Investigation of Association between Genetic Polymorphisms of *MMP2*, *MMP8*, *MMP9* and *TIMP2* and Development of Varicose Veins in the Slovak Population – Pilot Study

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Summary

Matrix metalloproteinases (MMPs) are a family of zinc-dependent metalloendopeptidases that degrades extracellular matrix (ECM) components. MMPs are associated with venous wall remodelling, proliferation, migration, phenotypic and functional transformation of vascular smooth muscle cells and ECM organization under the physiological and pathophysiological conditions. We investigated possible association of genetic promoter polymorphisms of *MMP2* (rs243866), *MMP8* (rs11225395), *MMP9* (rs3918242) and *TIMP2* (rs8179090) to varicose veins development in the Slovak population. Genomic DNA from 276 Slovak individuals (138 cases, 138 controls) was genotyped for selected SNPs (rs243866, rs11225395, rs3918242 and rs8179090) using the PCR-RFLP analysis. The data were analysed by chi-squared (χ^2) test, logistic regression, and Mann-Whitney test. The risk of varicose veins development was evaluated in dominant, codominant and recessive genetic models. The statistical evaluation of selected polymorphisms in patients in all three genetic models has not shown a significant risk of varicose veins development. Our study has not shown the association between selected polymorphisms and increased risk of varicose veins development in Slovak population. More evidence with broaden sample size is needed.

Key words

Varicose veins • Genetic polymorphisms • Matrix Metalloproteinases • *MMP2*, *MMP8*, *MMP9, TIMP2*

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Introduction

Chronic venous disease (CVD) including varicose veins belong to the most frequent diseases in developed countries in Europe and USA and affect from third to half of the population, especially women (Štvrtinová and Čelovská 2017). The socioeconomic importance of CVD is due to the cost of diagnostics and management, lowered quality of life, especially in higher clinical stages and loss of working days (Rabe and Pannier 2010). According to internationally used CEAP classification (C=Clinical picture, E=Etiology, A=Anatomy, P=Pathophysiology), CVD has seven stages (C0-C6). C0 indicates no visible signs of CVD or palpable varicose veins; C1, telangiectasia (spider veins); C2, varicose veins; C3, oedema; C4, skin damage due to

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varicose veins or venous reflux – hyperpigmentation, eczema, lipodermatosclerosis or atrophie blanche; C5, healed venous leg ulcer; C6, open and active venous leg ulcer (Salmhofer 2016).

The vein wall structure and function are partially regulated by matrix metalloproteinases (MMPs) (Chen *et al.* 2017). Matrix metalloproteinases (MMPs) are a family of zinc-dependent metalloendopeptidases that degrade extracellular matrix (ECM) components and non-ECM molecules including receptors, growth factors, cytokines and chemokines, all of which are determinants of the tissue microenvironment (Lauhio *et al.* 2016, Kunt *et al.* 2015, Shiomi *et al.* 2010).

The activity of MMPs is tightly regulated by a family of endogenous inhibitors termed as tissue inhibitors of metalloproteinases (TIMPs) (Johnson 2017). Both MMPs and TIMPs regulate the homoeostasis of the extracellular matrix. MMPs are involved in the degradation of the extracellular matrix components, while TIMPs influence vascular remodelling, causing an altered vein elasticity (Ellinghaus *et al.* 2017). MMPs can also influence the bioactive molecules present on the cell surface through the G-protein-coupled receptors (GPCRs) and regulate the cell environment (MacColl and Khalil 2015).

Multiple studies have suggested that imbalance between MMPs and TIMPs may contribute to the formation and development of varicose veins (Badier-Commander *et al.* 2000, Aravind *et al.* 2010, Kalinin *et al.* 2016, Johnson 2017, Hu *et al.* 2019). Results of these studies are inconsistent, disregard anatomical region the sample comes from and clinical stage of the disease (Serralheiro *et al.* 2017). Vascular remodelling represents the compensatory mechanism for the veins to adapt to pathological conditions such as venous hypertension and hypoxia (Zhao *et al.* 2020).

Elastin and collagen are important for the structural integrity of the vein wall (MacColl and Khalil 2015). In varicose veins decreased amount of elastin, and also structurally changed elastin were observed. MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are the main enzymes involved in elastin assembly (Görmüs *et al.* 2014, Nagase *et al.* 2006). MMP-2 can cleave many collagen substrates (I, II, III, IV, V, VII, X, XI, gelatine) and non-collagen substrates including aggrecan, elastin, fibronectin, laminin, nidogen, proteoglycan link protein and versican. Also, MMP-9 can degrade collagen type IV, V, VII, X, XIV, gelatine, and the same non-collagen substrates as gelatinase A. Similarly, MMP-8 (collagenase-2) breaks down collagen I, II, III, V, VII, VIII, X, gelatine, aggrecan, elastin, fibronectin, laminin, and nidogen (MacColl and Khalil 2015).

Different TIMPs inhibit different MMPs better than others, e.g. TIMP-1 inhibits MMP-1, MMP-3, MMP-7, and MMP-9 more effectively, whereas TIMP-2 inhibits MMP-2 better than TIMP-1. TIMP-2 is the only member of TIMP family that specifically interacts with both MT1-MMP and pro-MMP-2 on the cell surface, therefore functions as both MMP inhibitor and MMP activator (Bourboulia and Stetler-Stevenson 2010). Moreover, TIMP-2 has the ability to suppress the proliferation of endothelial cells directly (Seo *et al.* 2003).

The aim of the study was to investigate whether selected polymorphisms of *MMP2*, *MMP8*, *MMP9* and *TIMP2* genes are associated with a risk of varicose veins development in the Slovak cohort. All selected polymorphisms have the potential to influence gene expression.

Methods

Patients and controls

An approval for the study was granted by the Ethics Committee of Jessenius Faculty of Medicine in Martin, and all participants provided informed written consent before their inclusion. Participants in the study were of Caucasian origin recruited from the Clinic of Surgery and Transplant Center (University Hospital in Martin), ZILPO Ltd., GynMart Ltd. and JAVORKA Ltd. from October 2013 to December 2019. A 5 ml sample of venous peripheral blood was collected into EDTA tube. A total of 138 subjects (48 males and 90 females) diagnosed by qualified physicians as varicose veins patients based on the CEAP classification (C1-C3 stage) were recruited into the study. Varicose veins were surgically verified. At the same time, a total of 138 healthy individuals of comparable age and gender with no evidence of venous disease, venous ulcers, thrombophlebitis, thrombosis or history of cancer were enrolled into this study. Case-control groups were made up from individuals with a similar age and gender distribution and medians did not differ significantly (Table 1).

Genomic DNA extraction

Genomic DNA extraction was done by using PureLink® Genomic DNA Kit (Invitrogen™, Thermo

Fisher Scientific, New York, USA) from EDTA peripheral blood samples. Extracted DNA was stored at -20 °C until the analysis.

SNP genotyping

DNA was used for genotyping of the four selected SNPs (rs243866, rs11225395, rs3918242 and rs8179090) by using the PCR-RFLP method according to the modified protocols of Harendza *et al*. 2003, Xu *et al.* (2011), Izakovičová Hollá *et al.* (2012), and Kunt *et al.* (2015). The SNPs were identified from the database of single nucleotide polymorphisms from the National Centre for Biotechnology Information (NCBI) available online at http://www.ncbi.nlm. nih.gov/snp/.

All PCRs were performed in a 25 µl reaction mixture containing: 150 ng of the template DNA, 12.5 µl DreamTaq Green PCR Master Mix (2X), 0.25 µM of each primer and nuclease-free water. Technical details of PCR-RFLP are shown in Table 2.

Approximately 20 % of the samples were chosen at random for second PCR-RFLP analysis to confirm the genotypes from the amplified PCR product.

Table 1. Gender and age distribution in cases and controls.

	Cases	**Controls**	**P**
Number (males/females)	138 (48, 90)	138 (48, 90)	
Mean age	41.13	40.89	0.9189[a]
(95 % CI) (Range, SD)	(39.1-43.2) (17-65, ± 12.3)	(39.1-43.2) (17-65, ± 12.7)	
Males	37.23	36.23	0.6601[a]
(95 % CI) (Range, SD)	(34.0-40.5) (18-59, ± 11.1)	(32.6-39.5) (16-59, ± 11.4)	
Females	43.21	43.37	0.8953[a]
(95 % CI) (Range, SD)	(40.6-45.8) (17-65, ± 12.4)	(40.7-46.1) (17-65, ± 12.8)	

CI – confidence interval, [a] – Mann-Whitney U test.

Table 2. Technical details of PCR-RFLP analysis.

Polymorphism	**PCR primers (5′→3′)** **Thermal cycling parameters**	**Restriction enzyme**	**Gel electrophoresis**	**Fragment identifying genotypes (bp)**
MMP2 (rs243866)	F: ACCAGACAAGCCTGAACTTGTCTGA R: TGTGACAACCGTCTCTGAGGAATG	PagI	2 % agarose gel	WT=542 Het=542+460+82 Var=460+82
	95 °C – 5 min; 35 cycles: 95 °C – 30 s, 70 °C – 30 s, 72 °C – 60 s; 72 °C – 5 min			
MMP8 (rs11225395)	F: CTGTTGAAGGCCTAGAGCTGCTGCTCC R: GATCTTCTCTTCAAACTCTACCC	SfcI	1 % agarose gel	WT=893+74 Het=967+893+74 Var=967
	94 °C – 5 min; 34 cycles: 94 °C – 40 s, 61.5 °C – 45 s, 72 °C – 60 s; 72 °C – 10 min			
MMP9 (rs3918242)	F: GCCTGGCACATAGTAGGCCC R: CTTCCTAGCCAGCCGGCATC	PaeI	2.5 % agarose gel	WT=436 Het=436+242+194 Var=242+194
	94 °C – 4 min; 35 cycles: 94 °C – 60 s, 70 °C – 60 s, 72 °C – 60 s; 72 °C – 5 min			
TIMP2 (rs8179090)	F: CGTCTCTTGTTGGCTGGTCA R: CCTTCAGCTCGACTCTGGAG	Eco88I	10 % PAGE gel	WT=230+51+23 Het=253+230+51+23 Var=253+51
	95 °C – 5 min; 30 cycles: 95 °C – 30 s, 65.5 °C – 30 s, 72 °C – 60 s; 72 °C – 5 min			

F – forward primer, R – reverse primer, WT – wild type, Het – heterozygote, Var – variant.

Data analysis and statistical tests

To analyze the variance in selected risk factors between the case and control group, Fisher's exact test was used. The chi-squared (χ^2) test with Yates's continuity correction was used to evaluate differences from Hardy-Weinberg equilibrium and the independence of genotype and allele frequencies. Association of genotype combinations with the development of varicose veins was estimated by Fisher's exact test. The risk of varicose veins development was evaluated in codominant, dominant and recessive genetic models. Additionally, the odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated to identify the strength of association between selected SNP and varicose veins development. To verify the genotype distribution independence from age and gender chi-squared independence (χ^2_{indep}) test and logistic regression were used. A value of $P<0.05$ was considered statistically significant. The genotyping data were analysed by InStat (GraphPad Software, Inc.; Version 3.01) data analysis software and online statistical software package StatPages.net (https://statpages.info/). Sample size determination was performed in R (R Core Team 2020) ver. 4.0.2, using library pwr (Champely 2020). The power of 0.8 and significance level of 0.05 were used in the sample size determination for both the chi-squared test and the test of equality of two population proportions.

Results

We have conducted a case-control study determining whether selected genetic polymorphisms *MMP2* (rs243866), *MMP8* (rs11225395), *MMP9* (rs3918242) and *TIMP2* (rs8179090) present in the promoter regions were associated with varicose veins development and affect the occurrence and clinical stage of varicose veins in the Slovak population.

Similar age distribution was observed between the patients and healthy controls. However, family predisposition, history of thrombosis and thrombophlebitis, and working habits and smoking were different. Family history was positive in 106 (76.81 %) cases in the patient's group vs. 20 (14.49 %) cases in the control group. A positive history of thrombosis was observed in 23 (16.67 %) patients, thrombophlebitis in 5 (3.62 %) patients. More patients with varicose veins declared a prolonged standing, sitting or their combination during working hours, compared to the control group. Smoking was observed more often in the control group than in the group of varicose veins patients (28.99 % vs. 15.94 %). The studied population characteristics are shown in Table 3.

The genotype frequencies in patients and controls were in Hardy-Weinberg equilibrium. The frequency of minor allele A for the polymorphism of the *MMP2* gene (rs243866) was 0.25 in patients, and 0.28 in controls. Minor allele frequency (T allele) in polymorphism *MMP8* (rs11225395) was in patients 0.44, while in controls 0.46. The frequency of variant T allele for *MMP9* polymorphism (rs3918242) was 0.17 in patients *versus* 0.16 in controls. Variant allele C of *TIMP2* polymorphism (rs8179090) was detected in less than 1 % of all tested individuals. This frequency of the variant allele in the Slovak population was too low, therefore obtained results were not statistically processed. Minor allelic frequencies did not differ significantly between patients and controls. Distribution of alleles and genotypes were similar in comparison to average data for European Caucasian populations according to the Ensembl database (https://www.ensembl.org/index.html).

The evaluation of selected polymorphisms in patients in codominant, dominant and recessive genetic models did not show a significantly changed risk of varicose veins development. A similar distribution of genotypes of polymorphisms *MMP2* (rs243866), *MMP8* (rs11225395), and *MMP9* (rs3918242) was observed in both studied groups. These results are summed in Table 4. This is a pilot study. To reach the desired statistical power of 0.8 the total sample size needs to broaden (calculation of sample size determination is visible in Table 4 – SSD).

Additional analysis performed after classification of patients and controls based on sex, age, genetic predisposition, and clinical stage revealed no statistical significance ($p>0.05$; data not shown).

In contrast, risk assessment of selected genotypic combinations showed more interesting results. Genotype combination CC+TT of rs11225395 and rs3918242 polymorphisms indicates higher risk of varicose veins (OR=11.00; 95 % CI=0.56-215.51; p=0.0511), but the statistical power of this finding is very low. This observation was on the border of statistical significance and this genotype combination was found out only in 4 samples, what is insufficient to assume any trends (Table 5).

Discussion

Although varicose veins pathogenesis is multifactorial, understanding of the genetic and

Table 3. Characteristics of the study population.

Characteristics	Cases	Controls	OR	95 % CI	P
Total sample (females, males)	138 (90, 48)	138 (90, 48)			
Family history:			3.644	1.768-7.511	0.0005
Father	15 (10.87 %)	4 (2.90 %)			
Mother	63 (45.65 %)	14 (10.14 %)			
Both parents	28 (20.29 %)	2 (1.45 %)			
No history	32 (23.19 %)	22 (15.94 %)			
No answer	None	96 (69.57 %)			
History of thrombosis	23 (16.67 %)	None	56.359	3.384-938.66	<0.0001
History of thrombophlebitis	5 (3.62 %)	None	11.412	0.6245-208.55	0.0602
Working habits:			45.060	10.704-189.69	<0.0001
Prolonged standing	60 (43.48 %)	39 (28.26 %)			
Prolonged sitting	32 (23.19 %)	29 (21.01 %)			
Both standing and sitting	44 (31.88 %)	15 (10.87 %)			
BMI:			1.418	0.8826-2.279	0.1849
25.0-29.9	54 (39.13 %)	32 (23.19 %)			
30.0-34.9	14 (10.14 %)	23 (16.67 %)			
35.0-39.9	4 (2.90 %)	4 (2.90 %)			
>40.0	None	1 (0.72 %)			
Females:					
Hormonal contraception	19 (21.11 %)	9 (10.00 %)	2.408	1.024-5.663	0.0628
Number of deliveries:					
1	12 (13.33 %)	12 (13.33 %)			
2	43 (47.78 %)	39 (43.33 %)			
3	14 (15.56 %)	13 (14.44 %)			
4	3 (3.33 %)	3 (3.33 %)			
5	1 (1.11 %)	1 (1.11 %)			
Smoker	22 (15.94 %)	40 (28.99 %)	0.4647	0.2587-0.8347	0.0138

environmental factors which contribute to their formation is limited (Fukaya *et al.* 2018). The known risk factors include older age, positive family history, thrombosis and thrombophlebitis, prolonged standing or sitting, elevated BMI, female gender, pregnancy and smoking (Raetz *et al.* 2019, Zolotukhin *et al.* 2017, Yun *et al.* 2018). Because our study is oriented on genetic factors contributing to the disease development, we wanted to eliminate the influence of environmental risk factors as much as possible. This selection could have resulted in a higher portion of smokers present in the control, even though smoking is well-known risk factor for varicose veins (Gourgou *et al.* 2002). Our results show a statistically significant correlation between positive family history and the risk of varicose veins (p=0.0005).

A possible role of *MMP2* (rs243866), *MMP8* (rs11225395), *MMP9* (rs3918242) polymorphisms in varicose veins development was evaluated by analyzing SNPs within the promoter region assumed to affect the gene expression. In our study, frequencies of alleles and genotypes of selected polymorphisms of *MMP2*, *MMP8* and *MMP9* genes did not statistically significantly differ between the healthy controls and patients with varicose veins.

The role of polymorphism *MMP2* rs243864 (-1575 A/G) as a valuable marker for susceptibility to common genetically complex diseases is disputable, with both positive and negative findings. An association was reported in myocardial infarction (Peréz-Hernández *et al*. 2012), metabolic syndrome (Yadav *et al*. 2014) and colorectal cancer (Park *et al*. 2011). No association was observed with abdominal aortic aneurysm (Saracini *et al*.

Table 4. Analysis of genotypes of polymorphisms *MMP2* (rs243866), *MMP8* (rs11225395), and *MMP9* (rs3918242) in codominant, dominant and recessive genetic model.

Polymorphism	Genetic model	Case n=138 (%)	Controls n=138 (%)	SSD	P	χ^2	OR	95 % CI
MMP2 (rs243866)	Codominant:							
	Genotype GG	77 (55.80)	69 (50.00)				1.00	(Ref.)
	Genotype GA	52 (37.68)	62 (44.93)	1569.48	0.3100	1.0310	0.75	0.46-1.23
	Genotype GG	77 (55.80)	69 (50.00)				1.00	(Ref.)
	Genotype AA	9 (6.52)	7 (5.07)	17822.3	0.9974	1.0610^{E-05}	1.15	0.41-3.26
	Dominant:							
	Genotype GG	77 (55.80)	69 (50.00)				1.00	(Ref.)
	Genotype GA+AA	61 (44.20)	69 (50.00)	2327.68	0.3986	0.7125	0.79	0.49-1.27
	Recessive:							
	Genotype GG+GA	129 (93.48)	131 (94.93)				1.00	(Ref.)
	Genotype AA	9 (6.52)	7 (5.07)	8162.82	0.7967	0.0664	1.31	0.47-3.61
MMP8 (rs11225395)	Codominant:							
	Genotype CC	37 (26.81)	39 (28.26)				1.00	(Ref.)
	Genotype CT	80 (57.97)	71 (51.45)	1441.65	0.6380	0.2214	1.19	0.68-2.06
	Genotype CC	37 (26.81)	39 (28.26)				1.00	(Ref.)
	Genotype TT	21 (15.22)	28 (20.29)	2412.12	0.6498	0.2062	0.79	0.38-1.63
	Dominant:							
	Genotype CC	37 (26.81)	39 (28.26)				1.00	(Ref.)
	Genotype CT+TT	101 (73.19)	99 (71.74)	29825.67	0.8928	0.0182	1.08	0.63-1.82
	Recessive:							
	Genotype CC+CT	117 (84.78)	110 (79.71)				1.00	(Ref.)
	Genotype TT	21 (15.22)	28 (20.29)	1781.69	0.3446	0.8933	0.71	0.38-1.32
MMP9 (rs3918242)	Codominant:							
	Genotype CC	96 (69.57)	98 (71.01)				1.00	(Ref.)
	Genotype CT	37 (26.81)	37 (26.81)	369455.4	0.9399	0.0057	1.02	0.60-1.74
	Genotype CC	96 (69.57)	98 (71.01)				1.00	(Ref.)
	Genotype TT	5 (3.62)	3 (2.17)	3045.36	0.7183	0.1302	1.70	0.40-7.32
	Dominant:							
	Genotype CC	96 (69.57)	98 (71.01)				1.00	(Ref.)
	Genotype CT+TT	42 (30.43)	40 (28.99)	31214.92	0.8952	0.0174	1.07	0.64-1.80
	Recessive:							
	Genotype CC+CT	133 (96.38)	135 (97.83)				1.00	(Ref.)
	Genotype TT	5 (3.62)	3 (2.17)	4206.99	0.7198	0.1287	1.69	0.40-7.22

2011) or rheumatoid arthritis (Nemec *et al*. 2006). We did not observe an association between polymorphism rs243864 and varicose veins risk. Peréz-Hernández *et al*. (2012) presume that the functional effect of this polymorphism may vary depending on the cell type. Harendza *et al.* (2003) found out that in estrogen positive MCF-7 cells G allele showed increased, while A allele showed decreased transcriptional activity. On the other hand, Price *et al.* (2001) tried to search for naturally occurring polymorphisms within the promoter region of

Table 5. Association of genotype combinations with development of varicose veins.

Genotype combination	Case n=138 (%)	Controls n=138 (%)	SSD	P	OR	95 % CI
MMP2 + MMP8:						
GG+CC	20 (14.49)	15 (10.87)	1317.83		1.00	(Ref.)
GG+CT	46 (33.33)	39 (28.26)	1298.28	0.8412	0.88	0.40-1.96
GG+TT	11 (7.97)	15 (10.87)	1585.36	0.3056	0.55	0.20-1.54
GA+CC	15 (10.87)	23 (16.67)	548.86	0.1627	0.49	0.19-1.24
GA+CT	29 (21.01)	27 (19.57)	12083.79	0.6695	0.81	0.34-1.89
GA+TT	8 (5.80)	12 (8.70)	1243.71	0.2695	0.50	0.16-1.53
AA+CC	2 (1.45)	1 (0.72)	3122.84	1.0000	1.50	0.12-18.14
AA+CT	5 (3.62)	5 (3.62)	-	0.7310	0.75	0.18-3.07
AA+TT	2 (1.45)	1 (0.72)	3122.84	1.0000	1.50	0.12-18.14
MMP2 + MMP9:						
GG+CC	53 (38.41)	54 (39.13)	70963.96		1.00	(Ref.)
GG+CT	21 (15.22)	14 (10.14)	669.25	0.3321	1.52	0.71-3.32
GG+TT	3 (2.17)	1 (0.72)	996.58	0.6182	3.06	0.31-30.34
GA+CC	36 (26.09)	40 (28.99)	3725.91	0.8808	0.92	0.51-1.65
GA+CT	15 (10.87)	20 (14.49)	1317.83	0.5610	0.76	0.35-1.65
GA+TT	1 (0.72)	2 (1.45)	3122.84	1.0000	0.51	0.04-5.79
AA+CC	7 (5.07)	4 (2.90)	1247.60	0.5292	1.78	0.49-6.45
AA+CT	1 (0.72)	3 (2.17)	996.58	0.6187	0.34	0.03-3.37
AA+TT	1 (0.72)	0 (0.00)	540.26	1.0000	3.06	0.12-76.76
MMP8 + MMP9:						
CC+CC	22 (15.94)	27 (19.57)	1741.94		1.00	(Ref.)
CC+CT	11 (7.97)	12 (8.70)	22826.03	1.0000	1.13	0.42-3.04
CC+TT	4 (2.90)	0 (0.00)	134.08	0.0511	11.00	0.56-215.51
CT+CC	59 (42.75)	52 (37.68)	1465.39	0.3922	1.39	0.71-2.74
CT+CT	20 (14.49)	17 (12.32)	3850.13	0.5139	1.44	0.61-3.41
CT+CC	1 (0.72)	2 (1.45)	3122.84	1.0000	0.61	0.05-7.23
TT+CC	15 (10.87)	19 (13.77)	2011.65	1.0000	0.96	0.40-2.34
TT+CT	6 (4.35)	8 (5.80)	3580.85	1.0000	0.92	0.28-3.05
TT+TT	0 (0.00)	1 (0.72)	540.26	1.0000	0.41	0.01-10.50

the *MMP2* gene and to analyze the effect of these variations on the gene expression. Authors reported that this polymorphism had no functional effect when tested within the background of estrogen receptor negative cells, including vascular smooth muscle cells (A10), monocyte/macrophage-like cell line (RAW264.7) and human embryonic kidney cells (293). We are not sure to what extent this could have influenced our results.

According to our present knowledge, there was no published study regarding the relation between polymorphism *MMP8* rs11225395 (-790 C/T) and varicose veins. But there were studies testing the relation of this polymorphism to the development of arterial hypertension in men (Moskalenko *et al.* 2019) or carotid atherosclerosis (Djurić *et al.* 2011). Also, several studies have reported that this SNP influences the *MMP8* gene expression by alteration of its promoter activity. The T allele display higher promoter activity and protein expression than the C allele (Arechavaleta-Velasco *et al.* 2014, Feng *et al.* 2019, Decock *et al.* 2007, Dębniak *et al.* 2011, Wang *et al.* 2013). MMP-8 is overexpressed in long-lasting chronic venous ulcers. Chronic venous

ulceration occurs only in 1 % of the adult population in Western countries (Amato *et al.* 2015). Majority of patients enrolled in our study had varicose veins at CEAP stage C2. This could have influenced our results. It would be interesting to study the association of this polymorphism again with the group of C5-C6 patients.

Association of polymorphism *MMP9* rs3918242 (-1562 C/T) and varicose veins is controversial. Xu *et al.* (2010) found out that the C allele in *MMP9* polymorphism (rs3918242) predisposes to varicose veins in the Chinese population ($P<0.05$) (patients C3-C4). Previous studies show that T allele was associated with increased levels of MMP-9 in plasma (Raffetto and Khalil 2008, Xu *et al.* 2010). Kunt *et al.* (2015) obtained opposite results. Authors did not find out a statistically significant difference in allele or genotype distribution of *MMP9* polymorphism in the Turkish population (patients C2-C4). Slonková *et al.* (2017) reported that *MMP9* − 1562 C/T alleles, as well as the distribution of genotypes, significantly differed between CVD patient (C2-C6) and control groups. The T allele was observed more frequently in CVD patient group. We didn't obtain statistically significant results. Differences in these observations could have been influenced by various stages of CVD. SNPs are one of the numerous ways, through which the gene expression can be regulated.

TIMP-2 is an effective inhibitor of MMP-2 (Bourboulia and Stetler-Stevenson 2010). Kunt *et al.* (2015) observed an association of C allele of *TIMP2* rs8179090 (-418 G/C) polymorphism and a higher risk for varicose veins ($p=0.007$) in the Turkish population. The substitution of G→C allele may leads to lower transcription of *TIMP2* gene (Mikołajczyk-Stecyna *et al.* 2015). On the other hand allele and genotype frequencies of *TIMP2* (rs8179090) polymorphism were not significantly different between the patients with varicose veins and control group of Chinese origin ($p=0.069$) (Xu *et al.* 2010). Several studies have demonstrated racial or ethnic differences in alleles and genotypes frequencies (Hughes *et al*. 2006, Mattei *et al*. 2009). Due to the low frequency of a minor allele in the Slovak population, this polymorphism cannot serve as a marker for varicose veins development.

Unfortunately, none of our findings provide evidence that these polymorphisms are involved in the pathogenesis of varicose veins. Therefore, individually, they cannot serve as markers to this pathology. The analysis of genotypes combinations shows more promising results, although more data are needed.

Conclusions

Annually, varicose veins are diagnosed in 2.6 % of women and 1.6 % of men worldwide. The incidence of varicose veins (C2) is relatively variable (20 % to 64 %), the incidence of advanced stages of CVD (C3-C6) is approximately 5 % (Wittens *et al.* 2015). Despite the fact that varicose veins and CVD are generally recognized as medical problem, their profound effects on patient´s quality of life are largely underappreciated (Sutzko *et al.* 2018). Several studies have addressed this issue, effects of CVD and varicose veins on a patient's quality of life (Casana *et al.* 2018, Siribumrungwong *et al.* 2017, Onida and Davies 2016, Migdalski and Kuzdak 2015, Launois 2015).

Our analysis has not proven the association between selected polymorphisms and increased risk of varicose veins development in the Slovak population. More evidence with larger sample size is needed.

Conflict of Interest

There is no conflict of interest.

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