Cyclin D1 Polymorphism and Increased Risk of Colorectal Cancer at Young Age

Shouming Kong, Qingyi Wei, Christopher I. Amos, Patrick M. Lynch, Bernard Levin, Jihong Zong, Marsha L. Frazier

Colorectal cancer is the third most common cause of cancer and cancer mortality among men and women in the United States. It is estimated that there will be approximately 130,400 new colorectal cancer cases and 56,700 deaths in the year 2001 (1).

Cyclin D1 is involved both in normal regulation of the cell cycle and in neoplasia, where it is frequently overexpressed (2). It plays an important role in the transition from the G1 phase to the S phase of the cell cycle. Amplification or overexpression of the cyclin D1 gene (also known as CCND1) is common in a variety of different cancers and induces proliferation.

The cyclin D1 gene has a G to A polymorphism at codon 242 in exon 4 that increases the frequency of alternate splicing (3). In the alternately spliced RNA, intron 4 is not spliced out. Both the normal and altered transcripts encode a protein that contains amino acids 55–161, which are thought to be responsible for the cyclin D1 function (4). The protein encoded by the alternate transcript is missing the last 55 amino acids at the carboxy-terminus that are replaced by a shorter 43-amino-acid sequence encoded by intron 4. As a result, the carboxy-terminal end of the alternate transcript is missing sequences important in protein turnover; therefore, it may have a longer half-life (3). Slight elevations in the levels of cyclin D1 might make the cells less sensitive to signals by the cell-cycle checkpoint machinery.

The A allele leads to poorer clinical outcome in patients with non-small-cell lung cancer (3). In a previous study (5), we showed that patients with one or two copies of the polymorphic A allele of the cyclin D1 gene who also carried a mutation in a mismatch repair gene developed hereditary nonpolyposis colorectal cancer (HNPCC) on the average of 11 years earlier than mismatch repair gene carriers with the GG genotype.

To extend our findings, we conducted a hospital-based case-control study of nonsyndromic colorectal cancer to determine if the cyclin D1 polymorphism influences risk for this disease.

We studied a consecutive series of newly registered patients with nonsyndromic adenocarcinoma of the colon or rectum evaluated at The University of Texas M.D. Anderson Cancer Center, Houston, who agreed to participate in the study. Patients with nonsyndromic colorectal cancer are defined as individuals with no obvious physical characteristics such as occur in familial adenomatous polyposis coli (FAP) or Peutz-Jeghers syndrome. The patients were enrolled during an 11-month period that began in September 1994. From September 20, 1994, through August 17, 1995, a total of 321 patients with confirmed colon or rectal adenocarcinoma consented to participate in the study and provided a blood specimen, representing 69% of the patients originally contacted. Of these 321 patients, 188 (59%) were male and 133 (41%) were female. Most case subjects were Caucasian (75%), with African-American, Hispanic, and other ethnic groups accounting for 16%, 8%, and 1%, respectively. Thirty-four percent (n = 110) of the cases occurred in individuals less than 50 years old, and 23% (n = 75) occurred in individuals less than 45 years old. Because of possible heterogeneity in susceptibility to colorectal cancer among different racial and ethnic groups, we limited our study to Caucasians. Among the Caucasians, we limited the study to case subjects under the age of 60 years, because environmental effects are likely to dominate over genetic effects in older case subjects. This restriction led to our final tally of 156 case subjects, 28 (18%) of whom had rectal cancer (with two subjects having both rectal and colon cancers), and 11 patients (7%) met the Amsterdam criteria (6).
Control subjects were selected from a pool of control subjects to frequency match the case subjects by age (±5 years), sex, and ethnicity. This control subject pool consisted of healthy, unrelated, cancer-free subjects recruited from visitors accompanying dermatology and skin cancer patients in The University of Texas M.D. Anderson Cancer Center and The University of Texas Medical School, Houston. The exclusion criteria were previous cancer and any blood transfusion in the past year. There were 152 normal healthy control subjects. Each eligible subject, after giving written informed consent, completed a structured self-administered questionnaire to collect information on demographic data. The Institutional Review Boards of The University of Texas Medical School, Houston, and The University of Texas M.D. Anderson Cancer Center approved the study protocol. Written informed consent was obtained from all subjects.

From each subject, 10 mL of blood was drawn in Vacutainer tubes containing EDTA (Becton Dickinson Vacutainer System, Rutherford, NJ). DNA was isolated by one of two methods. In the first method, DNA was isolated with an Applied Biosystems 341 nucleic acid purification system according to the instructions of the manufacturer (Applied Biosystems, Foster City, CA). After each extraction, an extensive purge, as described by the manufacturer, was used to obtain polymerase chain reaction (PCR)-grade DNA. The alternate method was to use a DNA extraction kit (Qiagen Inc., Santa Clarita, CA) to isolate DNA from buffy coat isolated from whole blood. PCR and single-strand conformation polymorphism analyses were used to genotype the G/A cyclin D1 polymorphism in exon 4 as described previously (5).

Colorectal cancer was analyzed as the dichotomous dependent variable, and the independent variables were cyclin D1 genotypes (nominal), sex (dichotomous), and age (continuous). The Pearson χ² test was used to evaluate whether or not the cyclin D1 genotype proportions were identical in case subjects and control subjects. Univariate analysis was performed to calculate the crude odds ratios (ORs) and their 95% confidence intervals (CIs) for the cyclin D1 gene polymorphisms (AG, AA) with the use of the GG genotype as the reference. We tested for an association between colorectal cancer and cyclin D1 genotype by logistic regression analysis. We assigned indicator variables to the genotypes and adjusted for potential confounding variables from demographic factors. ORs and 95% CIs were calculated from the logistic regression analysis to determine the direction and strength of the association. We also used the likelihood ratio test to compare recessive or dominant models with a general model that included arbitrary effects for heterozygous individuals. These tests allowed us to evaluate recessive and dominant effects of cyclin D1 alleles. All statistical tests were two-sided, and the level of significance was set at α = .05. We performed all of the statistical analyses with SAS version 6.12 (SAS Institute, Inc., Cary, NC).

The allelic frequencies of the 156 case subjects (A, 0.54; G, 0.46) were statistically significantly different from those of the control subjects (A, 0.43; G, 0.57), with P = .005. The allelic frequencies of the 152 control subjects were similar to those reported previously by Betticher et al. (3) for 122 Europeans (A, 0.42; G, 0.58). The frequency distribution of the different genotypes for the cyclin D1 polymorphism is shown in Table 1. The AA genotype was more frequent among the case subjects than among the control subjects (31.4% and 15.1%, respectively). With the use of the χ² test, the difference in genotypic frequencies between case subjects and control subjects was statistically significantly different (P = .003). The difference in the genotypic frequencies between male and female case subjects was not statistically significant, nor was there a statistically significant difference in genotype frequency between young (<45 years) and old (>45 years) case subjects.

Using the GG genotype as the reference genotype and correcting for age and sex, we performed multivariable logistic regression (Table 1). In an initial analysis (results not shown in the table), we evaluated the general hypothesis of no effect of CCND1 genotypes on risk for colon cancer by comparing a model that contrasted both the AA and the AG genotypes jointly with the GG genotypes. We compared this model with a model without any genotypic effects and so constructed a likelihood ratio test with 2 df. Results from this preliminary analysis yielded a χ² deviate of 11.74 with P = .003, indicating a statistically significant global effect of CCND1 genotypes on risk for colorectal cancer.

We used two logistic regression models to specifically test for dominant versus recessive inheritance of the effects of the A allele. In the dominant model, we hypothesized that the cyclin D1 genotypes AG and AA contributed equally to the risk of colorectal cancer, and we coded GG = 0, AG = 1, and AA = 1. In the recessive model, we hypothesized that only the AA genotype contributed to the risk of the disease, and we coded GG = 0, AG = 0, and AA = 1. The log-likelihood statistics of each model were compared with the general logistic regression log likelihood that jointly fitted both recessive and dominant coding schemes. The χ² value of the dominant model was 9.95 (P = .002), and this model was rejected; however, the χ² value of the recessive model was 0.04 (P = .841). These find-

Table 1. Cyclin D1 genotype frequency by case–control status and logistic regression analysis

<table>
<thead>
<tr>
<th></th>
<th>Case subjects, No. (%)</th>
<th>Control subjects, No. (%)</th>
<th>Multivariate analysis*</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Adjusted OR†</td>
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<tr>
<td>General genotype</td>
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<tr>
<td>GG</td>
<td>36 (23.1)</td>
<td>45 (29.6)</td>
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<tr>
<td>AG</td>
<td>71 (45.5)</td>
<td>84 (55.3)</td>
<td>1.06</td>
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<tr>
<td>AA</td>
<td>49 (31.4)</td>
<td>23 (15.1)</td>
<td>2.68</td>
</tr>
<tr>
<td>Dominant genotype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>36 (23.1)</td>
<td>45 (29.6)</td>
<td>1</td>
</tr>
<tr>
<td>AG + AA</td>
<td>120 (76.9)</td>
<td>107 (70.4)</td>
<td>1.4</td>
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<td>Recessive genotype</td>
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<tr>
<td>GG + AG</td>
<td>107 (68.6)</td>
<td>129 (84.9)</td>
<td>1</td>
</tr>
<tr>
<td>AA</td>
<td>49 (31.4)</td>
<td>23 (15.1)</td>
<td>2.58</td>
</tr>
</tbody>
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*Adjusted for sex and age.
†OR = odds ratio.
‡CI = confidence interval. P values are for two-sided Wald test (11) that tests the hypothesis that the OR = 1.
Our previous study identified a polymorphism in the cyclin D1 gene, in which the A allele on cancer risk.

The AG genotypes were distributed approximately equally between case and control subjects and were associated with an OR of 1.06 (95% CI = 0.62 to 1.81; \( P = .845 \)). The AA genotype was associated with a statistically significantly elevated OR of 2.68 (95% CI = 1.38 to 5.19; \( P = .004 \)). These results are almost identical to those of our univariate logistic regression analysis (data not shown). We performed multivariable logistic regression again, using the GG genotype as the reference genotype and the AG genotypes as the reference genotype (Table 1). The OR for AA + AG genotype was 1.4 (95% CI = 0.84 to 2.34; \( P = .192 \)). Using the combined GG and AG genotypes as the reference genotype, we also performed multivariable logistic regression and found that the OR for the AA genotype was 2.58 (95% CI = 1.48 to 4.52; \( P = .001 \)), once again supporting a recessive model for the A allele (Table 1).

The major difference in the findings between our previous study on mismatch repair-deficient subjects (5) is the model of inheritance. In the earlier study, because increased risk was seen among those who were heterozygous for the A allele, the A allele appeared to have a dominant effect in HNPCC cases. In the current study, the effects of the A allele best fit a recessive model. This may be explained by the fact that patients carrying a germline mutation in one of the mismatch repair genes are more vulnerable to the effects of the cyclin D1 polymorphism as well as to other genetic or environmental influences that increase mutagenesis. Also, there may be an interaction between the mutated mismatch repair genes and cyclin D1, allowing the deleterious allele of cyclin D1 to influence age-associated risk, when either one or two copies are present. In the hospital-based case–control study, defects in DNA mismatch repair are expected to be uncommon among the case subjects; therefore, two copies of the cyclin D1 variant allele would be required to increase risk.

We realize that this hospital-based case–control study has limitations because the patients seen at The University of Texas M. D. Anderson Cancer Center are a referral population, and the case subjects may not be perfectly representative of colorectal cancer patients in the general population. Also, the control subjects were not selected from the same population as the case subjects. However, our comparison of the observed distribution (GG, AG, and AA: 30%, 55%, and 15%, respectively) of cyclin D1 genotype frequency with the expected one (33%, 49%, and 18%, respectively) from the Hardy–Weinberg equilibrium model suggested no selection bias (\( \chi^2 = 1.438; df = 2; P = .500 \)). In a different set of 248 healthy enrollees in a managed-care system in Houston (7), we observed genotype frequencies of 31%, 52%, and 16%, respectively, which is very similar to the results that we are reporting.

Epidemiologic studies on colorectal cancer suggest that as much as 15% of the cancer incidence is due to dominantly inherited genetic factors (8,9), the best characterized of these being FAP and HNPCC. Aside from a few unusual dominantly inherited syndromes, the contribution of genetic factors is thought to be relatively minor. In a recent study on twins (10), statistical modeling that estimates the relative importance of heritable and environmental factors in the development of cancers attributed 35% of the risk for colorectal cancer to heritable factors. Shared environmental factors accounted for 5%, and nonshared environmental factors accounted for 60%. Our findings in this hospital-based case–control study suggest that the cyclin D1 polymorphism is associated with an increased risk of colorectal cancer, and the data are most consistent with the influence of the polymorphism fitting a recessive model of inheritance.

**REFERENCES**


