# The *XRCC1* 399*Gln* Polymorphism and the Frequency of *p53* Mutations in Taiwanese Oral Squamous Cell Carcinomas[1]

**Ling-Ling Hsieh,[2] Huei-Tzu Chien, I-How Chen, Chun-Ta Liao, Hung-Ming Wang, Shih-Ming Jung, Pei-Feng Wang, Joseph Tung-Chieh Chang, Min-Chi Chen, and Ann-Joy Cheng**

Departments of Public Health [L-L. H., H-T. C., P-F. W., M-C. C.] and Otolaryngology Oncology, Head and Neck Surgery [I-H. C., C-T. L.], Division of Hematology/Oncology Department of Internal Medicine [H-M. W.], and Department of Medical Technology [A-J. C.], Chang Gung University, Tao-Yuan 333, Taiwan; Departments of Pathology [S-M. J.], and Radiation Oncology [J. T-C. C.], Chang Gung Memorial Hospital, Tao-Yuan 333, Taiwan; and Taipei Chang Gung Memorial Hospital Head and Neck Oncology Group, Tao-Yuan, 333 Taiwan [L-L. H., I-H. C., C-T. L., H-M. W., S-M. J., J. T-C. C., M-C. C., A-J. C.].

## 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 399*Gln* polymorphism in the DNA repair gene *XRCC1* 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 *XRCC1* 194*Trp*, 280*His*, and 399*Gln* 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 *XRCC1* genotype was determined by PCR-RFLP. Nineteen (8.02%) of the 237 OSCCs had a *Gln/Gln* genotype. One hundred six (43.88%) 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 *XRCC1 Arg*399*Gln* amino acid change may alter the phenotype of the *XRCC1* protein, resulting in a DNA repair deficiency. This study also suggests an important role for the *XRCC1* 399*Gln* 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 AQ[3] 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, betle 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). Safrole-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

Received 5/28/02; revised 1/16/03; accepted 1/31/03.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported by Grants NSC 89-2314-B182-096 and NSC 90-2320-B182-055 from the National Science Council and by Grants DOH88-HR-802, NHRI-GT-EX89P802P, NHRI-EX90-8802PP, and NHRI-CN-IN-9005P from the National Health Research Institute, Department of Health, The Executive Yuan, Republic of China.
[2] To whom requests for reprints should be addressed, at Department of Public Health, Chang Gung University, 259 Wen-Hwa 1 Road, Kwei-San, Tao-Yuan 33332, Taiwan, Republic of China.

[3] The abbreviations used are: AQ, areca quid; OSCC, oral squamous cell carcinoma; OR, odds ratio; CI, confidence interval; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PARP, poly(ADP-ribose) polymerase; AFB1-DNA, aflatoxin B1-DNA; SSCP, single-stranded conformation polymorphism; BRCT, BRCA1 C-terminal.

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, *XRCC1* 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 *XRCC1* 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 glycophorin 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 194*Trp* polymorphism did not seem to reduce DNA repair efficiency, the 399*Gln* 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 *XRCC1* 194*Trp*, 280*His*, or 399*Gln* 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 the buffy 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.** *XRCC1* 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-$\mu$l mixture containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 300 $\mu$M 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 *Msp*I 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-$\mu$l mixture containing 100 ng of genomic DNA, 1.5 mM $MgCl_2$, 200 $\mu$M 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 *Rsa*I 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 *XRCC1* genotype and cigarette smoking, alcohol drinking, AQ chewing, and the frequency of *p53* mutation was examined by the $\chi^2$ 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 *XRCC1* genotype. The interactions between *XRCC1* 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 bucca 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 194*Trp*, 280*His*, and 399*Gln* 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

*Table 1* Characteristics of the male patients with OSCCs ($n = 237$)

| Characteristics | |
|---|---|
| Age, yr | |
| Mean ± SD | 49.30 ± 11.03 |
| Range | 28–78 |
| Site of primary tumor, n (%) | |
| Oral cavity | 225 (94.94) |
| Lip | 6 (2.53) |
| Tongue | 74 (31.22) |
| Mouth floor | 12 (5.06) |
| Buccal mucosa | 82 (34.60) |
| Gingiva | 28 (11.81) |
| Hard palate | 9 (3.80) |
| Retromolar trigone | 14 (5.91) |
| Oropharynx | 6 (2.53) |
| Hypopharynx | 6 (2.53) |
| Clinical stage, n (%) | |
| Stage I | 27 (11.39) |
| Stage II | 60 (25.32) |
| Stage III | 37 (15.61) |
| Stage IV | 113 (47.68) |
| Cigarette smoker at some time, n (%) | 210 (90.13) |
| Alcohol drinker at some time, n (%) | 134 (57.26) |
| AQ chewer at some time, n (%) | 190 (81.20) |

shown). Furthermore, the distribution of all three of the polymorphisms was not associated with age, tumor TNM stage, and primary site.

Tumor samples from these 237 OSCC patients, including 187 samples published previously (12), were examined for mutations within exons 5–9 of the *p53* gene by PCR-SSCP. One hundred four (43.88%) of the 237 OSCCs showed *p53* gene mutations at exons 5–9. The frequency of *p53* mutations was not associated with age, TNM stage, cigarette smoking, or AQ chewing. However, alcohol drinkers had a significantly higher frequency (67 of 134, 50.00%) of *p53* mutations than nonusers of alcohol (34 of 100, 34.00%; $P = 0.01$).

Individuals with the 399 *Gln/Gln* genotype were more likely to have *p53* gene mutations OR, 4.29; 95% CI, 1.45–12.66; Table 2) than those with the 399 *Arg/Arg* genotype. After adjustment for age, cigarette smoking, alcohol drinking, and AQ chewing, individuals with 399 *Gln/Gln* genotype still had a higher frequency of *p53* mutations (OR, 5.03; 95% CI, 1.60–15.83) than those with the 399 *Arg/Arg* genotype. The adjusted OR of the 399 *Gln/Arg* heterozygous individuals was only slightly higher than that of individuals with the 399 *Arg/Arg* genotype (OR, 1.08; 95% CI, 0.61–1.89). After dichotomizing the data for the *XRCC1* 399 *Gln/Gln* genotype *versus* the *Gln/Arg* and *Arg/Arg* genotypes, the OR for the 399 *Gln/Gln* being associated with the frequency of *p53* gene mutation 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* mutation (OR, 4.50; 95% CI, 1.52–13.36). No statistically significant association was observed between the frequency of *p53* gene mutation and the 194 *Trp/Trp* or 280 *His/His* genotypes.

Table 3 shows that alcohol drinkers have a higher frequency of *p53* mutations despite their *XRCC1* codon 399 genotype after eliminating the interaction effect. Regardless of the cigarette smoking, alcohol consumption, and AQ chewing status of the individuals with 399 *Gln/Gln* genotype, they had a higher frequency of *p53* gene mutation than individuals with *Gln/Arg* or *Arg/Arg* genotypes. Because the sample sizes were small in the groups with 399 *Gln/Gln* genotype, the nonsignificant ORs that 95% CI covers should require further confirmation when more subjects are recruited. The interactions between *XRCC1* 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. However, the interactions were not statistically significant.

*Table 2* The association of *p53* gene mutations and *XRCC1* genotypes

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

[a] Adjusted for age, cigarette smoking, alcohol drinking, and AQ chewing.

## 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* mutation found in Taiwanese OSCCs were G:C to A:T transitions, and G:C to T:A transversions. G:C to A:T 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*-benzylnitrosamine, 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 $\beta$, 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* 399*Gln/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 AFB1-DNA adducts and glycophorin A variants in a normal population. Abdel-Rahman and El-Zein (23) found that the 399*Gln* polymorphism appeared to be associated with the reduced repair of NNK-induced genetic damage in cultured human lymphocytes. Taken

*Table 3* Stratification analysis of the *XRCC1* 399 genotype and the frequency of *p53* gene mutation

| Variable | *Gln/Gln* | | *Gln/Arg* and *Arg/Arg* | |
|---|---|---|---|---|
| | Mutations detected/tumors tested (%) | OR (95% CI) | Mutations detected/tumors tested (%) | OR (95% CI) |
| Smoking | | | | |
| Yes | 12/17 (70.59) | 3.20 (0.83–12.40) | 78/193 (40.41) | 0.90 (0.36–2.23) |
| No | 2/2 (100.00) | ∞ (0.21–∞)[a] | 9/21 (42.86 ) | 1 |
| Alcohol drinking | | | | |
| Yes | 9/10 (90.00) | 19.24 (2.33–159.11) | 58/124 (46.77) | 1.88 (1.07–3.30) |
| No | 5/9 (55.56 ) | 2.67 (0.67–10.69) | 29/91 (31.87 ) | 1 |
| AQ chewing | | | | |
| Yes | 11/16 (68.75) | 3.11 (0.91–10.58) | 70/174 (40.23) | 0.95 (0.48–1.90) |
| No | 3/3 (100.00) | ∞ (0.52–∞)[a] | 17/41 (41.46 ) | 1 |

[a] Exact OR by EGRET.

together, these findings provide evidence that the 399*Gln* 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 *Arg*399*Gln* 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 194*Trp* 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 194*Trp* with the frequency of *p53* mutations. But it is interesting to note that of the 19 individuals with 399*Gln/Gln* genotype in this series of OSCC patients, all were also of the 194*Arg/Arg* genotype; and of the 111 individuals carrying 194*Trp* alleles, all carried the 399*Arg* 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* 399*Gln/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 Arg*399*Gln* 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* 399*Gln* polymorphism on *p53* gene mutation in Taiwanese OSCCs.

## References

1. IARC. Betel-quid and areca-nut chewing. IARC Monogr., *37:* 141–291, 1986.

2. Ko, Y. C., Huang, Y. L., Lee, C. H., Chen, M. J., Lin, L. M., and Tsai, C. C. Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. J. Oral Pathol. Med., *24:* 450–453, 1995.

3. Hwang, L. S., Wang, C. K., Sheu, M. J., and Kao, L. S. Phenolic compounds of piper betel flower as flavoring and neuronal activity modulating agents. *In:* C. T. Ho, T. Osawa, M. T. Huang, and R. T. Rossen (eds.), Food Phytochemicals for Cancer Prevention I. pp. 186–191. Wash. DC: American Chemical Society, 1993.

4. Chen, C. L., Chi, C. W., Chang, K. W., and Liu, T. Y. Safrole-like DNA adducts in oral tissue from oral cancer patients with a betel quid chewing history. Carcinogenesis (Lond.), *20:* 2331–2334, 1999.

5. Hussain, S. P., and Harris, C. C. Molecular epidemiology of human cancer: contribution of mutation spectra studies of tumor suppressor genes. Cancer Res., *58:* 4023–4037, 1998.

6. Hsu, I. C., Metcalf, R. A., Sun, T., Welsh, J., Wang, N. J., and Harris, C. C. *p53* gene mutational hotspot in human hepatocellular carcinomas from Qidong, China. Nature (Lond.), *350:* 427–428, 1991.

7. Scorsone, K. A., Zhou, Y. Z., Butel, J. S., and Slagle, B. L. *p53* mutations cluster at codon 249 in hepatitis B virus-positive hepatocellular carcinomas from China. Cancer Res., *52:* 1635–1638, 1992.

8. Bressac, B., Kew, M., Wands, J., and Ozturk, M. Selective G to T mutations of *p53* gene in hepatocellular carcinoma from southern Africa. Nature (Lond.), *350:* 429–431, 1991.

9. Ozturk, M., and Collaborators. *p53* mutation in hepatocellular carcinoma after aflatoxin exposure. Lancet, *338:* 1356–1359, 1991.

10. Aguilar, F., Hussain, S. P., and Cerutti, P. Aflatoxin B1 induces the transversion of G → T in codon 249 of the *p53* tumor suppressor gene in human hepatocytes. Proc. Natl. Acad. Sci. USA, *90:* 8586–8590, 1993.

11. Mace, K., Aguilar, F., Wang, J. S., Vautravers, P., Gomez-Lechon, M., Gonzalez, F. J., Groopman, J., Harris, C. C., and Pfeifer, A. M. Aflatoxin B1-induced DNA adduct formation and *p53* mutations in CYP450-expressing human liver cell lines. Carcinogenesis (Lond.), *18:* 1291–1297, 1997.

12. Hsieh, L. L., Wang, P. F., Chen, I. H., Liao, C. T., Wang, H. M., Chen, M. C., Chang, J. T. C., and Cheng, A. J. Characteristics of mutations in the *p53* gene in oral squamous cell carcinoma associated with betel quid chewing and cigarette smoking in Taiwanese. Carcinogenesis (Lond.), *22:* 1497–1503, 2001.

13. Garro, A. J., Espina, N., Farinati, F., and Salvagnini, M. The effects of chronic ethanol consumption on carcinogen metabolism and on $O^6$-methylguanine transferase-mediated repair of alkylated DNA. Alcoholism Clin. Exp. Res., *10 (Suppl.):* 73S–77S, 1986.

14. Mufti, S. I. Alcohol acts to promote incidence of tumors. Cancer Detect. Prev., *16:* 157–162, 1992.

15. Loeb, K. R., and Loeb, L. A. Significance of multiple mutations in cancer. Carcinogenesis (Lond.), *21:* 379–385, 2000.

16. Rajewsky, M. F., Engelbergs, J., Thomale, J., and Schweer, T. Relevance of DNA repair to carcinogenesis and cancer therapy. Recent Results Cancer Res., *154:* 127–146, 1998.

17. Millar, A. L., Pal, T., Madlensky, L., Sherman, C., Temple, L., Mitri, A., Cheng, H., Marcus, V., Gallinger, S., Redston, M., Bapat, B., and Narod, S. Mismatch repair gene defects contribute to the genetic basis of double primary cancers of the colorectum and endometrium. Hum. Mol. Genet., *8:* 823–829, 1999.

18. Yao, X., Buermeyer, A. B., Narayanan, L., Tran, D., Baker, S. M., Prolla, T. A., Glazer, P. M., Liskay, R. M., and Arnheim, N. Different mutator phenotypes in Mlh1-*versus* Pms2-deficient mice. Proc. Natl. Acad. Sci. USA, *96:* 6850–6855, 1999.

19. Benhamou, S., and Sarasin, A. Variability in nucleotide excision repair and cancer risk: a review. Mutat. Res., *462:* 149–158, 2000.

20. Sturgis, E. M., Castillo, E. J., Li, L., Zheng, R., Eicher, S. A., Clayman, G. L., Strom, S. S., Spitz, M. R., and Wei, Q. Polymorphisms of DNA repair gene *XRCC1* in squamous cell carcinoma of the head and neck. Carcinogenesis (Lond.), *20:* 2125–2129, 1999.

21. Shen, M. R., Jones, I. M., and Mohrenweiser, H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. Cancer Res., *58:* 604–608, 1998.

22. Lunn, R. M., Langlois, R. G., Hsieh, L. L., Thompson, C. L., and Bell, D. A. *XRCC1* polymorphisms: effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. Cancer Res., *59:* 2557–2561, 1999.

23. Abdel-Rahman, S. Z., and El-Zein, R. A. The 399*Gln* polymorphism in the DNA repair gene *XRCC1* modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. Cancer Lett., *159:* 63–71, 2000.

24. Hsieh, L. L., Liou, S. H., Chen, Y. H., Tsai, L. C., Yang, T., and Wu, T. N. Association between aminolevulinate dehydrogenase genotype and blood lead levels in Taiwan. J. Occup. Environ. Med., *42:* 151–155, 2000.

25. Association of Directors of Anatomic and Surgical Pathology Recommendations for the reporting of specimens containing oral cavity and oropharynx neoplasms. Mod. Pathol., *13:* 1038–1041, 2000.

26. Belinsky, S. A., Devereux, T. R., White, C. M., Foley, J. F., Maronpot, R. R., and Anderson, M. W. Role of Clara cells and type II cells in the development of pulmonary tumors in rats and mice following exposure to a tobacco-specific nitrosamine. Exp. Lung Res., *17:* 263–278, 1991.

27. Oreffo, V. I., Lin, H. W., Padmanabhan, R., and Witschi, H. *K-ras* and *p53* point mutations in 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced hamster lung tumors. Carcinogenesis (Lond.), *14:* 451–455, 1993.

28. Chang, K. W., Lin, S. C., Koos, S., Pather, K., and Solt, D. *p53* and *Ha-ras* mutations in chemically induced hamster buccal pouch carcinomas. Carcinogenesis (Lond.), *17:* 595–600, 1996.

29. Ronai, Z. A., Gradia, S., Peterson, L. A., and Hecht, S. S. G to A transitions and G to T transversions in codon 12 of the *Ki-ras* oncogene isolated from mouse lung tumors induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and related DNA methylating and pyridyloxobutylating agents. Carcinogenesis (Lond.), *14:* 2419–2422, 1993.

30. Cloutier, J. F., and Castonguay, A. Modulation of DNA repair by various inhibitors of DNA synthesis following 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) induced DNA damage. Chem. Biol. Interact., *110:* 7–25, 1998.

31. Hecht, S. S. DNA adduct formation from tobacco-specific *N*-nitrosamines. Mutat. Res., *424:* 127–142, 1999.

32. Salles, B., Sattler, U., Bozzato, C., and Calsou, P. Repair of oxidative DNA damage *in vitro:* a tool for screening antioxidative compounds. Food Chem. Toxicol., *37:* 1009–1014, 1999.

33. Caldecott, K. W., Aoufouchi, S., Johnson, P., and Shall, S. *XRCC1* polypeptide interacts with DNA polymerase $\beta$ and possibly poly(ADP-ribose) polymerase, and DNA ligase III is a novel molecular 'nick-sensor' *in vitro.* Nucleic Acids Res., *24:* 4387–4394, 1996.

34. Masson, M., Niedergang, C., Schreiber, V., Muller, S., Menissier-de Murcia, J., and de Murcia, G. *XRCC1* is specifically associated with poly(ADP-ribose) polymerase and negatively regulates its activity following DNA damage. Mol. Cell. Biol., *18:* 3563–3571, 1998.

35. Soussi, T., and Beroud, C. Assessing *TP53* status in human tumours to evaluate clinical outcome. Nat. Rev. Cancer, *1:* 233–240, 2001.