The *hOGG1* mutant genotype is associated with prostate cancer susceptibility and aggressive clinicopathological characteristics in the Korean population

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**Background:** The gene encoding human 8-oxoguanine glycosylase 1 (*hOGG1*) is involved in DNA base excision repair from oxidatively damaged DNA. A case-control study was conducted to evaluate the correlation between the susceptibility and clinicopathological outcomes of prostate cancer (CaP) and *hOGG1* genotype.

**Patients and methods:** Subjects were recruited from 266 CaP patients and 266 age-matched benign prostatic hyperplasia patients. The *hOGG1* codon 326 genotype was determined by peptide nucleic acid-mediated PCR clamping and compared with Gleason score and tumor stage.

**Results:** The Cys allele at codon 326 of *hOGG1* was associated with an increased risk of CaP in comparison with the Ser allele (*P* = 0.005). Gleason scores of 8 or higher were observed more often in patients with the mutant genotypes Ser/Cys and Cys/Cys than in those with a wild-type genotype (*P* = 0.045), and the Cys/Cys homozygous genotype was associated with a significantly higher risk of metastatic disease in comparison with the Ser/Ser genotype (*P* = 0.017).

**Conclusions:** These results suggest that *hOGG1* is associated with the susceptibility to CaP and its aggressive clinicopathological characteristics.

**Key words:** *hOGG1*, prostatic neoplasms, single nucleotide polymorphism
introduction

Prostate cancer (CaP) is the most common malignancy among men in developed countries with an estimated 190,000 new cases diagnosed each year in Europe and the United States [1, 2]. Although the etiology and pathogenesis of CaP have not been identified clearly, genetic and environmental factors are associated with the risk of developing this type of malignancy [3]. Ethnic differences in the incidence of CaP are well established, and current data indicate a lower incidence of the disease among Asians compared with white and black men. Studies have shown that CaP incidence rates in the immigrant population are higher than those of the same population living in their homeland, which could be explained by the influence of environmental factors [4–6]. However, incidence rates are lower among immigrants than residents, even among USA-born Asian men, and African Americans have a 1.6 times greater risk of developing CaP than Caucasian Americans, indicating the important role of genetics in CaP risk [7]. The results of several studies have suggested that single nucleotide polymorphisms (SNPs) may determine the differences in the risk of CaP between ethnic groups [8, 9].

DNA damage can occur by various mechanisms, including the effect of byproducts of endogenous normal metabolism or the exposure to environmental mutagens, and damaged DNA, which is associated with carcinogenesis, is removed or repaired through different repair systems. Reactive oxygen species are one of the most common byproducts responsible for the induction of base modifications and single strand breaks [10]. Among known oxygen radicals, 8-hydroxy(oxo)-7,8-dihydrodeoxy(d)-guanine(G) (8-oxo-dG) is highly mutagenic and a major form of oxidative DNA damage [11]. Human 8-oxoguanine glycosylase 1 (hOGG1) is a multifunctional DNA glycosylase that performs the initial step of recognizing the 8-oxo-dG damage [12]. Previous reports have identified several SNPs at codon 326 in exon 7 of hOOG1 and, although this point is still controversial, the catalytic activity of the hOGG1 protein encoded by the wild-type Ser allele is higher than that of the variant type Cys allele [13]. An association between genetic polymorphisms in the hOGG1 gene and the incidence and clinical outcomes of various malignancies, such as lung, bladder and gastric cancer, has been reported [14–16]. Because these organs are characterized by a high risk of exposure to environmental mutagens, these findings imply that the activity of the DNA repair system could play an important role in the prevention of carcinogenesis. Infection or inflammation of the prostate gland draws interest as a mutagenic source of carcinogenesis [17]. Therefore, SNPs in the hOGG1 gene could be an important factor in the pathogenesis of CaP. Several epidemiologic studies have suggested the existence of an association between hOGG1 genotypes and the risk of CaP, but the results reported have been controversial [18–22]. Moreover, previous studies were based exclusively on Western populations, in which the distribution of the hOGG1 genotype differs from that in Asian men.

In the present report, a case–control study was designed to investigate the effect of hOGG1 genotypes on the risk of CaP and on the clinicopathological characteristics of CaP in Korean men using a benign prostatic hyperplasia (BPH) group as a control and peptide nucleic acid (PNA)-mediated PCR clamping to enhance the sensitivity of the system.

patients and methods

study population

A case–control study was conducted including 266 cases with newly diagnosed CaP and 266 controls among age-matched BPH patients. Cases were recruited from the patients with histologically confirmed primary adenocarcinoma of the prostate at our institution. Controls were selected from the database of BPH patients who underwent transurethral resection of prostate (TURP), and one-to-one matched with similar age and the closest date of blood sampling according to those of cases. Controls with serum PSA levels >3 ng/ml underwent a transrectal prostate biopsy before TURP to rule out the presence of cancer, and those with PSA levels >10 ng/ml were excluded from the study to rule out the possibility of prostate cancer. The Ethics Committee of Chungbuk National University approved this protocol, and written informed consent was obtained from each subject. Collection and analysis of all samples was approved by the Institutional Review Board of Chungbuk National University. Subjects with testosterone levels <1 ng/ml with a suspicious history of previous management for CaP or incomplete medical records were excluded from the study.

Gleason grade and TNM (tumor–node–metastasis) 2002 stage were used as prognostic factors. Gleason grade was measured from specimens of 6- or 12-core transrectal biopsies, TURP or radical prostatectomy. Tumor stage was estimated from the specimens of the radical prostatectomy, or from the computed tomography, magnetic resonance imaging or bone scan results.

genomic DNA extraction

Genomic DNA was extracted from human whole blood using a genomic DNA purification kit (Promega, Madison, WI). DNA was precipitated using isopropanol and then washed with 70% ethanol. The quality of the DNA was assessed by agarose gel electrophoresis. Genomic DNA samples were stored at −20°C until use.

PNA-mediated real-time PCR clamping method

All PNA oligomers were synthesized and purified using high performance liquid chromatography (Panagene, Daejeon, Korea). A specific 120 base-pair genomic fragment from hOGG1 exon 7 was amplified using the set of primers listed in Table 1. A Rotor-Gene 6000 (Corbett Research, Mortlake, Australia) was used for real-time PCR. PCR reactions were carried out in a final volume of 20 μl containing 10 μl of 2X SYBR GREEN master mix (TAKARA Bio Inc., Otsu, Japan), 1 μl of each of the forward and reverse primers (10 pmol/μl) and 8 μl of genomic DNA (2.5 ng/μl). To identify the codon 326 genotype, samples were analyzed without PNA probes and with 0.1 μM of Ser- or Cys-specific PNA probes (three different reactions for each sample). PCR thermal cycling parameters were as follows: one cycle at 95°C for 5 min followed by 45 cycles of 30 s at 95°C, 20 s at 70°C, 30 s at 63°C and 30 s at 72°C. Two control samples with known codon 326 genotypes (either Ser or Cys) were used to calculate the difference in the threshold cycle (ΔCt) for each reaction. Melting curve analysis was carried out by raising the temperature from 72°C to 99°C.

Table 1. DNA sequences of primers and PNA probes

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOGG1-F</td>
<td>TCAGTGGCCGACCTGGCGCAA</td>
</tr>
<tr>
<td>hOGG1-R</td>
<td>AGGTGCTTTGGGAGATTTTC</td>
</tr>
<tr>
<td>Ser PNA probe</td>
<td>TGGCGCAATCCCGCCATG</td>
</tr>
<tr>
<td>Cys PNA probe</td>
<td>TGGCGCAATCCCGCCATG</td>
</tr>
</tbody>
</table>

PNA, peptide nucleic acid; hOGG1, human 8-oxoguanine glycosylase 1; F, forward; R, reverse; Ser, serine; Cys, cysteine.
using a transition rate of 1°C per second. Amplified products were purified using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and quantified using a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). The sequences of the amplified hOGG1 products were then confirmed.

Ct values were obtained from all amplified products and nucleotide sequences were determined for samples with Act values >2 compared with the control (no PNA probe) Ct value. A PNA probe complementary to the wild-type sequence hybridizes specifically with the wild-type sequence and blocks amplification, while amplification of the variant sequence, which is an imperfect match to the PNA probe, proceeds. A single base mismatch is sufficient to cause differential amplification of mutant and wild-type sequences. Thus, the sample was designated as a 'Cys' genotype if its Ct value was higher than the Ser control sample when using the Cys PNA probe, and a 'Ser' genotype if the Ct value was higher than the Cys control sample when using the Ser PNA probe.

**Statistical analysis**

The chi-square test was used to detect genotype distribution deviations from the Hardy–Weinberg equilibrium. Clinical variables such as age, PSA, prostate size and BMI in patient and control groups were compared using the Student’s t-test. In the evaluation of CaP risk, Gleason scores were classified into ≤7 and >7, and the clinical stage was divided into ≤T4 and metastatic disease (any nodal or distant metastasis). Logistic regression models were used to estimate the odds ratio (OR) with the corresponding 95% confidence interval (CI). In the regression models, individuals who were homozygous for the wild-type allele were regarded as the reference group for the calculation of ORs for subjects heterozygous or homozygous for the variant allele. Statistical analysis was carried out using SPSS 12.0 software (SPSS Inc., Chicago, IL), and P < 0.05 was considered statistically significant.

**Results**

**Baseline characteristics**

Table 2 shows the baseline characteristics of the 266 CaP patients and 266 BPH controls included in the study. The prevalence of hOGG1 codon 326 polymorphism in prostate cancer cases and controls followed the Hardy–Weinberg equilibrium (P = 0.163 and P = 0.351, respectively). The mean age of CaP patients was 68.3 years (range 49–91) and that of the BPH controls was 68.9 years (range 46–90). The serum PSA level was higher in CaP patients than in BPH cases (132.1 ± 668.3 versus 3.0 ± 2.3; P = 0.002), and the size of the prostate was smaller in CaP patients than in BPH cases (37.9 ± 20.3 versus 42.6 ± 22.8; P = 0.013). There were no significant differences between cases and controls regarding age and BMI (P = 0.352 and P = 0.526, respectively). Of the 266 CaP patients, 170 (63.9%) cases underwent radical prostatectomy using open or laparoscopic procedure. Androgen blockage and radiation therapy were carried out in 92 (34.6%) and 30 (11.3%) patients, respectively. Thirty-two (12.0%) patients did not receive any treatment due to poor general condition or refusing further management. The number of subjects showing a Gleason score ≤7 and >7 was 125 (47.0%) and 141 (53.0%), respectively. Patients with a ≤T4 stage and metastatic disease were 167 (62.8%) and 99 (37.2%), respectively.

**Distribution of the hOGG1 genotype between controls and patients**

The hOGG1 genotype distribution for all patients is shown in Table 3. The distributions of Ser/Ser, Ser/Cys and Cys/Cys were 25.6%, 49.2% and 25.2% in BPH controls and 20.3%, 44.7% and 35.0% in CaP patients, respectively. The results of logistic regression analysis indicated that the risk of CaP was higher in Cys/Cys than in Ser/Ser homozygotes (OR = 1.81, 95% CI 1.12–2.91, P = 0.015) and in subjects with the 326Cys allele than in those with the 326Ser allele (OR = 1.35, 95% CI 1.10–1.67, P = 0.005).

**hOGG1 genotype and Gleason score in CaP patients**

Patients with a mutant genotype (Ser/Cys or Cys/Cys) showed a higher risk CaP with a Gleason score >7 compared with the wild-type genotype (OR = 1.86, 95% CI 1.01–3.42, P = 0.045) (Table 4).

**hOGG1 genotype and clinical stage in CaP patients**

When the Ser/Ser genotype was used as the reference group, Cys/Cys and the variant genotypes (Ser/Cys + Cys/Cys) had a significant risk of metastatic disease (OR = 2.49, 95% CI 1.18–5.24, P = 0.017 and OR = 2.15, 95% CI 1.09–4.26, P = 0.027, respectively) (Table 5).

**Discussion**

The current study reveals the association between the hOGG1 genotype and CaP susceptibility and the manifestation of clinicopathological characteristics in Korean men. Men carrying the Cys allele in codon 326 of hOGG1 have an increased risk of CaP in comparison with those carrying the Ser allele. Moreover, CaP patients who have a variant hOGG1 326Cys allele show an increased risk of a high-grade Gleason score and metastasis compared with those with the 326Ser allele.

Reports of the association between variant alleles such as Ser/Cys and Cys/Cys with the susceptibility to CaP have been conflicting. Chen et al. [19] suggested that subjects possessing the hOGG1 Cys allele had a significantly increased risk of CaP in
Table 3. *hOGG1* genotypes in patients with prostate cancer and controls

<table>
<thead>
<tr>
<th><em>hOGG1</em> genotype</th>
<th>No. of controls (%)</th>
<th>No. of cases (%)</th>
<th>OR (95% CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser326Ser</td>
<td>68 (25.6)</td>
<td>54 (20.3)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Ser326Cys</td>
<td>131 (49.2)</td>
<td>119 (44.7)</td>
<td>1.18 (0.77–1.83)</td>
<td>0.450</td>
</tr>
<tr>
<td>Cys326Cys</td>
<td>67 (25.2)</td>
<td>93 (35.0)</td>
<td>1.81 (1.12–2.91)</td>
<td>0.015</td>
</tr>
<tr>
<td>Ser326Cys + Cys326Cys</td>
<td>187 (70.3)</td>
<td>212 (79.7)</td>
<td>1.39 (0.93–2.09)</td>
<td>0.110</td>
</tr>
<tr>
<td>Ser allele</td>
<td>267 (50.2)</td>
<td>227 (42.7)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Cys allele</td>
<td>265 (49.8)</td>
<td>305 (57.3)</td>
<td>1.35 (1.10–1.67)</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*Logistic regression models were used to estimate OR with the corresponding 95% CI.*

*Reference.*

*hOGG1*, human 8-oxoguanine glycosylase 1; OR, odds ratio; CI, confidence interval; Ser, serine; Cys, cysteine.

Table 4. *hOGG1* genotypes and Gleason score in prostate cancer cases

<table>
<thead>
<tr>
<th><em>hOGG1</em> genotype</th>
<th>Gleason score</th>
<th>OR (95% CI)*</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser326Ser</td>
<td>32 (25.6)</td>
<td>22 (15.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>Ser326Cys</td>
<td>53 (42.4)</td>
<td>66 (46.8)</td>
<td>1.81 (0.94–3.48)</td>
</tr>
<tr>
<td>Cys326Cys</td>
<td>40 (32.0)</td>
<td>53 (37.6)</td>
<td>1.93 (0.98–3.81)</td>
</tr>
<tr>
<td>Ser326Cys + Cys326Cys</td>
<td>93 (74.4)</td>
<td>119 (84.4)</td>
<td>1.86 (1.01–3.42)</td>
</tr>
<tr>
<td>Ser allele</td>
<td>117 (46.8)</td>
<td>110 (39.0)</td>
<td>1.00</td>
</tr>
<tr>
<td>Cys allele</td>
<td>133 (53.2)</td>
<td>172 (61.0)</td>
<td>1.38 (0.97–1.94)</td>
</tr>
</tbody>
</table>

*Logistic regression models were used to estimate OR with the corresponding 95% CI.*

*Reference.*

*hOGG1*, human 8-oxoguanine glycosylase 1; OR, odds ratio; CI, confidence interval; Ser, serine; Cys, cysteine.

The results of the present study show an association between the Cys allele of *hOGG1* and high Gleason scores and metastatic stage. To date, there is only one published report on the relationship between *hOGG1* genotype and clinicopathological outcomes. Nock et al. [20] conducted a family-based case-control study (439 CaP cases, 479 brother controls) and suggested that the *hOGG1* 326 Cys/Cys genotype was inversely associated with disease in men with more aggressive CaP (Gleason score ≥7 or clinical tumor stage ≥T2C). Although the findings of this study were in disagreement with our results, the two studies included different populations with different characteristics. In the previous study, the proportion of cases with Gleason scores ≥7 and clinical tumor stage ≥T2c were 44% and 13%, respectively. However, the cases included in the current study had a more aggressive disease, with 53.0% of the cases showing Gleason scores ≥8 and 37.2% with a metastatic stage. In addition to the differences in the distribution of genotypes and cancer risk not only for CaP but also for bladder and lung cancer [14, 23–25]. Although the basis for these inconsistencies in reported data could not be explained, the present study differs from previous studies in several aspects. First, the present study included only Korean subjects, while previous studies addressing the *hOGG1* genotype in CaP were done exclusively with Western men. The distribution of the *hOGG1* codon 326 genotype was significantly different in Western and Asian individuals. Previous studies carried out with a Western population reported frequencies of the Ser/Ser, Ser/Cys and Cys/Cys genotypes of 60%, 30%–40% and 6%–21%, respectively, in the control group [20, 22]. The frequency of Ser/Ser in the Asian population was only 20%–30%, and the Ser/Cys and Cys/Cys types were found at frequencies of 40%–50% and 20%–30%, respectively [24–26]. Differences in the distribution of dominant *hOGG1* genotypes and the incidence of CaP between ethnicities could explain the discrepancies in the results of SNP studies of CaP. Second, different mutagens or carcinogens affect different organ-specific cancers. Smoking is an important risk factor for bladder cancer because its metabolites and chemicals directly attack the urothelium of the urinary bladder [27]. The correlation between prostate gland infection or inflammation and the development of CaP is possibly due to the associated release of mutagens [17]. Third, the present study used a PNA-mediated real-time PCR clamping method as a novel tool for the determination of genotype, which had a better detection rate and gave more accurate results than restriction fragment length polymorphism [28].
hOGG1 genotypes, the differences in the CaP characteristics between Western and Asian men could be associated with the discrepancies in the results of the two studies.

The present study has several limitations. First, the control group is not representative of the normal population because the controls were recruited from BPH patients who underwent TURP. However, the distribution of hOGG1 genotypes of the controls was similar to that found in previous studies carried out with Asian populations and followed the Hardy–Weinberg equilibrium, suggesting that selection bias may not be a major concern. Second, functional studies were not carried out and the activity of hOGG1 was therefore not evaluated in correlation to genotype in CaP tissue. To validate the findings of the current study, the enzymatic activity of hOGG1 in cancer should be assessed in the future.

In summary, the present study provides evidence of the association between the genotype of hOGG1 codon 326 and the risk and clinicopathological outcomes of CaP. Men carrying the Cys allele of hOGG1 showed increased susceptibility to CaP and poorer grade and stage of the disease. The findings of the present study are valuable to improve our understanding of the role of hOGG1 in CaP.

acknowledgements

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funding

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disclosure

The author declare no conflict of interest.

references