Vitamin D receptor polymorphisms in colorectal cancer in New Zealand: an association study

Robert W Bentley, Dayle A Keown, Richard B Gearry, Vicky A Cameron, Jacqui Keenan, Rebecca L Roberts, Andrew S Day

Abstract

Aim Polymorphisms of the vitamin D receptor (VDR) gene may be a risk factor for colorectal cancer (CRC). We investigated the association of three single nucleotide polymorphisms (SNPs) of the VDR gene with CRC in age and gender matched patients and controls of European origin in New Zealand.

Method CRC (N=200) and healthy control (N=200) samples were genotyped for the Fok1 (rs2228570), Taq1 (rs731236) and Cdx2 (rs11568820) polymorphisms using Taqman® SNP genotyping assays. Chi-squared analysis was used to test for overall association of VDR genotype with disease, and by age and gender subgroups.

Results There were no significant associations of the three VDR SNPs with disease either by allelic frequencies (p=0.43–0.73) or genotypic distribution (p=0.15–0.90). Furthermore, no significant differences for allelic frequencies of the three SNPs were revealed in subgroup analysis by age (above/below median age of 72 yrs; p=0.38–0.91), gender (p=0.22–0.88), or age/gender (p=0.33–0.93).

Conclusion: We found no evidence to suggest that the VDR SNPs Fok1, Taq1 and Cdx2 influence CRC risk in New Zealand Europeans.

Colorectal cancer (CRC) is the second most commonly diagnosed cancer in New Zealand (NZ), with over 2500 new cases of CRC registered in 2007. The role of vitamin D and its biological effects mediated through the vitamin D receptor (VDR) in the development of CRC is not entirely clear. Some studies have indicated that individuals with CRC have insufficient levels of vitamin D. Whilst vitamin D may be synthesised as vitamin D3 in the skin following exposure to ultraviolet light, it can also be obtained from dietary sources and it has been shown that vitamin D supplementation or an increase in the intake of foods with high vitamin D levels may play a role in the prevention of CRC.

The active form of vitamin D (1, 25-dihydroxyvitamin D3) is bound by the intracellular VDR. This complex binds and interacts with target-cell nuclei (at VDR elements) to produce a variety of biological effects.

Recent research has indicated that vitamin D may play a role as a key regulator of innate immunity in humans. Vitamin D is also shown to suppress CRC development and growth by affecting cell proliferation, differentiation, apoptosis, and angiogenesis.

The VDR gene maps to a region on chromosome 12. Association studies of single nucleotide polymorphisms (SNPs) in the VDR gene suggest that these variants may influence CRC risk.
Despite the high rates of CRC in the NZ population and associations of VDR gene polymorphisms with CRC risk reported elsewhere, very little research has been carried out in order to define the frequency of these variants in the general NZ population or in NZ CRC disease cohorts.

The aim of this study was therefore to screen for genetic variation of the three SNPs rs2228570 (also known as rs10735810; Fok1), rs731236 (Taq1), and rs11568820 (Cdx2) of the VDR gene in a well-defined population of individuals with CRC and compare their incidence to a healthy control population, in order to determine the contribution of VDR polymorphisms to CRC in NZ.

Method

Study participants—DNA from patients who had been diagnosed with CRC (N=200) was obtained from the Christchurch Tissue Bank (New Zealand). DNA was extracted from Whatman FTA Elute Cards (GE Healthcare, UK) using the manufacturers’ recommended protocols. Briefly, a 3.0 mm disc from the FTA Elute Card was washed with 500 µl of sterile H₂O by pulse vortexing and then incubated at 95°C for 20 minutes in 30 µl sterile H₂O. The eluted DNA was separated from the FTA matrix by centrifugation and stored at -20°C until analysed.

Control DNA (N=200) was obtained from the Canterbury Healthy Volunteers for the Study of Heart Disease project. Samples were selected by age- and gender-matching to the CRC patients. At the time of recruitment they had no personal history of cancer of any type or self-reported family history of cancer. Median follow-up was 5.9 years (range 0.1–8.7yrs).

Ethical considerations—Each participant provided written, informed consent. Ethical approval for use of these samples was covered by the Upper South A Ethics Committee (Reference CTY/01/05/062, and URA/10/09/068).

Genotyping—Genotyping of SNPs rs11568820 (Cdx2), rs2228570 (aka rs10735810, Fok1) and rs731236 (Taq1) was performed using pre-designed Taqman® SNP genotyping assays (Applied Biosystems, Foster City, CA) in a Lightcycler® 480 II (Hoffmann La Roche, Basel, Switzerland). 384-well plates with 4.8 µl reaction volumes (2 µl genomic DNA, 2.8 µl Taqman® master mix) were used.

Cycling conditions for all SNP assays were 10 minutes at 95°C, 40 cycles of 15 sec at 92°C and 1 min at 60°C, and 30 seconds of cooling at 40°C. Results were analysed using Lightcycler® 480 (version 1.5.0) software. The accuracy of the genotyping assay was confirmed by repeat analysis of 10% of samples. Concordance between original and repeat genotype calls was 99%.

Statistical analysis—A web-based calculator (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl) was used to test for deviations from Hardy-Weinberg Equilibrium (HWE) and to perform Chi-squared and odds ratio analyses. Associations were considered significant if p<0.05.

Results

Controls and CRC patients were age, gender and ethnicity matched. In the case and control groups, 94 samples (47%) were female. The median age by gender was the same in control and case groups (72 yrs). The average age by gender for case and control groups was 69.5±0.4 yrs. Samples were from New Zealand Caucasians of European origin.

DNA samples from 199 CRC patients, and 191(rs2228570) or 182 (rs731236 and rs11568820) DNA samples from healthy controls were successfully genotyped. Minor allele frequencies are shown in Table 1. Hardy-Weinberg equilibrium was seen for the three SNPs in case and control groups (p=0.14–0.73), indicating that allele and genotype frequencies do not deviate from expectation.

The allelic frequencies (p=0.43–0.73) and genotypic distribution (p=0.15–0.90) of the three VDR SNPs were not significantly associated with disease (Table 1).
Table 1. Genotype and allele frequencies of VDR SNPs in CRC patients and healthy controls

<table>
<thead>
<tr>
<th>VDR SNP</th>
<th>Phenotype</th>
<th>Genotype frequency n (%)</th>
<th>MAF a</th>
<th>Allelic P-value</th>
<th>OR [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1,1 b</td>
<td>1,2</td>
<td>2,2</td>
<td></td>
</tr>
<tr>
<td>Fok1 rs2228570</td>
<td>CRC</td>
<td>67 (33.7)</td>
<td>103 (51.8)</td>
<td>29 (14.6)</td>
<td>161 (40.5)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>79 (41.4)</td>
<td>80 (41.9)</td>
<td>32 (16.8)</td>
<td>144 (37.7)</td>
</tr>
<tr>
<td>Taq1 rs731236</td>
<td>CRC</td>
<td>34 (17.1)</td>
<td>101 (50.8)</td>
<td>64 (32.2)</td>
<td>169 (42.5)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>32 (17.6)</td>
<td>86 (47.3)</td>
<td>64 (35.2)</td>
<td>150 (41.2)</td>
</tr>
<tr>
<td>Cdx2 rs11568820</td>
<td>CRC</td>
<td>8 (4.0)</td>
<td>71 (35.7)</td>
<td>120 (60.3)</td>
<td>87 (21.9)</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>6 (3.3)</td>
<td>63 (34.6)</td>
<td>113 (62.1)</td>
<td>75 (20.6)</td>
</tr>
</tbody>
</table>

aMAF = Minor Allele Frequency.
bThe alleles constituting the genotype are denoted as 1 or 2.

Furthermore, no significant differences for allelic frequencies of the three SNPs were revealed in subgroup analysis by age (above/below median age of 72 yrs; p=0.38–0.91), gender (p=0.22–0.88), or age/gender (p=0.33–0.93).

Discussion

Association studies of VDR SNPs with different forms of cancer, including CRC, have indicated that they may influence disease risk risk$^{11-14}$ and that the frequency of these SNPs varies with ethnicity.$^{11}$

Little research has been performed in the New Zealand population to determine the distribution and association of VDR SNPs with CRC. The minor allele frequencies (MAFs) of Taq1 (CRC 42.5% and HC 37.7%) and Cdx2 (CRC 21.9% and HC 20.%) in our study were in agreement with the MAFs reported for these SNPs in other studies on populations of European origin.$^{13, 16}$

In contrast, the MAF of Fok1 was higher in our CRC patients (40.5%) and healthy controls (37.7%) than the MAF reported in French (33%)$^{16}$ and UK (31%)$^{17}$ populations. Reasons for this discordance are unknown, but may be due to subtle population stratification.

The lack of any significant overall or subgroup association of these SNPs of the VDR gene with CRC does not indicate a role for these variants in CRC in NZ Caucasians of European origin. However, previous studies have indicated that risk conferred by SNPs of the VDR gene may be modified by calcium intake, vitamin D uptake, dietary fat$^{18-20}$ and body mass index (BMI).$^{21}$ Our CRC samples were sourced from tissue bank samples lacking this supporting information.

Further research in a larger cohort taking these factors into account may clarify the nature of this gene/environment interaction in the NZ population.
Competing interests: None declared.

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