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Cyclin D1 gene polymorphism and susceptibility to lung cancer in a Chinese population

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The objective was to study the relationship between cyclin D1 gene (CCND1) polymorphism and lung cancer in the Chinese population. Blood samples of 182 cases and 185 controls were collected from a hospital based case-control study. PCR–SSCP was used to examine the G/A polymorphism in exon 4 of CCND1. The results showed that the frequencies of the CCND1 AA, GA and GG genotypes were 31.3, 46.7 and 22.0% respectively in cases, and 21.1, 53.0 and 25.9 respectively in controls. Adjusted by age (in years), sex and smoking status, multivariate logistic regression analysis showed that the AA genotype was associated with a significantly increased risk (OR = 1.87, 95% CI 1.01–3.45) for lung cancer. In the stratification analysis, the CCND1 AA variant genotype was associated with increased risk in individuals who were ≤50 years old (OR = 3.23, 95% CI 1.17–8.96) and males (OR = 2.46, 95% CI 1.18–5.10). According to histological types, there was significantly higher frequency of AA genotype in squamous cell lung cancer than that in controls (OR = 2.92, 95% CI 1.07–8.03). In conclusion, it is suggested that the CCND1 G/A polymorphism is associated with the early onset of lung cancer in men and contributes to susceptibility to lung cancer, especially squamous cell cancer, in this population.

Materials and methods

Study subjects
Eligible cases consisted of newly diagnosed patients with lung cancer who received treatment at the Beijing Institute of Chest Tumor from September 2001 to March 2002. All patients were diagnosed histologically with specimens obtained from biopsy or surgical resection. Demographic and epidemiological data, including tobacco use, were gathered by a questionnaire completed by trained interviewers and reviewed by a single reviewer during the hospitalization. The healthy controls were recruited from a community in Beijing during a similar time period. These control subjects were first surveyed by means of a short questionnaire to determine their willingness to participate in research studies. Using the same questionnaire as for the cases, the same interview group interviewed eligible subjects and collected the data including age, sex and smoking status.

After informed consent was obtained, a one-time 2 ml of blood sample was obtained from all participants and stored at −70°C for genotype analysis. The study was approved by our Institutional Review Committee.

Genotyping
Genomic DNA was obtained from blood by the routine high salt method (14). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm.

PCR-based single-strand conformation polymorphism (SSCP) analysis was used to genotype the CCND1 polymorphism in exon 4 (13). For PCRs the primers were 5′-TACTACCGCTCACAGCCTTC-3′ (sense), 5′-TTGGCCACACGCCTCGGCATTTC-3′ (antisense), which generated a 138 bp fragment. These fragments were amplified in 25 μl of reaction mixture containing 50 ng genomic DNA, 1× PCR buffer (50 mM KCl, 10 mM Tris–HCl, pH 9.0 at 25°C and 1% Triton X-100), 1.5 mM MgCl2, 0.2 mM each dATP, dTTP, dCTP and dGTP, and 0.05 units of Taq polymerase. 

Abbreviation: SSCP, single-strand conformation polymorphism.

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Results

There were 182 lung cancer cases and 185 controls included in this analysis. Table I summarizes selected characteristics of the subjects. The cases and controls were frequency matched by sex and smoking status. However, there were significantly more cases than controls in the >50 years’ group (P = 0.041). The observed distribution of CCND1 genotypes in controls (GG:GA:AA, 48:98:39) was not statistically different from those (51:92:42) expected from the Hardy–Weinberg equilibrium equation (P = 0.822).

Table I. Distribution of selected variables in lung cancer patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases n = 182</th>
<th>Controls n = 185</th>
<th>P*</th>
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<tbody>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>58</td>
<td>78</td>
<td>0.041</td>
</tr>
<tr>
<td>&gt;50</td>
<td>124</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male</td>
<td>131</td>
<td>105</td>
<td>0.568</td>
</tr>
<tr>
<td>Female</td>
<td>51</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
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<td></td>
</tr>
<tr>
<td>Never</td>
<td>68</td>
<td>80</td>
<td>0.432</td>
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<tr>
<td>Ever</td>
<td>114</td>
<td>105</td>
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<tr>
<td>Histological types</td>
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<tr>
<td>SCLCb</td>
<td>44</td>
<td>24.2</td>
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<tr>
<td>NSCLCc</td>
<td>138</td>
<td>75.8</td>
<td></td>
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<tr>
<td>Squamous cell lung cancer</td>
<td>48</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>76</td>
<td>55.1</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>14</td>
<td>10.1</td>
<td></td>
</tr>
</tbody>
</table>

*Two-sided χ² test.

Distributions of the CCND1 genotype and allele frequency in lung cancer patients and controls are shown in Table II. The A allele frequency was higher in cases (0.546) than that in controls (0.479), but this difference was only borderline statistically significant (P = 0.054). Although the CCND1 AA genotype was more frequent in the cases (31.3%) than in the controls (21.1%), no statistically significant difference was found in univariate analysis (OR = 1.75; 95% CI 0.98–3.15). After adjustment for age, sex and smoking status, the AA genotype was associated with a significantly increased lung cancer risk (adjusted OR = 1.87; 95% CI 1.01–3.45) compared with the GG genotype. The adjusted OR for the CCND1 AG type was 1.02 (95% CI 0.60–1.74).

Table III shows the CCND1 genotype frequencies in subjects stratified by age, sex and smoking status. In the younger
The associations between the $CCND1$ AA genotype and histological types of lung cancer are shown in Table IV. After adjustment with age, sex and smoking status, the $CCND1$ AA genotype was associated with a significantly higher risk in NSCLC ($OR = 1.95$; $95\% CI 1.00–3.78$), while there was no significant association in SCLC ($OR = 1.10$; $95\% CI 0.61–1.95$). Among NSCLC, the OR was $2.92$ ($95\% CI 1.07–8.03$) for the AA genotype and squamous cell lung cancer patients, but there was no significant association between $CCND1$ gene polymorphism and lung adenocarcinoma ($OR = 1.78$; $95\% CI 0.78–4.04$).

### Discussion

Cyclin D1 is one of the major cyclins involved in transition from $G_1$ to $S$ phase, being associated with CDK 4/6 in mid to late $G_1$ phase. Both gene activation (due to amplification or chromosomal rearrangement) and/or protein overexpression of cyclin D1 have been described in a variety of tumors, including lung cancer (15–17). Overexpression of cyclin D1 may lead to premature cell passage through the $G_1/S$ transition, resulting in propagation of unrepaired DNA damage, accumulation of genetic errors, and a selective growth advantage for the altered cells. Furthermore, in tumor tissues cyclin D1 overexpression or gene amplification is associated with a more rapid and frequent recurrence of cancer (18–20). In lung cancer, a high frequency of $CCND1$ amplification/overexpression was reported as one of the important steps in carcinogenesis (21).

The G/A single base pair polymorphism in $CCND1$ could reportedly influence tumor susceptibility in several cancers (22–25). The AA homozygotes express more altered transcript...
than the GG homozygotes and GA heterozygotes and the altered transcript fails to be spliced at the exon 4/intron 4 boundary, which does not contain exon 5 and terminates downstream of exon 4 (26). This results in an altered protein that lacks the destruction box encoded by exon 5, which is responsible for the increasing half-life of the cyclin D1 protein (11). Therefore, in individuals with the AA genotype, there are increasing steady-state levels of cyclin D1 protein, allowing cells to pass through the G1/S checkpoint more easily and promoting the transformation of cells. Zheng and colleagues (13) have shown that the frequency of the AA genotype was higher in SCCHN patients (23.6%) than in controls (16.5%) in non-Hispanic white population and that subjects with AA genotype tended to develop SCCHN at an earlier age than those with GG genotype. Recently the A allele of CCND1 was shown to be associated with an early age at onset of hereditary non-polysysis colorectal cancer, acting as a risk modifying gene (28). Furthermore, study in non-small cell lung cancer patients showed that the event-free survival was longer in patients with GG genotype (11), which indicated that the CCND1 G/A polymorphism was associated with clinical outcome of lung cancer. Similar results were found in ovarian cancer (29) and SCCHN (30). In agreement with those studies, we found that the CCND1 AA genotype was associated with a higher risk for lung cancer in a Chinese population and this risk is remarkably increased in younger subjects. This finding is consistent with Zheng’s result in SCCHN. For an early age of onset of disease is a hallmark of genetic predisposition, these results imply that the CCND1 G/A polymorphism may play a role in the susceptibility to carcinogenesis of lung cancer.

An interesting result was obtained when we divided the cases into subgroups by histological types. There were no significant associations in the subgroups except for squamous cell lung cancer patients. This finding is consistent with previous reports about cyclin D1 overexpression in squamous cell lung carcinomas (31,32). According to epidemiological studies, which have shown that smoking was the major cause of squamous cell lung cancer in Chinese (33) and the PAH-adduct was one of the main DNA damage of smoking-caused lung cancer (34), there was indication that the CCND1 G/A polymorphism might be due to the individual susceptibility to some specific environmental carcinogens or DNA damage, although the specific relationship between cyclin D1 protein and PAH has not been reported. This result also provides an explanation for the result of sex stratification. A significant higher risk of subjects with the AA genotype was found in squamous cell lung cancer cases, only 11.8% female patients in our study were squamous cell lung cancer, and smoking was not a risk factor in females. It seems that the CCND1 G/A polymorphism may not be a susceptibility marker for lung cancer of women in this population, but larger studies are needed to confirm this finding.

In conclusion, we found that the CCND1 G/A polymorphism may contribute to the susceptibility to lung cancer, especially in those who were young and male, in this Chinese population. In addition, the polymorphism may be a link in the smoking–DNA damage–lung cancer interaction. However, bias may arise from the unmatched age and limited numbers in the subgroups analyzed and possible selection bias inherent in a hospital-based case-control study. Therefore, these results remain preliminary and need to be validated in larger and well-designed studies.

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References


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