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Short Communication

Polymorphism of XRCC1 Codon Arg 399 Gln Is Associated with Higher Susceptibility to Endometriosis

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Abstract

Endometriosis shows some characteristics of malignancy, including local invasion and aggressive spread to distant organs. The pathology of endometriosis may involve a complex interaction among genetic defects, DNA repairing defects and environmental factors. Since DNA repair capacity is closely related to the sustaining of the genomic stability, an XRCC1 Arg399Gln polymorphism was performed to evaluate the possible association with endometriosis in this paper. Recruited adult females were divided into two groups: [1] endometriosis group (n = 141) and [2] non-endometriosis group (n = 100). Genomic DNA was obtained from their peripheral leukocytes. DNA fragment coding XRCC1 Arg399Gln polymorphism was amplified by PCR and subsequently digested with MspI, and then the genotypes and allelic frequencies in both groups were compared. The genotype distribution and allelic frequency of XRCC1 Arg399Gln polymorphism was significantly different ($P < 0.05$). The partition of the "GG" homozygote in the patient group was greater than that in the control group, which means that for those people with more G allele, they will have higher risk for endometriosis. We concluded that XRCC1 Arg399Gln polymorphism is associated with higher susceptibility to endometriosis and XRCC1 Arg399Gln polymorphism might be a useful biomarker for endometriosis.

Key Words: XRCC1, endometriosis, polymorphism, Arg399Gln

Introduction

Endometriosis, a common polygenic and multi-factorial disease, might be caused by an interaction between multiple genetic as well as environmental factors (5). Endometriosis, one type of metaplases of eutopic endometrial cells, displays some features of malignancy, including local invasion and aggressive spread to distant organs. Mutant or defected in DNA repairing system is essential for tumorigenesis. Then it is logical to suspect some genetic variants of DNA repair genes might contribute to endometriosis pathogenesis.

XRCC1 is a multifunctional protein that plays a central role in the repair of oxidative DNA base adducts, and also a negative regulator of apoptosis

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(6). *XRCC1* is thought to be involved in DNA single-strand beak (SSB) repair, and plays an important role in the base excision repair (BER) pathway (8). In coordinating BER, XRCC1 protein is proposed to interact with PARP and DNA ligase III in recognition and re-joining of DNA strand breaks. XRCC1 mutants display sensitivity to alkylating agents and ionizing radiation and exhibit elevated levels of sister chromatid exchange in the Chinese hamster ovary cell lines. Such alterations could be associated with increased cancer risk (20).

XRCC1 gene contains 17 exons, and the human gene maps to chromosome 19q13.2 (14). Polymorphisms within *XRCC1* gene was reportedly associated with increased risk for several cancers: gastric, head and neck, breast, and skin melanoma (15, 17, 21, 24). Furthermore, variants of *XRCC1* gene contributed to ionizing radiation susceptibility as measured by prolonged cell cycle G2 delay (13). The non-synonymous polymorphism in XRCC1 Arg399Gln (G/A allelic change) have been shown to alter DNA repair capacity in some phenotypic studies and have received considerable attention (19, 23).

It has been suggested that reduced DNA repair capacity may be a susceptibility factor predisposing women to breast cancer (3, 4). Genetic variations in DNA repair genes may lead to inter-individual variation in DNA repair capacity and modify the associations between exogenous and endogenous carcinogens and endometriosis risk. In the present study, we aimed primarily to evaluate whether *XRCC1* Arg399Gln polymorphism is attractive markers for moderate/severe endometriosis susceptibility. To our knowledge, this is the first survey in this field.

Materials and Methods

Pre-menopausal Taiwanese Chinese women who were surgically diagnosed of endometriosis were recruited in the study. All Taiwanese Chinese women were divided into two groups: group 1, women with endometriosis (n = 141); and group 2, women without endometriosis (n = 100). The free of endometriosis status was confirmed during cesarean section or diagnostic laparoscopy. All operations were performed by two experienced surgeons (Hsieh YY, Chang CC). All the women recruited have signed informed consents accepting the peripheral blood sampling for genotype analyses. This study was approved by the ethics committee of the China Medical University Hospital, Taichung, Taiwan.

The genomic DNA was isolated from peripheral blood using a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reactions (PCR) primer in a total volume of 25 μl containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magnesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin Elmer, Foster City, CA, USA.). For the *XRCC1* codon 399 polymorphism, a 242-base-pair (bp) fragment of *XRCC1* was amplified by PCR with the primers: forward 5'-CCCCAAGTACAGCCAGGTC-3' and reverse 5'-TGTCCCGCTCCTCTCAGTAG-3'.

The PCR conditions were fine-turned as: 1 cycle at 95°C for 5 min, 35 cycles at 95°C for 40 s, 55°C for 40 s, and 72°C for 40 s, and 1 final cycle of extension at 72°C for 7 min, then holding at 25°C. The polymorphisms were analyzed by PCR amplification followed by *MspI* restriction enzyme digestion. Two fragments measuring 147 bp and 95 bp will be present if the product is able to be excised. The uncut band shows up as a 242 bp length on the gel. The reaction was then incubated for overnight at 37°C, and then 10 μl of the digested products were loaded into a 3% agarose gel with ethidium bromide staining and separated by electrophoresis. The G/A 399 polymorphism of *XRCC1* was categorized as divisible homozygote (G/G), indivisible homozygote (A/A), and heterozygote (G/A).

The SAS package (Version 8.1, SAS Institute Inc., Cary, North Carolina, USA) with χ^2 and Fisher's extract tests were utilized for statistical analyses. A *P*-value of < 0.05 was considered statistically significant.

Results

No significant differences between control and endometriosis groups were found in terms of age, body mass index, and age of first menarche (Table 1). Representative PCR-based restriction analyses for the XRCC1 Arg399Gln polymorphisms were shown in Fig. 1. The frequencies of the genotypes in the endometriosis and control groups of the *XRCC1* Arg399Gln gene polymorphism were shown in Table 1. There was a statistically significant difference in the distribution of the *XRCC1* G/A polymorphism between non-endometriosis control and endometriosis patients ($P < 0.05$). The frequency of the "G" allele in the patient group (68.4%) was greater than that in the non-endometriosis group (57.5%), indicating that the "G" allele was indeed a risky one (Table 1). The frequency of the "GG" homozygote in the patient group was much greater than that in the non-endometriosis group (41.8% versus 30%). To sum up, all these results indicated that the G allele of *XRCC1* Arg399Gln polymorphism was associated with higher susceptibility to endometriosis.

Discussion

In this study, we have demonstrated an elevated

Table 1. Baseline characteristics, genotypes and allelic frequencies for XRCC1 Arg399Gln gene polymorphism in women with and without endometriosis

XRCC1 Arg399Gln	Endometriosis n = 141	Control n = 100	*P*-value
Age	31.4 ± 4.5	30.7 ± 5.4	NS
BMI	21.3 ± 2.1	20.9 ± 2.3	NS
First Menarche Age	15.1 ± 1.4	15.3 ± 1.3	NS
Genotype			0.0128
AA	7 (4.9%)	15 (15.0%)	
AG	75 (53.2%)	55 (55.0%)	
GG	59 (41.9%)	30 (30.0%)	
Allelic frequency			0.0161
Allele A	89 (31.6%)	85 (42.5%)	
Allele G	193 (68.4%)	115 (57.5%)	

NS: no significant difference

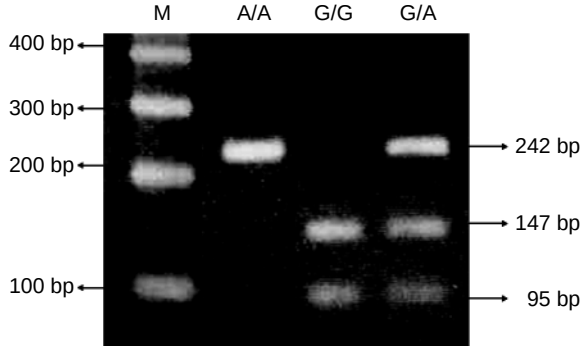

Fig 1. PCR-based restriction analysis of the Arg399Gln polymorphism of *XRCC1* gene shown on 3% agarose electrophoresis. M: 100 bp DNA size marker, A/A: indivisible homozygote, G/G: divisible homozygote, and G/A heterozygote.

risk of endometriosis associated with the frequency of "G" allele in Arg399Gln of *XRCC1* polymorphisms. In addition to Arg399Gln, there are two other polymorphisms identified to be related to carcinogenesis, Arg194Trp and Arg280His (23). We have also investigated the association of these two polymorphisms with endometriosis susceptibility, and found that neither of they were associated with endometriosis risk (unpublished data). The *XRCC1* 399Gln has been reported to be associated with higher levels of aflatoxin B_1-DNA adducts and glycophorin NN mutations in placental DNA, suggesting that the Arg399Gln polymorphism may result in deficient DNA repair (16). It was also reported to be associated with reduced repair of NNK-induced genetic damage in human lymphocytes (1), and also with the frequency of *p53* mutations in oral squamous cell carcinomas (9). The results of our study that those who carry 399Gln allele have a higher risk of endometriosis than those carrying 399Arg homozygotes, are consistent with previous findings in carcinogenesis (18, 21, 22).

Some gene polymorphisms are reported to be associated with endometriosis development, including those in estrogen receptor gene (7), and glutathione S-transferase M1 gene (2). In previous studies, our group has observed the correlation between endometriosis and a series of gene polymorphisms, including galactose-1-phosphate uridyl transferase, estrogen and androgen receptors, IL-1, IL-4, TNF, p53, and p21 polymorphisms (10, 11, 12).

Results from this study support the hypothesis that genetic variation in *XRCC1* may affect woman's endometriosis susceptibility. We found that the genotype proportions and allele frequencies of *XRCC1* gene polymorphisms were significantly different between the two groups (Table 1). We found a lower percentage of the AA homozygote and allele in the women with endometriosis compared with the non-endometriosis subjects, and the presence of the G allele was associated with an increased risk for endometriosis. With an individual systematic genotyping of Arg399Gln of *XRCC1* gene, together with other gene polymorphisms such as p53 and p21, the precise rate of diagnosis may increase, and those with higher risk may be screened out and advised to take some strategies for prevention of occurrences of endometriosis. Furthermore, the differential expression level and functional assays may also be useful evidence for the distinction of people who carry A or G genotypes. Thus, the A and G alleles may serve as biomarkers of the development of endometriosis as well as the targets for modulating or interfering of related pathogeneses.

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