisher aboutthis and interact with them in order to remove the publication as soon as possible or correcterrors. If the editor or publisher has received information from a third party that thepublication contains significant errors, the author must withdraw the work or correct theerrors as soon as possible.

CHIEF EDITOR

Dr. Fiona Egea

EDITORIAL BOARD

STUDY OF THE ASSOCIATION OF POLYMORPHISM rsl799750 OF THE MMP1 GENE AND rs2276109 OF THE MMP-12 GENE WITH THE DEVELOPMENT OF VARICOSE VEINS DISEASE OF THE LOWER LIMB AND ITS THROMBOTIC COMPLICATIONS

Yariev Alisher Alijonovich
surgeon of Syrdarya branch of the
Republican Scientific Center of
Emergency Medicine (RRCEM) MoH RUz
Sanjar Sobirovich Khudojberdiev
Director of the Syrdarya branch of the
Republican Scientific Center of
Emergency Medicine (RRCEM) MoH RUz.
Shernazarov Farhod Khaknazarovich
PhD, Gulistan State University
Department of General Medical Sciences
assistant professor, dekan
Boboev Kodirjon Tukhtaboevich
Head of Laboratory of Medical Genetics,
Republican Specialized Scientific-Practical
Medical Center of Hematology (RSSPMCH) MoH RUz,
https://orcid.org/0000-0002-0060-5638
Alimov Timur Raufovich
PhD, Researcher,
laboratory assistant
molecular genetics and cytogenetics
RSSPMC of Hematology MoH RUz
https://orcid.org/0000-0003-4432-5694

Aim. Assessment of the contribution of the rsl799750 polymorphism of the MMP1 gene of the rs2276109 and polymorphism of the MMP12 gene to the development of varicose veins disease of the lower limb (VVDLL) and its thrombotic complications.

Material and methods. A total of 316 people were enrolled in the study, 161 patients with VVDLL and venous thrombosis were included in the main group, and 155 conditionally healthy individuals were included in the control group. The frequency of detection of the rsl799750 polymorphism of the MMP1 gene and polymorphism rs2276109 of the MMP12 gene was investigated in all subjects using the Real-time PCR method.

Results. Study of the distribution of the mutant homozygous 2G/2G rs1799750 genotype of the MMP1 gene revealed a marked tendency for its prevalence in the subgroup of patients with venous thrombosis, where its proportion was 30.0%, versus the control group, where it was detected in 18.7% of cases, respectively ($\chi 2=3.5$; $p=0.06$; RR=1.8; 95%CI:0.97-3.5 OR=2.3; 95%CI:0.95-5.4).

The degree of detection of the mutant homozygous genotype G/G rs2276109 of the MMP12 gene was at a very low level - it was detected in only one patient with VVDLL complicated by venous thrombosis: in 2.0% of cases vs. 0.6%, respectively ($\chi 2=0.6$; $p=0.4$; RR =2.0; 95%CI:0.5-8.1; OR=3.0, 95%CI:0.2-48.2).

Conclusions: Carriage of 2G/2G genotypic variant of rs1799750 polymorphism of MMP1 gene is reliably associated not only with VVDLL formation but also with the development of venous thrombosis.

The presence of unfavorable allelic variant of rs7123600 polymorphism of MMP-12 gene may not serve as an early marker of development of structural changes of vein wall and VVDLL of lower limbs as well as development of thrombotic complications.

Keywords: rsl799750, MMP1 gene, rs2276109, MMP12 gene, VVDLL, venous thrombosis, thrombotic complications.

Introduction. The prevalence of chronic venous insufficiency of the lower extremities determines its relevance. The highest incidence of this pathology occurs in the age period over forty years. If on average in the world the prevalence of VVDLL reaches 10-15% of the working-age population, then in economically developed countries - for example, in the USA and countries of Western Europe - up to a quarter of the population [1, 2; 3; four]. It is known from other sources that, on average, clinically significant changes in the saphenous veins are recorded in 40% of the examined. According to the authors, up to a third of the population suffers from various forms of VVDLL [4]. At the same time, complications in the form of trophic disorders (ulcerative defects) can be detected in approximately 1% of the examined patients with VVDLL. The annual increase in the incidence of VVDLL is about 3.0% [4, 5, 6; 7].

A significant role in the etiopathogenesis of VVDLL is played by genetic disorders in the regulation of the components of the extracellular matrix, in particular, its degradation under the action of proteolytic enzymes synthesized by endothelial cells and macrophages. Among these enzymes, matrix metalloproteinases (MMPs) play a significant role [8; 9]. Regulation of expression and activity of MMPs is an important part of extracellular matrix homeostasis [10]. Despite the numerous works devoted to the study of the importance of matrix metalloproteinases in the development of VVDLL (in particular, the researchers described the results of studies of the transcriptional activity of metalloproteinase genes in vivo and in vitro), there is still insufficient data on the impact of MMPs gene defects on the clinical course and the occurrence of complications in this disease [11; 12; 13].

Another well-known metalloproteinase is MMP12, for which the association of the rs2276109 polymorphism of the MMP12 gene with the development of oncopathology, endometriosis, and coronary heart disease has already been shown [12; 14; 15].

The release of matrix metalloproteinase 12 (MMP12) by induced macrophages leads to the destruction of several components of the venous matrix, which facilitates the penetration of macrophages into the site of tissue damage [8; 16]. The developing local inflammatory reaction leads to the activation of free radical oxidation, and the release of protease enzymes leading to the destruction of the collagen fibers of the vein wall. This, in turn, leads to an increase in the activity of the proliferative activity of smooth muscle myocytes. However, newly synthesized young myocytes synthesize the components of the intercellular matrix in a much greater amount and vice versa, contractile elements in a smaller amount, which affects both the morphological structure and the functionality of the venous wall. Morphologically, this leads to thickening and disorganization of the connective tissue of the venous wall, which reduces its ability to perform a frame function and reduce the overall contractility of the vein. As a result, these changes lead to pathological restructuring and expansion of the veins [17, 23].

This paper presents the results of a study of the association of polymorphic loci rsl799750 of the MMP1 gene and rs2276109 of the MMP12 gene with the development of VVDLL and its thrombotic complications.

Purpose of the study. Evaluation of the contribution of polymorphisms rsl799750 of

the MMP1 gene and rs2276109 of the MMP12 gene to the development of VVDLL and its thrombotic complications.

Material and methods

In total, the study involved 316 people who were included in the following subgroups (Figure 1).

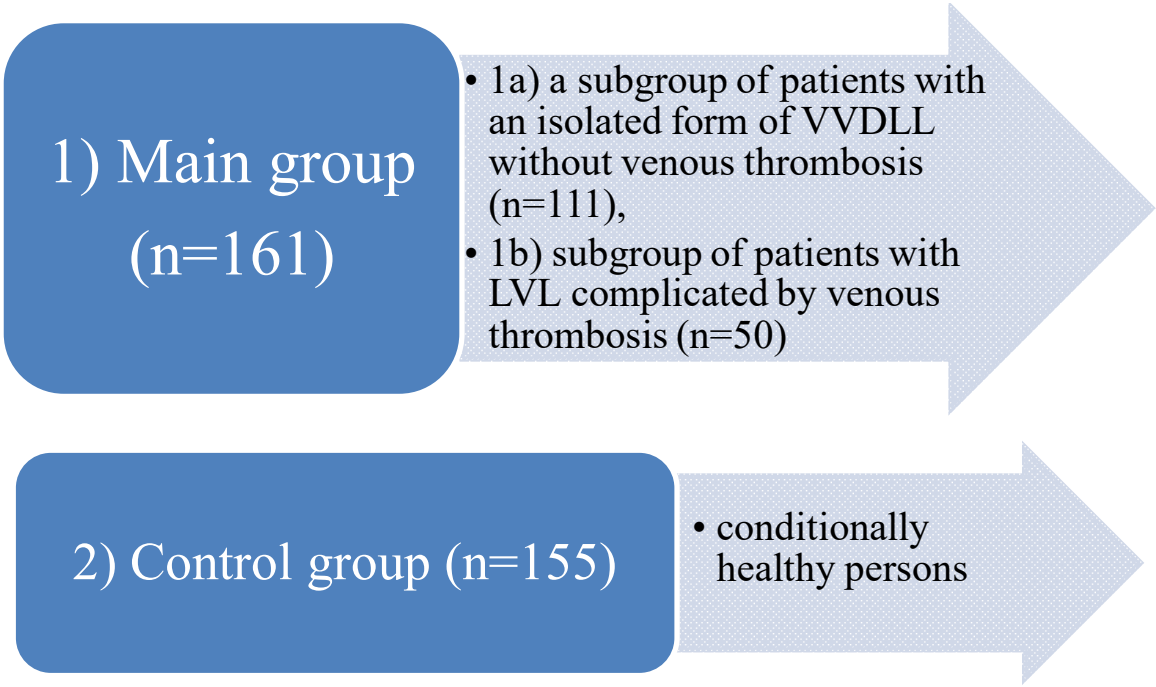

Figure 1. Scheme of the study - the distribution of the examined patients and control groups

In all examined individuals of the main and control groups, the frequency of detection of polymorphisms rsl799750 of the MMP1 gene and rs2276109 of the MMP-12 gene was studied. For the study, blood was taken from all subjects in vacuum tubes with EDTA. A PCR analysis of the selected biomaterial was carried out using the Real-Time PCR method. DNA isolation from peripheral blood lymphocytes was performed using the AmpliPrime RIBO-prep kit (Next Bio LLC, Russia). Detection of polymorphism was carried out using test systems of LLC research and production company Litech (Russia) according to the manufacturer's instructions. Amplification was performed using a Rotor Gene Q device (Quagen, Germany).

The software package "OpenEpi 2009, Version 2.9" was used as a tool for calculating the obtained data.

Results

Tables 1-4 present the results of a study of the frequency of distribution of alleles and genotypes of the rsl799750 polymorphism of the MMP1 gene in the main group of patients with VVDLL and phlebothrombosis compared with the control sample.

Table 1.

Distribution of alleles and genotypes of the rsl799750 polymorphism of the MMP1 gene in groups of patients and controls

№	Groups	n	Frequency distribution of:									
			alleles				genotypes					
			1G		2G		1G/1G		1G/2G		2G/2G	
			n	%	n	%	n	%	n	%	n	%
1	**Main group:**	161	157	48.8	165	51.2	43	26.7	71	44.1	47	29.2
1a	**VVDLL**	111	109	49.1	113	50.9	30	27.0	49	44.1	32	28.8
1b	**Venous thrombosis**	50	48	48.0	52	52.0	13	26.0	22	44.0	15	30.0
2	**Control group**	155	183	59.0	127	41.0	57	36.8	69	44.5	29	18.7

The frequency of the wild-type 1G allele of the rsl799750 polymorphism of the MMP1 gene was 48.8% in the main group versus 59.0% in the control group (χ^2=6.7; p=0.01; RR=0.8; 95%CI:0.7-0.95; OR=0.7, 95%CI:0.5-0.9) (Table 2). The frequency of the unfavorable 2G allele prevailed in the main group at 51.2%, which was higher than in the control sample at 41.0% (χ^2=6.7; p=0.01; RR=1.2; 95%CI:1.1-0.4; OR=1.5, 95%CI:1.1-2.1) (Table 2).

Table 2.

Associative association of the rsl799750 polymorphism of the MMP1 gene in the main group relative to the control group

Alleles и genotypes	Main group (n=161)	Control group (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
1G	48.8	59.0	0.8	0.7–0.95	0.7	0.5–0.9	6.7	0.01
2G	51.2	41.0	1.2	1.1–1.4	1.5	1.1–2.1		
1G/1G	26,7	36.8	0.8	0.6–1.1	0.6	0.4–1.0	3.7	0.05
1G/2G	44,1	44.5	1.2	0.9–1.6	1.4	0.8–2.3	1.4	0.2
2G/2G	29.2	18.7	1.4	1.1-1.9	2.1	1.2-4.0	6.1	0.01

The frequency of 1G/1G wild-type genotype carriage was lower (trend) in the main group compared to the control group, 26.7% versus 36.8%, respectively (χ^2=3.7; p=0.05; RR=0.8; 95%CI 0.6-1.1; OR=0.63; 95%CI:0.4-1.0). The detection rate of heterozygous 1G/2G genotype was statistically insignificantly prevalent among patients in the main group with VVDLL and venous thrombosis compared with the control group, 44.1% versus 44.5%, respectively. (χ^2=1.4; p=0.2; RR=1.2; 95%CI:0.9-1.6; OR=1.4; 95% CI:0.8-2.3). The unfavorable homozygous 2G/2G genotype was statistically significantly more prevalent in the main group patients with VVDLL and phlebothrombosis, 29.2% vs. 18.7%, respectively. (χ^2=6.1; p=0.01; RR=1.4; 95%CI: 1.1-1.9; OR=2.1, 95%CI 1.2-4.0).

The allele frequency and genotype distributions of the rs1799750 polymorphism of the MMP1 gene were examined for differences in the subgroups of patients with VVDLL and venous thrombosis and in the control sample. Carriage of the homozygous mutant 2G/2G genotype significantly increased the risk of disease development more than 2-x (OR=2.1; 95% CI:1.2-3.95; χ^2=6.1; p=0.01).

We also performed a comparative analysis of the allele and genotype frequency distribution of the rs1799750 polymorphism of the MMP1 gene in the subgroup of patients with VVDLL and in the control group, the results of which are presented in Table 3.

The wild-type 1G allele was detected in 49.1% and 59.0% of cases in the subgroup of patients with VVDLL and in the control sample, respectively (χ^2=5.1; p=0.02; RR=0.8; 95%CI: 0.7-0.97; OR=0.7; 95%CI:0.5-0.95). The frequency of the unfavorable 2G allele in patients with VVDLL and among conditionally healthy individuals was: 50.9% and 41.0%, respectively, (χ^2=5.1; p=0.02; RR=1.3; 95%CI:1.0-1.5; OR=1.5; 95%CI:1.1-2.1) (Tables 1 and 3).

Table 3.

Associative association of the rs1799750 polymorphism of the MMP1 gene in the subgroup of patients with VVDLL, relative to the control group

Alleles и genotypes	VVDLL (n=111), %	Control group (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
1G	49.1	59.0	0.8	0.7–0.97	0.7	0.5–0.95	**5.1**	**0.02***
2G	50.9	41.0	1.3	1.0–1.5	1.5	1.1–2.1		
1G/1G	27.0	36.8	0.8	0.6–1.2	0.7	0.4–1.2	1.4	0.2
1G/2G	44.1	44.5	1.2	0.8–1.7	1.4	0.8–2.4	1.0	0.3
2G/2G	28.8	18.7	1.5	1.1-2.2	2.1	1.1-4.1	**4.8**	**0.03***

The proportions of 1G/1G, 1G/2G, and 2G/2G rs1799750 genotypes in the MMP1 gene in the 1a subgroup consisting of patients with VVDLL were: 27.0%, 44.1%, and 28.8%, respectively, as compared with 36.8%, 44.5%, and 18.7% in the control group (Table 1). The 1G/1G genotype was detected insignificantly less frequently, with a frequency of 27.0%, among patients with VVDLL, relative to the control group, where its proportion was 36.8% (χ^2=1.4; p=0.2; RR=0.8; 95%CI:0.6-1.2; OR=0.7; 95%CI:0.4-1.2). The proportion of heterozygous 1G/2G genotype in VVDLL was 44.1%, which was highly insignificant and statistically insignificant lower than in the control group, where its frequency was 44.5% (χ^2=1.0; p=0.3; RR=1.2; 95%CI: 0.8-1.7; OR=1.4; 95%CI:0.8-2.4). The 2G/2G homozygous genotype was detected in the VVDLL group with a frequency of 28.8%, which was statistically significantly higher than the frequency of its detection in the control group, which was 18.7% (χ^2=4.8; p=0.03; RR=1.5; 95%CI:1.1-2.2; OR=2.1, 95%CI:1.1-4.1).

Further, Table 4 presents the results of a study of the frequency distribution of the rs1799750 polymorphism in the MMP1 gene among patients with an established diagnosis of venous thrombosis. The studies showed a pronounced tendency for the wild-type 1G allele to predominate in the control group, where its frequency was 59.03%, relative to 48.0% in the group of patients with venous thrombosis (χ2=3. 7; p=0.05; RR=0.7; 95%CI:0.5-1.0; OR=0.6; 95%CI:0.4-1.0), whereas the 2G allele predominated among patients with venous thrombosis, accounting for 52.0%, relative to 41.0% in the control group (χ2=3.7; p=0.05; RR=1.4; 95%CI:0.99-2.0; OR=1.6; 95%CI:0.99-2.5).

Table 4.

Associative association of the rs1799750 polymorphism of the MMP1 gene in a subgroup of VVDLL patients with thrombotic complications, relative to the control group

Alleles и genotypes	Thrombotic complications (n=50), %	Control group (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
1G	48.0	59.0	0.7	0.5–1.0	0.6	0.4–1.0	3.7	0.05
2G	52.0	41.0	1.4	0.99–2.0	1.6	0.99–2.5		
1G/1G	26.0	36.8	0.7	0.4–1.2	0.6	0.3–1.2	1.9	0.2
1G/2G	44.0	44.5	1.3	0.7–2.4	1.4	0.7–3.0	0.7	0.4
2G/2G	30.0	18.7	1.8	0.97-3.5	2.3	0.95-5.4	3.5	0.06

Study of genotype frequency distribution of polymorphic locus rs1799750 of MMP1 gene showed a lower detection rate of wild-type 1G/1G genotype among patients with thrombotic complications of 26.0%, compared with the control group, where it was detected with a non-significantly higher frequency of 36.8% (χ^2=1.9; p=0.2; RR=0.7; 95%CI:0.4-1.2; OR=0.6; 95%CI:0.3-1.2).

Statistically significant differences in the detection of heterozygous 1G/2G genotype in the group of patients with venous thrombosis relative to the control group were practically not detected. The incidence of 1G/2G genotype in the studied groups was 44.0% and 44.5% respectively (χ2=0.7; p=0.4; RR=1.3; 95%CI:0.7-2.4; OR=1.4, 95%CI:0.7-3.0).

The study of the distribution of the mutant homozygous 2G/2G genotype revealed a marked tendency for its prevalence in the subgroup of patients with venous thrombosis, where it accounted for 30.0%, versus the control group, where it was detected in 18.7%, respectively (χ2=3.5; p=0.06; RR=1.8; 95%CI:0.97-3.5 OR=2.3; 95%CI:0.95-5.4) (Table 4).

Tables 5-8 present the results of the frequency of alleles and genotypes of the rs2276109 polymorphism in the MMP12 gene for differences in their distribution in the main group of patients with hereditary predisposition to VVDLL and phlebothrombosis and in the control sample.

In the course of the study we found an equal distribution of A and G alleles of this polymorphic locus among the patients of the main group, in the main and control groups. The frequency of A/A, A/G and G/G genotypes in the main group of patients and controls was 85.7%, 13.6% and 0.6% versus 85.8%, 13.5% and 0.6% respectively. According to the data obtained, there were no statistically significant differences in the detection of A and G alleles (Table 6).

Table 5.

Frequency of alleles and genotypes of the rs2276109 polymorphism in the MMP12 gene in the main group of patients and in the control group

Group	n	Frequency distribution of:									
		alleles				genotypes					
		A		G		A/A		A/G		G/G	
		n	%	n	%	n	%	N	%	n	%
Main group	161	298	92.5	24	7.4	138	85.7	22	13.6	1	0.6
Control group	155	287	92.5	23	7.4	133	85.8	21	13.5	1	065

The A allele was almost equally predominant in the study and control groups, with a detection rate of 92.5%, and the G allele was detected significantly less frequently in 7.4% of cases (p=0.9; RR=1.0; 95%CI: 0.8-1.3; OR=1.0; 95%CI: 0.6-1.8) (Table 6).

The A/A genotype was detected with almost equal frequency in both the main group and control groups: 85.7% versus 85.8%, respectively (p=0.9; RR=1.0; 95%CI:0.7-1.4; OR=1.0; 95%CI:0.5-1.9).

The heterozygous A/G genotype occurred with approximately equal frequency among patients and controls: 13.6% versus 13.5%, respectively (p=0.9; RR=1.0; 95%CI:0.7- 1.4; OR=1.0; 95%CI:0.5-1.9).

Table 6.

Associative association between the rs2276109 polymorphism in the MMP12 gene in the main patient group and the control group

Alleles и genotypes	Main group (n=161)), %	Control group, (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
A	92.5	92.5	1.0	0.8–1.3	1.0	0.6–1.8	<3.84	0.9
G	7.4	7.4	1.0	0.8–1.3	1.0	0.6–1.8		
A/A	85.7	85.8	1.0	0.7–1.4	1,0	0.5–1.9	<3.84	0.9
A/G	13.6	13.5	1.0	0.7–1.4	1.0	0.5–1.9	<3.84	0.9
G/G	0.6	0.6	1.0	0.2–3.9	1.0	0.1–15.6	<3.84	0.9

The frequency of the mutant homozygous G/G genotype was also similar in both groups: 0.6% versus 0.6%, respectively (p=0.9; RR=1.0; 95%CI:0.2-3.9; OR=1.0, 95%CI:0.1-15.6) (Table 6).

Table 7 presents the results of the frequency of allele and genotype distributions of the rs2276109 polymorphism in the MMP12 gene in the groups of patients with VVDLL and in the control group.

Table 7.

Associative association between the rs2276109 polymorphism in the MMP12 gene in the groups of patients with VVDLL and controls

Alleles и genotypes	VVDLL (n=111), %	Control group, (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
A	91.9%	92.5%	1.0	0.7–1.4	0.9	0.5–1.7	0.1	0.8
G	8.1%	7.4%	1.1	0.7–1.5	1.1	0.6–2.1		
A/A	83.8%	85.8%	0.9	0.6-1.3	0.9	0.4-1.7	0.2	0.6
A/G	16.2%	13.5%	1.1	0.8–1.6	1.1	0.6–2.4	0.3	0.6
G/G	0.0%	0.6%	0.0	-	0.0	-	0.7	0.4

The results obtained during the study showed statistically insignificant differences in the distribution of A and G alleles of this polymorphism in the group of patients with varicose veins.

The proportion of the A allele in the VVDLL patient group and control group was 91.9% and 92.5%, respectively (χ2=0.1; p=0.8; RR=1.0; 95%CI:0.7-1.4; OR=0.9; 95%CI:0.5-1.7). The G allele was detected at a frequency of 8.1% versus 7.4% in the VVDLL patient group and control group, respectively (χ^2=0.1; p=0.8; RR=1.1; 95%CI:0.7-1.5; OR=1.1; 95%CI:0.6-2.1).

The frequency of wild-type A/A genotype detection was not significantly higher in the control group: 83.8% versus 85.8%, respectively (χ2=0.2; p=0.6; RR=0.9; 95%CI:0.6-1.3; OR=0.85; 95%CI:0.4-1.7). The heterozygous A/G genotype was detected more frequently among patients with VVDLL (16.2% versus 13.5%, respectively χ^2=0.34; p=0.5; RR=1.1; 95%CI:0.8-1.6; OR=1.1, 95%CI:0.6-2.4). The mutant homozygous G/G genotype was not detected in the VVDLL patient group (0.0% versus 0.6%, respectively, χ^2=0.7; p=0.4).

Table 8 shows the results of the distribution of allele and genotype frequencies of the rs2276109 polymorphism of the MMP12 gene in the groups of patients with venous thrombosis and in the control sample.

The proportion of the A allele in the venous thrombosis patient group and control group was 94.0% vs. 92.5%, respectively (χ^2=0.2; p=0.6; RR=1.2; 95%CI:0.6-2. 5), and the G allele in the venous thrombosis patient group and control group was 6.0% versus 7.4%, respectively (χ^2=0.2; p=0.6; RR=1.2; 95%CI:0.1-2.5; OR=1.3; 95%CI:0.5-3.2) (Figure 8).

Table 8.

Associative association between the rs2276109 polymorphism in the MMP12 gene in groups of patients with venous thrombosis and thrombotic complications in the control group

Alleles и genotypes	Thrombotic complications (n=50), %	Control group, (n=155), %	Statistical difference					
			Relative risk		Odds ratio		χ^2	p-value
			RR	95% CI:	OR	95% CI:		
A	94.0	92.5	1.2	0.57–2.48	1.3	0.50–3.18	0.2	0.6
G	6.0	7.4	1.2	0.12–2.48	1.3	0.50–3.18		
A/A	90.0	85.8	1.4	0.59–3.13	1.5	0.53–4.16	0.6	0.4
A/G	8.0	13.5	0.6	0.3–1.6	0.6	0.18–1.73	1.0	0.3
G/G	2.0	0.65	2.0	0.5-8.1	3.0	0.2-48.2	0.6	0.4

The A/A, A/G, and G/G genotypes of the rs2276109 polymorphism in the MMP12 gene in the VVDLL patient and control groups were distributed as follows: 90.0%, 8.0%, and 2.0% versus 85.8%, 13.5%, and 0.65%, respectively.

The frequency of detection of the wild-type A/A genotype was not significantly higher in the control group: 90.0% versus 85.8%, respectively ($\chi2$=0.6; p=0.4; RR=1.4; 95%CI:0.6-3.1; OR=1.5; 95%CI:0.3-4.2).

The frequency of detection of the heterozygous A/G genotype was statistically insignificantly higher in the control group compared with a sample of variceal patients: 8.0% versus 13.5%, respectively ($\chi2$=1.0; p=0.3; RR=0.6; 95%CI:0.3-1.6; OR=0.6; 95%CI:0.2-1.7).

A mutant homozygous G/G genotype was detected in one patient with VVDLL complicated by venous thrombosis: 2.0% versus 0.6%, respectively ($\chi2$=0.6; p=0.4; RR=2.0; 95%CI:0.5-8.1; OR=3.0, 95%CI:0.2-48.2) (Table 8).

Disscussion

Various studies have been devoted to the study of the processes mediating structural and morphological changes in the vein walls that impair their function [18]. A number of studies [19, 20] focused on the genes of various matrix metalloproteinases (MMP1, MMP2, MMP3, MMP9, MMP13).

According to D. Gillespie et al, 2002 [4] studied the samples of biomaterial obtained from the patients with VVDLL despite the absence of significant differences in the expression of genes MMP1 and MMP13 and absence of expression of MMP3 gene, an increase in the protein product MMP1 was determined by Western blotting. At the same time, according to P. Sansilvestri-Morel et al. (2005) [21] showed increased expression of the MMP2 gene and increased concentration of the MMP3? protein product was detected by enzyme immunoassay, and Y. Xu et al., 2017 [19] identified increased expression of candidate genes MMP2, MMP9 in patients with VVDLL.

Thus, according to Shadrina A.S. et al. (2019), none of the gene polymorphisms they studied, including the rs1799750 polymorphism of the MMP1 gene, was found to be associated with VVDLL [22]. However, this may be due to the chosen population, sample size, and peculiarities of the study methodology.

"In general, the extracellular matrix system in VVDLL has been much better studied in gene-candidate studies at the protein level than at the mRNA level, and despite some

inconsistencies in the results, the evidence for an imbalance in this system is very strong" [13].

In contrast, our studies revealed an association between individual alleles and genotypes of the rsl799750 polymorphism of the MMP1 gene and the development of VVDLL.

In our study of allele and genotype distribution frequencies of rs1799750 polymorphism of MMP1 gene, we found that carriage of homozygous mutant 2G/2G genotype increases the risk of venous thrombosis more than twofold in a subgroup of patients with VVDLL and in a control sample.

To date, there are many studies devoted to the role of matrix metalloproteinases in the development of diseases of various organs and systems, but in the structure of scientific papers there is very little material on the relationship between the activity disruption of extracellularly existing matrix metalloproteinase MMP-12, namely the representative of zinc-dependent endopeptidases. One of the important functions of MMP12 is elastin hydrolysis. Its hyperactivation and decrease of its tissue inhibitors activity under VVDLL conditions eventually leads to elastin loss and rupture in the structure of collagen fibers and reduction of tone and dilatation of venous walls [17].

According to the researchers' data, patients with relapsed VVDLL had a high incidence of the pathological gene MMP12 in 80.0%, both in homo- and heterozygous genotype variants, while in first-time patients - only in a third of cases (33.3%) [12, 22]. The authors found a statistically significant association between VVDLL and this gene (MMP12). These authors also determined the relationship between VVDLLB and the frequency of detection of a mutation in the MMP12 gene.

Differences in MMP-12 protein expression in patients with VVDLL and in normal patients were confirmed by foreign authors using immunoblotting and immunohistochemistry methods [23].

According to Slonkov? V.et al.(2017), who investigated the relationship between another polymorphism - rs7123600 of the MMP-12 gene and chronic venous insufficiency, it was found that the G allele was detected 2.1 times more often in SWD, in women, compared with controls, and there was also an association between the presence of this gene polymorphism studied in women with ulcers in SWD by 3.2 times, compared with women who did not have ulcers. A/G genotype detection of rs7123600 polymorphism of MMP-12 gene was 4.7 times higher in women with cardiovascular disease (stage C6) [9].

Conclusion

1.The 1G allele of the rs1799750 polymorphism of the MMP1 gene is a protective factor against the development of VVDLL and venous thrombosis.

2.A significant association between 2G-allele carriage of rs1799750 polymorphism of the MMP1 gene and the risk of VVDLL and venous thrombosis has been established. Carriage of 2G/2G genotypic variant of this polymorphism is reliably associated not only with VVDLL formation but also with the development of venous thrombosis.

3.Presence of unfavourable allelic variant of rs7123600 polymorphism of MMP-12 gene may not serve as an independent marker of development of structural changes of lower limbs vein walls and VVDLLTs as well as the development of DVT.

References.

1.Bharath V., Kahn S. R., Lazo-Langner A. Genetic polymorphisms of vein wall remodeling in chronic venous disease: a narrative and systematic review //Blood, The Journal of the American Society of Hematology. - 2014. - Т. 124. - №. 8. - С. 1242-1250.

2.Fukaya E, Flores AM, Lindholm D, Gustafsson S, Zanetti D, Ingelsson E, Leeper NJ. Clinical and Genetic Determinants of Varicose Veins. Circulation. 2018 Dec 18; 138(25): 2869-2880.

3.Ghaffarzadeh A., Bagheri M., Khadem-Vatani K., Abdi Rad I. Association of MMP-1 (rs1799750)-1607 2G/2G and MMP-3 (rs3025058)-1612 6A/6A Genotypes With Coronary Artery Disease Risk Among Iranian Turks. J. Cardiovasc Pharmacol. 2019;74(5):420-425.

4.Gillespie D, Patel A, Fileta B, Chang A, Barnes S, Flagg A, Kidwell M, Villavicencio J, Rich N. Varicose veins possess greater quantities of MMP-1 than normal veins and demonstrate regional variation in MMP-1 and MMP-13. J Surg Res. 2002;106(2):233-238.

5.Gomez I., Benyahia C., Louedec L., Leseche G., Jacob M.P., Longrois D., Norel X. Decreased PGE content reduces MMP-1 activity and consequently increases collagen density in human varicose vein. PLoS One. 2014;5;9(2):88021.

6.Gormus U, Kahraman O, Isbir S, Tekeli A, Isbir T. MMP2 gene polymorphisms and MMP2 mRNA levels in patients with superficial varices of lower extremities. In Vivo. 2011;25(3):387-391 [19]

7.Gormus U, Timirci-Kahraman O, Ergen A, Kunt A, Isbir S, Dalan A, Isbir T. Expression levels of elastin and related genes in human varicose veins. Folia Biol. 2014;60(2):68-73. [17]

8.Hollingsworth S.J., Powell G.l., Barker S.G., Cooper D.G. Primary varicose veins: altered transcri ption of VEGF and its receptors (KDR, fl t-1, soluble fl t-1) with sapheno-femoral junction incompetence // Eur J Vasc Endovasc Surg. 2004. Vol. 27, no. 3. pp. 259-268.

9.Slonkova V. et al. Genetic predisposition for chronic venous insufficiency in several genes for matrix metalloproteinases (MMP-2, MMP-9, MMP-12) and their inhibitor TIMP-2 //Journal of the European Academy of Dermatology and Venereology. - 2017. - Т. 31. - №. 10. - С. 1746-1752.

10.Jang-Young Kim. Polymorphisms of MMP-3, MMP-9, MMP-12, and TIMP-1 genes as a determinant of isolated coronary artery ectasia: дис. - Graduate School, Yonsei University, 2007. - С. 1-41.

11.Joseph N., Mohamed Faizan Thouseef A.B., Devi U.M., Abna A., Juneja I. A multicenter review of epidemiology and management of varicose veins for national guidance. Annals of medicine and surgery. 2016;8:21-27.

12.Klupp, F., Neumann, L., Kahlert, C. et al. Serum MMP7, MMP10 and MMP12 level as negative prognostic markers in colon cancer patients. BMC Cancer 16, 494 (2016) 1-9.

13.Kowalewski R., Sobolewski K., Wolanska M., Gacko M. Matrix metalloproteinases in the vein wall. Int Angiol. 2004;23(2):164-169.

14.Su L., Zhou W., Asomaning K., Lin X., Wain J.C., Lynch T.J., Liu G., Christiani D.C. Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cance // Carcinogenesis. 2006. no. 27. pp. 1024-1029.

15.Ye S., Eriksson P., Hamsten A., Kurkinen M., Humphries S.E., Henney A.M. Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression // J Biol

Chem. 1996. no. 271. pp. 13055-13060.

16.Shapiro S.D., Kobayashi8 D.K., Ley T.J. Cloning and characterization of a unique elastolytic metalloproteinase produced by human alveolar macrophages // J Biol Chem. 1993. no. 268. pp. 23824-23829.

17.Seryapina Y.U.V. et al. The genetic predictors of varicose veins of small pelvis: a pilot study //Flebologiya. - 2018. - T. 12. - №. 1. - C. 25-29.

18.Nullen H., Noppeney T. Diagnosis and treatment of varicose veins. Part 1: definition, epidemiology, etiology, classification, clinical aspects, diagnostic and indications. Der Chirurg; Zeitschrift fur AlleGebiete der Operativen Medizen. 2010;81(11):1035-1044.

19.Xu Y, Bei Y, Li Y, Chu H. Phenotypic and functional transformation in smooth muscle cells derived from varicose veins. J Vasc Surgery Venous Lymphat Disord. 2017;5(5):723-733.

20.Lim C, Davies A. Pathogenesis of primary varicose veins. Br J Surg. 2009;96(11):1231-1142. https://doi.org/10.1002/bjs.6798 [8]

21.Sansilvestri-Morel P, Rupin A, Jullien N, Lembrez N, Mestries-Dubois P, Fabiani J, Verbeuren T. Decreased production of collagen type III in cultured smooth muscle cells from varicose vein patients is due to a degradation by MMPs: possible implication of MMP-3. J Vasc Res. 2005;42(5):388-398. https://doi.org/10.1159/000087314 [15]

22.Shadrina A.S., Sodbo Z. Sharapov, Tatiana I. Shashkova, Yakov A. Tsepilov Varicose veins of lower extremities: Insights from the first large-scale genetic study. PLoS genetics. 2019;15(4):e1008110.

23.Woodside K. J. et al. Morphologic characteristics of varicose veins: possible role of metalloproteinases //Journal of vascular surgery. - 2003. - T. 38. - №. 1. - C. 162-169.