

# COL3A1 Gen Polymorphism in Women with Pelvic Floor Dysfunctions: A Case Control Study



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**Abbreviations:** POP: Pelvic Organs; SUI: Stress Urinary Incontinence; G: Guanine; A: Adenine

## Introduction

Pelvic floor dysfunction is a complex of disorders of the fascial structures and pelvic floor muscles that keep the pelvic organs in a normal position and provide continence of urine and feces [1-6]. The POP amounts 30.8% in the structure of urogynecological pathology in European countries [7], in the Middle East countries - 19.9-49.6% [4], in the countries of North and East Africa - 46-56% [8]. In recent years, the incidence of POP has been increasing worldwide [9]. However, despite the growing prevalence of pelvic floor dysfunction in women, there is no consensus on its etiology and pathogenesis to date. Herewith, there is no doubt that this is a multifactorial pathology, the development of which is determined by genetic and environmental factors. The environmental factors contributing to the development of pelvic floor dysfunction in women have been studied quite well. Those include traumatic and prolonged labor, estrogen deficient conditions, diseases accompanied with increased intra-abdominal pressure (bronchitis, bronchial asthma, constipation, etc.), disturbance of microcirculation of blood and lymph in the small pelvis, obesity, and sedentary lifestyle [5,10,11].

As for genetic factors, already at the beginning of the 20th century it was known about existence of family cases of POP. This fact served as the basis for conducting large-scale studies using classical methods of genetic analysis – clinical-genealogical, population-statistical and twin studies, aimed to elucidate the role of hereditary factors in the development of pelvic floor dysfunction in women. The generalized results of these studies have convincingly demonstrated that in families of probands (patients) a pelvic floor dysfunction occurs 3-5 times more often than it would be expected based on the frequency of this pathology in population. It was shown that for healthy relatives of a proband of 1 degree of kinship (mothers, sisters, daughters), the risk of developing of a similar pathology was increased 5-fold, compared to risk in general

population [12]. A formal-genetic analysis performed on the material of the Swedish and Danish twin registers showed that the correlative contribution of hereditary and environmental factors to the predisposition to POP is 43% and 57%, respectively [2]. It was found that in the general structure of POP the family forms (2 or more cases among relatives of 1 degree of kinship) make up 28% and that in such families the disease is inherited by an autosomal dominant type with high penetrance [12,13].

Therefore, genetic- epidemiological studies have justified the appropriateness for searching the specific molecular-genetic factors that form the structure of hereditary predisposition to pelvic floor dysfunction in women. The international gene disease database (Huge Navigator) 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 (COL1A1, COL3A1), matrix metalloproteinases 1, 3, 9 (MMP1, MMP3, MMP9) and laminin (LAMC1) (<http://www.cdc.gov/genomics/hugenet/hugenavigator.htm>). As early as in 1966, a hypothesis was stated about the role of disturbance of collagen metabolism in the development of POP, which was based on data on reduced content of total collagen by 25% in vaginal epithelial tissue in women with POP compared with healthy women [14].

For support of pelvic organs, the most important are collagen types I and III. The binding apparatus is mostly of type I collagen, giving strength to the ligaments due to the length and thickness of the fibers, and less- type III collagen, increased amount of which is associated with a decrease in the mechanical strength of the connective tissue. The role of polymorphism of the COL3A1 gene remains controversial. Some studies and meta-analysis found a

strict correlation between these genetic defects and POP, other investigators did not confirm it [3,15]. The aim of our study is to clarify the place of polymorphism of the COL3A1 gene in the development of POP and urinary incontinence.

## Materials and Methods

The study involved 250 women. All these women underwent treatment in the University urology department from September 2016 to June 2017. These women were divided into a control and research groups. The study group included 150 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). Inclusion criteria's a study and control groups were limited to having at least one or more risk factors, such as: two or more births through natural paths, traumatic births, births of 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. The control group included 100 patients without POP and SUI, matched with age and risk factors. These patients were admitted in a urological department for other reasons. Patients between the ages of 40 years and 70 years were included (average 63.2 years). All patients have at least 1 of the realized risk factors as in the study group. In both groups, saliva samples were taken for genetic analysis. Saliva samples were collected from all patients for detection of rs1800255 polymorphism of the COL3A1 gene. This polymorphism is a local mutation, which results in substitution of guanine (G) for adenine (A) in DNA sequence. Genotyping was performed by Sanger sequencing method, followed by estimation of sensitivity and specificity for POP and SUI.

## The Mechanism of Genetic Material Intake

An hour before the saliva was given the patient should not eat, drink or smoke. To collect saliva was used a sterile plastic tube. Before giving the saliva, the patients chewed the cheeks mucous during several seconds. Then spit into a test tube 3-4ml of saliva. On the test tube was written the individual number of the patient. And it was placed in the freezer. As the material accumulated, the material was sent to the laboratory.

## Sequencing in Laboratory

Saliva samples were collected from all patients for detection of rs1800255 polymorphism of the COL3A1 gene. This polymorphism is a point mutation which results in substitution of guanine (G) for adenine (A) in DNA sequence. Genotyping was performed by Sanger sequencing method, followed by estimation of sensitivity and specificity for POP and SUI. For SNP detection we design two primers ~200-350bp upstream and ~200-350bp downstream of needed position to amplify a 400-700bp fragment. Primers specificity was confirmed by 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 were.

- a) f primer TAGTTCACCCAGCTGTTC
- b) r primer ACCTTGTCACCTTTGGACC

## Statistical Analysis

The genotyping data were subjected to statistical processing using the PASW Statistics program 22. The descriptive statistics were presented as the mean and standard deviation, the median and the 25th and 75th percentile, the minimum and maximum values in the sample for the quantitative variables, as well as the frequencies of occurrence and shares in the sample for qualitative variables. To compare the quantitative data in two unrelated samples, the Mann-Whitney U test was applied. To compare the nominal variables in two unrelated sets, Fisher's exact test was used. The significance level (p) was assumed to be 0.05 in all comparisons.

## Results

The study involved 250 patients who received treatment at the urology clinics of the Moscow State Medical University. 150 patients with POP and / or SUI were included in the study group. These patients between the ages of 40 to 70 years (mean age 64.4 ± 2 years) had at least one external risk factor (two or more births through natural birthmarks, traumatic births, births of more than 4,000g, excessive physical activity, accompanied by an increase in abdominal pressure (bronchial asthma, chronic bronchitis, chronic constipation), the presence of operations on the pelvic organs). The control group included 100 patients, without pelvic floor dysfunction, between the ages 40 to 70 years (mean age 63.2 ± 2 years). These patients had the same external risk factors as the study group patients. All the genetic variants studied within the framework of the present work were in a state of equilibrium in the Hardy-Weinberg sense. The results of genotyping for the polymorphism rs1800255 in the COL3A1 gene are reflected in Table 1. Genotyping data were subjected to statistical analysis. Statistical analysis didn't reveal differences between the study group and the control group.

**Table 1:** Results of genotyping polymorphism rs1800255 in the gene COL3A1.

Groups	Genotype		
	GG	GA	AA
Study group	90 (60%)	50 (33,3%)	10 (6,7%)
Control group	64 (64%)	28 (28,0%)	8 (8,0%)

## Discussions

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 in 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 [15]. 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 confirmed the correlation of the polymorphic variant rs1800012 of the COL1A1 gene with the risk of developing POP [3]. Summarizing the analysis of world literature, it should be noted that classical genetic-epidemiological studies have provided evidence that pelvic floor dysfunction in women is

a pathology with hereditary predisposition, the development of which is determined by the interaction of multiple additive genetic factors (mutations and / or polymorphic alleles) and environmental factors.

The present study was carried out in order to elucidate the relationship between POP and SUI with the carrier of polymorphisms rs1800255 in the gene COL3A1 by genotyping the saliva samples of the patients of the analytical and control group by the Sanger sequencing method. Sequencing by Sanger is currently the gold standard in the genotyping of single polymorphisms, so this is the method chosen for this study. The genotyping of the patients in the study group (150 people) and the control group (100 people) and the subsequent statistical analysis didn't reveal the association of rs1800255 polymorphisms in the gene COL3A1 with pelvic floor dysfunction.

### Conclusion

Thus polymorphisms rs1800255 in the gene COL3A1 don't participate in the formation of a predisposition to POP and SUI. Further studies of these polymorphisms don't seem appropriate.

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