Study on vitamin D receptor gene polymorphisms in patients with urinary tract infections conducted in Northwestern Mexico

Estudio de los polimorfismos del gen del receptor de vitamina D en pacientes del noroeste de México con infección urinaria

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INTRODUCTION

Urinary tract infections are caused by the presence of uropathogens in the genitourinary system and are a severe public health problem. The clinical manifestations and their severity are produced by both an excessive inflammatory response in the host and microbial pathogenicity. The most common microbes are Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and Staphylococcus saprophyticus.

Human genetic factors have also been proposed to modulate the immune response. Vitamin supplements, such as vitamin D, have been reported to prevent urinary tract infections, whereas insufficient levels have been associated with risk and recurrence of urinary tract infections and a low expression of antimicrobial peptides in urine.

Vitamin D has a pleiotropic effect, acting as an immunomodulator of the innate immune response in the urothelium. It acts through its nuclear receptor, the vitamin D receptor (VDR), encoded by the VDR gene located at 12q13.11, whose single nucleotide polymorphisms (SNPs) have been previously associated with several infectious diseases, such as urinary tract infection, tuberculosis, leprosy, and pertussis in different populations.

In Mexico, the genetic background of urinary tract infections is unknown. The analysis of the VDR genetic polymorphisms, FokI, BsmI, ApaI, and TaqI, could be a starting point.

MATERIALS AND METHODS

Study subjects

We conducted a comparative, prospective, and cross-sectional study at the Hospital General de Culiacán, in the state of Sinaloa, Mexico, within the time frame of August 2016 to July 2017. A total of 325 individuals were included in the analysis and classified into two groups, according to their clinical features. Group 1 (n = 119) consisted of the patients with positive bacterial urine culture and urinary tract infection diagnosis made by internists, in accordance with the European Guidelines on Urological Infections of the European Association of Urology (EAU), and risk factors (e.g., diseases associated with urinary tract infections, previous urinary tract infection, hospitalization, and Foley catheter), excluding those patients with urine culture positive for fungi and mixed flora. Group 2 (n = 206) was composed of healthy controls. They were individuals that had no urinary tract infection and whose urine cultures were negative, but who also presented with the abovementioned risk factors.

The demographic data and the following clinical features were obtained from electronic medical record systems and entered into a database: clinical diagnosis e.g., asymptomatic bacteriuria, cystitis, pyelonephritis, catheter-associated urinary tract infections, and urosepsis; host risk factors according to the 2015 ONERUC classification system of the EAU; signs and symptoms, such as fever, lower urinary tract symptoms, abdominal pain, positive costovertebral angle percussion, nausea/vomiting, tenesmus, constipation, pyuria, proteinuria, and hematuria; associated diseases; previous urinary tract infection; hospitalization; and microbiologic test results.

All participants, as well as their parents and grandparents born in Sinaloa, were included in the analysis. Written statements of informed consent were obtained prior to the study, which was approved by the Office of Research Ethics.

DNA extraction and VDR genotyping

Genomic DNA was obtained from peripheral blood using the DTAB-CTAB method. The
VDR polymorphisms (FokI: rs2282570, BsmI: rs1544410, ApaI: rs7975232, and TaqI: rs731236) were genotyped using the polymerase chain reaction-restriction fragment length polymorphism method previously described by Shafia et al.\textsuperscript{14} and Li et al.\textsuperscript{15} and visualized in polyacrylamide gel electrophoresis at 6%. Ten percent of our results were randomly selected, then analyzed and confirmed by an independent observer.

**Statistical analysis**

For the association analysis between genotypes and urinary tract infection we used the chi-square test and odds ratio (OR) with a 95% confidence interval. To determine if there was a relationship between the clinical features and genotypes, we used the logistic regression method, adjusted by age and sex. The STATA version 13.0 program was utilized for both evaluations.

The SHEsisPlus free online software\textsuperscript{16} was used to analyze the haplotypes and linkage disequilibrium calculation between the two groups. The level of significance was defined as $p < 0.05$.

**RESULTS**

**Study subjects**

For the patients in group 1 (86 females and 33 males), the mean and standard deviation (SD) of age was $48 \pm 23.03$ and it was $44 \pm 22.38$ for the group 2 healthy controls (109 females and 97 males). The bacterial uropathogens found, in order of prevalence, were: *Escherichia coli*, *Pseudomonas sp*, *Klebsiella pneumonia*, *Enterococcus faecalis*, *Proteus mirabilis*, *Acinetobacter baumannii complex*, and *Serratia odorifera*. The clinical features of group 1 are shown in Table 1.

In the group 2 healthy controls, 43.69% of the subjects had associated diseases, such as diabetes, kidney disease, urolithiasis, sepsis, and prostatic hyperplasia. A total of 25.24% had presented with a previous urinary tract infection, 56.79% had been hospitalized, and 12.62% had required a Foley catheter.

**Allelic, genotypic, and haplotypic analysis**

All loci were in agreement with the Hardy-Weinberg equilibrium, except ApaI in group 1 and BsmI in both groups. Table 2 shows similar,

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**Table 1. Clinical characteristics of patients with urinary tract infections (group 1)**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic bacteriuria</td>
<td>60 (50.42)</td>
</tr>
<tr>
<td>Cystitis</td>
<td>17 (14.29)</td>
</tr>
<tr>
<td>Pyelonephritis</td>
<td>30 (25.21)</td>
</tr>
<tr>
<td>Catheter-associated urinary tract infections</td>
<td>7 (5.88)</td>
</tr>
<tr>
<td>Urosepsis</td>
<td>5 (4.20)</td>
</tr>
</tbody>
</table>

**Risk factors, classification system**

O: NO known/associated Risk Factor (RF); R: Recurrent urinary tract infection RF, but no risk of severe outcome; E: Extra-urogenital RF, with risk of more severe outcome; N: Nephropathic disease, with risk of more severe outcome; U: Urologic RF, with risk of more severe outcome, that can be resolved during therapy; C: Permanent urinary Catheter and non-resolvable urologic RF, with risk of more severe outcome.

**Table 2. Allelic, genotypic, and haplotypic analysis**

<table>
<thead>
<tr>
<th>Allele</th>
<th>FokI</th>
<th>FokI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.**
and therefore not statistically significant, allelic and genotypic frequencies between the two groups prior and subsequent to adjustment for age and sex, except for the CA genotype of ApaI, which was more prevalent in the group 2 healthy controls (p = 0.021, OR = 0.547, 95% CI = 0.327-0.912).

The logistic regression analysis found association between the FokI-C allele and lower urinary tract symptoms (p = 0.042, OR = 0.406, 95% CI = 0.169-0.969), as well as the Bsml-AA genotype and recurrent urinary tract infections (p = 0.008, OR = 8.488, 95% CI = 1.767-40.772). The remaining variables showed no association.

The haplotype analysis showed linkage disequilibrium between Bsml-Apal-TaqI (D’>0.90, R²-Bsml/Apal = 0.40, R²-Bsml/TaqI = 0.80, and R²-Apal/TaqI= 0.36). Six haplotypes were the most representative and showed no differences between the two groups (p > 0.05). The most common was TGCT (Table 3).

Finally, the comparison of haplotype frequencies in the group 1 patients, according to the presence/absence of clinical features through the logistic regression model showed an association of the TGAT haplotype with lower urinary tract symptoms (p ≤ 0.001, OR = 5.448, 95% CI = 1.890-15.707) and abdominal pain (p = 0.006, OR = 4.157. 95% CI = 1.401-12.326), and of the CGCT haplotype with lower urinary tract symptoms (p = 0.016, OR = 0.341, 95% CI = 0.137-0.849). The remaining variables showed no association.

**DISCUSSION**

The vitamin D endocrine system plays an important role in modulating the immune response. In fact, there are several studies that support the association between vitamin D and bacterial infections, implicating polymorphisms in the VDR gene. Nevertheless, there are presently only two reports on VDR polymorphisms and urinary tract infection. The first, by Garcia-Nieto et al.,

<table>
<thead>
<tr>
<th>SNP</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p-value, OR, [95%CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>35 (29.41)</td>
<td>44 (21.36)</td>
<td>ns</td>
</tr>
<tr>
<td>TC</td>
<td>53 (44.54)</td>
<td>111 (53.88)</td>
<td>ns</td>
</tr>
<tr>
<td>CC</td>
<td>31 (26.05)</td>
<td>51 (24.76)</td>
<td>ns</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>(48.31)</td>
<td>(51.69)</td>
<td>ns</td>
</tr>
<tr>
<td>Bsml Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>76 (63.87)</td>
<td>130 (63.11)</td>
<td>ns</td>
</tr>
<tr>
<td>GA</td>
<td>33 (27.73)</td>
<td>56 (27.18)</td>
<td>ns</td>
</tr>
<tr>
<td>AA</td>
<td>10 (8.40)</td>
<td>20 (9.71)</td>
<td>ns</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>(22.26)</td>
<td>(23.30)</td>
<td>ns</td>
</tr>
<tr>
<td>ApaI Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>48 (40.34)</td>
<td>63 (30.58)</td>
<td>ns</td>
</tr>
<tr>
<td>CA</td>
<td>45 (37.82)</td>
<td>108 (52.42)</td>
<td>0.021, 0.547, [0.327-0.912]</td>
</tr>
<tr>
<td>AA</td>
<td>26 (21.84)</td>
<td>35 (16.99)</td>
<td>ns</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>(40.75)</td>
<td>(43.20)</td>
<td>ns</td>
</tr>
<tr>
<td>TaqI Genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>75 (63.03)</td>
<td>128 (62.14)</td>
<td>ns</td>
</tr>
<tr>
<td>TC</td>
<td>41 (34.45)</td>
<td>65 (31.55)</td>
<td>ns</td>
</tr>
<tr>
<td>CC</td>
<td>3 (2.52)</td>
<td>13 (6.31)</td>
<td>ns</td>
</tr>
<tr>
<td>Allele</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>(19.74)</td>
<td>(22.08)</td>
<td>ns</td>
</tr>
</tbody>
</table>

OR: Odds ratio; CI: Confidence interval; ns: not significant
discarded the BsmI polymorphism as a candidate for a genetic biomarker in Spanish children with idiopathic hypercalciuria and urinary tract infection. The second, conducted by Aslan et al., proposed Apal as a protective factor against urinary tract infection and FokI as a predisposing factor in a Turkish pediatric population.

Our study is interesting because it is the first to offer suggestions and proposals about VDR gene polymorphisms in relation to clinical features of urinary tract infections. They are discussed below.

The Apal CA genotype could be a protective factor against bacterial urinary tract infections in individuals with risk factors, as is the case with the FokI C allele and the CGCT haplotype in lower urinary tract symptoms. In contrast, the BsmI AA genotype could predispose to urinary tract infection recurrence and the TGAT haplotype could increase the risk for developing lower urinary tract symptoms and abdominal pain in patients.

The functional role of the Apal and BsmI polymorphisms is still unknown, but it is possible that they are involved in the mRNA stability of the VDR. Due to its location in the 3' UTR, we suggest that both polymorphisms could deregulate vitamin D receptor expression in the urothelium. Thus, we assume that BsmI affects the capacity of vitamin D to enhance the expression of antimicrobial peptides, decreasing the full elimination of bacterial uropathogens, and in turn, favoring urinary tract infection recurrence.

On the other hand, the FokI genotypes may interact differentially with important transcription factors in the genes of the immune response. For example, once activated, NFκB enhances IL-6 transcription in bladder epithelial cells. We believe that could influence the beginning of lower urinary tract symptoms and their severity. Furthermore, Gurocak et al. have shown the hereditary predisposition of lower urinary tract symptoms in some families. In addition, a protective effect of the TaqI-VDR polymorphism against lower urinary tract symptom development in Japanese, Thai, and Indian men was reported by Cartwright et al. in their meta-analysis.

Moreover, for the haplotypic results, we suggest that the allelic variation in FokI (T or C allele) on the TGAT/CGCT haplotypes modulates the risk/protection for lower urinary tract symptom development. That has not been proved but could lay the groundwork for interesting future clinical investigations about VDR-vitamin D and its therapeutic potential in urinary tract infections.

Finally, the association found in the present study between abdominal pain in patients with urinary tract infections and the TGAT haplotypes of the VDR gene polymorphism was observed for the first time. However, there are reports on the participation of vitamin D levels and the VDR polymorphisms in the development of different kinds of pain, such as chronic pain, lower back pain, and knee pain in patients with osteoarthritis. The manifestation of pain has been explained by the role of vitamin D in nociceptive and inflammatory pain mechanisms, reducing the release of pro-inflammatory cytokines and suppressing T-cell response. In vitro studies have also shown that vitamin D inhibits the synthesis of prostaglandin E2.

These findings suggest that VDR gene polymorphisms have a possible role in the clinical features of urinary tract infection. Nevertheless, they raise more questions than answers. Clinical trials must be conducted to test the results of our study and rule out any random effects that may have occurred.

Acknowledgments

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