Polymorphism of the gene encoding α-1 chain of collagen type I and a risk of pelvic organ prolapse – a preliminary study

Polimorfizm genu kodującego łańcuch α-1 kolagenu typu I a ryzyko wystąpienia defektu statyki narządów dna miednicy mniejszej – wyniki wstępne

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Abstract

Introduction: *Polymorphism of the gene encoding α-1 chain of type I collagen (COL1A1) may influence the mechanical properties of the pelvic floor connective tissue.*

Aim of study: *We examined possible role of G→T substitution in transcription factor Sp1 binding site in the gene encoding α-1 chain of type I collagen (COL1A1) in the development of pelvic organ prolapse.*

Materials and methods: *The study group consisted of 37 women with pelvic floor defects graded according POPQ scale as stage II, III and IV. All study group patients underwent reconstructive surgery of the pelvic floor. We enrolled forty control subjects. All of them were treated for benign gynecological conditions other then stress urinary incontinence or pelvic organ prolapse. DNA was obtained from peripheral blood leukocytes. The fragment of the first intron of COL1A1 gene containing Sp1 binding site was amplified by PCR and analysis of restriction fragment length polymorphism was done.*

Results: *The GG polymorphism in COL1A1 gene was identified in 26 (70.3%), GT sequence in 10 (27%) and TT in 1 (2.7%) patient. The distribution of the investigated polymorphisms in the control group were: 27 (67.5%), 9 (22.5%) and 4 (10%), respectively. We do not found association between investigated polymorphic variants and pelvic organ prolapse (chi^2 test, p=ns).*

Conclusion: *G→T substitution in transcription factor Sp1 binding site in the COLIA1 gene does not increase the risk of development of pelvic floor defect (POPQ stages II, III, IV).*

Key words: **pelvic organ prolapse** / **collagen type I polymorphism** / **connective tissue** /

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Streszczenie

Wstęp: *Polimorfizm genu kodującego łańcuch α-1 kolagenu typu I (COL1A1) może mieć wpływ na właściwości mechaniczne tkanki łącznej, z której zbudowane są powięzie dna miednicy mniejszej.*

Cel badania: *Celem badania było ustalenie, czy istnieje związek między typami polimorfizmu miejsca wiązania czynnika transkrypcyjnego Sp-1 genu dla COL1A1 a ryzykiem wystąpienia defektów statyki narządów dna miednicy mniejszej.*

Materiał i metodyka: *Grupa badana liczyła 37 pacjentek hospitalizowanych z powodu zaburzeń statyki określanych w skali POPQ jako stopień II, III lub IV. Pacjentki zostały poddane leczeniu chirurgicznemu korygującemu zaburzenia statyki narządu płciowego. Grupa kontrolna: 40 kobiet ze schorzeniami nienowotworowymi, bez zaburzeń statyki (stopień 0, I POPQ) i nietrzymania moczu. Od każdej pacjentki pobrano 5 ml krwi, z której izolowano DNA. Następnie przeprowadzano reakcję amplifikacji z użyciem specyficznych dla badanego miejsca genomu starterów (fragment pierwszego intronu genu COL1A1).*

Wyniki: *Polimorfizm genu COL1A1 typu GG zidentyfikowano u 26 (70,3%), GT u 10 (27%), zaś TT u 1 (2,7%) pacjentki. W grupie kontrolnej dystrybucja polimorfizmów była następująca: GG 27 (67,5%), GT 9 (22,5%) TT 4 (10%). Nie wykazano istotnych różnic w częstości występowania typów polimorfizmu między porównywanymi grupami (chi^2 test, p=ns).*

Wnioski: *Wyniki wskazują, że substytucja typu G→T w obrębie miejsca wiązania czynnika transkrypcyjnego Sp-1 zlokalizowanego w obrębie promotora genu COL1A1 nie wpływa na ryzyko wystąpienia defektu statyki narządu rodnego (POPQ II, III, IV).*

Słowa kluczowe: **zaburzenia statyki narządu płciowego** / **polimorfizm genu kodującego** / / **kolagen typu I** / **tkanka łączna** /

Introduction

Pelvic organ prolapse is common condition with great influence on women's quality of life. It is estimated that 41% women with intact uterus develop some form of prolapse, with 34.3% having cystocele, 14.2% having uterine prolapse, and 18.6% having rectocele. Similar prevalence of pelvic floor defect is reported in hysterectomized women [1].

Overall lifetime risk of surgery because of prolapse is 11.1%. What is more, nearly 30% of surgically treated women require futher operation for the same or other pelvic floor defect [2]. The known risk factors for pelvic organ prolapse are vaginal birth, advanced age, obesity, cigarette smoking, chronic cough and previous hysterectomy [3, 4].

There are also data suggesting existence of familial predisposition for the development of this condition. This phenomenon may be explained by genetically inherited disturbances in connective tissue metabolism. This view is further supported by the results of previous study showed that the development of stress urinary incontinence (SUI) in women may be related to the diminished content of type I colagen in the pelvic floor connective tissue [5]. It has been postulated that the changes in the quantity and the quality of collagen may be involved in the pathogenesis of pelvic organ prolapse. One of the most intensively studied is the single-nucleotide polymorphism in the promoter region of first intron of COL1A1 gene. Recent data suggest that substitution of guanidine for the thymidine residue (G→T) at position 1240 in the first intron of COL1A1 gene can affect rate of its expression [6].

As a result three different genotypes exist i.e. homozygotes G/G, heterozygotes G/T and homozygotes T/T. These variations are of importance because affect a recognition site for the transcription factor Sp1 and thus are able to influence COL1A1 gene expression [7].

The clinical effect of Sp1 COL1A1 polymorphism is reduced mechanical strength of the connective tissue in G/T and especially in T/T individuals in comparison to G/G subjects.

Aim of the study

We checked hypothesis that the polymorphism in the transcription factor Sp1 binding site of gene encoding α-1 chain of collagen type I predisposes for the development of pelvic organ prolapse in women.

Material and methods

Study protocol was approved by Institutional Ethics Committee. All participants gave written informed consent. Thirty seven women presenting pelvic organ prolapse were included into study group. Detailed medical history was taken from each patient. The estimation of the of the defect was established during gynecological exmination. The severity of prolapse was graded according to POPQ scale [8].

Only patients with stages II, III and IV were included into the study group. Hysterectomized women, patients with neurological disorders, immobile or patients presenting symptoms suggesting stress, mixed or urge urinary incontinence were excluded from the study. The control group consisted of 40 women admitted to hospital and treated for benign gynecologic conditions. Vast majority of these patients had uterine myomas and underwent abdominal supracervical hysterectomy. All subjects were assessed with the same diagnostic workup as the study group. None of them reported symptoms of urinary incontinence or presented significant impairment (more then stage I in POPQ scale) of pelvic organ support.

Demographic and clinical characteristics of enrolled patients are given in Table I.

Table I. Demographic and clinical characteristics of study and control group

Characteristic	Women with pelvic organ prolapse (n = 37)	Control group (n = 40)	p
Mean (SD) age, y	57.1 (9,3)	54 (9,4)	0.2
Mean (SD) body mass index (kg/m^2)	27.5 (4,8)	27.9 (5,1)	0.8
Median (range) parity	2.6 (0-6)	2.4 (0-6)	0.5
Premenopausal	26	18	0.2
Postmenopausal	14	19	

Blood samples were taken into tubes containing anticoagulant EDTA. Genomic DNA was extracted from whole blood leukocytes using commercially available kit (GenomicPrep Blood DNA Isolation Kit, Amersham Biosciences, USA). DNA was stored at -20°C until used. Determination of COLIA1 polymorphism was done by PCR (Biometra T personal Thermocycler, Whatman Biometra, Germany) using 400 ng DNA. For amplification, the Taq DNA polymerase (Promega, Madison, WI, USA) and commercially obtained oligonucleotide primers were used (Serwis Sekwencjonowania i Syntezy DNA IBB PAN).

The first step PCR was carried out with the following primers set:
5'- GGAAGACCCGGGTTATTGCT - 3' (forward) and
5'- CGCTGAAGCCAAGTGAAATA - 3' (reverse) [9].

The 35 amplification cycles were preceded by denaturation at 99°C for 10 min. Annealing was carried out at 57°C for 1min, elongation at 72°C for 1min, and denaturation 94.5°C for 1min. A final primer extension was carried out at 72°C for 10min. The final recognition of Sp1 site was based on analysis of a restriction fragment length polymorphism (RFLP).

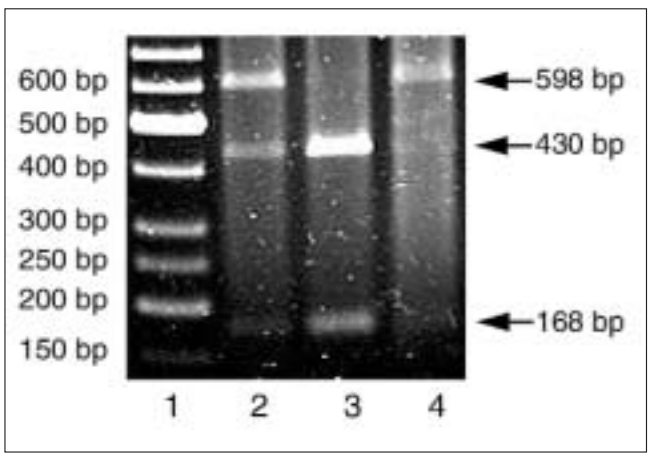

Figure 1. Representative gel showing restriction fragments after digestion of the PCR products with *Van91*I.
Lane 1 molecular weight markers; lane 2 heterozygote G/T; lane 3 homozygote G/G; lane 4 homozygote T/T.

PCR products (598 bp) underwent digestion with Van91I with subsequent separatation on 2% agarose gel. The sequence recognized by *Van91*I (5'-CCANNNN/NTGG-3') enables to identify nucleotide G in Sp1 polymorphic site.

Single band 598 bp corresponds to homozygote TT, three bands 598, 430 and 168 bp heterozygote GT and two bands 430, 168 bp enables to identify homozygote TT (Figure 1).

The *chi*^{2} test was used for the comparison of the genotypes prevalence between pelvic prolapse group and control patients. Because the distribution of age, parity and body mass index were skewed, differences in means and median were tested with the Mann-Whitney test. The influence of genotype on the risk of SUI was estimated by calculation of odds ratios and 95% confidence intervals. All statistics were performed with Statistica v.6.1 (StatSoft, USA).

Results

The compared groups were well matched with regard to demographic and clinical characteristics. In the study group the GG polymorphism in COL1A1 gene was identified in 26 (70.3%), GT sequence in 10 (27%) and TT in 1 (2.7%) patient. The distribution of the investigated polymorphisms in the control group were: 27 (67.5%), 9 (22.5%) and 4 (10%), respectively. The statistical analysis (*chi*^{2} test) did not show any significant differences between compared groups (p=ns).

Discussion

Analysis of clinical data showed that majority of parous women do not have pelvic organ prolapse, while this disturbance may occur in nulliparous women. There is also the evidence suggesting that genetic factors may play a role in the development of pelvic organ support failure in women. Jack et al. estimated risk of this defect in sisters with stage III and IV prolapse as 5 times greater then in general population [10].

Interesting results brought the study based on comparison of the occurence of pelvic floor defects within pairs of biological sisters, one of them parous, the other nulliparous. Authors found high concordance of pelvic organ prolapse in compared relatives. This finding indicates the existence of familial and inherited predisposition for the development of this disturbance [11].

Data linking genetic factors with the biomechanical impairment of pelvic floor support do not uncover pathogenesis of the defect. Based on findings of previous study which shed some light on genetic causes of SUI we proposed that similar mechanism may exist in the cases of pelvic organ prolapse [12].

However, in this study we did not found the association between COL1A1 gene transcription factor Sp1 binding site polymorphism and a risk of development of pelvic organ prolapse. This is in contrast to established link between GT and especially TT sequence in the Sp1 polymorphic site and incresed risk of SUI. The explanation of this finding is possible difference of pathogenesis of pelvic floor defects and SUI. However, the vast majority of clinical data suggest close links between SUI and pelvic organ prolapse. Due to complexity of connective tissue metabolism the involvement of other genetic factors in the development of pelvic organ prolapse is also possible.

Nikolova and co-workers suggest that the polymorphism of the promoter of laminin gamma1 (LAMC1) may increase risk of early-onset pelvic organ prolapse. Also, it is necessary to remember that pelvic floor defects are disturbances in which genetics plays probably minor role compared to environmental factors [13].

The main limitation of this study is number of enrolled patients. Much larger sample is necessary to obtain conclusive results regarding the role of Sp1 COL1A1 polymorphism in the development of pelvic organ prolapse.

Conclusion

G→T substitution in transcription factor Sp1 binding site in the COL1A1 gene does not increase the risk of development of pelvic floor defect (POPQ stages II, III, IV).

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Piśmiennictwo

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