Original Contribution

Association Between Plasma 25-Hydroxyvitamin D and Colorectal Adenoma According to Dietary Calcium Intake and Vitamin D Receptor Polymorphism

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Accumulating evidence has indicated that adequate levels of vitamin D may confer protection against the risk of colorectal cancer and adenoma, a well-established precursor lesion of colorectal cancer (1, 2). Recent meta-analyses of vitamin D intake and colorectal neoplasia have generally shown a weak inverse association (2, 3), while those of serum/plasma 25-hydroxyvitamin D in the circulation, have fairly consistently demonstrated a significant inverse association (3–5). This discrepancy in the magnitude of the association may reflect the fact that vitamin D in the body is derived not only from the diet but also from the skin, where a substantial amount of pre-vitamin D can be synthesized from 7-dehydrocholesterol through stimulation by solar ultraviolet B radiation (6).

The primary role of vitamin D is the maintenance of calcium homeostasis, the disruption of which is also related to colorectal carcinogenesis (2, 7). Vitamin D exerts its effects on calcium metabolism through binding to vitamin D receptor (VDR), a member of the nuclear receptor superfamily, which regulates the transcription of genes involved in calcium absorption from the small intestine. The VDR gene (VDR) has a number of single nucleotide polymorphisms (SNPs), including rs2228570 (previously rs10735810) and rs731236. These 2 polymorphisms, which correspond to the FokI and TaqI restriction sites, respectively, have been intensively explored over the last decade for their possible association with colorectal tumorogenesis. Vitamin D might protect against colorectal neoplasia, mainly through mechanisms other than the indirect mechanism via calcium metabolism.
Although several epidemiologic studies have investigated the association between circulating vitamin D levels and colorectal neoplasia in conjunction with total/dietary calcium intake (10–15), few have done so in consideration of VDR polymorphisms (13, 14), despite the fact that the anticarcinogenic potential of vitamin D might be mediated by not only calcium metabolism but also other mechanisms initiated by VDR. Here, we measured plasma concentrations of 25-hydroxyvitamin D in 1,520 middle-aged and elderly Japanese and evaluated its influence on colorectal adenoma, both alone and in interaction with dietary calcium intake and the \textit{FokI} and \textit{TaqI} polymorphisms of the \textit{VDR} gene.

**MATERIALS AND METHODS**

**Study population**

The Colorectal Adenoma Study in Tokyo (16, 17), a case-control study conducted by the Research Center for Cancer Prevention and Screening, a branch of the National Cancer Center of Japan, was specifically designed to investigate environmental and genetic factors related to the early stage of colorectal carcinogenesis among healthy volunteer examinees of a colorectal cancer screening program. The Research Center conducts its cancer screening programs on a research basis and accordingly requires all examinees to provide written informed consent prior to admission to the use of data and materials collected through the screening programs to be used for medical research. This means that virtually no examinee refuses to participate in medical research initiated by the Research Center. Examinees who attend the Research Center are primarily self-referred, and more than 90% reside in Tokyo and its 6 neighboring prefectures, collectively called the Kanto region. The study protocol was approved by the institutional review board of the National Cancer Center.

Eligible subjects were defined in advance as men aged 50–79 years and women aged 40–79 years who underwent total colonoscopy from the anus to the cecum and who were without a history of colorectal adenoma, any malignant neoplasm, ulcerative colitis, Crohn’s disease, familial adenomatous polyposis, carcinoid tumor, or colectomy. Of a consecutive series of 3,212 examinees undergoing magnifying colonoscopy with indigo carmine dye spraying between February 2004 and February 2005, 2,234 met these conditions. On the basis of the pit pattern of colorectal lesions, namely, the characteristics of mucosal crypts, 526 men and 256 women were determined to have at least 1 adenoma and were thus included as adenoma cases. Pit-pattern classification based on magnifying chromoendoscopy has been detailed elsewhere (18). Of the remaining 1,452 examinees, we identified 482 men and 721 women as potential controls who were also free from other benign lesions (e.g., hyperplastic polyps, inflammatory polyps, and diverticula). Because there were fewer potential controls than cases in men, all potential male controls were selected from potential controls and frequency matched to the female cases in 5 age categories (40–49, 50–54, 55–59, 60–64, and ≥65 years of age) and 2 screening periods (first and second halves). The screening period was matched because standard operating procedures were improved during the first half period after the establishment of the Research Center, which might have influenced, for example, the accuracy of diagnosis. Finally, the study enrolled 526 cases and 482 controls in men and 256 cases and 256 controls in women. A total of 242 male and 104 female cases had adenomas of ≥5 mm in diameter and were referred to clinical hospitals for definitive diagnosis and treatment. Of 1,362 adenomatous lesions referred to the National Cancer Center in 2004–2008, 1,221 (90%), 53 (4%), and 88 (6%) were histologically confirmed as adenoma, early cancer, and nonneoplastic lesions, respectively (unpublished data).

**Blood collection and laboratory procedures**

Blood is collected from all examinees of the Research Center for research purposes almost without exception. Examinees were scheduled for blood collection prior to any cancer screening procedures on the first day of screening. Fasting venous blood was drawn into a vacutainer tube with ethylene-diaminetetraacetic acid (EDTA). The vacutainer tubes were centrifuged to obtain the plasma and buffy coat layer, and the blood samples were preserved at −80°C until analysis. Plasma and buffy coat samples were available for all subjects of this study.

Plasma 25-hydroxyvitamin D concentrations were measured by a radioimmunoassay method by using a commercially available reagent (Kyowa Medex, Tokyo, Japan) with a minimum detection level of 6 ng/mL at an external laboratory (SRL, Tokyo, Japan). The laboratory reported intra- and interassay coefficients of variation of 5.96% and 5.31% for plasma 25-hydroxyvitamin D concentrations of 25.0 and 20.1 ng/mL, respectively. All laboratory personnel were blinded with respect to case and control status.

Genomic DNA was extracted from white blood cells in the buffy coat layer by using a FlexiGene DNA kit (Qiagen, Hilden, Germany) in our laboratory. More than 90% of buffy coat samples provided a sufficient amount of genomic DNA to perform genotyping. The \textit{FokI} and \textit{TaqI} polymorphisms of the \textit{VDR} gene were analyzed by using the TaqMan SNP genotyping assays (Applied Biosystems, Foster City, California). These analyses were carried out with blinding to case and control status.

**Self-administered questionnaire**

Prior to cancer screening, all examinees were encouraged to complete a self-administered questionnaire concerning lifestyle and socioeconomic characteristics, as well as personal and family medical history. Details of the questionnaire have been described elsewhere (16, 17). Although some examinees left individual items blank, no examinee refused to answer any substantial portion of the questionnaire.

The questionnaire also included a food frequency questionnaire of the present study was
essentially the same as that used in a large prospective cohort study among a Japanese population (20, 21). A validation study conducted among subsamples of cancer screening examiners revealed that the dietary calcium intake estimated from this food frequency questionnaire was relatively well correlated with that from 4-day dietary records, with deattenuated Spearman’s correlation coefficients for energy-adjusted calcium intake of 0.64 and 0.61 for men and women, respectively (unpublished data).

**Statistical analysis**

Odds ratios and 95% confidence intervals of colorectal adenoma for plasma 25-hydroxyvitamin D, dietary calcium intake, and the FokI and TaqI polymorphisms of the VDR gene were estimated by using an unconditional logistic regression model. Dietary calcium intake was energy adjusted for each sex by using a linear regression model with natural logarithm-transformed intakes of total energy and calcium as independent and dependent variables, respectively (22). Plasma 25-hydroxyvitamin D concentrations and dietary calcium intake were divided into sex-specific quintiles by cutoff points derived from the distribution among controls. Statistical adjustment was made in a manner similar to that in our previous studies of colorectal adenoma (16, 17). Model 1 controlled for sex, matching variables (i.e., age categories and screening periods), and season of blood collection (spring, summer, fall, and winter). Model 2 adjusted for the same variables as model 1 and additionally for cigarette smoking (never, ≤20, 21–40, and >40 pack-years), alcohol drinking (never, past, <150, 150–299, and ≥300 g/week), body mass index (<21.0, 21.0–22.9, 23.0–24.9, and ≥25.0 kg/m²), family history of colorectal cancer (yes or no), and nonsteroidal anti-inflammatory drug use (yes or no). Model 2 also adjusted for attained adult height, an indicator of gross energy intake in childhood and adolescence, and average daily energy intake in the past year. These variables were divided into quintiles, the cutoff points of which were based on the sex-specific distribution among controls. Linear trends in the odds ratios of colorectal adenoma were examined by assigning ordinal values to quintiles of plasma 25-hydroxyvitamin D and dietary calcium intake.

We then investigated the influence of plasma 25-hydroxyvitamin D on colorectal adenoma in interaction with dietary calcium intake and the FokI and TaqI polymorphisms of the VDR gene. Three genotypes of each VDR polymorphism were dichotomized on the basis of the dominant model, with the first homozygous for the major allele and the second heterozygous and homozygous for the minor allele combined. Similarly, quintiles of plasma 25-hydroxyvitamin D and dietary calcium intake were reduced to 2 levels, namely, lower and higher, on the basis of their association with colorectal adenoma. The likelihood ratio test with 1 df was used to evaluate whether dietary calcium intake and the VDR polymorphisms modified the association between plasma 25-hydroxyvitamin D and colorectal adenoma.

Of 1,443 subjects without extreme energy intakes (<800 or >4,200 kcal/day) or calcium supplement use, 3 subjects had missing information, 1 with regard to height and 2 for cigarette smoking. These were then excluded, and the analyses of plasma 25-hydroxyvitamin D and dietary calcium intake were conducted in the remaining 737 cases and 703 controls. Of note, we excluded calcium supplement users, who accounted for <4% of study subjects, and focused our analysis on dietary calcium intake. In the analyses of the FokI and TaqI polymorphisms of the VDR gene, 7 and 8 subjects with an undetermined genotype were excluded, respectively, from 1,332 subjects with a sufficient amount of genomic DNA to perform genotyping, leaving 1,325 (684 cases, 641 controls) and 1,324 (684 cases, 640 controls), respectively, for inclusion. Two-sided P values less than 0.05 were regarded as statistically significant. All statistical analyses were carried out using SAS, version 9.1, software (SAS Institute, Inc., Cary, North Carolina).

**RESULTS**

Table 1 shows selected characteristics of controls according to plasma 25-hydroxyvitamin D level. Increasing levels of plasma 25-hydroxyvitamin D were associated with older age and a higher intake of dietary vitamin D, while other selected characteristics were not related to plasma 25-hydroxyvitamin D levels.

Plasma 25-hydroxyvitamin D levels were inversely associated with the prevalence of colorectal adenoma (Table 2), albeit in a nonlinear manner. Compared with the lowest quintile of plasma 25-hydroxyvitamin D, only the highest showed a statistically significant decrease in the adjusted odds ratio of colorectal adenoma (odds ratio (OR) = 0.64, 95% confidence interval (CI): 0.45, 0.92). A similar pattern was noted when the analysis was replicated in men and women separately ($P_{interaction} = 0.30$) (Web Table 1, the first of 3 Web tables posted on the Journal’s Web site (http://aje.oupjournals.org/)). Given the well-known seasonal variation in circulating levels of 25-hydroxyvitamin D, we also conducted a stratified analysis by season of blood collection, which revealed that the association between plasma 25-hydroxyvitamin D levels and colorectal adenoma was not modified by season of blood collection ($P_{interaction} = 0.55$) (Web Table 2). A nonlinear inverse association was also observed for dietary calcium intake, although this differed from that for plasma 25-hydroxyvitamin D: Using the first quintile of dietary calcium intake as reference, we found that the second showed a significant decrease in the adjusted odds ratio of colorectal adenoma ($OR = 0.64, 95\% CI: 0.45, 0.90$), while the third to fifth showed no further decline. Again, we saw no apparent difference in the association by sex ($P_{interaction} = 0.70$) (Web Table 1). When mutually adjusted for plasma 25-hydroxyvitamin D and dietary calcium intake, the odds ratio for the highest quintile of plasma 25-hydroxyvitamin D was 0.66 (95% CI: 0.46, 0.95), whereas those for the second and fifth quintiles of dietary calcium intake were 0.65 (95% CI: 0.46, 0.92) and 0.69 (95% CI: 0.48, 0.99), respectively. The FokI and TaqI polymorphisms of the VDR gene were not associated with the prevalence of colorectal adenoma (Table 2). Genotype frequencies among controls were in agreement with Hardy-Weinberg equilibrium for both VDR polymorphisms ($P = 0.79$ and 0.82 for FokI and TaqI, respectively).

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Among 737 cases, 325 had a largest adenoma of \( \geq 5 \) mm in diameter (44.1%). Excluding 12 cases with missing information, 388 had the largest adenoma at the proximal colon (53.5%), 259 at the distal colon (35.7%), and 78 at the rectum (10.8%). We then investigated the association of plasma 25-hydroxyvitamin D and dietary calcium intake with the size and location of the largest adenoma using a multinomial logistic regression model (Table 3). The inverse association of plasma 25-hydroxyvitamin D and dietary calcium intake was even more striking in cases with a largest adenoma of \( \geq 5 \) mm in diameter. By location of the largest adenoma, the inverse association of plasma 25-hydroxyvitamin D was most pronounced in cases of proximal colon adenoma, whereas that of dietary calcium intake was most prominent in rectal adenoma cases.

We further evaluated the association of plasma 25-hydroxyvitamin D and dietary calcium intake with colorectal adenoma stratified by major risk factors of colorectal adenoma, namely, smoking and drinking habits and body fatness. Although no interaction of dietary calcium intake with body fatness was seen, such an interaction was suggested for plasma 25-hydroxyvitamin D \( (P_{\text{interaction}} = 0.05) \), in which the odds ratio of colorectal adenoma for the highest compared with lowest quintile was statistically significant in subjects with a body mass index of \( < 23 \) kg/m\(^2\) but not in those of \( \geq 23 \) kg/m\(^2\) (Web Table 3). With respect to smoking and drinking habits, we did not see any effect modification for either plasma 25-hydroxyvitamin D or dietary calcium intake (data not shown).

Table 4 shows the association of plasma 25-hydroxyvitamin D with colorectal adenoma according to dietary calcium intake and \( VDR \) polymorphism. Although we saw no multiplicative interaction, higher levels of plasma 25-hydroxyvitamin D and dietary calcium intake combined were related to the greatest decrease in odds ratio of colorectal adenoma \( (OR = 0.49, 95\% \text{ CI: } 0.33, 0.72) \). With regard to the \( VDR \) polymorphisms examined, we observed a significant interaction with the \( TaqI \) polymorphism \( (P_{\text{interaction}} = 0.03) \), for which an inverse association of plasma 25-hydroxyvitamin D was more evident in heterozygotes and homozygotes for the minor allele combined \( (P_{\text{trend}} = 0.001) \) than in homozygotes for the major allele \( (P_{\text{trend}} = 0.25) \). When examined in heterozygotes or homozygotes for the minor allele of \( TaqI \), the adjusted odds ratio of colorectal adenoma for higher compared with lower levels of plasma 25-hydroxyvitamin D was 0.32 \( (95\% \text{ CI: } 0.16, 0.65) \).

**DISCUSSION**

In this study, we found a nonlinear inverse association of plasma 25-hydroxyvitamin D and dietary calcium intake with colorectal adenoma. Moreover, we noted a significant

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**Table 1.** Selected Characteristics of Controls According to Plasma 25-Hydroxyvitamin D Level, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Quintile 1 (Lowest)</th>
<th>Quintile 3 (Middle)</th>
<th>Quintile 5 (Highest)</th>
<th>( P_{\text{difference}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>Median (IQR)</td>
<td>No.</td>
</tr>
<tr>
<td>Continuous variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma 25-hydroxyvitamin D, ng/mL</td>
<td>16</td>
<td>(14–19)</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Age, years</td>
<td>57</td>
<td>(54–63)</td>
<td></td>
<td>60</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165</td>
<td>(158–169)</td>
<td></td>
<td>163</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>1,855 (1,540–2,212)</td>
<td></td>
<td>1,829 (1,594–2,182)</td>
<td>1,894 (1,599–2,227)</td>
</tr>
<tr>
<td>Dietary vitamin D intake, ( \mu g/\text{day} )</td>
<td>6.0</td>
<td>(4.3–7.7)</td>
<td></td>
<td>6.6</td>
</tr>
<tr>
<td>Dietary calcium intake, mg/day</td>
<td>542</td>
<td>(383–685)</td>
<td></td>
<td>565</td>
</tr>
<tr>
<td>Categorical variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>86</td>
<td>66.6</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>64</td>
<td>49.6</td>
<td></td>
<td>74</td>
</tr>
<tr>
<td>Ever drinker</td>
<td>93</td>
<td>72.0</td>
<td></td>
<td>111</td>
</tr>
<tr>
<td>Overweight or obesity</td>
<td>33</td>
<td>25.5</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td>NSAID user</td>
<td>12</td>
<td>9.3</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td>Family history of colorectal cancer</td>
<td>19</td>
<td>14.7</td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations: IQR, interquartile range; NSAID, nonsteroidal antiinflammatory drug.

* Presenting characteristics of controls in quintiles 1, 3, and 5.
* Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (\( \geq 31 \)); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (\( \geq 28 \)).
* Based on the Wilcoxon rank-sum test for median difference and the Fisher exact test for percentage difference.
* History of colorectal cancer in parents and siblings.
interaction between plasma 25-hydroxyvitamin D and the TaqI polymorphism of the VDR gene. These findings underline the importance of vitamin D in colorectal carcinogenesis, at least in its early stage.

Circulating levels of 25-hydroxyvitamin D have been evaluated in at least 7 prospective studies of colorectal cancer and 6 observational studies of colorectal adenoma (best summarized by Gandini et al. (23)). However, only 2 of these were conducted in an Asian or, more specifically, Japanese population (6, 24). Although neither reported a straightforward overall association, the investigation of colorectal adenoma showed a nonlinear inverse association, similar to ours, but only in subjects who provided blood during the winter season (24). With respect to total/dietary calcium intake, we are aware of at least 4 observational studies of colorectal cancer in Asian populations (21, 25–27) but no study of colorectal adenoma in a similar population. Even when the lower consumption levels in Asian than Western populations were considered, all studies consistently reported an inverse association (21, 25–27).

A recent comprehensive review that estimated optimal concentrations of 25-hydroxyvitamin D for multiple health

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Subjects</th>
<th>Model 1a</th>
<th>Model 2b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td></td>
<td>Quintile 1 (lowest)</td>
<td>145</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>Quintile 2</td>
<td>132</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Quintile 3 (middle)</td>
<td>157</td>
<td>145</td>
</tr>
<tr>
<td></td>
<td>Quintile 4</td>
<td>175</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>Quintile 5 (highest)</td>
<td>128</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>P_trend</td>
<td>0.08</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td>Quintile 1 (lowest)</td>
<td>201</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Quintile 2</td>
<td>124</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Quintile 3 (middle)</td>
<td>141</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>Quintile 4</td>
<td>142</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Quintile 5 (highest)</td>
<td>129</td>
<td>142</td>
</tr>
<tr>
<td></td>
<td>P_trend</td>
<td>0.002</td>
<td>0.13</td>
</tr>
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<td></td>
<td>FF</td>
<td>274</td>
<td>260</td>
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<td></td>
<td>Ff</td>
<td>324</td>
<td>294</td>
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<tr>
<td></td>
<td>Ff/ff</td>
<td>410</td>
<td>381</td>
</tr>
<tr>
<td></td>
<td>TaqI genotypeef</td>
<td>523</td>
<td>492</td>
</tr>
<tr>
<td></td>
<td>Tt</td>
<td>156</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td>tt</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Tt/tt</td>
<td>161</td>
<td>148</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

a Model 1 was adjusted for sex, age, screening period, and season of blood collection.

b Model 2 was adjusted for the same variables as model 1 and additionally for cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.

c Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (≥31); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (≥28).

d Respective median (range) of each dietary calcium intake quintile by sex—for men, quintile 1: 288 mg/day (1–366); quintile 3: 514 mg/day (463–567); quintile 5: 667 mg/day (≥717); for women, quintile 1: 419 mg/day (1–498); quintile 3: 676 mg/day (613–742); quintile 5: 1,069 mg/day (≥881).

e The number of subjects providing sufficient genomic DNA to perform genotyping was 1,332.

f For FokI and TaqI, 7 and 8 subjects with undetermined genotype were excluded, respectively.
Table 3. Association of Plasma 25-Hydroxyvitamin D and Dietary Calcium Intake With the Size and Location of the Largest Adenoma, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

<table>
<thead>
<tr>
<th>Variable</th>
<th>Size of Largest Adenoma</th>
<th>Location of Largest Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≥5 mm in Diameter</td>
<td>&lt;5 mm in Diameter</td>
</tr>
<tr>
<td></td>
<td>No. of Cases</td>
<td>OR$^b$</td>
</tr>
<tr>
<td>Plasma 25-hydroxyvitamin D$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quintile 1 (lowest)</td>
<td>70</td>
<td>1</td>
</tr>
<tr>
<td>Quintile 2</td>
<td>56</td>
<td>0.75</td>
</tr>
<tr>
<td>Quintile 3 (middle)</td>
<td>67</td>
<td>0.81</td>
</tr>
<tr>
<td>Quintile 4</td>
<td>79</td>
<td>0.94</td>
</tr>
<tr>
<td>Quintile 5 (highest)</td>
<td>53</td>
<td>0.54</td>
</tr>
</tbody>
</table>

$^a$ Twelve cases had missing information on the location of the largest adenoma.

$^b$ Adjusted for sex, age, screening period, season of blood collection, cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.

$^c$ Respective median (range) of each plasma 25-hydroxyvitamin D quintile by sex—for men, quintile 1: 18 ng/mL (1–20); quintile 3: 25 ng/mL (24–26); quintile 5: 33 ng/mL (≥31); for women, quintile 1: 15 ng/mL (1–17); quintile 3: 23 ng/mL (22–24); quintile 5: 30 ng/mL (≥28).

$^d$ Respective median (range) of each dietary calcium intake quintile by sex—for men, quintile 1: 288 mg/day (1–366); quintile 3: 514 mg/day (463–567); quintile 5: 867 mg/day (≥717); for women, quintile 1: 419 mg/day (1–498); quintile 3: 676 mg/day (613–742); quintile 5: 1,069 mg/day (≥881).
outcomes, including colorectal cancer, concluded that the most
advantageous concentrations of 25-hydroxyvitamin D began
at around 30 ng/mL for all endpoints assessed (28), with
which our observations essentially agree. With regard to dietary
calcium intake, a pooled analysis of 10 cohort studies re-
ported a threshold effect of dietary calcium intake in which
all quintiles above the lowest showed a similar decrease in
the risk of colorectal cancer (7), which strongly supports our
present results.

We saw no multiplicative interaction between plasma 25-
hydroxyvitamin D and dietary calcium intake. Previous ob-
servational studies of primary colorectal cancer and adenoma
have also failed to identify such interaction (10–15). Al-
though these findings do not rule out the existence of biologic
interaction, they may suggest that vitamin D exerts an anti-
carcinogenic effect on the large intestine itself, and that its
interaction, in conjunction with vitamin D, as measured by dietary intake
and the observed associations might have been due to reverse
causality. In contrast to colorectal cancer, however, colorectal
adenoma likely does not affect circulating levels of vitamin D,
because colorectal adenoma is an asymptomatic benign tumor.

Although not nonsynonymous, the TaqI polymorphism of
the VDR gene appears to be in linkage disequilibrium with
a series of polymorphisms in the 3′ end of the VDR gene
(29), for example, the polyadenylated microsatellite in the
3′ untranslated region, the length of which likely determines
message RNA stability and hence likely affects intracel-
lular levels of VDR (30). To date, the 2 studies of colorectal
neoplasia that have examined the TaqI polymorphism in conjunc-
tion with vitamin D, as measured by dietary intake
(31) or circulating levels (14); the results were shown in the
text only), indicated the absence of any obvious interaction.

We investigated effect modification by the VDR gene using
2 traditional SNPs, although the gene spans approximately
100 kilobases and has numerous genetic polymorphisms. In
fact, sequencing of the VDR gene in a Japanese population
identified >20 SNPs with a minor allele frequency of >0.05,
including FokI and TaqI polymorphisms, at least some of
which would serve as tag SNPs to capture the common
variation in the gene (32). Further, recent genome-wide
scans revealed several genes associated with circulating
25-hydroxyvitamin D concentrations (33, 34). Our findings,
based on a limited number of SNPs in a single gene, provide
at most an intriguing insight into the gene-environmental
interaction in the vitamin D pathway.

The strengths of the present study include its measure-
ment of plasma 25-hydroxyvitamin D concentrations, which
may provide a relatively accurate classification of study
subjects by vitamin D status. In addition, the provision of
total colonoscopy to all study subjects likely decreased the
possibility of misclassification between cases and controls.
Conversely, a major limitation is its cross-sectional nature,
and the observed associations might have been due to reverse
causality. In contrast to colorectal cancer, however, colorectal
adenoma likely does not affect circulating levels of vitamin D,
because colorectal adenoma is an asymptomatic benign tumor.

Second limitation is that adenoma cases were not histo-
logically confirmed and necessarily included those with an
early cancer or nonneoplastic lesion. However, our preliminary
survey reported an accuracy of diagnosis based on magni-
fying chromoendoscopy of 90%, a result similar to those
previously reported (35, 36), and the influence of any mis-
classification caused by the technique is therefore likely to
have been minimal. Third, we were unable to analyze groups
of cases and their frequency-matched controls in single
batches, because single groups contained too many subjects
to allow placement in the same batch. Although the impact of
variability in assay performance was not reduced by si-
multaneously analyzing all subjects in a matching category,
blood samples were at least analyzed irrespective of case and
control status, reducing differential misclassification between
cases and controls. Fourth, blinded control samples from the
study population were not available and were therefore not

Table 4. Association of Plasma 25-Hydroxyvitamin D With Colorectal Adenoma According to Dietary Calcium Intake and Vitamin D Receptor Polymorphism, the Colorectal Adenoma Study in Tokyo, Japan, 2004–2005

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma 25-Hydroxyvitamin D</th>
<th>PInteraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quintiles 1–4 (Lower)</td>
<td></td>
</tr>
<tr>
<td>Dietary calcium intake</td>
<td>No. of Cases</td>
<td>No. of Controls</td>
</tr>
<tr>
<td>Quintile 1 (lower)</td>
<td>169</td>
<td>113</td>
</tr>
<tr>
<td>Quintiles 2–5 (higher)</td>
<td>440</td>
<td>433</td>
</tr>
<tr>
<td>FokI genotype&lt;sup&gt;a&lt;/sup&gt;</td>
<td>FF</td>
<td>228</td>
</tr>
<tr>
<td></td>
<td>Ff/ff</td>
<td>338</td>
</tr>
<tr>
<td>TaqI genotype&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>TT</td>
<td>423</td>
</tr>
<tr>
<td></td>
<td>Tt/tt</td>
<td>143</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.
<sup>a</sup> Adjusted for sex, age, screening period, season of blood collection, cigarette smoking, alcohol drinking, body mass index, family history of colorectal cancer, nonsteroidal antiinflammatory drug use, daily energy intake, and height.
<sup>b</sup> The number of subjects providing sufficient genomic DNA to perform genotyping was 1,332.
<sup>c</sup> For FokI and TaqI, 7 and 8 subjects with undetermined genotype were excluded, respectively.
incorporation of vitamin D into the measurement of plasma 25-hydroxyvitamin D; quality control for this measurement was performed by an external laboratory by using nonblinded controls. Accordingly, the reported intra- and interassay coefficients of variation would likely have underestimated the true underlying variations. Finally, we did not match cases and controls by season of examination or blood collection. If such matching had been conducted, we could have taken better account of the seasonal variation in plasma 25-hydroxyvitamin D concentrations.

In summary, we found that both plasma 25-hydroxyvitamin D and dietary calcium intake were inversely associated with the prevalence of colorectal adenoma, albeit in a non-linear manner. We further noted that plasma 25-hydroxyvitamin D levels interacted with the TaqI polymorphism of the VDR gene but not with dietary calcium intake. These observations highlight the importance of vitamin D in colorectal carcinogenesis, at least in its early stage. Vitamin D might protect against colorectal cancer and adenoma, mainly through mechanisms other than the indirect mechanism via calcium metabolism.

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REFERENCES


