The XRCC1 399Gln Polymorphism and the Frequency of p53 Mutations in Taiwanese Oral Squamous Cell Carcinomas

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Abstract
DNA repair gene polymorphisms have been implicated as susceptibility factors in cancer development. It is possible that DNA repair polymorphisms may also influence the risk of gene mutation. The 399Gln polymorphism in the DNA repair gene XRCCI has been indicated to have a contributive role in DNA adduct formation, sister chromatid exchange, and an increased risk of cancer development. Two hundred thirty-seven male oral squamous cell carcinomas (OSCCs) were included in a study to investigate the role of the XRCCI 1947Trp, 280His, and 399Gln polymorphisms on p53 gene mutation. PCR-single-strand conformation polymorphism and DNA sequencing were used to analyze the conserved regions of the p53 gene (exons 5–9). The XRCCI genotype was determined by PCR-RFLP. Nineteen (8.02%) of the 237 OSCCs had a Gln/Gln genotype. One hundred sixty-nine (70.8%) of the 237 OSCCs showed p53 gene mutations at exons 5–9. The OSCC patients with a Gln/Gln genotype exhibited a significantly higher frequency of p53 mutation than those with an Arg/Gln and an Arg/Arg genotype. After adjustment for age, cigarette smoking, areca quid chewing, and alcohol drinking, the Gln/Gln genotype still showed an independent association with the frequency of p53 mutation (odd ratio, 4.50; 95% confidence interval, 1.52–13.36). The findings support the hypothesis that XRCCI Arg399Gln amino acid change may alter the phenotype of the XRCCI protein, resulting in a DNA repair deficiency. This study also suggests an important role for the XRCCI 399Gln polymorphism in p53 gene mutation in Taiwanese OSCCs.

Introduction
Tobacco and alcohol are well-established risk factors for oral cancer. A dose relationship between the consumption of tobacco or alcohol or both and oral cancer has been demonstrated in the Western countries. On the basis of epidemiological studies in India, a working group of the IARC concluded that there was adequate evidence for an association between chewing AQ1 together with tobacco use (chewing or smoking) and oral cancer (1). In Taiwan, ~80% of all oral cancer patients are associated with the AQ chewing habit (2). In addition, most Taiwanese AQ chewers are also smokers and alcohol drinkers. AQ is a combination of areca nut, lime, betel leaf, and tobacco. The composition of the AQ varies in different geographical locations. In Taiwan, tobacco is not included in the preparation of AQ. As an alternative, Piper betle, which is not used elsewhere except Papua New Guinea, inflorescence is added to AQ, and it contains a high concentration of safrole (3). Saffrole-DNA adducts have been detected in 77% (23 of 30) of the OSCC tissues in a study of Taiwanese oral cancer patients with an AQ chewing history (4). Tobacco smoke contains an array of potent carcinogens including polycyclic aromatic hydrocarbons, aromatic amines, and tobacco-specific nitrosamines. These carcinogens can be metabolized in vivo and form adducts with DNA.

Previous studies have shown that certain carcinogens may induce a "fingerprint"-like pattern of mutations at the p53 gene, in terms of both mutation type and codon specificity (5). The most striking example is the p53 mutational spectrum found in hepatocellular carcinoma from either Qidong, People’s Republic of China (6, 7) or Southern Africa (8, 9). A G:C to T:A transversion at the third base position of codon 249 of the p53 gene is strongly associated with dietary aflatoxin intake and hepatitis B virus infection. This type of mutation is consistent with mutations caused in vitro by aflatoxin B1 (10, 11). Hence, the mutation spectrum associated with a human cancer can provide clues as to the nature of the incriminating carcinogens and the mutagenic mechanisms responsible for the genetic lesions that drive human carcinogenesis. Recently, we reported an important contributive role for tobacco carcinogens in p53 mutation for a series of Taiwanese patients with OSCCs (12). In addition, alcohol significantly increased the frequency of p53 mutations (OR, 2.24; 95% CI, 1.21–4.15) after adjustment for...
cigarette smoking and AQ chewing. Garro et al. (13) and Mufti (14) have demonstrated that chronic alcohol consumption interferes with the repair of alkylated DNA. Therefore, it is possible that alcohol interferes with the repair of DNA damaged by cigarette smoking, and this increases the possibility of p53 mutations in Taiwanese OSCCs.

DNA repair enzymes monitor DNA to correct damaged nucleotide residues generated by replication or exposure to carcinogens and cytotoxic compounds. Mutations are early events in carcinogenesis (15), and defective DNA repair is a risk factor for many types of cancer (16–19). Although DNA repair deficiencies often arise from mutations in genes that result in a functional loss of the DNA repair protein, DNA polymorphisms may alter the structure of the DNA repair enzyme and modulate repair capability. Mutations and polymorphisms have been identified in many of the genes coding for DNA repair enzymes. Among these, XRCCI polymorphisms have been suggested as playing a role in the etiology of smoking-related squamous cell carcinoma of the head and neck (20).

Shen et al. (21) reported five polymorphisms in the XRCCI gene, three of which occur at conserved sequences and resulted in amino acid substitutions. These three coding polymorphisms were detected at codons 194 (Arg-Trp), 280 (Arg-His), and 399 (Arg-Gln). Among these three polymorphisms, Lunn et al. (22) reported that the 399 Arg to Gln amino acid change was associated with high levels of AFB1-DNA adducts in a group of Taiwanese maternity subjects and with increased glycolipin A NN mutations in a mixed population of smokers and nonsmokers residing in North Carolina. Recently, Abdel-Rahman and El-Zein (23) found that, although the 194Trp polymorphism did not seem to reduce DNA repair efficiency, the 399Gln polymorphism seemed to be associated with the reduced repair of NNK-induced genetic damage in cultured human lymphocytes. In this study, we test whether the XRCCI 194Trp, 280His, or 399Gln polymorphisms are associated with an increased frequency of p53 mutations in Taiwanese OSCCs.

Material and Methods

Study Subjects. Two hundred sixty-four oral cancer patients were enrolled from Chang Gung Memorial Hospital, Lin-Kuo, between March 1999 and September 2000. All of the cases were histologically confirmed. Female patients (n = 17) were excluded from this study because of an insufficient number. Those who were diagnosed as non-squamous cell carcinoma (n = 10) were also excluded. Thus, a total of 237 male OSCC patients, including 187 patients previously studied (12), were included for the present analysis. After informed consent was obtained, 10 ml of blood were drawn into heparinized tubes (Vacutainer). The whole blood was separated into plasma, buffy coat cells, and red blood cells by centrifugation within 24 h of obtaining the blood, then stored in a −70°C freezer. Genomic DNA for genotyping was extracted and purified from theuffy coat cells as described previously (24).

Surgically removed samples were sent to the Department of Pathology, Chang Gung Medical Center, for examination and were scored according to the recommendations for the reporting of specimens containing oral cavity and oropharynx neoplasms by the Associations of Directors of Anatomic and Surgical Pathology (25). Histology diagnosis was defined as squamous cell carcinoma, verrucous carcinoma, cylindric cell carcinoma, adenoid cystic carcinoma, mucoepidermoid carcinoma, and adenocarcinoma.

Tobacco, AQ, and Alcohol Use. All of the patients were interviewed uniformly before surgery by a well-trained interviewer. Study participants were asked whether they had ever smoked cigarettes, chewed AQ, or drunk alcohol on a regular basis (at least once a week). Those who responded “yes” to these questions were classified as tobacco, AQ, and alcohol users.

Mutation Analysis of the p53 Gene. Mutation analysis of the p53 gene was performed as described previously (12). Briefly, SSCP analysis was used to analyze tumor samples for mutations within exons 5–9 of the p53 gene, which are the regions most frequently affected by mutations in human tumors. Cases displaying an altered electrophoretic mobility were reamplified in another reaction and were analyzed by direct sequencing of both strands to confirm and characterize the nature of the mutation.

Genotyping. XRCCI genotypes were detected using a PCR-RFLP technique as described by Lunn et al. (22). For codon 194 and 399, PCR was performed in a 25-µl mixture containing 100 ng of genomic DNA, 1.5 mM MgCl2, 300 µM each dNTPs, 1 unit of Taq, and 100 ng of each primer in 1 × PCR buffer using the running conditions: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The PCR products were digested overnight with MspI at 37°C, electrophoresed in 6% polyacrylamide gels, stained with ethidium bromide, and photographed under UV light. For codon 280 polymorphism, a separate PCR was performed in a 25-µl mixture containing 100 ng of genomic DNA, 1.5 mM MgCl2, 200 µM each dNTPs, 2 units of Taq, and 100 ng of each primer in 1 × PCR buffer using the running conditions: 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min. The PCR products were digested overnight with RsaI at 37°C, electrophoresed in 6% polyacrylamide gels, stained with ethidium bromide, and photographed under UV light.

Statistical Analysis. Statistical Analysis System (SAS) version 8.1 and EGRET were used for the statistical analysis. The association between XRCCI genotype and cigarette smoking, alcohol drinking, AQ chewing, and the frequency of p53 mutation was examined by the χ2 test. Logistic regression with an adjustment for age, cigarette smoking, alcohol drinking, and AQ chewing was used to estimate the OR and the 95% CI for the XRCCI genotype. The interactions between XRCCI 399 genotype and cigarette smoking, alcohol drinking, or AQ chewing on the frequency of p53 mutation were also tested in the multiple logistic regression model.

Results

Two hundred thirty-seven consecutive patients with a diagnosis of OSCC were enrolled in the study. The demographic data of the patients is shown in Table 1. The most common primary sites were the buccal and the tongue. Ninety % (210 of 233) of the patients had smoked at some time, 57.26% (134 of 234) were users of alcohol at some time, and 81.20% (190 of 234) had chewed AQ at some time.

The frequency of the 194Trp, 280His, and 399Gln allele was 0.30, 0.11, and 0.28, respectively. The frequency of the Trp/Trp, Trp/Arg, and Arg/Arg genotypes for codon 194 was 9.91, 40.09, and 50.00%, respectively. The frequency of the His/His, His/Arg, and Arg/Arg genotypes for codon 280 was 1.30, 19.13, and 79.57%, respectively. The frequency of the Gln/Gln, Gln/Arg, and Arg/Arg of codon 399 was 8.02, 39.66, and 52.32%, respectively. All of the distributions were in Hardy-Weinberg equilibrium. After stratifying for smoking, alcohol drinking, and AQ chewing, no differences in genotype frequencies were noted between subgroups of patients (data not
The frequency of p53

One hundred four (43.88%) of the 237 OSCCs showed p53 mutations. However, alcohol drinkers had a significantly higher frequency of p53 mutations (OR, 1.60; 95% CI, 1.45–12.66) than those with the 399 Arg/Arg genotype. The adjusted OR of the 399 Gln/Gln genotype, when more subjects are recruited, was 3.98 (95% CI, 1.39–11.45). After adjustment for age, cigarette smoking, alcohol drinking, and AQ chewing, the 399 Gln/Gln genotype still showed an independent association with the frequency of p53 mutations (OR, 5.03; 95% CI, 1.60–15.83). In addition, the most prevalent types of p53 gene mutations found in Taiwanese OSCCs were G:C to A:T transitions, and G:C to T:A transversions. G:C to T:A transitions are the most common mutations observed in lung adenocarcinoma in rodents treated with NNK (26, 27) and in hamster buccal pouch carcinomas induced by N-methyl-N-benzyl-nitrosamine, a potent alkylating carcinogen that is similar to tobacco nitrosamine (28). G:C to T:A transversions are attributed to NNK in experimental animal models (29). Studies have shown that NNK increases the levels of 8-hydroxydeoxyguanosine (8-OHdG) in DNA (30, 31). 8-OHdG is removed from DNA by the base excision repair pathway (32). Furthermore, evidence from the literature also indicates that NNK-induced methylated and pyridyloxobutylated DNA adducts, in addition to being repaired by the nucleotide excision repair pathway, are also repaired by base excision repair (30). XRCC1 plays an important role in the base excision repair pathway, and interacts with DNA polymerase β, PARP, and DNA ligase III. It also has a BRCT domain, which is characteristic of proteins involved in cycle checkpoint functions, and this domain can be responsive to DNA damage (33, 34). Thus, XRCC1 enzyme may play a role in the carcinogenesis pathway of Taiwanese oral cancer.

This study demonstrated a significant association between the XRCC1 399Gln/Gln genotype and the frequency of p53 gene mutations in Taiwanese OSCCs. This finding suggests that polymorphism at XRCC1 codon 399 plays a role relative to p53 gene mutation in chemical carcinogen-associated OSCCs. Lunn et al. (22) reported that XRCC1 codon 399 polymorphism was associated with higher levels of both AFBI-DNA adducts and glycophorin A variants in a normal population. Abdel-Rahman and El-Zein (23) found that the 399Gln polymorphism appeared to be associated with the reduced repair of NNK-induced genetic damage in cultured human lymphocytes. Taken

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>49.30 ± 11.03</th>
<th>28–78</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of primary tumor, n (%)</td>
<td>225 (94.94)</td>
<td>6 (2.53)</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>74 (31.22)</td>
<td>72 (50.6)</td>
</tr>
<tr>
<td>Lip</td>
<td>82 (34.60)</td>
<td>28 (11.81)</td>
</tr>
<tr>
<td>Glossa</td>
<td>9 (3.80)</td>
<td>14 (5.91)</td>
</tr>
<tr>
<td>Retromolar trigone</td>
<td>1/3 (33.33)</td>
<td>17/44 (38.64)</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>53/111 (47.75)</td>
<td>82/183 (44.81)</td>
</tr>
<tr>
<td>Hard palate</td>
<td>20/90 (22.22)</td>
<td>1/3 (33.33)</td>
</tr>
<tr>
<td>Buccal mucosa</td>
<td>15/78 (19.58)</td>
<td>5/25 (20.00)</td>
</tr>
<tr>
<td>Tongue</td>
<td>28/150 (18.66)</td>
<td>1/3 (33.33)</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>4/21 (19.05)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Clinical stage, n (%)</td>
<td>237</td>
<td>100</td>
</tr>
<tr>
<td>Stage I</td>
<td>27 (11.39)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Stage II</td>
<td>60 (25.32)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Stage III</td>
<td>37 (15.61)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>113 (47.68)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Cigarette smoker at some time, n (%)</td>
<td>210 (90.13)</td>
<td>134 (57.26)</td>
</tr>
<tr>
<td>Alcohol drinker at some time, n (%)</td>
<td>190 (81.20)</td>
<td>190 (81.20)</td>
</tr>
<tr>
<td>AQ chewer at some time, n (%)</td>
<td>190 (81.20)</td>
<td>190 (81.20)</td>
</tr>
</tbody>
</table>

Discussion

Recently, we reported that tobacco carcinogens play an important contributory role with respect to the p53 mutation in Taiwanese OSCCs (12). In addition, the most prevalent types of p53 gene mutations found in Taiwanese OSCCs were G:C to A:T transitions, and G:C to T:A transversions. G:C to T:A transitions are the most common mutations observed in lung adenocarcinoma in rodents treated with NNK (26, 27) and in hamster buccal pouch carcinomas induced by N-methyl-N-benzyl-nitrosamine, a potent alkylating carcinogen that is similar to tobacco nitrosamine (28). G:C to T:A transversions are attributed to NNK in experimental animal models (29). Studies have shown that NNK increases the levels of 8-hydroxydeoxyguanosine (8-OHdG) in DNA (30, 31). 8-OHdG is removed from DNA by the base excision repair pathway (32). Furthermore, evidence from the literature also indicates that NNK-induced methylated and pyridyloxobutylated DNA adducts, in addition to being repaired by the nucleotide excision repair pathway, are also repaired by base excision repair (30). XRCC1 plays an important role in the base excision repair pathway, and interacts with DNA polymerase β, PARP, and DNA ligase III. It also has a BRCT domain, which is characteristic of proteins involved in cycle checkpoint functions, and this domain can be responsive to DNA damage (33, 34). Thus, XRCC1 enzyme may play a role in the carcinogenesis pathway of Taiwanese oral cancer.

This study demonstrated a significant association between the XRCC1 399Gln/Gln genotype and the frequency of p53 gene mutations in Taiwanese OSCCs. This finding suggests that polymorphism at XRCC1 codon 399 plays a role relative to p53 gene mutation in chemical carcinogen-associated OSCCs. Lunn et al. (22) reported that XRCC1 codon 399 polymorphism was associated with higher levels of both AFBI-DNA adducts and glycophorin A variants in a normal population. Abdel-Rahman and El-Zein (23) found that the 399Gln polymorphism appeared to be associated with the reduced repair of NNK-induced genetic damage in cultured human lymphocytes. Taken

### Table 2

<table>
<thead>
<tr>
<th>XRCC1 genotype</th>
<th>Mutations detected/tumors tested (%)</th>
<th>OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Codon 194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trp/Trp</td>
<td>9/22 (40.91)</td>
<td>0.76 (0.30–1.92)</td>
<td>0.74 (0.28–1.95)</td>
</tr>
<tr>
<td>Trp/Arg</td>
<td>38/89 (42.70)</td>
<td>0.82 (0.47–1.43)</td>
<td>0.82 (0.46–1.46)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>53/111 (47.75)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Codon 280</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>His/His</td>
<td>1/3 (33.33)</td>
<td>0.62 (0.06–6.91)</td>
<td>0.49 (0.04–5.71)</td>
</tr>
<tr>
<td>His/Arg</td>
<td>17/44 (38.64)</td>
<td>0.78 (0.40–1.52)</td>
<td>0.75 (0.37–1.50)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>82/183 (44.81)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Codon 399</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>14/19 (73.68)</td>
<td>4.29 (1.45–12.66)</td>
<td>5.03 (1.60–15.83)</td>
</tr>
<tr>
<td>Gln/Arg</td>
<td>41/94 (43.62)</td>
<td>1.18 (0.69–2.04)</td>
<td>1.08 (0.61–1.89)</td>
</tr>
<tr>
<td>Arg/Arg</td>
<td>49/124 (39.52)</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*Adjusted for age, cigarette smoking, alcohol drinking, and AQ chewing.
together, these findings provide evidence that the 399Gln polymorphism of the XRCC1 gene is associated with reduced DNA repair efficiency. This hypothesis is biologically plausible. In theory, amino acid changes at conserved sites may alter enzyme function. The Arg399Gln polymorphism occurs in a region of the XRCC1 gene that contains biologically important domains (the PARP binding and the BRCT domain), and these domains have homology with other DNA repair-related genes (34).

Our previous study demonstrated that alcohol has a significant association with the frequency of p53 mutations (OR, 2.24; 95% CI, 1.21–4.15) after adjustment for cigarette smoking and AQ chewing (12). It has been suggested that alcohol may have an effect on DNA repair mechanisms. Garro et al. (13) and Mufti (14) have demonstrated that chronic alcohol consumption interferes with the repair of alkylated DNA. In the present study, we found that alcohol drinkers have a higher frequency of p53 mutation irrespective of their XRCC1 codon 399 genotype (Table 3). Furthermore, alcohol has a significant association with the frequency of p53 mutations (OR, 2.11; 95% CI, 1.21–3.68) after adjustment for age, cigarette smoking, AQ chewing, and XRCC1 399 genotype in the present series of OSCCs. This finding supports the hypothesis that alcohol may have a significant real inhibitory effect on the DNA repair mechanisms.

Lunn et al. (22) found that individuals carrying a 194Trp allele were slightly more common in the nondetectable AFB1-DNA adduct group. Furthermore, Sturgis et al. (20) demonstrated that the 194 Arg/Arg genotype was a significant risk factor specifically for cancers of the oral cavity and pharynx (adjusted OR, 2.46; 95% CI, 1.22–4.97). However, Abdel-Rahman and El-Zein (23) found that there was no significant difference in NNK-induced sister chromatid exchange between cells with the codon 194 Arg/Arg genotype and cells with the codon 194 Arg/Trp genotype at all concentrations of NNK tested. Our present study did not observe a significant association of 194Trp with the frequency of p53 mutations. But it is interesting to note that of the 19 individuals with 399Gln/Gln genotype in this series of OSCC patients, all were also of the 194Arg/Arg genotype; and of the 111 individuals carrying 194Trp alleles, all carried the 399Arg allele. Therefore, this suggests that, in the future, a study of the association between the haplotype for this gene and DNA adduct formation, sister chromatid exchange, and risk of cancer development would be useful.

This study is limited because it analyzed only p53 mutations within exons 5–9. Soussi and Beroud (35) analyzed 158 studies that screened the entire p53 gene and found that 13.6% of mutations were located outside exons 5–8, with a significant number of mutations in exons 4, 10, and, to a lesser extent, 9. Although the frequency of p53 mutations in our series of OSCCs may be biased, this should not affect our findings, even if the XRCC1 399Gln/Gln genotype is not associated with the p53 mutations outside exons 5–9.

In conclusion, after adjustment for smoking, AQ chewing, and alcohol drinking, the XRCC1 399 Gln/Gln genotype still showed an independent association with the frequency of p53 mutations (OR, 4.50; 95% CI, 1.52–13.36). The findings support the hypothesis that XRCC1 Arg399Gln amino acid change may alter the phenotype of the XRCC1 protein, resulting in deficient DNA repair. Our study also suggests an important role for the XRCC1 399Gln polymorphism on p53 gene mutation in Taiwanese OSCCs.

References


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