DNA repair gene \(\textit{XRCC1}\) Arg399Gln polymorphism is associated with increased risk of uterine leiomyoma

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\textbf{BACKGROUND:} DNA repair gene \(\textit{XRCC1}\) Arg399Gln polymorphism has been associated with the risk of several human tumours. In the present study we investigated whether the \(\textit{XRCC1}\) polymorphism is related to the risk of uterine leiomyoma, the most common neoplasm of the female genital tract.

\textbf{METHODS:} Three hundred and twenty-seven patients with uterine leiomyoma and 197 normal controls were enrolled, and \(\textit{XRCC1}\) genotyping was determined by PCR and restriction fragment length polymorphism.

\textbf{RESULTS:} The proportions of individuals homozygous for 399Arg allele, heterozygous and homozygous for the 399Gln allele were 85.8%, 13.7% and 0.5% among the control group, and 46.2%, 53.2% and 0.6% in those with leiomyoma (\(P<0.001\)), respectively. Logistic regression analysis (after adjusting for age, parity, menarche age and body mass index) showed a significant increased risk of uterine leiomyoma in women with the Arg/Gln genotype versus the Arg/Arg genotype (odds ratio 6.79; 95% confidence interval 4.20–10.99; \(P<0.001\)).

\textbf{CONCLUSIONS:} In Korean women, the 399Gln polymorphism of \(\textit{XRCC1}\) is associated with an increased risk of uterine leiomyoma.

\textit{Key words:} DNA repair gene \(\textit{XRCC1}/\text{leiomyoma/neoplasm/polymorphism/uterus}

\section*{Introduction}

Uterine leiomyoma is the most common neoplasm of the female genital tract, and despite their high prevalence, little attention has been paid to the cause and pathogenesis of this disease because it seldom undergoes malignant transformation. Regardless of their generally benign neoplastic character, uterine leiomyomas are responsible for significant morbidity and can cause a variety of symptoms including menometrorrhagia, dysmenorrhea, pelvic pain, compression of adjacent pelvic viscera and reproductive failure (Haney, 2000).

Several predisposing factors of this tumour have been identified, including age (late reproductive years), African-American ethnicity, nulliparity and obesity. Estrogen and progesterone are recognized as promoters of tumour growth. Several growth factors are elevated in uterine leiomyomas and may be the effectors of estrogen and progesterone promotion (Flake et al., 2003). Although the initiator(s) of leiomyomas remains unknown, considering their extremely high incidence, initiating conditions must be common to most or all women (Flake et al., 2003). In fact if this is the case, one could hypothesize that leiomyoma development is related to individual susceptibilities to a common initiator(s). In addition, the fact that leiomyomas are monoclonal tumours derived from a single myometrial cell suggests that DNA repair failure may be an early event in myoma development (Townsend et al., 1970).

In this study we have focused on the \(\textit{XRCC1}\) gene, the product of which plays an important role in DNA repair. Indeed, although lacking any known enzymatic activity itself, \(\textit{XRCC1}\) interacts with enzymatic components of each stage of DNA strand break repair, including PARP-1, AP endonuclease-1, polynucleotide kinase, DNA polymerase-\(\beta\) and DNA ligase \(\text{III}a\) (Caldecott, 2003). Thus, polymorphisms in \(\textit{XRCC1}\) that cause amino acid substitutions may impair the interaction of \(\textit{XRCC1}\) with the other enzymatic proteins and consequently alter DNA strand break repair.

Hence, we undertook this hospital-based case-control study to determine the association between the Arg399Gln polymorphism of \(\textit{XRCC1}\) and susceptibility to uterine leiomyoma in Korean women.

\section*{Materials and methods}

\textbf{Subjects}

All patients (\(n=334\)) who had been treated by myomectomy or hysterectomy due to uterine leiomyoma at the Department of Obstetrics and Gynecology, Seoul National University Hospital in 1999 were included in this study. All surgical specimens were confirmed as leiomyoma by histopathological examination. The non-tumour

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control group (n = 206) was composed of healthy Korean women that visited our hospital to participate in a routine gynecological cancer detection program. Control women had no evidence of leiomyoma by transvaginal ultrasonography and a normal cervical cytology in at least two consecutive annual examinations. Women with any malignant disease, those who had received blood transfusion and those with any systemic problem, such as chronic liver disease, were also excluded from the control group. The patients and controls were all Korean, meaning that they belonged to the same ethnic group. Informed consent and 10 ml of peripheral blood were obtained from each participant. Before beginning this study, the study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

Genotyping
DNA was extracted from peripheral blood samples. XRCC1 genotypes were determined by PCR restriction fragment length polymorphism (PCR-RFLP). The codon 399 region was amplified using the following primers: forward, 5‘-CCCCAAGTACAGCCAGGTC-3’ and reverse, 5‘-TGTTCCCGTCTCCTCAGTAG-3’. The PCR was started with a mixture containing the following reagents: 0.1 µg of DNA, 5 µmol/l of dNTPs, 5 µmol/l of primer and 1 U of Taq polymerase, which was added into PCR buffer containing 10 µmol/l of Tris–HCl, 1.5 µmol/l MgCl₂ and 50 µmol/l KCl. PCR conditions were 95°C for 3 min, followed by 40 cycles of 94°C for 30 s, 57°C for 45 s, 72°C for 45 s and a final elongation step at 72°C for 5 min. Following PCR, 20 µl aliquots were removed and subjected to restriction digestion with MspI (New England Biolabs Inc., Beverly, MA, USA). The digested products were resolved on 1.5% agarose gels and stained with 0.5 µg/ml ethidium bromide. To test the reliability of the assay, 50 randomly selected samples were re-tested with identical results being obtained. Genotyping failures resulted from the DNA extraction error led to the exclusion of seven (2.1%) subjects from the control group. The patients and controls were all Korean, meaning that they belonged to the same ethnic group. Informed consent and 10 ml of peripheral blood were obtained from each participant. Before beginning this study, the study protocol was approved by the Institutional Review Board of Seoul National University Hospital.

Statistical analysis
Statistical analyses were carried out using the SPSS version 11.0 for Windows (SPSS Inc., Chicago, IL, USA). The χ²-test and the t-test were used to compare variables. A logistic regression model was used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding P-values for each genotype controlling age, parity, age at menarche and body mass index (BMI) as covariates. All the variables listed were forced into the equation when running logistic regression. P-values of <0.05 were considered statistically significant.

Results
Selected case and control demographics are presented in Table I. The mean ages and parity of the two groups were similar (P = 0.623 and 0.770). However, mean ages at menarche and BMI were significantly different (both P < 0.001).

Genotype distribution results are presented in Table II. The genotype distribution of the control group was in Hardy–Weinberg equilibrium (P = 0.995). The proportions of individuals homozygous for 399Arg allele, heterozygous, and homozygous for the 399Gln allele were 85.8%, 13.7% and 0.5% in the control group, and 46.2%, 53.2% and 0.6% in patient group (P < 0.001), respectively. Women with the Arg/Gln genotype were found to have an elevated risk of uterine leiomyoma compared with those with the Arg/Arg genotype (OR 6.79; 95% CI 4.20–10.99; P < 0.001). However, no significant increased risk of uterine leiomyoma was found for those homozygous for the 399Gln allele (OR 2.97; 95% CI 0.26–34.35; P = 0.384).

Discussion
This study indicates that the Arg/Gln genotype is associated with higher risk of uterine leiomyoma than the Arg/Arg genotype. No risk could be assigned to subjects with the 399Gln homozygote due to its rarity in the Korean population. To the best of our knowledge this is the first report on the XRCC1 polymorphism and the risk of uterine leiomyoma. Other studies on the Arg399Gln variant phenotype suggest that the 399Gln allele is associated with higher levels of DNA adducts, mutations and sister chromatid exchanges, which theoretically would increase the incidence of malignancy (Lunn et al., 1999; Duell et al., 2000). Based on the fact that most tumours are clonal in origin, it was estimated that five events in humans and two or three in rodents are required to transform a normal cell into a cancer cell (Nowell, 1976; Hahn and Weinberg, 2002). The results of the present study suggest that DNA damage/repair mechanisms related to XRCC1 are associated with the genesis of uterine leiomyomas.

Genetic aspects of leiomyomas have been suggested by studies that viewed the issue from different perspectives, i.e. ethnic predisposition, twin studies, familial aggregation and cytogenetic changes (Kurbanova et al., 1989; Marshall et al., 1997; Ligon and Morton, 2000; Luoto et al., 2000; Sato et al., 2002; Van Voorhis et al., 2002; Dal Cin et al., 2003). In terms of the association between genetic polymorphisms and susceptibility to uterine leiomyoma, the estrogen receptor gene polymorphism has been investigated by several researchers (Kitawaki et al., 2001; Massart et al., 2001; Hsieh et al., 2003; Massart et al., 2003); however, these studies have produced inconsistent findings.

Table I. Selected characteristics of leiomyoma cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases (n = 327)</th>
<th>Controls (n = 197)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47.3 ± 7.7</td>
<td>46.9 ± 10.5</td>
<td>NS</td>
</tr>
<tr>
<td>Parity</td>
<td>2.4 ± 1.3</td>
<td>2.3 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Menarche age (years)</td>
<td>15.2 ± 1.6</td>
<td>14.5 ± 1.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.5 ± 3.2</td>
<td>22.2 ± 2.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
BMI = body mass index; NS = not significant.

Table II. XRCC1 genotype frequencies for cases and controls and their ORs

<table>
<thead>
<tr>
<th>XRCC1 genotype</th>
<th>Cases (n = 327)</th>
<th>Controls (n = 197)</th>
<th>Adjusted OR (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg (%)</td>
<td>151 (46.2)</td>
<td>169 (85.8)</td>
<td>1.00 (reference)</td>
<td></td>
</tr>
<tr>
<td>Arg/Gln (%)</td>
<td>174 (53.2)</td>
<td>27 (13.7)</td>
<td>6.79 (4.20–10.99)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gln/Gln (%)</td>
<td>2 (0.6)</td>
<td>1 (0.5)</td>
<td>2.97 (0.26–34.35)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Adjusted for age, parity, menarche age and BMI.
NS = not significant.
The results of the present study, on the other hand, corresponded well with those of other studies on the XRCC1 polymorphism and proliferative disease: carcinoma. Various types of carcinoma were investigated, namely esophageal cancer, prostate cancer, colorectal cancer, lung cancer and hepatocellular carcinoma (Park et al., 2002; Yu et al., 2003; Zhou et al., 2003; Krupa and Blasiak, 2004; Rybicki et al., 2004; Yu et al., 2004). All of these studies found that the 399Gln allele is associated with a higher risk of cancer than the 399Arg allele. In addition, these conclusions corresponded well with the finding that subjects with the 399Gln allele have higher numbers of chromosomal breaks per cell than those with other genotypes (Wang et al., 2003), and that these subjects are more susceptible to chemically induced genetic damage (Li et al., 2003).

However, an almost equally impressive number of studies have reported that the XRCC1 399Gln allele has no or an inverse relationship with the risk of neoplasms (Olshan et al., 2002; Mort et al., 2003; Shen et al., 2003; Smith et al., 2003; Kelsey et al., 2004; Matsuo et al., 2004; Mertens et al., 2004; Sanyal et al., 2004; Wu et al., 2004). A credible explanation for this discrepancy is that the variant protein has an altered repair efficiency; the resultant increased levels of damage might give rise to enhanced apoptosis at the time of cell division, which would finally manifest as reduced risk for exposure-induced cancer (Nelson et al., 2002) and the possibility of XRCC1-independent single-strand break repair (Caldecott, 2003). However, the exact mechanism by which the XRCC1 399Gln allele can have apparently opposite effects on the risk of neoplasm development is not known, and this should be the subject of further investigation.

In this study, age and parity were similar between the two groups, although we expected low parity in the case group because the nulliparity is a known predisposing factor for leiomyomas. A possible explanation for this unexpected result is that nulliparous women in Korea generally are unwilling to have an operation on their uterus and clinicians also have a similar attitude. Therefore, our inclusion criteria—patients who had an operation and histopathological confirmation of leiomyoma—might not have led to the enrollment of a sufficient number of nulliparous women in this study.

We included menarche age in the analysis on the grounds that early menarche leads to increased duration of estrogen exposure and could result in high susceptibility to leiomyomas. A possible explanation for this discrepancy is that the variant protein has an altered repair efficiency; the resultant increased levels of damage might give rise to enhanced apoptosis at the time of cell division, which would finally manifest as reduced risk for exposure-induced cancer (Nelson et al., 2002) and the possibility of XRCC1-independent single-strand break repair (Caldecott, 2003). However, the exact mechanism by which the XRCC1 399Gln allele can have apparently opposite effects on the risk of neoplasm development is not known, and this should be the subject of further investigation.

In conclusion, our study is the first to determine the significance of the XRCC1 polymorphism in uterine leiomyoma, and suggests that the XRCC1 399Gln allele is associated with an increased risk of uterine leiomyoma in Korean women. Although further studies are needed, we hope that this work increases knowledge about the pathogenesis of uterine leiomyomas and may give clues for the primary and secondary prevention of the disease.

Acknowledgements
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