Collagen gene polymorphisms relative to stress urinary incontinence

Associação entre polimorfismos do gene do colágeno e Incontinência urinária de esforço

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ABSTRACT

Objective: To investigate the possible association between the single-nucleotide polymorphism G→T at the Sp1-binding site at the first intron of the COLIA1 gene and Stress Urinary Incontinence. Study design: We compared the allelic and genotypic frequencies of the COLIA1 gene in patients with SUI (n=29) to those with healthy individuals (n=22). Our strategy was the amplification and the restriction fragment length polymorphism of a DNA fragment with Sp1-binding at the first intron of COLIA1 gene. The results were statistically assessed by Fisher and Mann Whitney’s tests. Results: There were no significant differences between the allelic and genotypic frequencies of COLIA1 gene when comparing individuals with SUI to healthy ones (p=1). Conclusion: The results showed no association between the G→T single-nucleotide polymorphism at the Sp1 binding site at the first intron of the COLIA1 gene and Stress Urinary Incontinence.

Keywords: colia1 gene, sp1 polymorphism, stress urinary incontinence.
RESUMO
Objetivo: Investigar a possível associação entre o polimorfismo de nucleotídeo único G→T no site de ligação Sp1 no primeiro intron do gene COLIA1 e a Incontinência Urinária de Estresse. Projeto do estudo: Comparamos as frequências alélicas e genotípicas do gene COLIA1 em pacientes com IUE (n=29) com aqueles com indivíduos saudáveis (n=22). Nossa estratégia foi a amplificação e o polimorfismo do comprimento do fragmento de restrição de um fragmento de DNA com ligação Sp1 no primeiro intron do gene COLIA1. Os resultados foram avaliados estatisticamente pelos testes de Fisher e Mann Whitney. Resultados: Não houve diferenças significativas entre as frequências alélicas e genotípicas do gene COLIA1 ao comparar indivíduos com SUI com indivíduos saudáveis (p=1). Conclusão: Os resultados não mostraram associação entre o polimorfismo de nucleotídeo único G→T no site de ligação Sp1 no primeiro intron do gene COLIA1 e a Incontinência Urinária de Estresse.

Palavras-chave: gene colia1, polimorfismo sp1, incontinência urinária de esforço.

1 INTRODUCTION
Stress Urinary Incontinence (SUI) is defined as the involuntary leak of urine caused by work, physical exercises, sneezing and/or coughing (ABRAMS et al, 2018). The etiopathogenesis of this disease is both complex and not fully understood. Nevertheless, it seems that there is a synergism between environmental and genetic factors in its development, characterizing it as a multifactor disease. Epidemiological studies suggest that obesity, aging, vaginal and instrumental deliveries increase the risk of this condition (TÄHTINEN et al, 2016). Genetic factors involvement has also been suggested by studies with family members of patients with incontinence and by the records of this disease in women with no risk factors (ZHANG et al 2021; ANDRADA HAMER e PERSSON, 2013).

According to the integral theory, the endopelvic fascia forms a suburethral support that ensures the female urinary continence (BERGSTRÖM, 2022). It is known that the main component of the fascia is type I collagen, which is responsible for much of its strength. Also, a large number of studies have shown both qualitative and quantitative changes of this protein in SUI patients (LIAPIS et al, 2000; HAN et al, 2014; FRANIC e FISTONIC, 2019). The type I collagen comprises three peptide chains: 2 α1 (I) and 1 α2 (I), (α1 (I ) 2α2 (I)), which in turn are encoded by genes COLIA1 and COLIA2, respectively (BORNSTEIN et al, 1987). There are reports where a single-nucleotide polymorphism (SNP) G→T (rs1800012) at the regulatory Sp1 binding site at the first intron 1 of COLIA1 gene is related to changes in the type I collagen structure (BORNSTEIN et al, 1987). The association of this SNP was firstly reported in the bone
tissue, with an increase in osteoporotic fractures in individuals carrying the allele T (UITTERLINDER et al, 1998; COUSMINER et al, 2019). SKORUPSKI et al (2006) and SIOUTIS et al (2011) also reported the association of this SNP with SUI in a Caucasian population.

The investigation of the polymorphism of the COLIA1 gene in different populations is an important contribution to understand the involvement of collagen in the pathogenesis and clinical manifestation of SUI. The aim of this study was to investigate the possible association between the SNP G→T at the Sp1 binding site at the first intron of COLIA1 gene and Stress Urinary Incontinence in a admixed Brazilian population.

2 MATERIALS AND METHODS

This is a case-control study, which compared allelic variants of the COLIA1 gene of SUI patients (n = 29) and continent women (n = 22), seen at Hospital São Marcos, Teresina, Piauí, Brazil. The SUI diagnosis was established after the urodynamic investigation (ROSIER et al, 2017). Patients diagnosed with either detrusor hyperactivity or previous neurological disorders were excluded from the study. The control group was comprised of continent women, whose medical history and effort test were negative for SUI. Patients who had undergone surgery of the pelvic floor were excluded from the control group. The following variables were evaluated: COLIA1 gene’s genotypic and allelic frequencies, ethnicity, age, number of pregnancies, number of vaginal deliveries, body weight index (BWI), menopausal status, current or previous smoking and pelvic organ prolapse measured by the Pelvic Organ Prolapse Quantification (POP-Q) (BUMP et al, 1996). The studied individuals’ ancestry was established by examining the following criteria: morphological face features (shape of the nose, mouth and eyes), type of hair and skin color. Individuals were classified as Caucasian, African-descendants, Admixed and Amerindians. The study was approved by the Ethics Committee of Piauí Federal University (CAAE: 0189.0.045.000-07). All of the patients had the term of consent read and explained to them, and afterwards they all signed the form.

Peripheral blood was collected from all cases and controls. Genomic DNA was extracted from blood leucocytes with a Wizard Genomic DNA Purification Kit (Promega, Madison, USA). Determination of the COLIA1 polymorphism was carried out by 2-step polymerase chain reaction (PCR), as described by Skorupski et al. The first step of PCR was carried out with the following primers set: P1, 5'-GGAAGACCCGGTTATTGCT-3' (forward) and P2, 5'-CGCTGAAGCCAAAGTGAAATA-3' (reverse). The primers used
for the nested PCR reaction were: P3, 5'-TAACTTCTGGACTATTTGCGGACTTTTTGG-3' (forward) and P4, 5'-GTCCAGCCCTCATCCTGGCC-3' (reverse). The recognition of the type of polymorphism at the Sp1 site was based on analysis of a restriction fragment length polymorphism. PCR products were digested with BalI (MscI) (Biolabs, New England) that recognize the sequence 5'-TGGCCA-3'.

Allele assignments were based on the size of the resulting fragments, being 262 base pairs (bp) for a wild genotype (GG), 262 bp and 242 bp for variant GT and 242 bp for variant TT.

Genital prolapse stages, the prevalence of menopause, smoking, frequency of genotypes between cases and controls were compared using Fisher's exact test. The variables related to age, number of vaginal deliveries, number of pregnancies and body weight index (BWI) were compared using the Mann-Whitney’s test. Statistical tests were performed using the Graph Pad Prism software version 5. A 5%-alpha error was adopted as a criterion for rejection of invalidity.

3 RESULTS

All patients in this study were from Piauí and Maranhão, and were all of admixed ethnicities. There was no significant difference between cases and controls regarding age, BWI, pregnancies, menopause, smoking and genital prolapse stages. The number of vaginal deliveries was significantly higher in continent patients (P = 0.0345) (Table 1 and 2).

Table 1. Demographical and clinical characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Women with SUI (n = 29)</th>
<th>Women without SUI (n = 22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) †</td>
<td>50 (28-60)</td>
<td>42 (35-67)</td>
<td>0.08</td>
</tr>
<tr>
<td>Parity (range) †</td>
<td>3 (0-5)</td>
<td>4 (2-7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Vaginal deliveries (number) †</td>
<td>2 (0-5)</td>
<td>3 (1-7)</td>
<td>0.02</td>
</tr>
<tr>
<td>BWI(Kg/m²) †</td>
<td>26.3(19.6-34.7)</td>
<td>23.9 (16.5-38.1)</td>
<td>0.2</td>
</tr>
<tr>
<td>Menopausal (%)</td>
<td>41.4</td>
<td>36.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>34.5</td>
<td>22.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

* P values determined by Mann-Whitney test for age, pregnancies, vaginal deliveries and BWI and by Fisher test for menopause and smoking.
† values given in medians and variations
Table 2. Distribution of POP-Q stage for incontinent and continent women.

<table>
<thead>
<tr>
<th>POP-Q stage</th>
<th>Women with SUI (n=29)</th>
<th>Women without SUI (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

The genotypic and allelic frequencies of the COLIA1 gene are shown in table 3. There was no significant difference regarding the distribution of allelic and genotypic frequencies between cases and controls (p = 1). None of the studied cases had the type TT variant.

The distribution of genotypes COLIA1 gene was in Hardy-Weinberg equilibrium in the total group of patients, with 56.8% for the GG genotype and 43.1% for the GT genotype.

Table 3. Allelic and genotypic frequencies of COLIA1 variants in women with SUI and controls.

<table>
<thead>
<tr>
<th>Allele-genotype</th>
<th>Women with SUI (%) (n=29)</th>
<th>Women without SUI (%) (n=22)</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P* value</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>77.5</td>
<td>79.5</td>
<td>0.89</td>
<td>0.341-2.320</td>
<td>1</td>
</tr>
<tr>
<td>T</td>
<td>22.4</td>
<td>20.4</td>
<td>1.12</td>
<td>0.431-2.928</td>
<td>1</td>
</tr>
<tr>
<td>GG</td>
<td>55.2</td>
<td>59.1</td>
<td>0.85</td>
<td>0.277-2.616</td>
<td>1</td>
</tr>
<tr>
<td>GT</td>
<td>44.8</td>
<td>40.9</td>
<td>1.17</td>
<td>0.382-3.604</td>
<td>1</td>
</tr>
<tr>
<td>TT</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* P value determined by Fisher test.

4 COMMENT

This study found no association between the SNP G→T at the Sp1 of COLIA1 gene and SUI for the admixed Brazilian population.

These results should be interpreted with great caution, since studies of association between the SNP of the COLIA1 gene and SUI are still at the beginning. Indeed, there is only two reports in the literature (SKORUPSKI et al, 2006; SIOUTIS et al 2011) showing this association, contrasting with another work which shows no association between the polymorphism and genital dystopias (SKORUPSKI et al, 2006).

The fact that our results differ from those found by SKORUPSKI et al (2006) and SIOUTIS et al (2011) may be partly explained by the difference in ethnicity. While patients in the Polish and Greek study were Caucasian, the sample of this study was...
admixed. This is an important factor that may have been conclusive for the results, since the occurrence of a particular genetic variation may differ among the different ethnicities (FUTUYMA, 1992). Studies on ancestry, based on mitochondrial DNA revealed that the gene pool of admixeds in northeastern Brazil is trihybrid: 34% Caucasians, 44% Africans and 22% Amerindians (PENA, 2002). Similarly, our group showed that the admixed population of Teresina had a predominantly mixture of Caucasian Portuguese and African-descendants and a lower pool of Amerindians (MONTE et al, 2004). Therefore, if the variability of the gene COLIA1 is associated with the Caucasian ethnicity, the low presence of this ethnicity in our sample may have weakened the chances of detecting a higher occurrence of polymorphic GT genotype within the SUI patients.

The TT genotype was not found in our samples. This may be due to the very low T allelic frequency. Previous studies carried out with the Dutch and Brazilian Caucasian population have shown 3% and 2.27% frequency of TT genotype, respectively (VAN DER SLUIS et al, 2002; BARROS et al, 2002).

Therefore, in order to detect the TT genotype in a trihybrid population with a low frequency of Caucasian genes, it would be necessary a larger sample size.

It is true that there are many contradictions in the literature as for the complexity of endo-pelvic fascia constitution, about which proteins might be involved in the SUI pathogenesis, making difficult the search for the genes responsible for this condition. It is unlikely that a single gene or a protein be involved in this disease, since it is a multifactorial disease. Microarray studies, for example, comparing gene expression between patients with SUI and healthy subjects, have shown a change of expression of other genes involved in the activity of extracellular matrix (ECM), mainly related to the metabolism of collagen and elastin. Moreover, there are comparative studies between patients with SUI and without which emphasize the role of other ECM proteins in the pathogenesis of this disease, such as proteoglycans, elastic fibers, actin and tissue inhibitors of metalloproteinases, proteins responsible for degradation of collagen (CHENG YEH, 2011; ISALI et al, 2020).

In conclusion, it is possible to suggest that the genetic diversity of the COLIA1 gene is not one of the main factors determining differences among individuals on SUI susceptibility in the studied population. However, the lack of association does not exclude the role of this SNP in the pathogenesis of the disease. On the contrary, it only suggests that it has no impact on the risk of developing SUI in the studied population.
Since SUI is a complex disease, of multi-genetic inheritance, studies such as genomic wild are necessary to show the correlation of SUI with genomic regions until now unexplored. This would allow the investigation of new genes in the pathogenesis of this disease.

**ABBREVIATIONS**

SUI: Stress Urinary Incontinence  
POP-Q: Pelvic Organ Prolapse Quantification  
BWI: Body weight index  
PCR: Polymerase chain reaction  
RFLP: Restriction fragment length polymorphism  
SNP: Single-nucleotide polymorphism  
CAAE: Ethics Committee Evaluation Certificate
REFERENCES


