Short Communication

No Association between Polymorphisms of the DNA Repair Gene *XRCC1* and Cervical Neoplasm Risk

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Abstract

Objectives: To investigate the association between genetic polymorphisms of *X-ray repair cross-complementing group 1 (XRCC1)* codons 194, 280, and 399 and cervical neoplasm susceptibility.

Methods: A community-based nested case-control study was conducted. The study population consisted of women living in Chiayi City, located in southwestern Taiwan, who had received pap smear screening between October, 1999, and December, 2000 (n=32,466). The potential cases were women having lesions greater than cervical intraepithelium neoplasm II (CIN2) reconfirmed by cervical biopsy. The potential controls (case : control=1 : 2) were age matched (± 2 yrs) and residency matched women who had had normal pap smears. In total, 100 cases (39 CIN2, 12 CIN3, 46 carcinoma in situ (CIS), and 3 invasive cancer) and 196 controls had the information on both questionnaire and data of *XRCC1* polymorphisms.

Results: The frequency of *Arg/Arg*, *Arg/Gln*, and *Gln/Gln* in codon 399 among cases and controls was 54% (54/100), 38% (38/100), and 8% (8/100) and 58% (114/196), 37% (73/196), and 5% (9/196), respectively, which were not significantly different. No associations were also observed between *XRCC1* codon 194 and 280 genotypes and cervical neoplasm. While dichotomized by age (<40 vs. ≥ 40 yrs), smoking status (active and passive smokers vs. non-smokers), and disease status (CIN2 and CIN3 vs. CIS and invasive cancer), the results remained insignificant.

Conclusions: The present findings suggest that *XRCC1* codon 194, 280 and 399 genotypes may not influence cervical neoplasm risk in the Taiwanese population.

Key words: cervical neoplasm, *XRCC1*, genetic polymorphism

Introduction

Cervical cancer (23.8 per 100,000 females) was the most prevalent malignancy in Taiwanese women in 2000 (1). High-grade squamous intraepithelium neoplasms are known to be precancer cervical lesions, which include moderate dysplasia (cervical intraepithelium neoplasm II: CIN2) and severe dysplasia (CIN3). They are often found by exfoliative cytology screening (pap smear). If not treated, at least 25% will progress to carcinoma in situ (CIS) or invasive cancer (2).

X-ray repair cross-complementing 1 (XRCC1) is one of the major base-excision repair genes, located on the long arm of

human chromosome 19 (19q13.2–13.3) with 17 exons (3). Shen et al. (4) sequenced its coding regions in 12 healthy Caucasians and found three genetic variants resulting in amino acid changes at codons 194, 280 and 399, respectively. Cancer epidemiologic studies revealed that *XRCC1* codon 399 *Gln* was significantly associated with increased risks of lung cancer and breast cancer, but reduced or no risks of skin cancer, esophageal cancer or bladder cancer (5–9). In addition, it was suggested that a lack of the *XRCC1* 194 *Trp* allele is associated with bladder cancer, gastric cancer, and oral cavity and pharynx risks (10–12), but not with colorectal cancer and breast cancer (5, 13). Therefore, in the present study, we examined the effect of *XRCC1* genetic polymorphisms in codons 194, 280 and 399 on cervical neoplasm risk in Taiwan.

Materials and Methods

Participants. Between October, 1999 and December, 2000, 32,466 women above 19 years of age at the time of

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examination underwent pap smear screening in Chiayi City, on the southwestern coast of Taiwan. Of these 32,466 women, 420 were newly-diagnosed as having lesions greater than cervical intraepithelium neoplasm I ($\geq$ CIN1). Of these 420 women, 349 were followed up by biopsy. The remaining 71 women were treated as follows: follow up by pap smear: 24; direct therapy: 3; uncooperative: 5; no information: 5; others: 34. Among the 349 subjects with biopsy follow up, 116 women had lesions greater than CIN2 by biopsy confirmation, and were selected as potential cases for inclusion in this study. The potential controls were randomly selected from women who lived at the same area of Chiayi City and whose pap smear results were negative in the first screening of the study period ($n=32,046$). The case-control ratio was 1:2 with matching on age (± 2 yrs), residency (eastern or western administrative district), and time that the pap smear was performed (within 6 months of each other). The findings of previous pap smears taken prior to this study were found to be normal in both cases and controls. Using a structured questionnaire, 19 trained public health nurses interviewed these study subjects in their homes between October, 2000, and March, 2001. A current or former smoker was defined as a person who had been smoking more than one cigarette per day for at least one year. Subjects were considered passive smokers if they were exposed to the smoke from more than one cigarette per day for at least one year from the home or the workplace.

In total, 100 study cases and 197 study controls were completely interviewed. Reasons given for the lack of response in the 16 non-respondent study cases included: refusal to be interviewed ($n=10$), at least two unsuccessful visits ($n=5$), and death ($n=1$). The mean ages ($\pm$ SD) of respondent women ($n=100$) and non-respondent women ($n=16$) (yrs) were 52.5 (13.1) and 56.5 (12.1), respectively, which were not significantly different (p -value=0.16). This study was approved by the Human Subjects Committee of the Kaohsiung Medical University.

Genotyping of XRCC1. One study control did not provide a blood specimen. Thus, DNA specimens from 100 cases and 196 controls were genotyped. The genotype of XRCC1 was determined by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and has been described in detail elsewhere (6). In brief, the following primers were used for PCR: 26240F: GTT CCG TGT GAA GGA GGA GGA and 26377R: CGA GTC TAG GTC TCA ACC CTA CTC ACT for codon 194; and 27405F: TTG ACC CCC AGT GGT GCT AA and 28265R: GGC TGG GAC CAC CTG TGT T for codons 280 and 399. After the completion of PCR, the products were, respectively, digested by restriction enzymes of PvuII (for codon 194), RsaI (for codon 280), and MspI (for codon 399) (the restriction enzymes from B.M.). After digestion, the Arg allele of codon 194 gave a segment of 138-bp, while the Trp allele gave the products of 63-bp and 75-bp. For the PCR product of codon 280, a restriction site of RsaI was created in the Arg allele and gave rise to products of 63, 201 and 597-bp, while the His allele gave the products of 201 and 660-bp. For the PCR product of codon 399, a restriction site of MspI was created in the Arg allele and gave the products of 115, 285 and 461-bp, while the Gln allele gave the products of 285 and 576-bp. Blind genotyping of XRCC1 was performed by a technician. About 10% of the samples ($n=30$) were repeated and the results

were exactly the same as the first ones.

Statistical analysis. Conditional logistic regression models were performed to assess the association between the XRCC1 genetic polymorphism and cervical neoplasm risk after adjusting for other covariates. Covariates in the models included variables which differed significantly between cases and controls in the univariate analyses, including education levels ($\geq$ high school, junior high school, and $\leq$ primary school), cigarette smoking (current and former smokers, passive smokers, and non-smokers), and age at first intercourse (quartiles) as categorical variables. We also dichotomized age (<40 vs. ≥ 40 yrs), smoking status (active and passive smokers vs. non-smokers), or disease status (CIN2 and CIN3 vs. CIS and invasive cancer) to examine their associations. In addition, joint effects were evaluated among XRCC1 genetic polymorphisms.

The frequency of XRCC1 codon 399 Arg/Gln and Gln/Gln among the 196 controls was 41.8%. Assuming detection of a significantly increased risk of 2-fold in these genotypes at the level of $p<0.05$, the present study could detect at $\sim 80\%$ power among 100 cases and 196 controls. The data were analyzed using the SAS statistical package; all p -values were two-sided.

Results and Discussion

Among the 100 cases with completed questionnaires, 39 (39%), 12 (12%), 46 (46%), and 3 (3%) were CIN2, CIN3, CIS, and invasive cancer, respectively. Mean ages (range) of cases and controls were 51.5 (24–82) and 51.7 (22–81) years old, respectively. The most significant predictors of cervical neoplasm risk were educational levels and age at first intercourse (Table 1). Cigarette smoking was marginally associated with cervical neoplasm risk. Neither polymorphism was associated with cervical neoplasm risk, without or with adjusting for potential confounders (Table 2). In addition, XRCC1 polymorphisms did not modify the associations between cervical neoplasm and age (<40 vs. ≥ 40 yrs), smoking status (active and passive smokers vs. non-smokers), or disease status (CIN2 and CIN3 vs. CIS and invasive cancer). We also did not find any significant joint effects among these three XRCC1 polymorphisms (data not shown).

Epidemiologic studies revealed that XRCC1 codon 399 Gln was significantly associated with increased risks of lung cancer and breast cancer, but reduced risks of skin cancer and esophageal cancer (5–13). Duell et al. (5) reported a positive association between XRCC1 codon 399, but not codon 194, and breast cancer risk among 253 cases and 266 controls of African-Americans. However, similar results were not found in 386 cases and 381 controls of Caucasians. African-Americans with Arg/Gln or Gln/Gln showed a 1.7-fold greater risk to develop breast cancer than those with Arg/Arg (5). A wide racial variation between Africans-Americans and Caucasians at XRCC1 codon 399 polymorphism was also noted in the study. The frequencies of Arg/Arg, Arg/Gln, and Gln/Gln were 74%, 24%, and 2% in 266 controls of African-Americans and 43%, 41%, and 16% in 381 controls of Caucasians, respectively. In contrast, the frequency of Arg/Arg, Arg/Gln, and Gln/Gln among the present 196 controls was 58.2%, 37.2%, and 4.6%. Based on

Table 1 Distributions of demographic characteristics and other potential confounders

Variables	Cases (n=100)	Controls (n=197)	p-value
Age (yrs)			
<42	25 (25.0)	49 (24.9)	0.90
42–51	27 (27.0)	47 (23.9)	
52–62	21 (21.0)	48 (24.4)	
>62	27 (27.0)	53 (26.9)	
Cigarette smoking ^a			
non-smoker	12 (12.0)	45 (22.8)	0.07
passive smoker	83 (83.0)	146 (74.1)	
current & former smoker	5 (5.0)	6 (3.1)	
Education levels			
≥high school	32 (32.0)	109 (55.3)	<0.01
junior high school	23 (23.0)	22 (11.2)	
≤primary school	45 (45.0)	66 (33.5)	
Times of prior pap smears (times)			
one	18 (18.0)	36 (18.3)	0.95
>1	82 (82.0)	161 (81.7)	
Number of lifetime sexual partners			
≤1	93 (93.0)	189 (95.9)	0.27
>1	7 (7.0)	8 (4.1)	
Age at first intercourse (yrs)			
>26	11 (11.0)	43 (21.8)	<0.01
25–26	11 (11.0)	43 (21.8)	
22–24	42 (42.0)	60 (30.5)	
≤21	36 (36.0)	51 (25.9)	

^a Passive smokers: exposed to environmental tobacco smoke from home or the workplace.

Table 2 Association between cervical neoplasm risk and XRCC1 polymorphisms

Variables	Cases (n=100)	Controls (n=196)	OR (95% CI)	AOR (95% CI) ^a
<i>XRCC1</i> codon 194				
<i>Arg/Arg</i>	48 (48.0)	87 (44.4)	1.00	1.00
<i>Arg/Trp</i>	43 (43.0)	93 (47.4)	0.82 (0.47–1.41)	0.70 (0.38–1.28)
<i>Trp/Trp</i>	9 (9.0)	16 (8.2)	1.00 (0.42–2.40)	1.12 (0.43–2.92)
<i>XRCC1</i> codon 280				
<i>Arg/Arg</i>	74 (74.0)	140 (71.4)	1.00	1.00
<i>Arg/His</i>	24 (24.0)	55 (28.1)	0.85 (0.50–1.46)	0.90 (0.49–1.64)
<i>His/His</i>	2 (2.0)	1 (0.5)	3.80 (0.34–42.18)	2.96 (0.24–36.20)
<i>XRCC1</i> codon 399				
<i>Arg/Arg</i>	54 (54.0)	114 (58.2)	1.00	1.00
<i>Arg/Gln</i>	38 (38.0)	73 (37.2)	1.13 (0.66–1.92)	1.09 (0.59–2.01)
<i>Gln/Gln</i>	8 (8.0)	9 (4.6)	2.07 (0.71–6.09)	1.29 (0.41–4.02)

^a Adjusting for education levels, cigarette smoking, and age at first intercourse.

the calculation of the frequency of *Arg/Gln* or *Gln/Gln* (41.8%), the present sample size could detect ~80% power at an increased risk of 2-fold in the genotypes of *Arg/Gln* or *Gln/Gln* vs. *Arg/Arg*. However, the present study may not have an adequate power, which was only 52%, to detect the risk of 1.7. An alternative possible explanation of the present negative findings is: no actual effect of *XRCC1* codon 399 on cervical neoplasm risk in Taiwanese population, which is similar to the findings in Caucasians, but not in the African-Americans (5).

Several limitations are noted in this study. There was no information about the status of human papillomavirus infection (HPV) infections among our subjects. HPV is well-documented to be associated with cervical neoplasm risk (14). However, in the present analyses, we accounted for the variable of age at first intercourse which may be related to the status of HPV infection. Another limitation is that only 3 of 100 subjects had invasive cervical cancer. Genetic polymorphisms of *XRCC1* may play a vital role in neoplastic lesions, instead of precancer

lesions. Finally, the present sample size is probably small to detect the slight increase of risk in the genotypes of *XRCC1* polymorphisms.

In conclusion, the present study was the first to investigate the relation between genetic polymorphisms of *XRCC1* and cervical neoplasm risk. The present findings show that a polymorphism in codon 194, 280 or 399 of *XRCC1* was not associated with cervical neoplasm susceptibility.

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