Contribution of DNA Repair Xeroderma Pigmentosum Group D Genotype to Gastric Cancer Risk in Taiwan

HONG-XUE JI1,2*, WEN-SHIN CHANG1,2,*, CHIA-WEN TSAI2†, JU-YU WANG3, NAI-KUEI HUANG4, AN-SHENG LEE5,6, MING-YI SHEN1,6, WEI-YU CHEN6,7, YAO-CHANG CHIANG8, TZU-CHING SHIH9, CHIN-MU HSU2 and DA-TIAN BAU1,2,10

Graduate Institutes of Clinical Medical Science and Basic Medical Science, and Department of Biomedical Imaging and Radiological Science, China Medical University, Taichung, Taiwan, R.O.C.; Terry Fox Cancer Research Laboratory, L5 Research Center, and Center for Drug Abuse and Addiction, China Medical University Hospital, Taichung, Taiwan, R.O.C.; Basic Medical Science, Department of Nursing, Hung-Kuang University, Taichung, Taiwan, R.O.C.; National Research Institute of Chinese Medicine, Taipei, Taiwan, R.O.C.; Department of Medicine, Mackay Medical College, New Taipei, Taiwan, R.O.C.; Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.

Abstract. Aim: It has been proposed that genetic variations of DNA repair genes confer susceptibility to cancer, and the DNA repair gene xeroderma pigmentosum group D (XPD), the caretaker of genome stability, is thought to play a major role in the nucleotide excision repair system. We investigated three genotypes of XPD, at promoter -114 (rs3810366), and codon 312 (rs1799793), 751 (rs13181), and their associated with gastric cancer susceptibility in a Taiwanese population. Materials and Methods: In the present study, 121 patients with gastric cancer and 363 gender- and age-matched healthy controls were recruited and genotyped for XPD by polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) methodology, and the association of XPD genotype with gastric cancer risk was investigated. Results: We found a significant difference in the distribution of A allele-bearing XPD codon 312 genotypes [odds ratio (OR)=1.64, 95% confidence interval (CI)=1.20-2.25, p=0.0019] and 1.87-fold (95% CI=1.14-2.95, p=0.0159) increased risk of gastric cancer compared to those with G/G. The risk for G/A and A/A genotypes had synergistic effects with alcohol drinking (OR=11.27, 95% CI=3.72-34.17, p=0.0001), cigarette smoking (OR=23.20, 95% CI=6.24-86.23, p=0.0001) and Helicobacter pylori infection (OR=5.38, 95% CI=2.76-10.52, p=0.0001) on gastric cancer susceptibility. Conclusion: Our findings suggest that the A allele of XPD codon 312 may contribute to gastric carcinogenesis and may be useful for early detection and prevention of gastric cancer.

Gastric cancer is the fourth most common type of cancer and the second most frequent cause of death of cancer worldwide (1). In 2008, it was estimated that a total of 989,600 new stomach cancer cases and 738,000 deaths occurred, accounting for 8% of the total cases and 10% of total deaths worldwide (2, 3). The precise mechanisms for gastric carcinogenesis remain unknown. In addition to known factors such as unhealthy diet, infectious agents (e.g. Helicobacter pylori) and pre-existing conditions (e.g. pernicious anemia, atrophic gastritis, and intestinal polyps) (4), inherited genetic variations may also play a role in determining individual susceptibility to gastric cancer but are largely uninvestigated (5-8). In recent years, several studies evaluating the associations of DNA repair genes with the risk of gastric cancer have been conducted and published (9-11). The rationale for these studies is that the responses of the cell to genetic injury and its ability to maintain genomic stability through a variety of DNA repair mechanisms are essential in preventing tumor initiation and progression.

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau, Chin-Mu Hsu and Tzu-Ching Shih, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 40447 Taiwan, R.O.C. Tel. +886 422052121 Ext. 7534, e-mail: artbau2@gmail.com; e12013@gmail.com; shih@mail.cmu.edu.tw

Key Words: Alcohol consumption, gastric cancer, genotype, polymorphism, smoking, XPD.

0250-7005/2015 $2.00+.40
Mutations and defects in the DNA repair system may be closely related to tumorigenesis (12).

From the cellular physiological viewpoint, the DNA repair system plays a vital role in maintaining the homeostasis of overall cellular functions. It keeps the genome from somatic and inherited mutations through the removals of all types of DNA adducts induced by endogenous and exogenous damage during daily life (13). Inherited functional polymorphisms or accumulated mutations of DNA repair genes may influence the capacity to repair damaged DNA and thus explain the etiology of familial and sporadic cancer, respectively (14). Since the single-nucleotide polymorphism (SNP) is the most frequent and subtle type of genetic variation in the human genome and has a great potential for application to association studies of complex disease (15), we investigated SNPs of the xeroderma pigmentosum group D (XPD, also known as ERCC2) gene in order to evaluate their contribution to gastric cancer development.

XPD gene, which is important in the nucleotide excision repair sub-pathway in the DNA repair system, is believed to be in charge of removing the helix-distorting base lesions produced by ultraviolet light and other carcinogenic agents (16). The protein it encodes participates in DNA unwinding during both nucleotide excision repair and transcription, which may be due to its capacities as single-strand DNA-dependent ATPase and DNA helicase (17, 18). It has been reported that mutations of the XPD gene diminish its helicase activity, resulting in defective nucleotide excision repair capacity and transcriptional activity, and in an abnormal response to apoptosis (19). The most widely investigated XPD polymorphisms in associations with cancer susceptibility comprise a non-synonymous A to C substitution in exon 23 causing a lysine (Lys) to glutamine (Gln) substitution in codon 751 (Lys751Gln, rs13181), and a non-synonymous G to A substitution in exon 10 leading to an aspartic acid (Asp) to asparagine (Asn) substitution in codon 312 (Asp312Asn, rs1799793), respectively (14). Since the single-nucleotide polymorphism (SNP) is the most frequent and subtle type of genetic variation in the human genome and has a great potential for application to association studies of complex disease (15), we investigated SNPs of the xeroderma pigmentosum group D (XPD, also known as ERCC2) gene in order to evaluate their contribution to gastric cancer development.

Accordingly, we aimed to explore whether the genotypes of XPD are associated with gastric cancer risk among Taiwanese. To test this hypothesis, we determined the genotypic frequency of three polymorphisms of the XPD gene at codon 312 (Asp312Asn, rs1799793), codon 751 (Lys751Gln, rs13181), and the promoter (rs3810366, also merged from rs17587357 and rs58794467) in a Taiwanese population, and analyzed their contribution to gastric cancer susceptibility and interactions with alcohol consumption, cigarette smoking, and Helicobacter pylori infection. To our knowledge, this is the first study to evaluate XPD genotypes in a Taiwanese population.

Materials and Methods

Study population and sample collection. One hundred and twenty-one patients diagnosed with gastric cancer were recruited at the outpatient clinics of general surgery between 2005-2007 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The mean age of the patients was 51.26 (SD=9.42) years. There were 56 females and 65 males. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. H. pylori status was determined by standard enzyme-linked immunosorbent assay, histological examination, and biopsied urease test. Positivity in any one of these tests was defined as presence of H. pylori infection. Matched by age and gender, 363 healthy people were selected from the Health Examination Cohort of the hospital as controls, and the same questionnaires were recorded. Our study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants with the help of the Tissue Bank.

Genotyping assays. Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to our previous publications (43-45). Briefly, for XPD codon 312, the primers 5’-GGGCCCTCTGCTGACTTGTCCC-3’ and 5’-GACGGGGAGGCCGAAAGGGACT-3’ were used, for XPD codon 751, the primers 5’-ACTTCATAAGACCTTCTAGC-3’ and 5’-GATTATGCGGGATCTTCCA-3’ were used, and for XPD promoter -114, the primers 5’-ATGAATATTCAGCGAGAGGC-3’ and 5’-CTGGGTTCGATCAATACTCAAT-3’ were used. The following cycling conditions were performed: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension at 72°C for 10 min. The PCR products were studied after digestion with Hpy99I, EarI, and Bme1580I, restriction enzymes for XPD codon 312 (cut from 250 bp A type into 188+62 bp G type), 751 (cut from 326 bp C type into 127+199 bp A type) and promoter -114 (cut from 303 bp G type into 101+202 bp C type), respectively.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of XPD SNP in the controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson’s chi-square test or Fisher’s exact test (when the expected number in any cell was less than five) was applied to compare the distribution of the XPD genotypes between case and control groups. Cancer risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data was recognized as significant when the statistical p-value was less than 0.05.
Results

The clinical characteristics of the gastric cancer patient group together with the control group are shown in Table I. The BMI was at the same level (p>0.05) in both control and gastric cancer groups. The percentage of alcohol consumers seemed to be higher in the patient group (32.2%) than the control group (23.1%), and the percentage of heavy drinkers in the gastric cancer group (9.9%) was more than twice than that in the control group (4.4%). For the cigarette smoking habit analysis, there were significant trends indicating that the patient group had a higher percentage of cigarette consumers, especially heavy smokers, than the control group (34.7% vs. 19.6%, and 10.7% vs. 1.7%, respectively). Regarding infection with Helicobacter pylori, 70.2% of the patients with gastric cancer were positive, much higher than 51.8% for the controls (p<0.05). To sum up, heavy consumption of alcohol and cigarettes, in addition to infection with Helicobacter pylori, were risk factors contributing to increased gastric cancer risk in Taiwanese.

The frequencies of the genotypes of XPD codon 312, codon 751 and promoter -114 polymorphisms in the gastric cancer and control groups are shown in Table II. Compared to the G/G genotype of codon 312 as the reference group, there was an obvious increased risk for the G/A and A/A groups (G/A: OR=1.83, 95% CI=1.14-2.95, p=0.0159; A/A: OR=1.87, 95% CI=1.04-3.34, p=0.0378). XPD codon 312 A allele carriers had a 1.84-fold risk for gastric cancer (Table II). Neither hetero- nor homozygotes of variant C allele of XPD codon 751, seemed to be risky genotypes for gastric cancer, as was also the case in the variant G allele of XPD promoter -114 polymorphic site (Table II).

The frequencies of the alleles for the XPD codon 312, codon 751 and promoter -114 polymorphisms between gastric cancer and control groups are presented in Table III. The distributions of all the three polymorphisms were in Hardy–Weinberg equilibrium and were similar between patients with gastric cancer and controls (data not shown). The variant A allele at XPD codon 312 was clearly significantly associated with increased gastric cancer risk.
In the present study, we investigated the association of XPD codon 312, XPD codon 751 and promoter -114 genotypes, with gastric cancer susceptibility in Taiwanese. The results demonstrated that the G/A and A/A genotypes of XPD codon 312 were associated with higher risk of developing gastric cancer in Taiwanese (Table II). To support this finding, the results of allelic frequency analysis also showed that the A allele of XPD codon 312 was associated with higher risk of gastric cancer development (Table III). To the best of our knowledge, this is the first epidemiological study based on molecular genetics to find a significant association between XPD genotype and the susceptibility to gastric cancer with the analysis of a gene–lifestyle interaction. Interestingly, the contribution of variant A allele to gastric cancer was more obvious than the single lifestyle factors, such as alcohol drinking, cigarette smoking, and the infection status of Helicobacter pylori infection in the development of gastric cancer.

**Table IV. Combined analysis of xeroderma pigmentosum group D (XPD) genotype and alcohol consumption lifestyle for gastric cancer risk.**

<table>
<thead>
<tr>
<th>XPD codon 312 allele</th>
<th>Alcohol consumption</th>
<th>Controls/ Cases</th>
<th>Odds ratio (95% CI)</th>
<th>p-Value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>No A carrier</td>
<td>No</td>
<td>155/33</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>No</td>
<td>124/49</td>
<td>1.86 (1.13-3.06)</td>
<td>0.0169</td>
</tr>
<tr>
<td>No A carrier</td>
<td>Yes</td>
<td>79/27</td>
<td>1.61 (0.90-2.86)</td>
<td>0.1315</td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>Yes</td>
<td>5/12</td>
<td>11.27 (3.72-34.17)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

CI, Confidence interval; ^a based on \(\chi^2\) test. Significant values are shown in bold.

**Table V. Combined analysis of xeroderma pigmentosum group D (XPD) genotype and cigarette consumption lifestyle for gastric cancer risk.**

<table>
<thead>
<tr>
<th>XPD codon 312 allele</th>
<th>Cigarette consumption</th>
<th>Controls/ Cases</th>
<th>Odds ratio (95% CI)^a</th>
<th>p-Value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>No A carrier</td>
<td>No</td>
<td>166/31</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>No</td>
<td>126/48</td>
<td>2.03 (1.23-3.39)</td>
<td>0.0074</td>
</tr>
<tr>
<td>No A carrier</td>
<td>Yes</td>
<td>68/29</td>
<td>2.28 (1.28-4.08)</td>
<td>0.0057</td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>Yes</td>
<td>3/13</td>
<td>23.20 (6.24-86.23)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

CI, Confidence interval; ^a based on \(\chi^2\) test. Significant values are shown in bold.

**Table VI. Combined analysis of xeroderma pigmentosum group D (XPD) genotype and Helicobacter pylori infection for gastric cancer risk.**

<table>
<thead>
<tr>
<th>XPD codon 312 allele</th>
<th>Helicobacter pylori infection</th>
<th>Controls/ Cases</th>
<th>Odds ratio (95% CI)</th>
<th>p-Value^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>No A carrier</td>
<td>No</td>
<td>94/16</td>
<td>1.0 (reference)</td>
<td></td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>No</td>
<td>81/20</td>
<td>1.45 (0.71-2.94)</td>
<td>0.3615</td>
</tr>
<tr>
<td>No A carrier</td>
<td>Yes</td>
<td>140/44</td>
<td>1.85 (0.98-3.46)</td>
<td>0.0721</td>
</tr>
<tr>
<td>Yes A carrier</td>
<td>Yes</td>
<td>48/41</td>
<td>5.38 (2.76-10.52)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

CI, Confidence interval; ^a based on \(\chi^2\) test. Significant values are shown in bold.

Discussion

In the present study, we investigated the association of XPD codon 312, XPD codon 751 and promoter -114 genotypes, with gastric cancer susceptibility in Taiwanese. The results demonstrated that the G/A and A/A genotypes of XPD codon 312 were associated with higher risk of developing gastric cancer in Taiwanese (Table II). To support this finding, the results of allelic frequency analysis also showed that the A allele of XPD codon 312 was associated with higher risk of gastric cancer development (Table III). To the best of our knowledge, this is the first epidemiological study based on molecular genetics to find the interaction of genotype of XPD codon 312 and alcohol consumption for the risk of gastric cancer is presented in Table IV. Among non-alcohol drinkers, the variant A allele significantly increased the risk of gastric cancer (OR=1.64, 95% CI=1.20-2.25, \(p=0.0019\)). In contrast, the variant C allele at XPD codon 751, or the variant G allele at XPD promoter -114, were not differentially distributed in the patient and control groups \((p>0.05)\) (Table III).

The interaction of genotype of XPD codon 312 and alcohol consumption for the risk of gastric cancer is presented in Table IV. Among non-alcohol drinkers, the variant A allele significantly increased the risk of gastric cancer (OR=1.86, 95% CI=1.13-3.06, \(p=0.0169\)). The contribution of alcohol consumption behavior to gastric cancer risk was borderline among those without an A allele at XPD codon 312 (OR=1.61, 95% CI=0.90-2.86, \(p=0.1315\)), while it synergistically enhanced the effects of A allele of XPD codon 312 on increasing gastric cancer risk (OR=11.27, 95% CI=3.72-34.17, \(p=0.0001\)) (Table IV).

The interaction of genotype of XPD codon 312 and cigarette consumption for the risk of gastric cancer is presented in Table V. Among non-cigarette smokers, the variant A allele significantly increased the risk of gastric cancer (OR=2.03, 95% CI=1.23-3.39, \(p=0.0074\)). The contribution of cigarette consumption to gastric cancer risk was 2.28-fold for those without (OR=2.28, 95% CI=1.28-4.08, \(p=0.0057\)) and 23.20-fold for those with A allele at XPD codon 312 (OR=23.20, 95% CI=6.24-86.23, \(p=0.0001\)) (Table V).

Among people not infected with Helicobacter pylori, the carriage of XPD allele A at codon 312 was not associated with an increased risk of gastric cancer (OR=1.45, 95% CI=0.71-2.94, \(p=0.3615\)). Helicobacter pylori infection was of borderline significance for its association with an increased risk of gastric cancer among those without variant A allele of XPD codon 312 (OR=1.85, 95% CI=0.98-3.46, \(p=0.0721\)). Helicobacter pylori-infected individuals who were carriers of XPD allele A at codon 312 exhibited an increased risk of gastric cancer (OR=5.38, 95% CI=2.76-10.52, \(p=0.0001\)) (Table VI). The results in Tables IV, V and VI indicate a synergistic interaction between XPD allele A at codon 312 and alcohol consumption, cigarette smoking and Helicobacter pylori infection in the development of gastric cancer.
three common \textit{XPD} polymorphisms, codon 156, codon 312 and codon 751, with gastric cancer risk among different ethnicities, but with conflicting and inclusive results (33, 35-42, 47). To date, there is only one article reporting that genotype at \textit{XPD} codon 156 was associated with gastric cancer risk in China (40), and no positive association was found in our study (data not shown). Our positive findings for \textit{XPD} codon 312 are consistent with previous investigations reporting that the A allele of \textit{XPD} codon 312 is a risky genetic factor (33, 35, 47).

From Table I, we observed that alcohol consumers were at higher risk of gastric cancer \((p=0.0538)\), especially those who were heavy drinkers \((p=0.0049)\). Specifically, we note that non-alcohol consumers with the risky A allele at \textit{XPD} codon 312 were at 1.86-fold increased risk (95\% CI=1.13-3.06, \(p=0.0169\)) of developing gastric cancer, and the risk was dramatically elevated to 11.27-fold for those alcohol consumers with the risky A allele at \textit{XPD} codon 312 (95\% CI=3.72-34.17, \(p=0.0001\)) (Table IV). The limited sample size of patients and detailed records of their cigarette and alcohol habits restricted our further analysis accordingly, for instance, to investigate the correlation of alcohol intake and gastric cancer risk. Given that different drinking habits, types of alcoholic beverages consumed and genetic background of genes for alcohol metabolism among Eastern and Western populations, the epidemiological findings for the role of alcohol consumption in the etiology of gastric cancer remain even more controversial (48, 49). In Taiwan, people are used to drinking socially without considering themselves as alcohol consumers, or monitoring their alcohol intake. In 2007, the International Agency for Research on Cancer (IARC) concluded that alcoholic beverages are causally related to cancer of the oral cavity, pharynx, larynx, esophagus, liver, colorectum, and female breast. However, the IARC has not determined the contribution of alcohol consumption to gastric cancer. The issue of alcohol consumption as a risk factor for gastric cancer needs further investigation, and it is believed that the association may be population-dependent.

Some studies have provided evidence for increased risk of gastric cancer among cigarette smokers (50-55), while some others have not (56, 57). In the present study, we found that cigarette smoking may indeed contribute to an increased risk of gastric cancer \((p=0.0012)\), especially in heavy smokers \((p=0.0001)\) (Table I). Similarly, we found that cigarette consumption, or carrying the risky A allele at \textit{XPD} codon 312 led to 2.03- (95\% CI=1.23-3.39, \(p=0.0074\)) and 2.28-fold (95\% CI=1.28-4.08, \(p=0.0057\)) increased risk of developing gastric cancer, and the risk was dramatically elevated to 23.2-fold for cigarette consumers with the risky A allele at \textit{XPD} codon 312 (95\% CI=6.24-86.23, \(p=0.0001\)) (Table V). In 2012, Smyth and colleagues investigated the contribution of tobacco usage history to 5-year survival status, finding that smoking was a risk factor of gastric cancer and was associated with worse 5-year survival (58). For those who smoked fewer than 20 pack-years (defined as light smokers) or 20 or more pack-years (defined as heavy smokers), their gastric cancer disease-specific, 5-year disease-free and overall survival rates were less than those for the non-smokers (58). To sum up, cigarette smoking may not only contribute to individual gastric cancer risk, but also to death after undergoing surgical resection.

Regarding \textit{Helicobacter pylori} infection, Table I shows that 51.8\% of the controls were infected, which was significantly lower compared to 70.2\% of the gastric cancer patients \((p=0.0005)\) (Table I). The stratified analysis showed that among those without the risky A allele at \textit{XPD} codon 312, \textit{Helicobacter pylori} infection added a non-significant 1.85-fold risk of gastric cancer (95\% CI=0.98-3.46, \(p=0.0721\)) (Table VI). \textit{Helicobacter pylori} infection synergistically increases gastric cancer risk for those with the risky A allele at \textit{XPD} codon 312 (OR=5.38, 95\% CI=2.76-10.52, \(p=0.0001\)) (Table VI). Our results show that although \textit{Helicobacter pylori} infection has been documented as a risk factor for gastric cancer, only 1-2\% of \textit{Helicobacter pylori}-infected patients develop gastric cancer (5, 59).

These results suggest that genetic variants of \textit{XPD} at codon 312 may influence the DNA repair capacity and play a critical role in gastric cancer etiology. One possible mechanism for this is that those with risky genotypes, such as carriers of the A allele of \textit{XPD}, may have a lower capacity in their excision repair of DNA damage after cigarette smoking and alcohol consumption. As time goes by, people with these habits and the risky A allele at \textit{XPD} codon 312 in their genome might more easily accumulate genetic defects in their genome, which may lead to gastric carcinogenesis.

In conclusion, our findings suggest that the A allele of \textit{XPD} codon 312 is associated with higher risk for gastric cancer, and the A allele of \textit{XPD} is a useful marker combined with alcohol drinking, cigarette smoking, and \textit{Helicobacter pylori} infection, for individualized early detection, prevention and anticancer intervention.

\textbf{Acknowledgements}

This study was supported by research grants from the Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW104-TDU-B-212-113002). The Authors appreciate all the participants who contributed their samples and all the doctors, nurses, and colleagues at the Tissue Bank for their efforts in the collection of samples and questionnaires. The authors declare no conflict of interest.
References


Received May 7, 2015
Revised June 15, 2015
Accepted June 17, 2015