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THE COMBINATIONS OF SINGLE NUCLEOTIDE POLYMORPHISMS OF THE PROMOTERS OF THE MMP-1 AND MMP-3 GENES ARE ASSOCIATED WITH THE INCREASED RISK OF PELVIC ORGAN PROLAPSE.

Hypothesis / aims of study
The previous studies showed that the single nucleotide polymorphism (SNP) of the promoter of the matrix metalloproteinase type 1 (MMP-1) gene and the SNP of the promoter of the matrix metalloproteinase type 3 (MMP-3, stomelysin-1) could influence the connective tissue activity of both enzymes. The insertion of the extra guanine (G) base (position -1607/1608) forms transcription factor Ets binding site and upregulates MMP-1 transcription. The addition of the adenosine (A) base in the promoter of the MMP-3 gene (position – 1612/1617) creates a run of six adenosines (6A) while the other allele has only five (5A). The presence of 6A allele enables binding of the represor ZBP-89 that downregulates the expression of MMP-3 gene. The aim of the study was to estimate the associations between the combinations of the genotypes created by polymorphisms in the promoters of MMP-1 and MMP-3 genes and the risk of the pelvic organ prolapse (POP).

Study design, materials and methods
One hundred fifty five patients with significant defects of the pelvic floor static (grades II, III and IV; POPQ) were included into the study group. The control group consisted of 111 women without POP (grades 0, 1; POPQ). Vast majority of these patients were admitted with uterine myomas and subsequently underwent abdominal hysterectomy. All subjects in both study and control group were assessed with the same diagnostic work-up. The compared groups were well matched with regard to demographic and clinical characteristics. Genomic DNA was extracted from whole blood leukocytes. Determination of MMP-1 and MMP-3 polymorphisms was done by two steps PCR and RFLP. *MMP-1 polymorphism:* PCR products were digested with *Alu*I restriction endonuclease according to manufacturer instructions and separated on 3% agarose gel. The patterns of DNA fragments obtained in the electrophoresis enable to identify type of polymorphism. Single band 269 bp corresponds to 2G/2G homozygote, two bands 241, 28 bp to 1G/1G homozygote and 3 bands 269, 241, 28 bp to heterozygote 1G/2G.. *MMP-3 polymorphism*: PCR products were digested with *Tht*111 restriction endonuclease and separated on 3% agarose gel. Single band 129 bp corresponds to 6A/6A homozygote, 3 bands 129, 97, 32 bp to 5A/6A heterozygote and 2 bands 97, 32 bp to 5A/5A homozygote.

Results
The distributions of MMP-1 and MMP-3 polymorphisms are shown in table 1 and table 2, respectively. No statistically significant differences were found.

Table 1

Group (n)	MMP-1 polymorphism (position -1607-1608)						
	1G1G	%	1G2G	%	2G2G	%	Pearson's chi^2 test
control	45	34.1	54	40.9	33	25	
study	47	35.3	54	40.6	32	24.1	
							chi^2=0.07, p=1

Table 2

Group (n)	MMP-3 polymorphism (position -1612-1617)						
	5A5A	%	5A6A	%	6A6A	%	Pearson's chi^2 test
control	34	25.8	79	59.9	19	14.4	
study	28	22.2	81	64.3	17	13.5	
							chi^2=0.6, p=0.8

However, the comparison of the frequencies of the combination of the 2 genotypes, one for MMP-1 and the other for MMP-3, showed that some of them are overrepresented (e. g. **1G/2G – 5A/6A, 2G/2G – 5A/6A, 1G/1G – 6A/6A**) in women with POP (Pearson's chi^2 test =28.6, p=0.005), (table 3).

Table 3

Control group, freq. of polymorphisms (%)		MMP-3		
		5A/5A	5A/6A	6A/6A
MMP-1	1G/1G	39.5	58.1	2.3
	1G/2G	26.4	56.6	17

	2G/2G	6.2	68.7	25
Study group, freq. of polymorphisms (%)				
MMP-1	1G/1G	39.5	51.2	**9.3**
	1G/2G	14	**68**	18
	2G/2G	10.3	**75.9**	13.8

Interpretation of results

The association between combinations of the single nucleotide polymorphisms of the promoters of MMP-1 and MMP-3 genes and the increased risk of POP was proved.

Concluding message

The modification of the connective tissue activity of collagen remodeling enzymes – MMP-1 and MMP-3 could be of importance in the pathogenesis of the pelvic organ prolapse.

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What were the subjects in the study?	**HUMAN**
Was this study approved by an ethics committee?	**Yes**
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Was the Declaration of Helsinki followed?	**Yes**
Was informed consent obtained from the patients?	**Yes**