Association of the Cyclin D1 A870G Polymorphism With Advanced Colorectal Cancer

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Cyclin D1 (CCND1) is a key regulatory protein of the cell cycle, promoting the transition through the restriction point in the G1 phase beyond which the cell is committed to divide. Overexpression of the CCND1 gene, which has been shown to occur in 30% to 50% of breast and colorectal cancers, has been associated with increased cell proliferation and poor prognosis for a number of human malignancies, including colorectal cancer. A common adenine-to-guanine (A/G) substitution at nucleotide 870 in the conserved splice donor region of exon 4 has been shown to modulate splicing of CCND1 messenger RNA. The G allele preferentially splices transcript a, whereas the A allele mainly splices transcript b, which encodes a protein with a longer half-life. The A allele has been associated with poor prognosis for several cancers and with increased risk of colorectal cancer in hereditary non-polyposis colorectal cancer families, as well as in two small hospital-based case-control studies. Because the A allele is common and may preferentially affect progression, it is important to further clarify its association with colorectal cancer, a neoplasm that is particularly difficult to cure once it has spread outside the intestines. We investigated the association of the CCND1 870A allele with colorectal cancer in a population-based case-control study originally conducted to test gene-diet interactions in the multiethnic population of Hawaii.

Methods
The participants and data collection methods for this study have been described in detail elsewhere. Cases were identified through the rapid reporting system of the Hawaii Tumor Registry, a member of the Surveillance Epidemiology and End Results program of the National Cancer Insti-
The questionnaire was administered during an in-person interview and included detailed information on demographics; a quantitative food-frequency questionnaire; a lifetime history of tobacco, alcohol, and aspirin use; a history of recreational sports activities since age 18 years; a personal history of various relevant medical conditions; a family history of colorectal cancer in parents and siblings; information on height and weight at different ages; and for women, a history of reproductive events and hormone use. The Surveillance Epidemiology and End Results summary staging-information was extracted from the Hawaii Tumor Registry and is defined as follows: in situ tumors (10% of cases) had remained intraepithelial, localized tumors (47%) were confined to the colon or rectum, regional tumors (37%) had either extended through the muscularis to adjacent tissue or metastasized to regional mesenteric lymph nodes, and distant tumors (6%) had metastasized to distant sites.

DNA was extracted from blood lymphocytes using a standard method (QIAamp DNA Blood Midi Kit, Qiagen, Valencia, Calif.). Genotyping for CCND1 was performed with forward primer (CCND1F): AGTTCATTTC-CAATCCGCC and reverse primer (CCND1R): TTTCCGTGGCACTAG-GTGT. Polymerase chain reaction conditions consisted of an initial de-naturation of 94 °C for 5 minutes, followed up with 35 cycles of 94 °C for 30 seconds, 60 °C for 30 seconds, and 72 °C for 30 seconds, with a final extension of 72 °C for 10 minutes. The resulting 212 base pair (bp) polymerase chain reaction product was digested with the restriction enzyme MspI and run on a 3% MetaPhor gel (Cambrex, Rockland, Me), yielding 2 bands for the A allele (175 and 37 bp) and 3 bands for the G allele (141, 37, and 34 bp) (FIGURE).

The statistical analysis used unconditional logistic regression to compute odds ratios (ORs) and 95% confidence intervals (CIs). All models were adjusted for the matching variables (age, sex, and ethnicity) and for potential confounders (pack-years of cigarette smoking, lifetime recreational physical activity [hours], body mass index [calculated as weight in kilograms divided by the square of height in meters] 5 years ago, lifetime use of aspirin [months], years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements). Because the ORs were similar in men and women, results are presented for both sexes combined. Gene-dosage effects were modeled by assigning a value of 1, 2, or 3 to the genotype variable according to the number of A alleles (0, 1, and 2 A alleles, respectively). The likelihood ratio test was used to statistically test interaction among certain variables with respect to colorectal cancer. The test compares a main effects model with a fully parameterized model containing all possible interaction terms for the variables of interest. Polytomous logistic models were performed, comparing cases by stage and subsite of cancer to all eligible controls. Such models were used to compare the risk of in situ/localized and regional/distant cancer, and the risk of colon and rectal cancer by stage. The risk between groups was compared statistically by a Wald test. Genotype frequencies were tested for deviation from the Hardy Weinberg equilibrium with the χ² test. Statistical significance was
defined as P<.05. All analyses were performed with SAS statistical software version 8.2 (SAS Institute Inc, Cary, NC).

RESULTS

The characteristics of colorectal cancer cases and controls are shown in Table 1.12-14 Cases were comparable with controls with regard to age, sex, and ethnicity but were somewhat less educated, more likely to have a family history of colorectal cancer, and less likely to have used aspirin regularly. Cases had smoked more cigarettes and exercised less in their lifetimes. They were also heavier and consumed more calories, less calcium, less folate, and less dietary fiber (measured as nonstarch polysaccharides) from vegetable sources than controls.

Table 2 presents the distributions of cases and controls with colorectal cancer by CCND1 genotype. Based on the controls, the frequency for the putative high-risk A allele was 0.49, 0.43, and 0.57, in Japanese, white, and Hawaiian participants, respectively. The genotype distributions were consistent with Hardy Weinberg equilibrium in each ethnic group (Japanese, P=.31; white, P=.60; Hawaiian, P=.21). Overall, the A allele was associated with a 30% increase in colorectal cancer risk (OR, 1.3; 95% CI, 1.0-1.7) and the CCND1 870 AA genotype was associated with a 50% increased risk of colorectal cancer (OR, 1.8; 95% CI, 1.8-2.1) and 1.9 (95% CI, 1.2-3.1) for the GA and AA genotype, respectively, compared with the GG genotype. This association showed a statistically significant gene-dosage effect (P=.008). In contrast, no statistically significant association was found with the A allele for early-stage colorectal cancer among all participants combined. The OR for the presence of the

![Table 1](http://jama.jamanetwork.com/pdfaccess.ashx?url=/data/journals/jama/4906/)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n = 504)</th>
<th>Controls (n = 624)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>66.5 (10.5)</td>
<td>66.8 (10.5)</td>
</tr>
<tr>
<td>Men, No. (%)</td>
<td>307 (60.9)</td>
<td>363 (58.2)</td>
</tr>
<tr>
<td>Ethnicity, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Japanese</td>
<td>296 (58.7)</td>
<td>380 (60.9)</td>
</tr>
<tr>
<td>White</td>
<td>138 (27.4)</td>
<td>161 (25.8)</td>
</tr>
<tr>
<td>Hawaiian</td>
<td>70 (13.9)</td>
<td>83 (13.3)</td>
</tr>
<tr>
<td>Education, y</td>
<td>13.1 (3.0)</td>
<td>13.8 (3.2)</td>
</tr>
<tr>
<td>Family history of colorectal cancer, No. (%)†</td>
<td>85 (16.9)</td>
<td>63 (10.1)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoker, No. (%)</td>
<td>294 (58.3)</td>
<td>333 (53.4)</td>
</tr>
<tr>
<td>Pack-years</td>
<td>22.4 (31.5)</td>
<td>15.8 (25.7)</td>
</tr>
<tr>
<td>Body mass index, 5 years ago‡</td>
<td>25.6 (5.4)</td>
<td>24.8 (4.8)</td>
</tr>
<tr>
<td>Dietary intake, per d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total calories, kcal</td>
<td>2211 (1048)</td>
<td>2056 (948)</td>
</tr>
<tr>
<td>NSP from vegetables, g</td>
<td>2.84 (2.27)</td>
<td>3.48 (2.52)</td>
</tr>
<tr>
<td>Ethanol, g</td>
<td>8.97 (18.30)</td>
<td>7.69 (16.07)</td>
</tr>
<tr>
<td>Total calcium, mg§</td>
<td>1772 (2171)</td>
<td>2361 (3002)</td>
</tr>
<tr>
<td>Total folate, µg§</td>
<td>1070 (1236)</td>
<td>1346 (1590)</td>
</tr>
<tr>
<td>Red meat, g</td>
<td>86.1 (80.6)</td>
<td>74.6 (67.7)</td>
</tr>
<tr>
<td>Processed meats, g</td>
<td>31.2 (36.8)</td>
<td>23.1 (26.2)</td>
</tr>
<tr>
<td>Lifetime aspirin use, mo</td>
<td>16.8 (56.2)</td>
<td>30.5 (81.8)</td>
</tr>
<tr>
<td>Lifetime recreational activity, h</td>
<td>7792 (12 225)</td>
<td>9001 (12 576)</td>
</tr>
</tbody>
</table>

Abreviation: NSP, nonstarch polysaccharides.
*Data are presented as mean (SD) unless otherwise specified.
†Among parents and siblings.
‡Calculated as weight in kilograms divided by square of height in meters.
§Total calcium and total folate are from foods and supplements. Nutrients are adjusted for calories.

Table 2. Colorectal Cancer and Ethnicity by CCND1 A870G Genotype*

<table>
<thead>
<tr>
<th>GG</th>
<th>GA</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Cases</td>
<td>No. of Controls</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>All</td>
<td>109</td>
<td>164</td>
</tr>
<tr>
<td>Japanese</td>
<td>75</td>
<td>96</td>
</tr>
<tr>
<td>White</td>
<td>29</td>
<td>50</td>
</tr>
<tr>
<td>Hawaiian</td>
<td>5</td>
<td>18</td>
</tr>
</tbody>
</table>

Abbreviations: A870G, adenine 870 guanine; CCND1, cyclin D1; CI, confidence interval; OR, odds ratio.
*Adjusted by unconditional logistic regression for age, sex, ethnicity (when appropriate), pack-years of cigarette smoking, lifetime recreational physical activity (hours), lifetime aspirin use (months), body mass index 5 years ago, years of schooling, and intakes of nonstarch polysaccharides from vegetables and calcium from foods and supplements.
†For genetic trend, based on a variable assigned the value 1, 2, or 3 according to the patient’s number of A alleles (0, 1, and 2, respectively).
A allele among patients with advanced colorectal cancer was significantly different from that in patients with early-stage disease \( (P = .048) \). In the corresponding race-specific analyses, a nonsignificant association was suggested for early-stage disease in white and Hawaiian participants, and the association with advanced disease was observed or suggested in all ethnic groups. The number of cases with distant stage at diagnosis was too small to allow for separate analyses.

**Table 4** compares the effect of the A allele on colorectal cancer risk stratified by stage at diagnosis and anatomical subsite with the use of polytomous logistic regression. Associations were suggested for the A allele with late-stage colon cancer and early-stage rectal cancer; however, none were statistically significant. The strongest effect for presence vs absence of the A allele was found for late-stage rectal cancer (OR, \( 2.8; 95\% \text{ CI}, 1.3-6.0 \)), which was also significantly different from the early-stage colon cancer (OR, \( 1.0; 95\% \text{ CI}, 0.7-1.5 \); \( P = .02 \)).

Analyses for interaction showed no modifying effect of age or family history on the association of the A allele with colorectal cancer.

**COMMENT**

In this population-based case-control study, we found that the **CCND1** 870 AA genotype was associated with a 50% increased risk of colorectal cancer, with a statistically significant gene-dosage effect \( (P = .03) \). The association with the A allele was significantly stronger for advanced stage disease than for early stage disease. The observed effect was consistent between sexes, across ethnic groups (particularly for advanced disease), and stronger for rectal cancer.

Amplification and/or overexpression of the **CCND1** gene have been described in several forms of human cancer and associated with increased cell proliferation and poor prognosis. \(^3\)\(^-\)\(^5\) With regard to colorectal cancer, overexpression of **CCND1** is observed in 30% of the tumors and expression of an antisense to **CCND1** complementary DNA has been shown to inhibit the proliferation of human colon cancer cells, as well as their tumorigenicity in nude mice. \(^1\) The 870G polymorphism in exon 4 of the **CCND1** gene is associated with a splice site variation coding for 2 messenger RNA transcripts. \(^6\) Transcript B, which skips exon 5 and reads into intron 4, does not contain the exon 5 destruction box sequence, resulting in a protein with a longer half-life. It has been shown that, although both the A and G alleles encode the 2 transcripts, the A allele preferentially encodes the altered transcript leading to a state of increased **CCND1** level, even in the heterozygous state. \(^6\)\(^,\)\(^18\)

Five previous studies have reported on the association of the **CCND1** 870G polymorphism and colorectal cancer. Kong et al\(^9\) found that patients with 1 or 2 copies of the **CCND1** 870A allele who also carry a mutation in a DNA mismatch repair gene develop hereditary nonpolyposis colorectal cancer an average of 11 years earlier than mismatch repair gene mutation carriers with the GG genotype. In contrast, Bala...
and Peltomäki\textsuperscript{19} found no correlation between the A allele and age of onset among 146 affected mismatch repair mutation carriers; however, the presence of the variant transcript \(b\) in blood or healthy mucosa was associated with a significantly lower age of onset compared with individuals with transcript \(a\) only (35 vs 46 years; \(P = .02\)). Porter et al\textsuperscript{11} also showed that the CCND1 870A allele was overrepresented in 107 non-hereditary nonpolyposis colorectal cancer familial cases of colorectal cancer compared with 171 patients without cancer. In the same study, an overrepresentation of the A allele was also observed in 128 “sporadic” colorectal cancer cases, which did not quite reach statistical significance (\(P = .08\)). Kong et al\textsuperscript{10} recently reported on a hospital-based case-control study of 156 white patients with colorectal cancer younger than 60 years and 152 matched dermatology control patients. Compared with the GG genotype, Kong et al\textsuperscript{10} found that the AA genotype was associated with an elevated OR of 2.6 (95% CI, 1.4-5.2), whereas the GA genotype was unassociated with risk (OR, 1.1; 95% CI, 0.6-1.8). Finally, in a hospital-based study of 100 patients and 101 blood donors, McKay et al\textsuperscript{3} reported a lack of association between the CCND1 870A allele and colorectal cancer; however, survival was significantly shorter in patients with a high level of CCND1 expression in their tumors (>50% cells demonstrating immunoreactivity). Overall, these past studies, which were hospital-based and relatively small, were suggestive of a possible association of the CCND1 G870A allele with progression of colorectal tumors.

Our study expands these findings with a population-based design and a larger sample size. We also report ethnicity, which has not been studied before. The A allele is particularly common in Native Hawaiians, a group of patients who often present at a late stage and experience a poorer stage-adjusted cause-specific survival for various cancers, including colorectal cancer, compared with white patients.\textsuperscript{20,21} Similarly, the weaker effect for the A allele observed among Japanese participants in this study would, if confirmed, be consistent with the early presentation and better cause-specific survival of Japanese patients with colorectal cancer in Hawaii.\textsuperscript{20,21} Colorectal cancer is rarely curable when the disease has spread outside the large intestine. Given the high frequency of the A allele (0.43-0.57 in the 3 ethnic groups) and the stronger association for advanced disease, this polymorphism may be responsible for a sizable portion of the morbidity and mortality from colorectal cancer. If confirmed, this association may have implications for the colorectal cancer screening and treatment of the A allele carriers.

Other methodological aspects of our study deserve consideration. Differences in detection rate by genotype appears to be an unlikely explanation for our results because the association was observed in 2 ethnic groups with markedly different socioeconomic status and screening practices.\textsuperscript{21,22} This is reflected in the proportions of in situ tumors in our population-based case-series (Japanese, 10%; white, 9%; Hawaiian, 4%). Confounding by other variables is also unlikely because the effects of known risk factors were thoroughly investigated in the analysis. The association was observed in several ethnic groups, arguing against residual confounding by ethnicity. Moreover, the frequency of the CCND1 870A allele in our white control participants (0.43) is very similar to that from previous reports,\textsuperscript{10,11} and the genotype frequencies were in Hardy-Weinberg equilibrium, arguing against selection bias. This is consistent with the fact that characteristics of participants who gave blood were very similar to those of all interviewed participants in this study.\textsuperscript{13}

In conclusion, these data provide strong evidence that the CCND1 870A allele may be associated with colorectal cancer, and particularly with forms of the disease that result in severe morbidity and mortality.

**Author Contributions:** Dr Le Marchand had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study concept and design:** Le Marchand, Donlon, Wilkens.

**Acquisition of data:** Le Marchand, Seifried, Lun-Jones, Wilkens.

**Analysis and interpretation of data:** Le Marchand, Lun-Jones, Wilkens.
Drafting of the manuscript: Le Marchand, Donlon, Wilkens.
Critical revision of the manuscript for important intellectual content: Le Marchand, Seifried, Lun-Jones, Wilkens.
Statistical expertise: Le Marchand, Wilkens.
Obtained funding: Le Marchand.
Administrative, technical, or material support: Le Marchand, Seifried, Lun-Jones, Donlon.
Study supervision: Le Marchand.

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2. Musgrove EA, Lee CSL, Buckley MF, Sutherland RL. Cyclin D1 induction in breast cancer cells shorten G1 and is sufficient for cells arrested in G1 to complete the cell cycle. Proc Natl Acad Sci U S A. 1994;91:8022-8026.

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