Association of Vitamin D Receptor Gene Polymorphisms with Prostate Cancer Risk in the Pakistani Population

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Abstract

Background: Vitamin D receptor (VDR) gene has been a subject of extensive pharmacogenetic research recently. Association studies between different types of cancers including prostate cancer (PCa) and VDR gene polymorphism have also been conducted. The objective of this study was to find possible associations between PCa and VDR gene polymorphisms in the Pakistani population. Materials and Methods: A total of 162 subjects, including prostate cancer patients and controls, were genotyped for Apa I, Taq I and Fok I polymorphisms in the VDR gene using allele specific PCR, PCR-RFLP and direct DNA sequencing. Allelic frequencies were tested for Hardy-Weinberg equilibrium and associations between the genetic markers and PCa were calculated using logistic regression. Results: Apa I CC genotype was found to have strongest association with PCa risk, and “A” genotype was found to have protective effect. Fok I and Taq I did not have appreciable levels of association with PCa, although Taq I “TC” heterozygotes seemed to have some protective effect. Similarly the “C” allele of Fok I also seemed to have protective effect. Conclusions: To our knowledge, this is the first report showing association between VDR gene polymorphisms and PCa in Pakistan. Our findings may be somewhat skewed because of small sample size and tendency of consanguineous marriages in Pakistani society; nevertheless, it shows the trend of association and protective effects of certain VDR gene polymorphisms against PCa.

Keywords: Prostate cancer - VDR gene - polymorphisms - Pakistan

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Introduction

Prostate Cancer (PCa) is one of the most common cancer in men above 60 years of age (Stangelberger et al., 2008). The National Cancer Institute predicts 233,000 new cases in year 2014 alone. In Pakistan, PCa is reported as the most common cancer in elderly males (Arshad and Ahmad, 2013). Another recently study conducted, places prostate cancer at the third position of all the most commonly encountered malignancies among all Pakistani males (Jamal et al., 2014). Pathology of PCa is mainly chalked up to imbalance between cell growth and apoptosis in prostate tissue. Other cancers, PCa is multi-step and multi-factorial disease. Age, genetics and ethnicity are the factors most commonly implicated in the pathogenesis of PCa (Sporn and Suh, 2002; Nelson et al., 2003). Low serum levels of vitamin D are found to be associated with risk of different types of cancers (Giovannucci, 2009). The link between calcitriol and the inhibition of PCa is already well-established (Krishnan et al., 2003; Beer and Myrthue, 2004). Antiproliferative effect of calcitriol has been ascertained on many PCa cell lines (Peehl et al., 1994; Miller et al., 1995). Similarly, reports have been unanimous in hailing anticancer effect of calcitriol on prostate cancer mice models (Feldman et al., 1995; Trump et al., 2004). The actions of calcitriol require intact signaling pathways, which are all initiated by binding of active vitamin D to vitamin D receptor (VDR) (Vanoirbeek et al., 2011). VDR belongs to a steroid nuclear receptor super-family, which is a ligand-activated transcription factors (Haussler et al., 1995). Vitamin D- Vitamin D receptor complex bind to retinoid X receptor to form a heterodimer, which is responsible for expression/repression of genes involved in cell cycle arrest, apoptosis and differentiation (Jensen et al., 2001). Vitamin D receptors are present in bone, intestine,
kidney and the parathyroid gland, but more importantly, high levels of VDR are taken as a positive indicators of prostate cancer regression (Krishnan and Feldman, 2010; Hendrickson et al., 2011). This discovery led to the idea that variation in VDR gene might have some effect in the risk &/or prognosis of PCA. VDR gene is more than 100 kb in size and is located on q arm of chromosome 12 (Uitterlinden et al., 2004). Four most commonly studied VDR polymorphisms are Fok I, Taq I, Apa I and Bsm I. The Fok I polymorphism lies in the exonic region and is associated with change in the reading frame of VDR gene (Gross et al., 1998). On the other hand, Bsm I, Apa I and Taq I polymorphisms are located in the 3’-UTR region and have no direct effect on the protein sequence. It is are however, reported that 3’-UTR region may change the mRNA stability (Whitfield et al., 2001).

Association between VDR gene polymorphisms and prostate cancer risk and/or prognosis has been a matter of debate. Several studies have shown correlation between prostate cancer and VDR polymorphisms are (Mishra et al., 2005; Onen et al., 2008; Bai et al., 2009; Raimondi et al., 2009; Oh et al., 2014; Xu et al., 2014). However, there are other studies that could not find any significant association (Guo et al., 2013). One of the reason for these conflicting reports may be differences in the population as it has been observed that SNPs bearing positive association in Asian population have little or no effect on prostate cancer risk in Caucasians and vice versa (Dianat et al., 2009). Association between VDR gene polymorphisms and prostate cancer risk has not been established in Pakistani population. In view of these facts, we designed this study to identify any possible association of VDR gene polymorphisms with prostate cancer risk in Pakistani population.

Materials and Methods

Study design and population

We designed our research as a case control study and non-probability purposive sampling was employed to obtain the samples. The study was approved by institutional ethical committee and was in concordance to Helsinki declaration. In total 162 samples of Pathan descent, including 114 normal healthy controls (age: 50 years±10) and 48 prostate cancer patients (age: 55 years±4.5) were collected. Informed written consent was obtained from all the participants. The patients were collected from Institute of Radiotherapy and Nuclear Medicine (IRNUM), Peshawar. All patients had confirmed prostate cancer following histopathological examination. They were at advanced stage of the disease and were under radiation therapy. Prostate-specific antigen levels of the patients was also obtained.

DNA extraction and SNP genotyping

Genomic DNA was extracted from the venous blood samples of selected patients using standard DNA extraction kit (Vivantis, CA, USA). Allele specific primers were designed for SNPs in VDR gene region i.e. rs731236 (Ta I), rs2228570 (Fok I) and rs7975232 (Apa I) using Primer 3 online tool (Koreesaar and Remm, 2007; Untergasser et al., 2012). Standard polymerase chain reaction (PCR) of 50µl was carried out using 50ng of genomic DNA and 2 units of Taq DNA polymerase. Primer annealing temperatures were different for each SNP and are mentioned in Table 1. The PCR product was run on 2% agarose gel prestained with ethidium bromide and alleles were assigned based on the presence or absence of allele specific band. Alleles that could not be characterized through allele specific PCR were confirmed through PCR-RFLP using respective restriction enzymes. Finally, the results were confirmed through direct DNA sequencing of the regions of VDR gene flanking the polymorphism.

Statistical analysis

We used OEGE online tool to test the distribution of different alleles (Rodriguez et al., 2009) Web-based tool (http://www.socscistatistics.com/tests/chisquare/Default2.aspx) was used for calculation of Chi-square statistics and the odds ratio was calculated using Medcalc easy-to-use software (http://www.medcalc.org/calc/odds_ratio.php). A p-value of less than 0.05 was considered as statistically significant.

Results

The genotype distribution in control samples of three SNP (rs7975232, rs731236, rs2228570) were not

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Table 1. Sequences of Primers Used for Genotyping by Allele Specific PCR

<table>
<thead>
<tr>
<th>#</th>
<th>Primer ID</th>
<th>Sequence</th>
<th>Annealing Temperature (°C)</th>
<th>Exp. Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs731236 (Ta I)</td>
<td>1. VDR3-36-F</td>
<td>GCCAGCGAGACTCTCCACAGCAG</td>
<td>61</td>
<td>592</td>
</tr>
<tr>
<td>2. VDR3-36T-R</td>
<td>CCGCTCCTGAGATGGCCACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. VDR3-36C-F</td>
<td>CAGGACGAGGCCGCTTTC</td>
<td>60</td>
<td>396</td>
<td></td>
</tr>
<tr>
<td>4. VDR3-36-R</td>
<td>GCCCAACACTCTCAGGACACAAG</td>
<td></td>
<td>231</td>
<td></td>
</tr>
<tr>
<td>rs7975232 (Apa I)</td>
<td>5. VDR3-32-F</td>
<td>CCAAACACTTCGAGCACAAGG</td>
<td>60</td>
<td>589</td>
</tr>
<tr>
<td>6. VDR3-32-R</td>
<td>AGACGAGAGTTCCAAGGAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. VDR3-32T-R</td>
<td>GGTGGAAGTTGAGCAGTAGGAAT</td>
<td>58</td>
<td>313</td>
<td></td>
</tr>
<tr>
<td>8. VDR3-32G-R</td>
<td>GTGGATTGAGCAGTAGGATGG</td>
<td></td>
<td>312</td>
<td></td>
</tr>
<tr>
<td>rs2228570 (Fok I)</td>
<td>9. VDR3-70-F</td>
<td>AAACACCTCTCCTAATAGCAGCA</td>
<td>57</td>
<td>519</td>
</tr>
<tr>
<td>10. VDR3-70T-R</td>
<td>TGGACGCCATCTTTCACTACA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. VDR3-70C-F</td>
<td>CTCGTCCTGCTCTTCATAGTAC</td>
<td>58</td>
<td>217</td>
<td></td>
</tr>
<tr>
<td>12. VDR3-70-R</td>
<td>CTCGTCCTGCTCTTCCTCAC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13. VDR3-70G-F</td>
<td>CTCGTCCTGCTCTTCCTCACACAGTAG</td>
<td>58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14. VDR3-70-R</td>
<td>CTCGTCCTGCTCTTCCTCACACAGTAG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
in HWE (p>0.00001). The associations of allele and genotypes for all the three SNPs are presented in Tables 2-4. We observed statistically significant association between rs7975232 genotype ‘CC’ and prostate cancer (p=0.041). The ‘CC’ genotype exhibit a protective role for the disease (OR=0.383; 95% CI=0.149-0.984). These results are further strengthened from the ‘C’ allele carriers (OR=0.536; 95% CI=0.305-0.943). For rs731236, a decrease in risk for prostate cancer was found for ‘CC’ genotype (OR=0.352; 95% CI=0.114-1.087) and for the ‘C’ allele carriers (OR=0.681; 95% CI=0.389-1.194). However, these results did not reach a statistical significance.

### Table 2. Apa I Polymorphism Frequencies and Effect Estimation by Age in Prostate Cancer Cases and Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>27 (61.36)</td>
<td>76 (63.86)</td>
<td>2.61 (1.01-6.70)</td>
<td>0.04</td>
</tr>
<tr>
<td>TC</td>
<td>13 (29.54)</td>
<td>11 (9.24)</td>
<td>0.3 (0.12-0.75)</td>
<td>0.01</td>
</tr>
<tr>
<td>CC</td>
<td>4 (9.09)</td>
<td>32 (26.89)</td>
<td>2.84 0.91-8.78</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table 3. Taq I Polymorphism Frequencies and Effect Estimation by Age in Prostate Cancer Cases and Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>27 (61.36)</td>
<td>76 (63.86)</td>
<td>2.61 (1.01-6.70)</td>
<td>0.04</td>
</tr>
<tr>
<td>TC</td>
<td>13 (29.54)</td>
<td>11 (9.24)</td>
<td>0.3 (0.12-0.75)</td>
<td>0.01</td>
</tr>
<tr>
<td>CC</td>
<td>4 (9.09)</td>
<td>32 (26.89)</td>
<td>2.84 0.91-8.78</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Table 4. Fok I Polymorphism Frequencies and Effect Estimation by Age in Prostate Cancer Cases and Controls

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>2 (4.87)</td>
<td>13 (12.03)</td>
<td>2.66 (0.57-12.38)</td>
<td>0.21</td>
</tr>
<tr>
<td>TC</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CC</td>
<td>39 (85.12)</td>
<td>85 (87.96)</td>
<td>1.00 0.95-1.04</td>
<td>0.89</td>
</tr>
</tbody>
</table>

### Table 5. Apa I, Taq I and Fok I Haplotype Frequencies and Risk Estimates in Prostate Cancer

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Patients</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA/TC/CC</td>
<td>35</td>
<td>21</td>
<td>0.375 0.081-13.00</td>
<td>0.074</td>
</tr>
<tr>
<td>AA/CC/CC</td>
<td>17</td>
<td>3</td>
<td>0.889 0.83-13.00</td>
<td>0.074</td>
</tr>
<tr>
<td>CA/TC/CC</td>
<td>1</td>
<td>5</td>
<td>0.12 0.013-1.098</td>
<td>0.06</td>
</tr>
</tbody>
</table>

### Discussion

There are various factors that contribute to the basic pathology of prostate cancer. Clinical diagnosis of the disease is helped by PSA and biopsy, but none of these methods provide definitive diagnosis and/or credible account of progression of the disease (Scher and Heller, 2000; Harnden et al., 2008). Lately, research has been tilting towards genetics to provide a better account of disease risk and progression. Several report have shown positive association between the genetic polymorphisms and prostate cancer risk, including those on androgen receptor (AR) gene (Sun et al., 2010), angiotensin I-converting enzyme gene (Wang et al., 2012) and VDR gene. The reason why VDR gene polymorphism has attracted so much attention is because of the overall anticancer effect of the vitamin D itself (Lou et al., 2010). Analysis for the rs228570 revealed that this SNP also plays a protective role for homozygous form ‘TT’ (OR=0.375; 95% CI=0.081-1.739). However, the effect was not statistical significant. We could not detect any heterozygous individual in control or patient group for this SNP.

As, all the three SNPs have protective effective therefore we also performed diplotype analysis to estimate the combination effect of these SNPs (Table 5). The diplotypes ‘AA/CC/CC’ is associated with the disease (OR=3.4; 95% CI=0.889-13.00) and ‘CA/TC/CC’ have shown protective role (OR=0.12; 95% CI=0.013-1.098); however, the effect could not reach the significance level.
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