Vitamin D receptor start codon polymorphism and colorectal cancer risk: effect modification by dietary calcium and fat in Singapore Chinese

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Vitamin D has been implicated as a protective agent against colorectal cancer. We hypothesized that a functional start codon polymorphism in the vitamin D receptor (VDR) influences the risk of colorectal carcinoma. We conducted a case-control study nested within a large cohort of Singapore Chinese. VDR genotypes, determined by FokI restriction endonuclease digestion of PCR-amplified DNA, were performed on 217 colorectal cancer cases and 890 controls. We found that compared with individuals carrying the FF genotype, those with Ff genotype had a 51% increase in risk of colorectal cancer and those with the ff genotype, an 84% increase in risk (P for trend = 0.01). The effect of the VDR genotype on risk appeared to be modified by both dietary calcium and fat. Among those with either low calcium or low fat intake (below the median values in controls), the risk for colorectal cancer increased in a gene–dose-dependent manner such that individuals possessing the ff genotype displayed a ~2.5-fold increased risk that was statistically significant. There was little evidence of a VDR genotype-colorectal cancer association among subjects with higher than median values of either dietary fat or calcium.

Introduction

Vitamin D has been implicated as a protective agent against colorectal cancer. Ecological data have indicated an inverse correlation between solar (UV) exposure, and thus cutaneous colorectal cancer. Ecological data have indicated an inverse incidence (2). Two cohort studies (3,4) and one case-control study (5) found an inverse association between colon cancer and dietary fat or calcium.

Of interest to this study, many individuals consume much lower quantities of calcium and fat than are seen in western populations; thus a much broader range of dietary variation exists among subjects than is observed in populations with more liberal dietary intake. However, dietary calcium and fat may modify any effect of the VDR polymorphism on colorectal cancer risk. Calcium, the first factor, regulates the formation of the active vitamin D metabolite [1,25(OH)2D3] in a feedback loop via the parathyroid hormone (reviewed in ref. 23 and references therein). The second factor, dietary fat, increases bile acid secretion, which may predispose individuals consuming a high fat diet to colorectal cancer and adenomas.

We examined the hypothesis that VDR genotype influences colorectal cancer, with possible modification by dietary calcium and fat, in a population-based prospective cohort of 63 000 middle-aged and older Chinese of both sexes in Singapore (the Singapore Chinese Health Study). Singapore is a rapidly westernizing population; thus a much broader range of dietary intakes are observed compared with US whites and blacks. Of interest to this study, many individuals consume much higher quantities of calcium and fat than are seen in western populations (24).

Materials and methods

Study population

The subjects were participants of the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk (24). Briefly, between April 1993 and December 1998, we recruited 63 257 Chinese men and women from two major dialect groups in Singapore (Hokkien and Cantonese). Subjects were between the ages of 45 and 74 years, and resided in government housing estates. Eighty-six percent of the Singapore population lived in such facilities. Each subject completed a structured questionnaire administered in-person by a trained interviewer.

In April 1994 we began collecting blood and single-void urine specimens from a random 3% sample of study participants. A 20-ml blood sample is obtained from each subject. Immediately after blood collection, the tubes are put on ice during transport from the subjects’ homes to the laboratory. The specimens are then separated into their various components (plasma, serum, red blood cells and buffy coat). The specimens used in this study were subsequently stored in a liquid nitrogen tank at −180°C until August 2001, when they were moved to a −80°C freezer for long-term storage. Subjects who are unwilling to donate blood are asked to donate buccal cells through the use of a mouthwash protocol based on published methods (25,26). These subjects are provided with a new toothbrush and asked to clean their teeth thoroughly. After an interval of 20 min, during which no food or drink is consumed, they are given 10 ml of commercially purchased ‘Listerine’ mouthwash and asked to swish the liquid vigorously in their mouths for 60 s. The mouthwash is then collected in a sterile 50 ml polypropylene tube, put on ice and brought back to the laboratory within 5 h, where it is stored at −30°C.

Abbreviations: Ca2+, calcium; CI, confidence intervals; OR, odds ratio; VDR, vitamin D receptor.

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Case ascertainment
We identified incident colorectal cancer cases through the population-based Singapore Cancer Registry (27). As of December 31, 2000, 482 cases of incident colorectal cancer (ICD-O C18-C20) had developed among cohort members. Histological information on each colorectal cancer diagnosis was confirmed by reviewing the pathology report. Urine and blood or buccal samples were obtained on cases as described for controls above.

The study protocol was approved by the Institutional Review Boards of the National University of Singapore and the University of Southern California. All participants gave written, informed consent at the time of recruitment and at collection of blood (or buccal cells) and urine specimens.

Information on diet and other background variables
The development and validation of the Singapore Chinese Health Study food frequency questionnaire (FFQ) have been described previously (24). At recruitment, information on usual diet over the last year was obtained via a semi-quantitative FFQ, which was administered in person at the subject’s home. The questionnaire listed 165 food items, and the respondent was asked to select from eight frequency categories (ranging from ‘never’ to ‘two or more times a day’) and three portion sizes with accompanying photographs. Average daily intake of 96 nutrients and non-nutrient compounds, including calcium and fat, was computed for each study subject via linkage to the Singapore Food Composition table. The dietary component of the questionnaire was subsequently validated against a series of 24 h diet recalls (24). Apart from dietary history, the questionnaire also elicited information on lifetime tobacco use, usual physical activity, medical history, family history of cancer, and menstrual and reproductive history (women only).

Genotyping
DNA was purified from buffy coats of peripheral blood and buccal cell samples using standard, published methods (28) with modification. The forward primer was 5’-AGCTGGCCCTGCGACCTCTGCTCTC-3’ and reverse primer was 5’-ATGGAAACACCTTGCTTCTTCTCCCT-3’ and annealing temperature was 64°C. The VDR start site polymorphism was assayed by FoE restriction endonuclease digestion of PCR-amplified DNA as described previously (29). All genotype batches included at least one ff homozygote to ensure complete restriction enzyme reaction and one non-template control for detection of contamination.

Statistical analysis
As of July 1999, blood (n = 908) or buccal cells (n = 286) had been collected from 678 female and 516 male subjects in the 3% randomly sampled subcohort. Eight of these subjects developed incident colorectal cancer by December 31, 2000. The 900 cohort subjects for whom blood was drawn and who were free of a history of colorectal cancer on December 31, 2000 comprised the control group for this nested case-control analysis.

As of December 31, 2000, 482 cases of colorectal cancer had developed from the entire cohort. Blood (n = 170) or buccal (n = 55) specimens were available on 47% (225/482) of the colorectal cancer cases. Compared with those who had no formal education, a higher proportion of subjects who had primary school or higher education donated a blood or buccal cell specimen (51 versus 39%). More females donated specimens (52%) compared with males (41%). Similar proportion of Cantonese and Hokkien cases donated specimens (48 versus 46%). The average ages at diagnosis of cancer were comparable between cases with and without specimen (65.0 versus 65.6 years).

Among the 225 cases and 900 controls, VDR genotypes were not available in eight cases and nine controls due to PCR failure. In addition, one control with a missing value on calcium supplement was excluded. Therefore, the present analysis included 217 cases and 890 controls.

Allele frequencies were determined by gene counting. The observed allele frequencies among controls were used to calculate the expected genotype frequency under Hardy–Weinberg equilibrium. Departures from Hardy–Weinberg equilibrium were assessed with a χ² test where sample size permitted.

We used conditional logistic regression methods (30) with matched sets defined jointly by year of birth (1917–1925, 1926–1930, 1931–1935, 1936–1940, 1941–1945, 1946–1954), year of recruitment (1993–1995, 1996–1998), gender and dialect group (Cantonese, Hokkien) to estimate odds ratio (ORs) and their corresponding 95% confidence intervals (CIs) and two-sided P values. Gene dose effect was examined with test for trend by assigning an ordinal value of 0 to each of the categorical genotypes and treating the variable as continuous in the logistic regression model.

Colorectal cancer was coded into anatomic subsites as per the International Classification of Disease Oncology (2nd Edn): proximal colon (C18.0–C18.5), distal colon (C18.6–C18.7), not otherwise specified (C18.8–C18.9) and rectal (C19.0–C20.0). To test for heterogeneity of odds ratios across anatomic subsites, we fitted a polychotomous logistic regression model, adjusted for the four matching covariates (year of birth, year of recruitment, gender, dialect group) detailed in the previous paragraph.

Possible effect modification by dietary fat, dietary saturated fat, dietary fiber, red meat as well as gender and dialect group were tested by including respective multiplicative interaction terms in the conditional logistic regression model and conducting likelihood ratio tests.

Results
We have described previously the baseline characteristics of the Singapore Chinese Health Study cohort (31). Briefly, the mean age of cohort subjects at enrolment was 56.5 years. Fifty-six percent of the cohort subjects were women and 54% belonged to the Hokkien dialect group. Most were married (83%) at the time of recruitment. Eighty-eight percent of cohort subjects were born in Singapore or Malaysia (Singapore and Malaysia are neighboring countries with similar sociocultural groups), whereas virtually all of the remaining 12% were born in China. The cohort was relatively uneducated; 27% of its members had no formal education and 44% received only a primary school education. Compared with US whites and blacks, cohort members had lower intakes of dietary fats, calcium and alcohol.

Table I presents selected characteristics of study cases and controls. Controls were comparable with the whole cohort. Cases differed from the controls in some respects. Females made up 56.7% of the control group as opposed to only 41.5% of cases. The Hokkien dialect group comprised 50.5% of the controls but 42.9% of the cases. Cases were less likely to have a secondary or higher education. Controls and cases did not differ in terms of physical inactivity (data not shown), body mass index (weight in kilograms divided by height in meters squared) or intakes of total calories, calcium, fat and fiber.

Neither did they differ on family history of cancer or colorectal cancer, alcohol intake, smoking, age at menarche, age at menopause, parity or age at first birth. The age at diagnosis for cases ranged from 47 to 79 years (median = 66 years).

Table I. Characteristics of cases and controls, Singapore Chinese Health Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (%)</th>
<th>Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>890 (100)</td>
<td>217 (100)</td>
</tr>
<tr>
<td>Gendera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>385 (43.3)</td>
<td>127 (58.5)</td>
</tr>
<tr>
<td>Female</td>
<td>505 (56.7)</td>
<td>90 (41.5)</td>
</tr>
<tr>
<td>Dialect groupa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hokkien</td>
<td>450 (50.5)</td>
<td>93 (42.9)</td>
</tr>
<tr>
<td>Cantonese</td>
<td>440 (49.5)</td>
<td>124 (57.1)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;22</td>
<td>146 (16.4)</td>
<td>38 (17.5)</td>
</tr>
<tr>
<td>22–&lt;25</td>
<td>493 (55.4)</td>
<td>103 (47.5)</td>
</tr>
<tr>
<td>25–&lt;28</td>
<td>205 (23.0)</td>
<td>62 (28.5)</td>
</tr>
<tr>
<td>28+</td>
<td>46 (5.2)</td>
<td>14 (6.5)</td>
</tr>
<tr>
<td>Educationb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No education</td>
<td>213 (23.9)</td>
<td>64 (29.5)</td>
</tr>
<tr>
<td>Primary education</td>
<td>381 (42.8)</td>
<td>110 (50.7)</td>
</tr>
<tr>
<td>Secondary or higher</td>
<td>296 (33.3)</td>
<td>43 (19.8)</td>
</tr>
<tr>
<td>Total calories (kcal/day)b</td>
<td>1491.6 (837.2, 2454.4)</td>
<td>1510.8 (808.3, 2662.0)</td>
</tr>
<tr>
<td>Calcium (g/d)</td>
<td>387.8 (60.5, 853.3)</td>
<td>352.0 (128.7, 775.1)</td>
</tr>
<tr>
<td>Total dietary fat (g/d)</td>
<td>41.5 (19.8, 81.2)</td>
<td>39.9 (18.3, 84.4)</td>
</tr>
<tr>
<td>Dietary fiber (g/d)b</td>
<td>12.3 (5.3, 23.2)</td>
<td>11.8 (11.8, 22.9)</td>
</tr>
</tbody>
</table>

aCases versus controls P < 0.05.
bMedian intake (5th percentile, 95th percentile).

dietary and supplementary.
Among the controls, the longer VDR allele (f allele) had a frequency of 46.7%, similar to that reported previously in Asian populations (20,32,33), and genotype frequencies were in Hardy–Weinberg equilibrium. Colorectal cancer risk was positively associated with the f allele in a gene–dose manner (Table II). Compared with individuals carrying the FF genotype, those with Ff genotype had a 51% increase in risk and those with the ff genotype, an 84% increase in risk (P for trend = 0.01). Odds ratios for colon and rectal cancer risk did not differ significantly (P for heterogeneity = 0.23). Odds ratios for proximal, distal and not otherwise specified colorectal cancer were similar (data not shown). Therefore, the remaining analyses were performed on all colorectal cancers as a single group.

To determine whether dietary factors (fat and calcium) modify the effect of VDR genotype on colorectal cancer risk, we stratified on high versus low nutrient intake (based on the median of the nutrient distribution in the controls) (Table III). In the lower dietary fat category, risk increased with increasing copies of the f allele (P for trend = 0.01); the OR associated with the ff genotype was 2.5 (95% CI, 1.3–4.7). In the higher fat category, the corresponding ORs were not significantly different from the null value (P = 0.19). Effect-modification analyses performed with saturated fats (rather than total fats) yielded comparable results (data not shown). Similarly for calcium (dietary plus supplementary), the effect of VDR was observed only in the lower calcium category. Analyses using calcium intake from diet only was similar (data not shown).

**Discussion**

In this cohort of Singapore Chinese, we observed a significant effect of the VDR start codon (FokI) genotype on risk of colorectal cancer. This effect appeared to be confined to those with relatively low fat and calcium intakes. This study, to the authors’ knowledge, is the first to demonstrate an association between a VDR variant and colorectal cancer. Additionally, this is the first report of the VDR FokI variant as a risk factor for a major cancer, including cancers of the breast (35–37) and prostate (38,39).

The protective effect of the VDR F allele is consistent with reported functional data. The FokI polymorphism changes the first of two start codons from ATG to ACG, resulting in a receptor protein that is shorter by three amino acids (19). The shorter allele, designated as the F variant, was shown previously (20,21) to be more efficient at transactivating vitamin D target genes. Thus, the F allele is expected to transmit stronger anti-proliferative and pro-differentiation signals.

The VDR effect appeared to be modified by dietary fat. This is consistent with the recent finding that VDR acts as a bile acid sensor in colonic cells. Binding of VDR to the secondary acid sensor in colonic cells. Binding of VDR to the secondary bile acid, lithocholic acid, increases expression of CYP3A which catabolizes bile acids (17). This detoxification pathway is presumably overwhelmed by the high levels of bile acids resulting from a high fat diet. Indeed, we were able to detect a difference between genotypes only among those consuming a low fat diet. Similarly, for calcium, the effect of VDR was observed only in the low calcium group. Presumably, the subtle difference among the genotypes is overwhelmed when calcium intake is higher.

Sparse epidemiologic literature exists on the VDR FokI polymorphism and colorectal cancer. In the only reported

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**Table II. ORs and 95% CIs for VDR FokI genotypes and colorectal cancer, Singapore Chinese Health Study**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (%)</th>
<th>Cases (%)</th>
<th>OR (95%CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>242 (27.2)</td>
<td>42 (19.3)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>Ff</td>
<td>456 (51.2)</td>
<td>116 (53.4)</td>
<td>1.51 (1.00, 2.29)</td>
</tr>
<tr>
<td>ff</td>
<td>192 (21.6)</td>
<td>59 (27.3)</td>
<td>1.84 (1.15, 2.94)</td>
</tr>
</tbody>
</table>


**Table III. ORs and 95% CIs for VDR FokI genotypes and colorectal cancer by dietary factors, Singapore Chinese Health Study**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (%)</th>
<th>Cases (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary fat &lt;41.54&lt;sup&gt;a&lt;/sup&gt; g/day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>139 (31.2)</td>
<td>21 (18.4)</td>
<td>1.00 (referent)</td>
</tr>
<tr>
<td>Ff</td>
<td>227 (50.8)</td>
<td>62 (54.4)</td>
<td>1.76 (0.99, 3.11)</td>
</tr>
<tr>
<td>ff</td>
<td>80 (18.0)</td>
<td>31 (27.2)</td>
<td>2.43 (1.25, 4.71)</td>
</tr>
</tbody>
</table>


<sup>bMedian values in controls.</sup>
study addressing any VDR variant and colorectal cancer susceptibility, no association with FokI genotype was found in a US white population (40). A possible reason for the inconsistent finding with our study is the difference between US whites and Singaporean Chinese in terms of dietary intakes, specifically calcium, that are metabolically related to vitamin D. The median calcium intake among US whites (10) was nearly 4-fold higher than that in Singapore Chinese (804 versus 243 mg/day). Three previous studies have examined VDR FokI variants in relation to the colorectal cancer precursor lesion, colorectal adenoma (10, 33, 41). Only one study noted a VDR genotype effect (33), but contrary to our findings, increased risk of adenomas was associated with the postulated more active F allele. Fewer than 10% of adenomas are thought to progress to adenocarcinomas (42). The reasons behind the seemingly contradictory findings between colon adenomas and colorectal cancer are unclear.

The strengths of the present study include the epidemiologic study design. Because this study is nested within an ethnically homogeneous, well-defined cohort, selection bias is unlikely to explain the findings. Further, dietary information, including major sources of fat and calcium, was collected prospectively using a validated questionnaire, eliminating differential recall between cases and controls. A second strength is the inclusion of a biomarker of genetic susceptibility. In contrast to previous studies—which focused on vitamin D status, which is difficult to capture—this study assayed VDR genotype, which is less susceptible to measurement error and residual confounding that could attenuate modest risk ratios. The finding that genetic variation in the vitamin D pathway contributes to risk supports a role for vitamin D in colon cancer etiology. Finally, it supports a role for vitamin D in colon cancer etiology. Finally, the relatively low calcium and fat intake in the Singapore Chinese population may have contributed to the unmasking of the effect of VDR genotype on colorectal cancer risk. High calcium and fat intake in western diets could have precluded detection of a VDR effect in a previous study (40). Limitations of the present study include the relatively short duration of follow-up of the Singapore Chinese Health Study cohort, and the resultant relatively small number of colorectal cancer cases. However, more cases will accrue over time within the cohort to definitively address this novel VDR genotype-colorectal cancer association.

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References


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