Abstract. Background: The tumor suppressor p53 protein plays a critical role in different cellular processes in response to DNA damage and it is responsible for transcriptional induction of the p21 (CDKN1A/WAF1/CIP1) gene. Both p53 and p21 are thought to play major roles in the development of human malignancy. Polymorphic variants of p53 at codon 72, and CDKN1A at codon 31, have been found to be associated with cancer susceptibility, but few studies have investigated their effect on endometriosis risk. Materials and Methods: In this hospital-based case–control study, we investigated the association of p53 codon 72 and CDKN1A codon 31 polymorphisms with endometriosis susceptibility in a Taiwanese population. In total, 180 patients with endometriosis, and 330 age-matched controls in Central Taiwan were recruited and genotyped. Results: We found a significant difference in the distribution of the p53 genotype, but not the CDKN1A genotype, between the endometriosis and control groups. Individuals with the C (Pro) allele at p53 codon 72 had a 1.6-fold increased odds ratio of endometriosis, and those with Arg/Pro and Pro/Pro genotypes for p53 codon 72 had a 1.84- and 2.74-fold (95% confidence interval=1.17-2.92 and 1.58-4.74) increased risk of endometriosis compared to those with Arg/Arg, respectively. The distribution of haplotype combinations of p53 codon 72 and CDKN1A codon 31 was statistically different in the endometriosis and control groups. The percentages of the three subgroups with p53 CC homozygote were all higher in the endometriosis group than in the control group. Conclusion: Our findings suggest that the C (Pro) allele of p53 codon 72 may be associated with the development of endometriosis, and could serve as a potential biomarker for early prediction of this disease.

Endometriosis is a chronic gynecological disease characterized by growth of endometrial tissue in sites other than the uterine cavity, most commonly in the pelvic cavity, including the ovaries, the uterosacral ligaments, and pouch of Douglas (1). Endometriosis possesses many features of a benign neoplastic process with the potential for malignant transformation (2). Although the overall mechanisms and even the exact prevalence are unknown, several factors are thought to be involved in the development of endometriosis. Generally speaking, retrograde menstruation remains the dominant theory for the development of pelvic endometriosis (3). Recently, genetic studies have reported that some specific genotypes were associated with endometriosis in selective populations, such as Brazil (4), Turkey (5), and Taiwan (6, 7); however, the exact genomic and proteomic factors that play a role in endometriosis are not very clear. Similar to carcinogenesis, genomic alterations may represent important events in the development of endometriosis. Tumor suppressor genes are known to play a role in the regulation of cell growth and prevention of carcinogenesis. Thus, altered tumor suppressor genes might be related with the development of endometriosis (8).

The p53 gene is a tumor suppressor gene whose function is partially mediated by transactivating the cyclin-dependent kinase inhibitor 1A (CDKN1A) promoter, to control the cell cycle and prevent tumor formation (9). It was found that premalignant lesions with mutant p53 protein overexpressed...
p21 (10), p21-immunopositive well-differentiated tumors with p53 missense mutations probably harbor a p21-dependent differentiation pathway activated through a p53-independent mechanism (11). There is discrepancy about this presentation of p53 polymorphisms in various tumor types. The p53 Arg72 homozygote is considered to be a risk factor in the development of cancer (12). In contrast, some investigators demonstrated no association between the different p53 polymorphisms and individual cancer development (13). Still other studies revealed a higher risk in individuals homozygous for p53 Pro72 (14, 15). High frequency of p53 locus deletion was observed in endometriosis specimens (16). p53 protein abnormalities and chromosomal aberrations may be involved in malignant transformation of ovarian endometriosis (17). In contrast, some investigators have demonstrated no expression of p53 in the endometriosis specimens (18-20).

The protein p21 (CDKN1A/WAF1/CIP1), encoded by the CDKN1A locus, is a cyclin-dependent kinase inhibitor which play a role in cell cycle regulation. The human CDKN1A gene contains three exons of 68, 450, and 1600 bp (21). In normal cells, p21 exists predominantly in quaternary complexes with cyclins, cyclin-dependent kinases (CDKs), and proliferating cell nuclear antigen (PCNA) to inhibit the activity of CDKs and control the G1 to S phase transition (22). The CDKN1A gene has a p53 transcriptional regulatory motif, and cells lacking functional p21 express very low levels of p21, suggesting that p53 regulates CDKN1A expression directly (23). p21 controls the differentiation of normal and transformed cells, and the involvement of p21 in terminal differentiation has been observed in several studies (24, 25). Differential regulation of p21 by p53 and retinoblastoma protein has been reported in cellular response to oxidative stress (26). In addition, several studies suggest a critical role for p21 in apoptosis (27).

In view of the central role of p21 in inducing growth arrest, terminal differentiation, or apoptosis, aberrant CDKN1A genomic and proteomic regulation may play a vital role in the pathogenesis of cancer. Alterations in p21 expression have been observed in specific types of human cancer, including ovarian, uterine, cervix, colorectal, hepatocellular, and head and neck carcinomas (28-30). As is well known, in response to DNA damage, p21 is a key mediator of the G1-S cell cycle arrest induced by tumor suppressor p53. It has been revealed that p21 also interacted with PCNA to cause both G1 and G2 cell cycle arrest in p53-deficient cells (24, 31, 32). Recently, a novel polymorphism of CDKN1A gene in codon 149 was found in an Indian population and was considered as a genetic susceptible marker of esophageal and oral cancer (10, 33). Soon after, bioinformatics analysis revealed that the so-called polymorphism of p21 codon 149 is not a susceptible site (34). Although the p21 genotype was found not to be associated with endometriosis, the limited population investigated and this being the only literature is not convincing (35). Thus in this study, we wished to investigate the association of combined genotypes of p53 and p21 with endometriosis.

Taken together, these effects reflect the complexity of the p53/p21 pathways of cell cycle regulation and differentiation in overall carcinogenesis. Mutations in either p53 or CDKN1A are detected in some tumor cells (9, 36), and polymorphisms of p53 codon 72, and CDKN1A codon 31 were found to be associated with many tumors (37-40).

Since p53 gene mutations are the most common cancer-related genetic alterations, being found in ~50% of human cancer cases (41), and p53 regulates p21 expression (23), we were interested to check the susceptible site in p53 and CDKN1A gene together in Taiwan endometriosis patients. Thus, the main goal of the study was to check the joint effect of genotypes in p53 tumor suppression gene codon 72, and CDKN1A gene codon 31, with endometriosis in a Taiwan population.

### Materials and Methods

#### Study population and sample collection.

We recruited 180 individuals diagnosed with endometriosis at the Outpatient Clinics of General Surgery at the Chung-Shan Medical University Hospital, Taichung, Taiwan, Republic of China. All patients voluntarily participated, completed a self-administered questionnaire and provided their peripheral blood. Three hundred and thirty non-endometriosis and healthy individuals as controls were selected by matching for age and gender, from people who voluntarily visited the health-screening clinic at the same hospital. A questionnaire administered to the volunteers included questions on alcohol consumption habit and smoking habits were evaluated and classified as categorical variables. Information on these factors was obtained more than twice a week for years. Our study was approved by the Institutional Review Board of Chung-Shan Medical University Hospital and written informed consent was obtained from all participants.

#### Genotyping assays.

Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan), and further processed according to a previous published paper (42). Briefly, for p53 codon 72, the primers 5'-

### Table 1. Allelic frequencies for p53 codon 72 and CDKN1A codon 31 polymorphisms in the endometriosis and control groups.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>p-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53 codon 72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele G (Arg)</td>
<td>167 (46.4)</td>
<td>383 (58.3)</td>
<td>1.00 (ref)</td>
<td></td>
</tr>
<tr>
<td>Allele C (Pro)</td>
<td>193 (53.6)</td>
<td>277 (42.0)</td>
<td>1.60 (1.23-2.07)</td>
<td>0.00036</td>
</tr>
<tr>
<td>p53 codon 31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele A (Arg)</td>
<td>171 (47.5)</td>
<td>334 (50.6)</td>
<td>1.00 (ref)</td>
<td></td>
</tr>
<tr>
<td>Allele C (Ser)</td>
<td>189 (52.5)</td>
<td>326 (49.4)</td>
<td>1.13 (0.88-1.46)</td>
<td>0.3431</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval. *Based on χ² test.
TCCCCCTTGCCGTCCCAA-3' and 5'-CGTGCAAGTCACA-3' were used, and for CDKN1A codon 31, the primers 5'-GTCAGAACCGGCTGGGGATG-3' and 5'-CTCCTCCCAACTCATCCCGG-3' were used. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 20 s, 58˚C for 20 s, and 72˚C for 20 s; and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with BstUI restriction enzyme for p53 codon 72, and with BlpI for CDKN1A codon 31, respectively.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, deviation of the genotypic frequencies of p53 codon 72 and CDKN1A codon 31 single nucleotide polymorphisms in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s χ² test or Fisher’s exact test (when the expected number in any cell was less than five) was used to compare the distribution of the p53 and CDKN1A genotypes between cases and controls. We estimated the endometriosis risk associated with the genotypes as odds ratio (ORs) and 95% confidence intervals (CIs) by using unconditional logistic regression with adjustment for age, smoking, alcohol consumption and betel quid (BQ) chewing habits. Data was recognized as significant when the statistical p-value was less than 0.05.

Results

The mean ages of the endometriosis patients and the controls were 31.3 (standard deviation SD=4.12) and 32.2 (SD=4.58) years, respectively. There were no differences in the age, body mass index (BMI), smoking and alcohol drinking status between the two groups (data not shown). The frequency of the alleles for p53 codon 72 and CDKN1A codon 31 in the endometriosis and control groups is shown in Table I. The Pro allele at p53 codon 72 was significantly associated with endometriosis risk (p=0.00036, OR=1.60, 95% CI=1.23-2.07). In contrast, neither Arg nor Ser at CDKN1A codon 31, was differently distributed between the endometriosis patients and control groups (p>0.05).

The frequency of the genotype of p53 codon 72 and CDKN1A codon 31 polymorphisms in the endometriosis and control groups is shown in Table II. These data indicate that there was an obvious association between carrying the C allele (72Pro) of p53 and endometriosis risk. As for these significant stratifications, after controlling for age, smoking, and alcohol drinking status, the adjusted OR was still significant (Table II). On the contrary, neither hetero- nor homozygotes of 31Ser of CDKN1A seemed to be risky genotypes for endometriosis (p>0.05) (Table II).

Since p53 and CDKN1A may be closely related to each other in a same pathway, the gene–gene interaction was also investigated. The result of analysis of the haplotype combinations of p53 codon 72 and CDKN1A codon 31 polymorphisms in the endometriosis and control groups is shown in Table III, and there was a significant difference between endometriosis patients and control groups (p>0.05).

Discussion

Cell proliferation and death are essential aspects in the understanding of carcinogenesis. Considerable evidence now links the activities of the p53 gene to regulation of the cell cycle and mutations in this gene are the most common

Table II. Association of p53 codon 72 and CDKN1A codon 31 polymorphisms with endometriosis risk.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>34 (18.9)</td>
<td>107 (32.4)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Arg/Pro</td>
<td>99 (55.0)</td>
<td>169 (51.2)</td>
<td>1.84 (1.17-2.92)b</td>
<td>1.79 (1.15-2.84)b</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>47 (26.1)</td>
<td>54 (16.4)</td>
<td>2.74 (1.58-4.74)b</td>
<td>2.76 (1.54-5.03)b</td>
</tr>
<tr>
<td>With Pro</td>
<td>146 (81.1)</td>
<td>223 (67.6)</td>
<td>2.06 (1.33-3.20)b</td>
<td>2.03 (1.29-3.23)b</td>
</tr>
<tr>
<td>With Arg</td>
<td>133 (73.9)</td>
<td>276 (83.6)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>47 (26.1)</td>
<td>54 (16.4)</td>
<td>1.80 (1.16-2.81)b</td>
<td>1.78 (1.14-2.83)b</td>
</tr>
<tr>
<td>CDKN1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>35 (19.4)</td>
<td>69 (20.9)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Aer/Ser</td>
<td>101 (56.1)</td>
<td>196 (59.4)</td>
<td>1.01 (0.63-1.63)</td>
<td>0.98 (0.64-1.70)</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>44 (24.4)</td>
<td>65 (19.7)</td>
<td>1.34 (0.76-2.33)</td>
<td>1.37 (0.69-2.35)</td>
</tr>
<tr>
<td>With Ser</td>
<td>145 (80.6)</td>
<td>261 (79.1)</td>
<td>1.10 (0.70-1.73)</td>
<td>1.01 (0.69-1.86)</td>
</tr>
<tr>
<td>With Arg</td>
<td>136 (75.6)</td>
<td>265 (80.3)</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>Ser/Ser</td>
<td>44 (24.4)</td>
<td>65 (19.7)</td>
<td>1.32 (0.85-2.04)</td>
<td>1.37 (0.79-2.07)</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval. aAdjusted for age and habits (smoking and alcohol drinking habits). b p<0.05.
Based on χ² chi-square test. bSignificant difference. cChi square = 172.6228, degree of freedom = 8.

Table III. Distribution of haplotype combinations of p53 codon 72 and CDKN1A codon 31 polymorphisms in the endometriosis and control groups.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>Crude OR (95% CI)</th>
<th>p-Valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>P53/CDKN1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG/AA</td>
<td>7 (3.9)</td>
<td>22 (6.7)</td>
<td>1.00 (ref)</td>
<td></td>
</tr>
<tr>
<td>GG/AC</td>
<td>19 (10.6)</td>
<td>64 (19.4)</td>
<td>1.07 (0.40-2.89)</td>
<td></td>
</tr>
<tr>
<td>GG/CC</td>
<td>8 (4.4)</td>
<td>21 (6.4)</td>
<td>0.84 (0.26-2.71)</td>
<td></td>
</tr>
<tr>
<td>GC/AA</td>
<td>19 (10.6)</td>
<td>36 (10.9)</td>
<td>0.60 (0.22-1.67)</td>
<td></td>
</tr>
<tr>
<td>GC/AC</td>
<td>56 (31.1)</td>
<td>100 (30.3)</td>
<td>0.57 (0.23-1.41)</td>
<td></td>
</tr>
<tr>
<td>GC/CC</td>
<td>24 (13.3)</td>
<td>33 (10.0)</td>
<td>0.44 (0.16-1.19)</td>
<td></td>
</tr>
<tr>
<td>CC/AA</td>
<td>9 (5.0)</td>
<td>11 (3.3)</td>
<td>0.39 (0.11-1.32)</td>
<td></td>
</tr>
<tr>
<td>CC/AC</td>
<td>26 (14.4)</td>
<td>32 (9.7)</td>
<td>0.39 (0.14-1.06)</td>
<td>&lt;0.0001b</td>
</tr>
<tr>
<td>CC/CC</td>
<td>12 (6.7)</td>
<td>11 (3.3)</td>
<td>0.29 (0.09-0.95)</td>
<td></td>
</tr>
</tbody>
</table>

aBased on χ² chi-square test. bSignificant difference. cChi square = 172.6228, degree of freedom = 8.

In conclusion, this study shows that p53 codon 72 polymorphism may be involved in the development of endometriosis.

Acknowledgements

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References