Study of the polymorphism rs3025058 of the MMP-3 gene and risk of pelvic organ prolapse in Brazilian women

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ABSTRACT

Objective: To analyze the polymorphism -1171 5A / 6A rs3025058 of the MMP-3 gene and the risk for pelvic organ prolapse (POP).
Study Design: This is a cohort study. All patients attended the Urogynecology and Vaginal Surgery Section of the FMABC, from 2014 to 2016 and they were randomly recruited by the researchers at the first medical appointment. We selected 112 patients with symptomatic POPs and 180 patients with normal pelvic floors. The single nucleotide polymorphism (SNP) 5A / 6A of MMP-3 was determined by polymerase chain reaction (PCR) and analysis of the restriction fragments in both groups. Chi-squared test was used to compare the frequencies of polymorphisms between the groups. For those characteristics with statistical relevance, the crude odds ratio (OR) and its respective 95% confidence intervals were calculated; and, by logistic regression, were adjusted for each of the other characteristics, obtaining the adjusted OR. Hardy-Weinberg gene balance was determined using Pearson's Chi-squared test. Values of $p < 0.05$ were considered statistically significant.
Results: Logistic regression of factors associated with genital prolapse showed that age (adjusted OR = 11.89, 95% CI, 3.53–40) and home delivery (adjusted OR = 9.645, 95% CI, 3.35–27.7) remained risk factors for genital prolapse in the sample studied. There was no statistically significant difference between the groups in the distribution of genotypes, even after calculating the contribution of the 5A recessive allele in the aggregated genotypes (5A / 5A + 5A / 6A).
Conclusion: The polymorphism -1171 5A / 6A rs3025058 of the MMP-3 gene was not associated with the risk for POP. Age and home delivery were significantly associated with increased risk for the disease.

Introduction

Pelvic organ prolapse (POP) is defined as the displacement, slippage or descent of the uterus and / or different vaginal compartments and its neighboring organs, such as the bladder, rectum, or intestine. It is, therefore, the anatomical change of the pelvic organs. [1] It affects approximately 40% of women after menopause. Its etiology is multifactorial, and includes age, hypoestrogenism, genetic, racial factors, those associated with increased intra-abdominal pressure, parity and other obstetric risk factors, vaginal hysterectomy and connective tissue diseases [2].

Collagen is one of the main constituents of the pelvic floor, with types I and III being the most prevalent. The fibers of type I collagen are well structured and are part of the ligaments. The fibers of type III collagen, however, make up the adventitia of the vaginal wall and involve the pelvic organs [3]. Recent studies have shown that the content, components and structure of collagen are modified in patients with POP. [4,5],

In the human species, metalloproteinases (MMPs) are a family of at least 20 proteolytic enzymes that are fundamental for extracellular matrix (ECM) turnover and are capable of regulating collagen metabolism, besides having an important role in the catabolism of most matrix components. [4,6], MMP-3 is capable of activating other MMPs and degrading proteoglycans, fibronectin, laminin, gelatin, elastin and collagens type II, III, IV and IX. [7,8], On the other hand, tissue inhibitors of metalloproteinases combine with inactive forms (pre-enzymes) or active forms to inhibit their

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production and activation, thus interfering with collagen degradation [4].

The gene expression of these proteins depends on several factors, mainly the different polymorphisms of the promoter. A single nucleotide polymorphism (SNP) is a site in the DNA where a single base pair varies from person to person in a given population. Most of the polymorphisms do not have clinical repercussions, but another part is related to differences in transcription and expression of proteins, which confers phenotypic differences and individual susceptibility or resistance to the development of diseases. [8,9], According to a survey in the public database of SNPs (dbSNP), more than one thousand polymorphisms of the MMP-3 gene are described in the human species.

The polymorphism rs3025058 results in the insertion of the adenosine (A) base in the promoter of the MMP-3 gene at position -1612/-1617 and creates a polymonomeric run of six adenosines, while the other variant has five adenosines. The presence of the 6A allele downregulates the expression of the MMP-3 gene. Thus, in 5A/5A the degradation of collagen and ECM may be accelerated. [6]

The interrelationship of epidemiologic, environmental, and genetic risk factors for POP constitutes the genetic epidemiology of prolapse. The motivation for our study is that with improved understanding of these relationships, there may be a role for individual risk assessment in future. Perhaps, women at high-risk for prolapse may choose her treatment. Maybe, in the future, this information may be incorporated into patient counseling and treatment decisions.

Given this, we hypothesized that variations in the MMP-3 gene may alter gene expression and increase the risk of POP. In the present study we evaluated the polymorphism -1171 5A / 6A rs3025058 of the MMP-3 gene in a small sample of Brazilian postmenopausal women. We also assessed the associated clinical features and the risk for prolapse.

Materials and methods

The present study was reviewed and approved by the Research Ethics Committee of the Faculdade de Medicina do ABC (FMABC), Santo André, São Paulo, Brazil under number 554.670 / 2014. Participants were informed about the study protocol and procedures and written informed consent forms were obtained.

This is a cohort study, and all patients attended the Urogynecology and Vaginal Surgery Section of the FMABC, from 2014 to 2016 and they were randomly recruited by the researchers at the first medical appointment. All the women were subjected to history and physical examination. The POP-Q (Pelvic Organ Prolapse Quantification) classification proposed by Bump et al in 1996, a consensus statement from ICS- International Continence Society, AUGS -American Urogynecologic Society and SGS- Society of Gynecologic Surgeons, was used. [10]

The group (A) consisted of 112 patients with POPs in stages III and IV. The control group (B) consisted of 180 patients with stages 0 and I. In both groups, patients were in the postmenopausal period. Those who did not have POP had urinary incontinence, hypertrophy of labia minora, Bartholin's gland cyst and Skene's gland cyst, desire for contraception by vaginal ligature and Müllerian duct anomalies. Patients who refused blood collection after they were provided with clarification about the study and those with previous vaginal surgery were excluded.

In the clinical history, the following data were collected: age, ethnicity, body mass index (BMI), parity, place of birth and delivery, obstetric interventions (analgesia and episiotomy), weight of newborns, previous diseases, hypertension, dyslipidemia, chronic cough and constipation), life habits (physical activity with physical exertion and smoking) and previous hysterectomy. In terms of hormone replacement therapy (HRT), those patients who

regularly used vaginal or systemic hormones for at least six months were considered as users. The frequencies of polymorphism genotypes at the MMP-3 gene site -1171 5A / 6A were compared between cases and controls.

Molecular analysis of the -1171 5A / 6A polymorphism of the MMP3 gene

Genomic DNA was extracted from 5 ml of venous blood from each patient collected in an EDTA anticoagulant bottle. DNA extraction was conducted from the leukocyte phase using the commercial kit, Illustra blood genomicPrep mini spin (GE Healthcare). The obtained DNA was stored at $-20\,°C$ until use.

Amplification of the DNA by polymerase chain reaction (PCR) was performed in a volume of 10 μL with PCR Master Mix reagent (Promega) and 1 μM of each primer.

The primers used were described by Gnasso et al (2000): 5′-GGTTCTCCATTCCTTTGATGGGGGGAAAGA-3′(sense); 5′-CTTCCTGG AATTCACATCACTGCCACCACT-3′(antisense). [7] The reaction in the thermal cycler was programmed as follows: one cycle at $94\,°C$ with a duration of 5 min; 45 cycles of $94\,°C$ for 30 s, $65\,°C$ for 30 s, $72\,°C$ for 1 min; 1 cycle of $72\,°C$ with a duration of 10 min and finally, the products were kept at $10\,°C$. The PCR products were subjected to digestion with 2 U of the restriction enzyme, Psyl (Thermo Scientific), under the conditions described by the supplier for 16 h. For homozygous patients, 6A / 6A, a 129 bp DNA band was expected; for the 5A / 5A homozygous patients, two bands of 97 and 32 bp were expected, while for the heterozygous patients the combination of bands of both alleles (129, 97 and 32 bp) was expected. Restriction fragment length polymorphism (RFLP) analysis was performed by 2.5% agarose gel electrophoresis plus ethidium bromide for the development of DNA bands.

Statistical analysis

The continuous quantitative variables are presented as the means. Qualitative variables are presented as numbers or percentages. Chi-squared test was used to compare the frequencies of polymorphisms between the groups of cases and controls. For those characteristics with statistical relevance, the crude odds ratio (OR) and its respective 95% confidence intervals were calculated; and, by logistic regression, were adjusted for each of the other characteristics, obtaining the adjusted OR. Hardy-Weinberg gene balance was determined using Pearson's Chi-squared test. Values of $p < 0.05$ were considered statistically significant. Statistical analysis was performed using GraphPad Prism version 7.0 and SPSS version 23.

Results

The clinical characteristics of the patients are presented in Table 1. The patients in the study group were older than the patients in the control group. In addition, they also had on average a greater number of pregnancies and births, and among those, the vaginal route was the prevalent one. Home delivery contributed significantly to the occurrence of pelvic organ prolapse in the studied group.

The logistic regression of factors significantly associated with genital prolapse adjusted for each of the other factors showed that age (adjusted OR = 11.89, 95% CI, 3.53–40) and home birth (adjusted OR = 9.645; 95% CI, 3.35–27.7) remained risk factors for the occurrence of genital prolapse in the studied samples (Table 2).

The frequencies of 5A / 5A, 5A / 6A and 6A / 6A genotypes were in Hardy-Weinberg equilibrium. Table 3 shows the frequency of MMP-3 genotypes in the study and control groups. There was no statistically

Table 1

Analysis of the clinical characteristics of women with and without pelvic organ prolapse.

	Group A (n = 112) Mean or %	Group B (n = 180) Mean or %	p*
Age, mean (years)	68.4	57.8	< 0.0001[a]
White	69.90%	64.80%	0.422[b]
Non-White	30.10%	35.20%	
Body mass index (kg/m²)	28.8	28.9	0.874[a]
Age of menopause (years)	48.8	46.6	0.07[a]
Hormonal therapy	10.70%	18.10%	0.09[b]
Smoking	13.10%	20.10%	0.15[b]
Arterial hypertension	57.80%	49.40%	0.186[b]
Diabetes mellitus	24.50%	23.70%	0.888[b]
Dyslipidemia	25.40%	24.70%	0.889[b]
Chronic cough	1.80%	6.80%	0.08[b]
Constipation	14.30%	10.40%	0.35[b]
Pregnancy	5.6	3.5	< 0.0001[a]
Parity	4.8	2.9	< 0.0001[a]
Vaginal delivery	4.1	2.3	< 0.0001[a]
C-section	0.08	0.12	0.377[a]
Heaviest birth weight (g)	3516	3059	0.147[a]
Episiotomy	8.30%	9.20%	> 0.99[b]
Labor analgesia	3.70%	4.80%	0.768[b]
Home birth	25.90%	3.05%	< 0.0001[b]
Hysterectomy	15.20%	15.60%	> 0.99[b]

[a] Unpaired *t*-test.
[b] Fisher's exact-test.

Table 2

Logistic regression of factors associated with risk of pelvic organ prolapse.

Factors	Crude OR CI 95%	p	Adjusted OR CI 95%
Age ≥ 51	15.57 (4.73 – 51.2)	< 0.0001	11.89 (3.53 – 40)
Pregnancy ≥ 3	2.02 (1.24 – 3.28)	0.004	0.656 (0.283 – 1.51)
Vaginal delivery ≥ 3	3.12 (1.86 – 5.23)	< 0.0001	1.91 (0.7 – 5.22)
Parity ≥ 3	2.64 (1.62 – 4.31)	< 0.0001	2.01 (0.665 – 6.1)
Home birth	11.1 (4.14 – 29.7)	< 0.0001	9.645 (3.35 – 27.7)

OR: Odds ratio. CI: Confidence Interval.

Table 3

Distribution of the frequencies of MMP-3 genotypes between cases and controls.

MMP3 genotypes	Group A (n = 106)	Group B (n = 169)	p
6A/6A	55 (51.9%)	83 (49.1%)	0.895[a]
5A/6A	38 (35.9%)	65 (38.4%)	
5A/5A	13 (12.2%)	21 (12.5%)	
MMP3 aggregated genotypes			
6A/6A	55 (51.9%)	83 (49.1%)	0.7105[b]
5A/6A + 5A/5A	51 (48.1%)	86 (50.9%)	

[a] Chi-square test.
[b] Fisher's exact-test.

significant difference between the groups in the distribution of genotypes, even after calculating the contribution of the 5A recessive allele in the aggregated genotypes (5A / 5A + 5A / 6A).

Discussion

POP negatively affects the quality of life of women, especially after the age of 50, and among these, approximately 10% will require surgery until the age of 80. Its etiology is multifactorial, and lately, genetic factors have been extensively studied. [11,12],

SNPs at specific sites of genes that encode the synthesis and protein degradation of extracellular matrix components are a possibility that seek to explain this genetic origin for POP and other diseases.

The current information suggests that changes in ECM turnover play an important role in the pathogenesis of genital prolapse. Studies have already evaluated the synthesis, degradation, types and amount of collagen in patients with POPs. [5,13,14],

The polymorphism -1171 5A / 6A rs3025058 of the MMP-3 gene has already been related to certain diseases, mainly cancer and cardiovascular diseases. It is associated with an increased risk of development and metastasis of esophageal, lung and colorectal cancer. MMPs stimulate the proteolysis of ECM and basement membrane components, thus facilitating tumor dissemination. [15,16], This polymorphism was also associated with increased risk of atherosclerosis and restenosis in 6A / 6A homozygous individuals, probably due to failure of tissue remodeling [17]. In another study, the 5A allele was related to increased risk of deep vein thrombosis [18].

Some studies were conducted based on the various genomic polymorphisms of MMPs in women with genital prolapse and obtained contradictory results. According to the systematic reviews by Ward et al (2014) and Cartwright et al (2015), only two previous studies specifically addressed the rs3025038 polymorphism of MMP-3 in women with genital prolapse. [2,6,8,19],

In the first systematic review, 21 articles pertained to the genetic epidemiology of POP. Ten candidate genes were studied. All of the case-control studies defined the control as POP-Q stage 0 or 0-I. Only two studies looked at Brazilian population (9.5%, 2/21). Age was similar between cases and controls for nine studies. Two studies preferentially recruited controls from an older population; all other studies with an age discrepancy had controls that were younger than the prolapse cases. The metaanalysis suggests that COL3A1 rs1800255 genotype AA is associated with POP (OR, 4.79; 95% confidence interval [CI], 1.91–11.98; P < .001); compared with the reference genotype GG in the same populations. The similar finding in both Asian and Dutch populations increases the likelihood that this is a true association. [2]

Cartwright et al (2015) evaluated 34 studies that provided data on polymorphisms in or near 32 different genes. In pooled analysis, the rs4994 polymorphism of the ADRB3 gene was associated with overactive bladder (odds ratio [OR], 2.5; 95% confidence interval [CI], 1.7–3.6; n = 419). The rs1800012 polymorphism of the COL1A1 gene was associated with prolapse (OR, 1.3; 95% CI, 1.0–1.7; n = 838) and stress urinary incontinence (OR, 2.1; 95% CI, 1.4–3.2; n = 190). Other metaanalyses, including those for polymorphisms of COL3A1, LAMC1, MMP-1, MMP-3, and MMP-9 did not show significant effects. In this systematic review and meta-analysis, other epidemiological characteristics of the populations studied were not addressed. [19]

When we deal specifically with the polymorphism in question, we have two articles in the literature that address its relationship with POP.

Ferrari et al (2012) studied 137 women with stage II pelvic organ prolapse or more, while controls (96) were those defined as having stage 0–1. Controls were matched for POP risk factors such as age, BMI, smoking habits, parity and rate of instrumental deliveries. Previous hysterectomy and/or surgery for POP or urinary incontinence as well as a past history of malignancy were exclusion criteria. Family history showed that 35% of patients with POP had a first-degree relative affected by the same disease compared with a prevalence of 16% in the control group (p = 0.002; OR: 2.9, 95% CI 1.5–5.7). They concluded that MMP-3 polymorphism -1171 5A / 6A did not increase the risk for prolapse in the group of cases compared to controls, both including Italian women. The groups were homogeneous for all the clinical characteristics studied. They also found that

positive first-degree family history for POP constituted a factor significantly associated with risk of prolapse. [8]

Shorupski et al (2013) studied 133 women with stage II pelvic organ prolapse or more. The patients were subjected to pelvic floor repair procedures with mesh. The control group consisted of 132 women without significant POP. The vast majority of these patients were admitted with uterine myomas and subsequently underwent total abdominal hysterectomy or supracervical abdominal hysterectomy. The rest of the control group consisted of patients with dysfunctional uterine bleeding. The patients in the control group were younger than their counterparts in the study group (p < 0.05). Not surprisingly, fewer patients in the control group had entered the menopause (p < 0.05). Besides that, they found overexpression of MMP-1 1 G / 2 G 1 G / 2 G SNPs and MMP-3 -1612 / -1617 5A / 6A SNPs in Polish women with genital prolapse, but when analyzed in isolation, both polymorphisms were not associated with increased risk for POP. [6]

Thus, our study matches the findings of previous studies that investigated the same MMP-3 polymorphism associated with risk of genital prolapse. This is the first study developed with Brazilian postmenopausal women that seeks to relate the occurrence of genital prolapse with the polymorphism rs3025038 of the MMP-3 gene. We also evaluated a greater number of clinical characteristics of the sample than the previously mentioned studies.

Our samples were not fully comparable in some clinical characteristics, probably because the occurrence of more advanced degrees of prolapse occurs in older women with more gestations and deliveries, and in this case, in those of the study group. The relatively young age of the controls increases the risk for misclassification of women who have not yet manifested prolapse. Horst et al (2016) also found that age greater than 35 years, normal delivery and fetal weight greater than 4 kg are risk factors for genital prolapse in Brazilian women. [20] Vergeldt et al (2015) concluded that parity, vaginal delivery, age, and BMI are factors significantly associated with disease risk [21].

As a limitation, our study evaluated only one polymorphism and one gene alone. Shorupski et al found a correlation between polymorphisms of two different genes (MMP-1 and MMP-3) in patients with genital prolapse, further suggesting the complexity of genetic factors, such as etiology for the disease. In addition, history collection may present a significant recall bias. [6]

In conclusion, the polymorphism rs3025058 of the MMP-3 gene was not associated with the risk for POP in this sample. Age and home delivery were significantly associated with increased risk for the disease.

The negative findings of the study stimulate new research that analyzes polymorphisms of other genes related to the synthesis of collagen and other constituents of the ECM. The identification of patients at increased risk for developing POP would revolutionize the prevention of this change; guiding the choice of the birth route for example. In addition, it would assist in the identification of patients with greater risk of recurrence of POP, allowing a better evaluation of patients who are candidates for vaginal surgeries.

Conflict of interest

The authors have no conflicts of interest to declare.

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