Lack of association of vitamin D receptor gene polymorphisms with susceptibility to type 1 diabetes mellitus in the Portuguese population

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Summary The vitamin D receptor (VDR) gene is a candidate gene for susceptibility to autoimmune disorders. Association studies of VDR polymorphisms and risk of type 1 diabetes often produced conflicting results in different ethnic backgrounds. The aim of this study was to test for association between common VDR polymorphisms and the genetic susceptibility to type 1 diabetes in the Portuguese population. We genotyped 207 patients with type 1 diabetes and 249 controls for the FokI T>C (rs10735810), BsmI A>G (rs1544410), Apal G>T (rs7975232), and TaqI C>T (rs731236) single nucleotide polymorphisms by polymerase chain reaction and restriction fragment length polymorphism analysis. The distribution of VDR genotype, allele, and haplotype frequencies did not differ significantly between patients and controls. These data suggest that the single nucleotide polymorphisms of the VDR gene are unlikely to contribute significantly to type 1 diabetes susceptibility in the Portuguese population.

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KEYWORDS
Vitamin D receptor; VDR; Single nucleotide polymorphism; SNP; Type 1 diabetes mellitus; Genetic susceptibility

Introduction
Vitamin D is a potent modulator of the immune system and is involved in the regulation of cell proliferation and differentiation [1]. Vitamin D is an effective immunosuppressant via inhibition of lymphocyte activation and cytokine production [1] and prevents or markedly suppresses the development of several autoimmune diseases in animal models [1]. The administration of vitamin D protects against the development of insulitis and type 1 diabetes in nonobese diabetic mice [2]. In humans, epidemiological studies indicated that dietary vitamin D supplementation during early childhood decreases the risk of type 1 diabetes.
Genotyping

Genomic DNA was extracted from whole blood using standard protocols. DNA was amplified by polymerase chain reaction (PCR) using previously described primer sequences [14]. Amplified fragments were digested with the appropriate restriction enzyme (New England Biolabs, Beverly, MA, USA) according to the manufacturer’s instructions and visualized on a 3% agarose gel. The FokI T>C (rs10735810) SNP was analyzed by digestion of a 267-base pair (bp) PCR product with FokI, which resulted in two fragments of 206 and 61 bp in the presence of the T allele and in an uncut fragment in the presence of the C allele. The BsmI A>G (rs1544410) SNP was analyzed by digestion of a 191-bp PCR product with BsmI, which resulted in two fragments of 115 and 76 bp in the presence of the G allele and in an uncut fragment in the presence of the A allele. The Apal G>T (rs7975232) and TaqI C>T (rs731236) SNPs were analyzed by digestion of a 745-bp PCR product with Apal, which resulted in two fragments of 528 and 217 bp in the presence of the G allele and in an unfragmented fragment in the presence of the T allele, and by digestion with TaqI, which resulted in three fragments of 293, 251, and 201 bp in the presence of the C allele and in two fragments of 494 and 251 bp in the presence of the T allele. VDR haplotypes derived from FokI, BsmI, Apal, and TaqI polymorphisms were constructed using informative combinations of genotypes (e.g., an individual genotyped as CT/AA/GG/TT was considered to possess haplotypes C/A/G/T + T/A/G/T). Individuals heterozygous for more than one polymorphic site were not considered for haplotype frequency analysis, because the distribution of the alleles between the two homologous chromosomes could not be unequivocally defined (e.g., an individual genotyped as CT/AG/GG/TT could possess either haplotypes C/A/G/T + T/G/G/T or C/G/G/T + T/A/G/T).

Statistical analysis

Pearson’s χ² test of independence, with one degree of freedom, was used to examine differences of genotype, allele, and haplotype frequencies between patients and controls. When expected values were less than 5, Fisher’s exact test was used. Two-tailed p values were calculated and statistical significance was set at p < 0.05. Odds ratios and the corresponding 95% confidence intervals were calculated for each genotype, allele, and haplotype. Hardy-Weinberg equilibriums were assessed using the χ² goodness of fit test to compare the observed and allele-based expected genotype frequencies. Power calculation was analyzed using the program Power and Sample Size Calculations (Version 2.1.30).
allele, with an estimated power of 0.8 and a type 1 error probability of 0.05.

Discussion

The VDR locus has been studied in different populations for association with susceptibility to immune-mediated diseases, but with inconsistent findings in type 1 diabetes mellitus [11]. To clarify the contribution of VDR polymorphisms to genetic susceptibility to type 1 diabetes mellitus among Portuguese patients, we conducted a retrospective case-control study by analyzing four well-characterized VDR polymorphisms. We reported no evidence of allelic or genotypic association of the FokI $T>C$ (rs10735810), BsmI $A>G$

<table>
<thead>
<tr>
<th>Haplotype$^a$</th>
<th>Controls, $n$ (%)</th>
<th>Patients, $n$ (%)</th>
<th>Odds ratio (95% CI)</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FokI/BsmI/Apol/TaqI</td>
<td>n = 223</td>
<td>n = 186</td>
<td>1.04 (0.69-1.57)</td>
<td>0.840</td>
</tr>
<tr>
<td>C/G/G/T</td>
<td>77 (34.5)</td>
<td>66 (35.5)</td>
<td>1.04 (0.69-1.57)</td>
<td>0.840</td>
</tr>
<tr>
<td>C/A/T/C</td>
<td>56 (25.1)</td>
<td>52 (28.0)</td>
<td>1.16 (0.75-1.80)</td>
<td>0.516</td>
</tr>
<tr>
<td>T/G/T/G</td>
<td>50 (22.4)</td>
<td>35 (18.8)</td>
<td>0.80 (0.50-1.30)</td>
<td>0.371</td>
</tr>
<tr>
<td>T/A/T/C</td>
<td>16 (7.2)</td>
<td>10 (5.4)</td>
<td>0.74 (0.33-1.63)</td>
<td>0.458</td>
</tr>
<tr>
<td>C/G/T/T</td>
<td>11 (4.9)</td>
<td>13 (7.0)</td>
<td>1.45 (0.65-3.25)</td>
<td>0.378</td>
</tr>
<tr>
<td>T/G/T/T</td>
<td>5 (2.2)</td>
<td>4 (2.2)</td>
<td>0.96 (0.27-3.35)</td>
<td>1.000</td>
</tr>
<tr>
<td>C/A/T/T</td>
<td>4 (1.8)</td>
<td>1 (0.5)</td>
<td>0.30 (0.04-1.99)</td>
<td>0.382</td>
</tr>
<tr>
<td>C/A/G/T</td>
<td>3 (1.3)</td>
<td>2 (1.1)</td>
<td>0.80 (0.16-4.04)</td>
<td>1.000</td>
</tr>
<tr>
<td>T/A/G/T</td>
<td>1 (0.4)</td>
<td>0</td>
<td>0.00 (0.00-4.61)</td>
<td>1.000</td>
</tr>
<tr>
<td>C/A/G/C</td>
<td>0</td>
<td>2 (1.1)</td>
<td>0.63 (0.63-∞)</td>
<td>0.206</td>
</tr>
<tr>
<td>T/A/G/C</td>
<td>0</td>
<td>1 (0.5)</td>
<td>0.31 (0.31-∞)</td>
<td>0.455</td>
</tr>
</tbody>
</table>

CI = confidence interval.

$^a$ Haplotypes deduced from informative combinations of genotypes.
VDR polymorphisms in type 1 diabetes (rs1544410), Apal G→T (rs7975232), or TaqI C→T (rs731236) SNPs of the VDR gene with type 1 diabetes mellitus in our population.

Such associations have been reported in populations from India [15], Taiwan [16], Germany [17–19], Hungary [14], Japan [20,21], the Netherlands [22], Croatia [23,24], Spain [25,26], and Chile [27]. However, no associations were detected in other populations from Brazil [28], Romania [29,30], Finland [30,31], Norway [30], the United States [30], and the United Kingdom [30].

The apparent discrepancies between this and other studies could be a result of the effect of ethnic differences related to the distribution of VDR polymorphisms in these populations, as well as to interactions with other genetic or environmental factors involved in the pathogenesis of type 1 diabetes mellitus. Human leukocyte antigen studies indicated that although the Portuguese are genetically related to Spaniards [32], they seem to have some ethnic-specific characteristics that distinguish them from other Europeans [32], and this may have contributed to the outcome of this study. Furthermore, because these polymorphisms, with the potential exception of the FokI variant [33], have no known functional effects, the VDR itself may not be the disease-affecting locus, but rather a marker locus in linkage disequilibrium with the real disease locus, and the discrepant findings may reflect variable strengths of linkage disequilibrium in different populations.

In conclusion, our case-control study indicates that the four SNPs of the VDR gene studied are not associated with type 1 diabetes mellitus in the Portuguese population.

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References


