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Collagen type 3A1 and 1A1 polymorphisms in women with pelvic organ prolapse and urinary incontinence assessed with Sanger sequencing method

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Introduction This case-control trial investigates the prevalence of COL3A1 and COL1A1 gene polymorphisms in female patients suffering pelvic organ prolapse (POP) and stress urinary incontinence (SUI) in comparison with controls.

Material and methods Inclusion criteria were having one or more risk factors for SUI and POP. Exclusion criteria were hereditary connective tissue diseases as well as surgeries for POP/SUI for the control group. The rs1800255 polymorphism in COL3A1 gene was considered as a local substitution of guanine (G) for adenine (A). The rs1800012 polymorphism in COL1A1 gene was considered as a local substitution of guanine (G) for thymine (T). Genotyping was performed by Sanger sequencing method, followed by estimation of sensitivity and specificity for POP and SUI.

Results Fifty-two patients with POP and SUI (mean age 64.4 years) and 21 women were included in the control group (mean age 63.2 years). Homozygous genotype (AA) in COL3A1 was found in 10% of patients suffering from POP or SUI. No women in the control group had this genotype. The single nucleotide polymorphism (SNP) had high specificity (1.0) for POP/SUI, but low sensitivity (0.1). Heterozygous genotype (AG) in COL3A1 had a sensitivity equal to 0.47 and specificity of 0.62. Homozygous genotype (TT) in COL1A1 was found in only 2% of patients with POP/SUI, but was not found in controls. Heterozygous genotype (TG) in COL1A1 has sensitivity equal to 0.25 and specificity of 0.74.

Conclusions POP/SUI patients have specific SNPs in COL1A1 and COL3A1 sequenced by Sanger method.

Key Words: female urethral reconstruction ‹› Martius flap ‹› urethro-vaginal fistula ‹› complications of midurethral sling

INTRODUCTION

Pelvic organ prolapse (POP) and stress urinary incontinence (SUI) have a mixed etiology – hereditary and acquired. During the last decade, the role of genetics in POP and SUI has become profoundly obvious. Twin studies have shown that there is a high concordance for pelvic disorders and that genetic factors are the cause in up to 40% of patients [1, 2]. The global gene-disease database (HuGENavigator) contains about 30 publications on the exploration for genetic factors associated with pelvic floor dysfunction in women. Most of the works are devoted to the studying of genes controlling the synthesis and degradation of connective tissue, and particularly to collagen genes, such as collagen type I alpha 1 (COL1A1), collagen type III alpha 1 (COL3A1), matrix metalloproteinases 1, 3, 9 (MMP1, MMP3, MMP9) and laminin (LAMC1) [3, 4]. Collagen plays a major role in pelvic floor supportive structures. Connective tissues are composed mostly

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of type I collagen, giving strength to the ligaments due to the length and thickness of the fibers, and to a lesser degree, of type III collagen, an increased amount of which is associated with a decrease in the mechanical strength of the connective tissue. The role of single nucleotide polymorphism (SNP) of the COL1A1 or COL3A1 genes remain controversial. Some studies and meta-analysis found a strict correlation between these genetic defects and POP; other investigators did not confirm this [5].

Kluivers et al. reported on a polymorphism in the alpha one chain of the type III collagen protein-encoding gene (rs1800255, COL3A1 2209 G>A) [6]. To detect these polymorphisms, authors used polymerase chain reactions followed by restriction fragment length polymorphism analysis for DNA analysis. That study showed that the homozygous form of one of these polymorphisms (rs1800255, COL3A1 2209 G>A polymorphism) was associated with POP with an odds ratio (OR) of 5.0 (95% confidence interval 1.4–17.1) compared to the controls. Chen et al. have shown similar results for POP patients using the same analysis technique [7]. Lince et al. presented the results of the study that included 354 women. According to their data, there is no association between POP and the homozygous polymorphism with high-resolution melting analysis.

This inconsistency has resulted in the current study in which the rs1800255 polymorphism of the COL3A1 gene and rs1800012 polymorphism in COL1A1 in saliva samples of women were investigated using the Sanger sequencing method [8].

MATERIAL AND METHODS

This case-control trial investigates the prevalence of COL3A1 and COL1A1 gene polymorphisms in female patients suffering POP and SUI in comparison with women without these symptoms. The number of the patients in the study and control groups were designed in a 2:1 ratio. Patients of the control group were selected by the case control method to the patients of the study group, taking into account 2 main signs: the correspondence of age and the presence in the anamnesis of the same risk factors for the development of pelvic floor dysfunction (2 or more births, traumatic childbirth, increased physical activity, diseases accompanied by an increase in intraperitoneal pressure, menopause, obesity stage 2–3). Inclusion criteria for the study and control groups were limited to having at least one or more POP/SUI risk factors, such as: two or more natural births, traumatic births, births of children weighing more than 4,000 grams, excessive physical activity, diseases accompanied by increased intra-abdominal pressure (bronchial asthma, chronic bronchitis, chronic constipation), history of pelvic surgery. All women included for both treatment and control groups were Caucasian of Russian descent.

Exclusion criteria for both groups were hereditary diseases with a known increased risk of POP, such as Marfan or Ehlers-Danlos syndrome as well as previous surgeries for POP/SUI for the control group. University Ethics committee approved (№ 05–17, June 2016) the study and every patient signed informed consent about study terms and conditions.

During the first visit, data about age, parity, history of POP and SUI surgery were collected from all patients. All women underwent gynecologic examination for the assessment of the POP-Q stage and stress urinary incontinence. Patients were examined performing maximum Valsalva followed by cough test with a full bladder.

Laboratory tests

All patients collected saliva into a sterile plastic container after at least 1 hour of being without any meal or drinks. Before giving the saliva probes, the patients chewed their cheek mucous for several seconds and then collected 3–4 ml of saliva. All samples were numerated and frozen until transportation to the laboratory was arranged.

The rs1800255 polymorphism of the COL3A1 gene was considered as a local substitution of guanine (G) for adenine (A) in DNA sequence of this gene. The rs1800012 polymorphism of the COL1A1 gene was considered as a local substitution of guanine (G) for thymine (T) in DNA sequence of this gene. Genotyping was performed by the Sanger [8] sequencing method, followed by estimation of sensitivity and specificity for POP and SUI. To study SNP in COL1A1 and COL3A1, we design two primers ~200-350 bp upstream and ~200–350 bp downstream of needed position to amplify a 400–700 bp fragment. Primers' specificities were confirmed by polymerase chain reaction (PCR) with subsequent electrophoresis in agarose gel. Sanger sequence of PCR product was performed on ABI 3730XL (Life Technologies) from one end, one reaction per template on ABI. Final primers for COL3A1 were f primer TAGTTCCCACCCAGCTGTTC and r primer ACCTTGTCACCCTTTGGACC. The final primers for COL1A1 were f primer ACTCCAACCTCAGCCCATTG and r primer GACACCTAGTGGCCGTCTG

RESULTS

Seventy-three women were involved in the study. All these women underwent treatment in the Urol-

ogy department from September 2016 to May 2017. The study group included 52 patients with prolapse of pelvic organs (POP) and stress urinary incontinence (SUI). Patients between the ages of 40 years and 70 years were included (average 64.4 years).
The control group included 21 patients without POP and SUI, matched with age and risk factors. These patients were admitted to a urological department for other reasons with a mean age of 63.2 years. All of them have at least 1 of the realized risk factors similar to those in the study group. The prevalence of polymorphisms among the patients is presented in Table 1.
According to results of our study, homozygous genotype (AA) in COL3A1 was found in 10% of patients suffering from POP or SUI. No women in the control group had that type of SNP in COL3A1. The detection of this mutation has high specificity (1.0) for POP and SUI patients along with low sensitivity (0.1). Heterozygous genotype (AG) in COL3A1 was found in 37% of patients suffering from POP or SUI. At the same time, 38% of women in the control group have similar SNP. Detection of that mutation has sensitivity equal to 0.47 and specificity of 0.62.
Homozygous SNP genotype (TT) in COL1A1 was found in 2% of patients suffering from POP or SUI, but no women in the control group have it. Detection of this SNP had high specificity (1.0) for patients suffering from POP and SUI but showed low sensitivity (0.01). Heterozygous genotype (TG) in COL1A1 was found in 23% of patients with POP or SUI. At the same time, 28.6% of women in the control group have similar SNP. Detection of this mutation has a sensitivity equal to 0.25 and specificity of 0.74. The data is presented in Table 2.

Table 1. *Prevalence of genotypes of the rs1800255 polymorphic variant of the COL3A1 gene among patients with pelvic floor dysfunction and the control group*

Genotype	Number of patients			
	POP	SUI	POP+SUI	Control group
AA (%)	1 (5.9)	2 (10.5)	2 (11.7)	0
AG (%)	4 (23.5)	10 (52.6)	5 (29.5)	8 (38.1)
GG (%)	12 (70.6)	7 (36.9)	10 (58.8)	13 (61.9)
Total	17 (100,0)	19 (100)	17 (100)	21 (100)

POP – pelvic organ prolapse; SUI – stress urinary incontinence

Table 2. *Genotyping of polymorphism 1800255 of the COL3A1 gene with calculation of sensitivity and specificity*

	Allele	Sensitivity	Specificity	AUC
POP	AA	0.05	0.93	0.49
	AG	0.4	0.52	0.46
SUI	AA	0.1	0.96	0.53
	AG	0.55	0.63	0.59
POP or SUI	AA	0.09	1.00	0.54
	AG	0.47	0.62	0.54
POP+SUI	AA	0.00	0.93	0.46
	AG	0.50	0.56	0.53

POP – pelvic organ prolapse; SUI – stress urinary incontinence; AUC – area under the curve

DISCUSSION

It should be noted that classical genetic-epidemiological studies have provided evidence that pelvic floor dysfunction in women is a pathology with a hereditary predisposition, the development of which is determined by the interaction of multiple additive genetic factors (mutations and/or polymorphic alleles) and environmental factors [1, 2, 3].
This study was performed to clarify the association between POP/SUI and the homozygous polymorphism in the alpha one chain of collagen type III (rs1800255, COL3A1 2209 G >A poly-morphism) after analysis of saliva samples with Sanger sequencing techniques.
The authors showed that the development of POP is associated not with this polymorphic variant, but with the carrier of the genotype rs1800255-A/A of the COL3A1 gene, which increases the probability of the development of POP by 4.79 times. According to the authors, the nucleotide substitution of 2092G>A (rs1800255) leads to the replacement of alanine with threonine (Ala698Thr), which may affect the strength of collagen fibers [9].
Another meta-analysis performed in 2015 by Cartwright and co-authors, did not confirm the association of the rs1800255-A/A gene of COL3A1 with the development of POP but upheld the correlation of the polymorphic variant rs1800012 of the COL1A1 gene with the risk of developing POP [10].
In one review of the literature describing clinical studies on hereditary factors in POP, a discrepancy between the different studies on COL3A1 polymorphism and POP was found [11]. Chen and colleagues [12] reported an association between the homozygous COL3A1 2209 G>A polymorphism as assessed with PCR and POP. Jeon et al. [13] found that the GG genotype of this polymorphism, instead of the AA genotype, was significantly associated with POP. Martins et al. [14], however, did not find any association with POP. Our initial explanation for these differences was that all studies were performed in different ethnical populations, i.e., Korean, Dutch, Taiwanese, and Brazilian, with different back-ground risks of POP. However, with the present knowledge,

these differences may also be explained by the fact that the methodology to detect this single nucleotide variant was not sufficiently accurate for the analyses in these studies. Furthermore, it should be noted that the use sequencing is the gold standard method for analyzing DNA. For this reason, we decided to use this method instead of others, sometimes less time and resource consuming ones.

In conclusion, for patients suffering from POP or/and SUI with homozygous genotype (AA) in COL3A1, the estimated sensitivity was low (0.1), but specificity was high (1.0). For the same patients, sensitivity calculated for the heterozygous genotype (GA) was 0.47, and specificity was 0.62. The patients, suffering only from SUI with homozygous genotype (AA) have shown a test sensitivity of 0.1 and specificity of 0.96. The same test performed for only POP patients showed similar results: AA mutation sensitivity equal to 0.05 and specificity - 0.93. As for COL1A1 positive homozygous mutation (TT) test was highly specific for POP and SUI patients but has low sensitivity. Heterozygous mutation in the same collagen has a specificity of 0.73 and sensitivity of 0.25.

Much of the data on the genetic nature of pelvic dysfunction is highly controversial. This may be due to the fact that pelvic organ prolapse and urinary incontinence are multifactorial diseases and genetic studies must be conducted with strict consideration of external risk factors. For example, delivery in patients is not always taken into account [15]. Also, most of the studies are devoted to individual genetic polymorphisms, while it is more reasonable to search for a combination of genetic markers. Further studies of the role of genetic factors in the development of pelvic floor dysfunction should shed light on the etiology / this pathology and contribute to the development of an individual approach to its prevention and treatment.

Limitations of the study were related to the small number of participants in both study and control groups. Bigger sample size may give more information about the prevalence of collagen mutations in females with or without pelvic disorders. Since POP is likely to be a multigenic disorder, where the relatively small influence of multiple genes adds up to the risk of developing POP, a follow-up study with a larger sample size would be advisable. According to the results of the study, we may hypothesize that some patients with pelvic organ disorders, such as POP and SUI have specific single nucleotide polymorphisms in alpha chains of collagen type 1 and 3 sequenced by the Sanger method indeed detection of these mutations have low sensitivity but high specificity.

CONCLUSIONS

Our statistically significant data confirm that carriage of the rs1800255-A/A genotype of the COL3A1 gene is associated with the risk of developing pelvic floor dysfunction in women. The small number of included patients is the main limitation of this study, which requires further study on a wider sample of patients. Nevertheless, it is hard to suggest genetic testing for screening as prophylaxis of POP or urinary incontinence. We need more clinical trials and systematic reviews to have more evidence-based solutions to this issue.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

CONTRIBUTION TO THE MANUSCRIPT

George Kasyan: Project Development, Data Collection, Manuscript writing

Dmitry Vishnevsky: Data Collection

Larissa Akulenko: Data collection, Sequencing and Laboratory Workup

Bagrat Grigoryan: Data Collection

Laura Pivazyan: Data collection

Dmitry Pushkar: Critical revision of the manuscript for scientific and factual content

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