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Original Research

# Differences in efficacy and toxicity in relation to genetic polymorphisms of the cytochrome P450 2C8 gene after chemotherapy in epithelial ovarian cancer

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**Summary**

*Purpose of investigation:* To survey genetic polymorphisms in the promoter region of the cytochrome P450 2C8 family gene (CYP2C8) in patients with epithelial ovarian cancer, and to determine whether the frequency of genetic polymorphisms and haplotypes in CYP2C8 are associated with efficacy and toxicity of anticancer drugs. *Materials and Methods:* The authors enrolled 43 patients diagnosed with epithelial ovarian cancer. They performed direct sequencing PCR using specific primers to detect single nucleotide polymorphisms (SNPs). They analyzed the efficacy and toxicity of chemotherapy in relation to SNP allele and haplotype patterns in patients. *Results:* The mutant alleles CYP2C8*1D (-411T>C), CYP2C8*1C (-370T>G), and CYP2C8*1B (-271C>A) were found in the patient group, with an allele frequency of 0.37, 0.32, and 0.09, respectively. Of the 35 patients with advanced epithelial ovarian cancer, the CYP2C8*1D mutation group had a significantly shorter disease-free interval after treatment ($p = 0.020$). *Conclusions:* CYP2C8*1D mutation group had poorer prognosis and earlier onset of neurotoxicity.

*Key words:* CYP2C8; Genetic polymorphism; Ovarian cancer.

## Introduction

Advanced epithelial ovarian cancer is associated with a severely poor prognosis, and research efforts continue to identify measures to improve survival. Postoperative paclitaxel and platinum combination chemotherapy is the current gold standard for treating epithelial ovarian cancer. Recently, advances in genetics and proteomics have led to rigorous attempts to shed light on individual differences in the diagnosis and treatment of disease and to personalize treatment regimens. Such attempts stem from the fact that there is inter-individual variability towards drug response that may be because of genetic or protein differences among individuals. Although several factors contribute to inter-individual variability towards drug toxicity and efficacy, it is believed that genetic polymorphisms, which have decisive roles in the transcription of enzymes involved in drug metabolism, are a major contributing factor. In particular, Nebert and Gonzalez [1] argued that genetic polymorphisms of genes from the cytochrome P450 (CYP) family, which is an important enzymatic family for drug metabolism, are one of the key factors that lead to variations in the toxicity and efficacy of a drug. Isoenzymes of CYP are the major enzymes involved in drug metabolism, and have central roles in the fate of drugs within the body [2]. Most clinically prescribed drugs are metabolized by isoenzymes in the CYP3A, CYP2D6, or CYP2C subfamily. Researchers have continued efforts to investigate interracial and inter-individual differences in genetic variability in the genes that encode these CYP isoenzymes, with specific efforts to determine the phenotypes and pharmacokinetics associated with single nucleotide polymorphisms (SNPs) of CYP2C9 and CYP2C19 [3-6].

The structure of CYP2C8, which encodes an isoenzyme of the CYP2C subfamily, has been recently reported that has enabled research in determining the phenotypes and pharmacokinetics related to genetic variations at this locus. CYP2C8 is an important enzyme for the metabolism of a number of therapeutic drugs, including carbamazepine, diazepam, diclofenac, ibuprofen, mephobarbital, naproxen, omeprazole, tolbutamide, s-warfarin, and paclitaxel, and several in vitro and in vivo studies have found that the enzyme mainly acts in the metabolic pathway of these drugs [5, 6]. Furthermore, it has been detected that CYP2C8 metabolizes arachidonic acid to epoxyeicosatrienoic acid, which is an endogenous bioactive substance, in the liver and kidneys [7].

Paclitaxel is metabolized to 6α-OH-paclitaxel (85%) by CYP2C8, and to 3-p-OH-paclitaxel (15%) by CYP3A4. The metabolite, 6α-OH-paclitaxel, has been associated with

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an up to 30-fold reduction in toxicity activity, which has prompted researchers to shift their interest from pharmacological associations between drugs and CYPs to investigating differences in cytotoxicity and the effects of drugs in relation to genetic polymorphisms in CYPs [4, 8]. A study in gynecological oncology found an increased prevalence of breast cancer and ovarian cancer in patients that have the CYP1A1 allele, which is involved in the metabolism of estrogen [9]. Variant alleles of CYP2C8 include CYP2C8*2 (805A>T, I269F) and CYP2C8*3 (416G>A, R139K; 1196A>G, K399R), which occur in Caucasians at allele frequencies of 0.18 and 0.02, and of 0 and 0.13, respectively. In particular, activity of the enzymes encoded by the CYP2C8*2 and CYP2C8*3 alleles to metabolize paclitaxel to 6α-OH-paclitaxel in vitro were reduced by 50% and 100% respectively, however these variants have not been reported in Asians [8].

A recent phenotypic and pharmacokinetic study of CYP2C8 haplotypes in healthy Korean adults found 11 SNPs in CYP2C8. Of these, CYP2C8*1B (-271C>A), CYP2C8*1C (-370T>G), and CYP2C8*1D (-411T>C), all of which have allele frequency of ≥ 0.1, were found in the 5′-flanking region of CYP2C8, with evidence of strong linkage. Furthermore, IVS2-13insT was identified as a common intronic polymorphism, but was a synonymous mutation, and therefore, was not predicted alter the amino acid sequence [10].

In the present study, the authors examined common genetic polymorphisms in CYP2C8 in Korean patients with epithelial ovarian cancer to determine whether genetic polymorphisms at this locus are associated with either therapeutic efficacy or toxicity following postoperative paclitaxel chemotherapy.

## Materials and Methods

Patients diagnosed with epithelial ovarian cancer at the Obstetrics and Gynecology Department of the University Medical Center between March 2010 and June 2016 were enrolled in this study. Written informed consent was obtained from each patient using consent forms and protocols approved by the Review Board for Human Research of Inje Medical Center. Patients who received neoadjuvant chemotherapy before cytoreductive surgery and patients with did not undergo at least six cycles of adjuvant chemotherapy were excluded. Patients with concurrent endometrial cancer found after surgery were also excluded. Finally, 43 patients were enrolled in this study. DNA was extracted from peripheral blood samples to genotype SNPs in the promoter region of CYP2C8. In addition, to identify differences in the expression of SNPs and haplotypes in the 5′-flanking region of CYP2C8, the authors specifically examined 35 patients in the International Federation of Gynecology and Obstetrics (FIGO) Stage III and IV who had received cytoreductive surgery and had undergone at least six cycles of paclitaxel/carboplatin chemotherapy after being diagnosed with epithelial cancer (advanced cancer group). Furthermore, the authors analyzed the efficacy, toxicity, and clinical presentation of chemotherapy in relation to differences in SNP expression and haplotype patterns. National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) grading scale was a method used to evaluate neurotoxicity. For analysis of differences of hematological toxicity in SNPs, patients who had clinically developed grade 4 neutropenia within the third cycle were assigned to the toxicity group. Disease free interval was calculated from the time of surgery to the time of recurrence.

Peripheral blood samples from patients were obtained and stored with ethylenediaminetetraacetic acid (EDTA) in a freezer at -20°C. Genomic DNA was isolated with a blood kit and underwent polymerase chain reaction (PCR) sequencing, followed by PCR sample purification using a PCR kit.

To detect the three most frequent SNPs in the 5′-flanking region of CYP2C8 in a Korean cohort, the authors specially designed and used the following primers: (F) 5′-CAC TAT TTC ATG TTT AGG CAG-3′ and (R) 5′-TTG GCT GGA GGA ACA TAA G-3′ (Table 1).

The reaction mixture (24 μL) for PCR sequencing consisted of 1 μL each of forward and reverse primers (10 pmol/μL), 3 μL of dNTPs (2.5 mM each), 0.5 μL of SP-Taq DNA polymerase (2.5 U/μL), 2.5 μL of 10 × SP-Taq buffer, and 16 μL of distilled water ($dH_2O$). PCR involved 30 cycles with an initial denaturation for two minutes at 94°C, followed by denaturation at 94°C for one minute, annealing at 63°C for one minute, and extension at 72°C for one minute, with a final elongation for 10 minutes at 72°C. Following PCR, a 1 μL sample was used for 1% agarose gel electrophoresis to confirm the PCR product (Figure 1).

PCR purification of amplicons was performed with the LaboPass PCR kit. First, the PCR product was suspended in PBS buffer (5 × the sample volume), transferred to a spin column, and centrifuged for one minute. The supernatant was removed, 700 μL of NW buffer (provided from the kit) was added, and the mixture was centrifuged for one minute. The supernatant was removed and 300 μL of NW buffer was added followed by centrifugation for two minutes. The spin column was transferred to a new 1.5 mL tube and, after adding 30 μL of dH2O to the center of the column and incubating for one min at room temperature, the mixture was centrifuged a final time for one minute and the eluate was collected.

The sequence of PCR products was verified via automated sequencing (Figure 2). Three SNPs in CYP2C8 were confirmed via direct sequencing using 5′-flanking region-specific primers. Genotyping and haplotyping were performed through individual variations and groupings.

All statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) version 20.0. Age, chemotherapy toxicity, and CA-125 values for each haplotype group were compared using the Mann-Whitney test. Genotype and allele frequencies were analyzed with the Chi-square test and Fisher's exact test, and disease-free intervals were compared with the Kaplan-Meier test. Values of $p < 0.05$ were considered significant.

## Results

The mean age of the patients with epithelial ovarian cancer tested in this study was 57.6±13.4 years with a mean CA-125 of 1321.7 ± 410.1 IU/mL. The mean age of the patients in the advanced cancer group (advanced ovarian carcinoma; n = 35) was 59.1 ± 11.1 years with a mean CA-125

Table 1. — *Sequences of primers used in the analysis of CYP2C8 polymorphisms.*

| Primers | |
|---|---|
| *CYP2C8*-seq1F (Forward) | 5'-CAC TAT TTC ATG TTT AGG CAG-3' |
| *CYP2C8*-seq1B (Reverse) | 5'-TTG GCT GGA GGA ACA TAA G-3' |

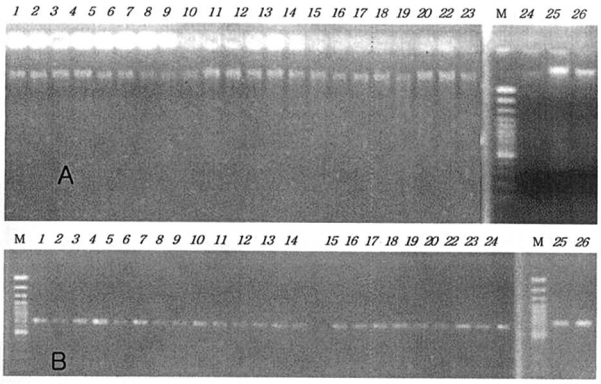


Figure 1. — Two-dimensional gel electrophoresis of (A) genomic DNA from patients with epithelial ovarian cancer or (B) corresponding PCR products after sequencing. Band size is 730 bp.

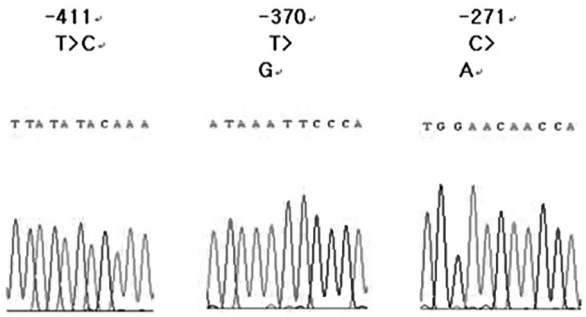


Figure 2. — Common CYP2C8 SNPs in Koreans confirmed by automated sequencing of PCR product.

of 1548.3 ± 563.7 IU/mL. There were no significant differences observed between the two groups (Table 2).

Three SNPs were genotyped in the 5′-flanking region of CYP2C8 (760 bp) in 43 patients using direct sequencing (Figure 3), of which -271C>A and -370T>G were identified as CYP2C8*1B and CTP2C8*1C, respectively, in the Human Cytochrome P450 (CYP) Allele Nomenclature Database (http://www.cypalleles.ki.se/) [11] and -411T>C was previously identified as CYP2C8*1D by Cho *et al.* [10]. The mutant allele frequencies of these three SNPs in this cohort of the 43 patients with ovarian cancer were 0.37 for CYP2C8*1D (-411T>C), 0.32 for CYP2C8*1C

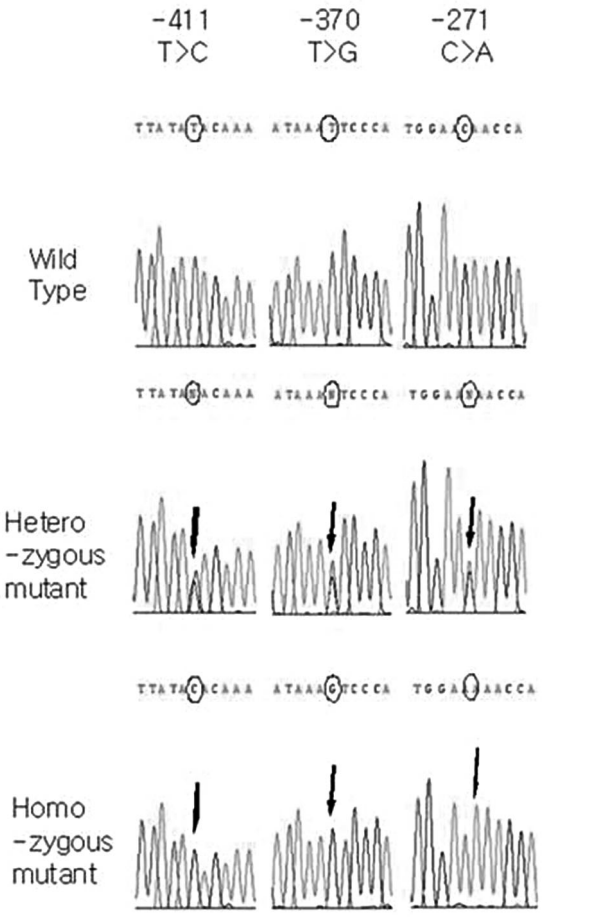


Figure 3. — Electropherograms of three SNPs in the 5′-flanking region of CYP2C8 genotyped in this cohort using direct sequencing.

Table 2. — *Patient characteristics.*

| | *Total (n=43)* | *Study group* (n=35)* |
|---|---|---|
| Age (years) | 57.6±13.4 | 59.1±11.1 |
| Height (cm) | 143±6.1 | 154.3±6.1 |
| Weight (kg) | 55.9±10.1 | 55.6±10.1 |
| FIGO Stage | | |
| I | 5 | |
| II | 3 | |
| III | 30 | 30 |
| IV | 5 | 5 |
| Pathology | | |
| Serous | 33 | 27 |
| Mucinous | 3 | 2 |
| Endometrioid | 3 | 3 |
| Clear | 3 | 3 |
| Mixed | 1 | |
| CA125 (IU/ml) | 1321.7±410.1 | 1548.3±563.7 |

**FIGO Stages III, IV, epithelial ovarian cancer with cytoreductive surgery*

(-370T>G), and 0.09 for CYP2C8*1B (-271C>A), which were not significantly different compared to those found in

Table 3. — *Frequencies of CYP2C8 SNPs in:*
*a) epithelial ovarian cancer patients.*

| Location | Nucleic acid substitution | Nucleotide sequence | Genotype (*n*=43) | | | Allele frequencies | |
|---|---|---|---|---|---|---|---|
| | | | w/w | w/m | m/m | w* | m* |
| Promoter | -411 T→C | TTATA(T/C)ACAAA | 16 | 22 | 5 | 0.63 | 0.37 |
| | -370 T→G | ATAAA(T/G)TCCCA | 19 | 21 | 3 | 0.68 | 0.32 |
| | -271 C→A | TGGAA(C/A)AACCA | 37 | 5 | 1 | 0.91 | 0.09 |

** Nucleotide numbering begins from the translation start site of the CYP2C8 gene, * w: wild type, m: mutation type, w/w: homogenous wild type, w/m: heterogeneous mutation, m/m: homogenous mutation.*

*b) healthy group vs. ovarian cancer group.*

| Nucleic acid substitution | Healthy group* (n=306) | | Ovarian cancer group (n=43) | | p-value |
|---|---|---|---|---|---|
| | w | m | w | m | |
| -411 T→C | 0.617 | 0.382 | 0.63 | 0.37 | NS |
| -370 T→G | 0.670 | 0.330 | 0.68 | 0.32 | NS |
| -271 C→A | 0.884 | 0.116 | 0.91 | 0.09 | NS |

** Healthy group data[10]*

*c) FIGO Stages III and IV ovarian cancer groups.*

| Location | Nucleic acid substitution | Nucleotide sequence | Genotype (*n*=35) | | | Allele frequencies | |
|---|---|---|---|---|---|---|---|
| | | | w/w | w/m | m/m | w* | m* |
| Promoter | -411 T→C | TTATA(T/C)ACAAA | 13 | 18 | 4 | 0.62 | 0.38 |
| | -370 T→G | ATAAA(T/G)TCCCA | 15 | 18 | 2 | 0.68 | 0.32 |
| | -271 C→A | TGGAA(C/A)AACCA | 30 | 4 | 1 | 0.91 | 0.09 |

** w: wild type, m: mutation type*

a previously reported cohort (*n* = 306) of normal Korean adults (Table 3). In addition, from the present analysis using FIGO Stage, the authors found no evidence of an association between these SNPs and patients with Stage III or IV ovarian cancer (Table 3).

Six haplotypes were derived from the nucleotide positions of the tested polymorphisms (-411, -370, and -271 bp from the transcription start site) (Table 4). The authors found that four haplotype pairs contained the most prevalent haplotypes observed in this cohort. The -411| -370 heterotype (H1 + H2) and the -411 heterotype (H1 + H3) were the most prevalent, with a frequency of 0.13 in this study group, followed by the -370 heterotype (H1 + H4) with a frequency of 0.07, and -370| -271 heterotype (H1 + H6), which had a frequency of 0.05 (Table 5).

For this investigation in chemotherapy toxicity, patients who had clinically developed grade 4 neutropenia within the third cycle were assigned to the toxicity group. The authors found that hematological toxicities were significantly less prevalent in patients with the CYP2C8*1C allele ($p$ = 0.038) and more prevalent in patients carrying the CYP2C8*1D allele (p = 0.014) (Table 6).

The disease-free interval for the CYP2C8*1D mutation group was significantly shorter (22.8 ± 4.5 months) compared to that found in the wild-type group (46.0 ± 8.0 months) ($p$ = 0.02) (Figure 4). In contrast, the authors found no statistically significant difference ($p$ = 0.385) in the disease-free interval for patients with the CYP2C8*1C mutation (38.3 ± 9.1 months) and those with the normal allele (22.8±6.3 months) (Figure 5).

The disease free interval between CYP2C8*1C and CYP2C8*1B mutation group was longer (32.2 ± 9.1 months) compared to wild type group (23.2 ± 3.3 months), but it had no statistical significance ($p$ = 0.582) (Figure 6).

Table 4. — *Frequencies of CYP2C8 SNP haplotypes in the study group.*

| Haplotype | Nucleotide position* | | | *n* | Frequency |
|---|---|---|---|---|---|
| | -411 | -370 | -271 | | |
| H1 | T | T | C | 31 | 0.44 |
| H2 | C | G | C | 9 | 0.13 |
| H3 | C | T | C | 15 | 0.21 |
| H4 | T | G | C | 9 | 0.13 |
| H5 | T | T | A | 2 | 0.03 |
| H6 | T | G | A | 4 | 0.06 |
| | | | | 70 | 1.00 |

** Nucleotide numbering begins from the translation start site of the CYP2C8 gene.*

Table 5. — *Common haplotype pairs for the CYP2C8 SNP in the study group.*

| Haplotype pair | *n* | Freqency | -411 | -370 | -271 | |
|---|---|---|---|---|---|---|
| H1+H2 | 9 | 0.13 | TC | TG | CC | -411,-370 hetero |
| H1+H3 | 9 | 0.13 | TC | TT | CC | -411, hetero |
| H1+H4 | 5 | 0.07 | TT | TG | CC | -370 hetero |
| H1+H6 | 4 | 0.05 | TT | TG | CA | -370,-271 hetero |

## Discussion

Approximately 75% of all cases of epithelial ovarian cancer are discovered at advanced stages because the disease is generally asymptomatic in earlier stages. Prognosis de-

Table 6. — *Differences in hematologic toxicity* and characteristics between SNP groups.*

*a) SNP group CYP2C8*1D (-411 T→C).*

| | Mutation(*n*=21) | Wild (*n*=14) | *p* value |
|---|---|---|---|
| Age (years) | 59.7±10.3 | 59.4±12.8 | 0.23 |
| Height (cm) | 153.4±6.5 | 153.4±6.0 | 0.60 |
| Weight (kg) | 57.0±11.1 | 53.3±8.1 | 0.28 |
| Hematologic toxicity | 16 (76.2%) | 4 (30.8%) | **0.014** |
| CA-125 (IU/ml) | 1545.1±82.7 | 1545.9±741.3 | 0.93 |

*b) SNP group CYP2C8*1C (-370 T→G).*

| | Mutation(*n*=19) | Wild (*n*=16) | *p* value |
|---|---|---|---|
| Age (years) | 59.5±12.2 | 59.8±10.1 | 0.79 |
| Height (cm) | 154.5±5.4 | 153.9±7.4 | 0.94 |
| Weight (kg) | 57.3±10.6 | 53.5±9.3 | 0.28 |
| Hematologic toxicity | 8 (42.1%) | 12 (80.0%) | **0.038** |
| CA-125 (IU/ml) | 1284.0±1084.3 | 2261.9±609.1 | 0.20 |

*c) SNP group CYP2C8*1B (-271 C→A).*

| | mutation(*n*=5) | wild (*n*=30) | *p* value |
|---|---|---|---|
| Age (years) | 56.4±14.0 | 60.2±10.7 | 0.47 |
| Height (cm) | 152.4±5.9 | 154.6±6.3 | 0.49 |
| Weight (kg) | 50.8±6.0 | 56.5±10.5 | 0.25 |
| Hematologic toxicity | 2 (40.0%) | 18 (62.1%) | 0.62 |
| CA-125 (IU/ml) | 192.8±44.9 | 1656.8±2636.5 | 0.44 |

*d) SNP group -370 T→G with -271 C→A.*

| | Mutation(*n*=20) | wild (*n*=15) | *p* value |
|---|---|---|---|
| Age (years) | 58.9±12.1 | 60.9±9.9 | 0.59 |
| Height (cm) | 154.8±5.3 | 153.6±7.5 | 0.64 |
| Weight (kg) | 56.7±10.7 | 54.1±9.3 | 0.48 |
| Hematologic toxicity | 9 (45.0%) | 11 (78.6%) | **0.07** |
| CA-125 (IU/ml) | 977.5±1054.3 | 2261.9±609.9 | 0.20 |

**Grade 4 hematologic toxicity within 3rd cycle chemotherapy*

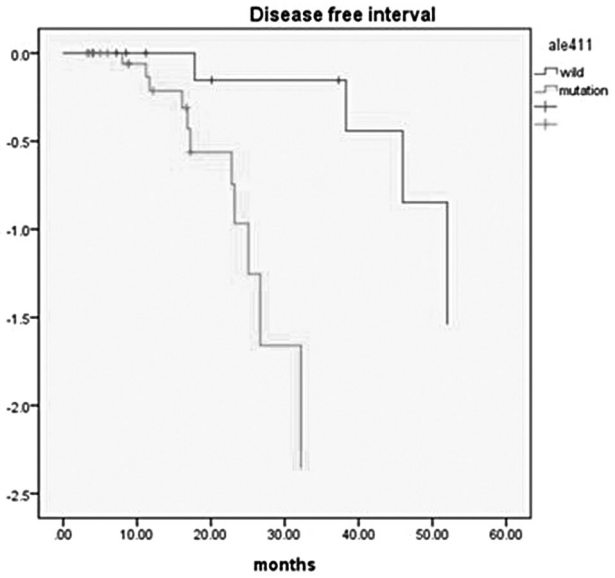


Figure 4. — Disease-free interval between the -411 mutation group and the -411 wild-type group with medians of 22.8 ± 4.5 months and 46.0 ± 8.0 months, respectively (log rank 5.41, $p$ = 0.020).

pends critically on the stage of the disease at initial treatment with a five-year survival reaching 90% for early epithelial ovarian cancer, but dropping to 25–30% for more advanced stages [12]. Causative factors of epithelial ovarian cancer include genetic factors (e.g., family history), hormones, virility, and environmental factors. However, no risk factor has yet been definitively linked, and survival is extremely low, which has prompted epidemiological studies to elucidate the disease etiology. Surgical staging is essential to determine treatment plans and predict prognosis for epithelial ovarian cancer. One of the most important determinants of prognosis of advanced epithelial ovarian cancer is the size of the residual tumor after cytoreductive surgery [13, 14]. Furthermore, combination chemotherapy is performed postoperatively to improve patient survival, with the most frequently recommended chemotherapeutics being paclitaxel and carboplatin. However, paclitaxel is associated with side effects, such as inhibition of bone marrow function, alopecia, myalgia, arthralgia, hypersensitivity, and peripheral neuropathy [15, 16]. Therefore, determination of the appropriate doses for chemotherapy to produce adequate efficacy, but not exceeding the toxicity limit is extremely important yet very challenging. With recent reports suggesting that several genetic polymorphisms in cytochrome P450-endcoding genes alter enzymatic activities, many studies have attempted to identify genetic polymorphisms of enzymes that are involved in the metabolism of chemotherapy drugs [17, 18]. Drug metabolism may vary according to the drugs concomitantly used, the patient's general health condition, and genetic factors. There has been extensive research to elucidate changes in the roles of drugs induced by genetic factors, with more than 100,000 reports published annually in the United States alone. In particular, there have been numerous reports that have investigated changes of drug concentration and toxicity caused by genetic polymorphisms in the CYP2C family. Studies have been performed to determine whether there was evidence of an association between CYP2D6 and tamoxifen [19, 20], CYP2B6 and cyclophosphamide [21, 22], and CYP3A4 and irinotecan [23,24].

Many cases of advanced epithelial ovarian cancer have poor prognosis despite cytoreductive surgery and adjuvant chemotherapy. A low response to chemotherapy may be a contributing factor, which could be influenced by polymorphisms in genes that transcribe the enzymes involved in drug metabolism. CYP2C8 is an important human liver enzyme that metabolizes paclitaxel to 6α-OH-paclitaxel [25], a metabolite that is approximately 30 times less effective than paclitaxel. For this reason, CYP2C8 activity is critical as it reduces the concentration of paclitaxel, and therefore, diminishes the drug's cytotoxicity [26, 27]. In addition, CYP2C8 metabolizes arachidonic acid to epoxye-

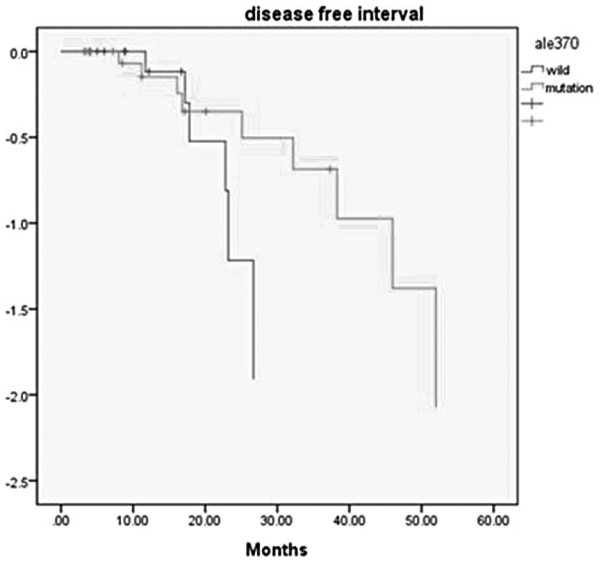


Figure 5. — Disease-free interval between the -370 mutation group and the -370 wild-type group with medians of 38.3 ± 9.1 months and 22.8 ± 6.3 months, respectively (log rank 0.75, $p$ = 0.385).

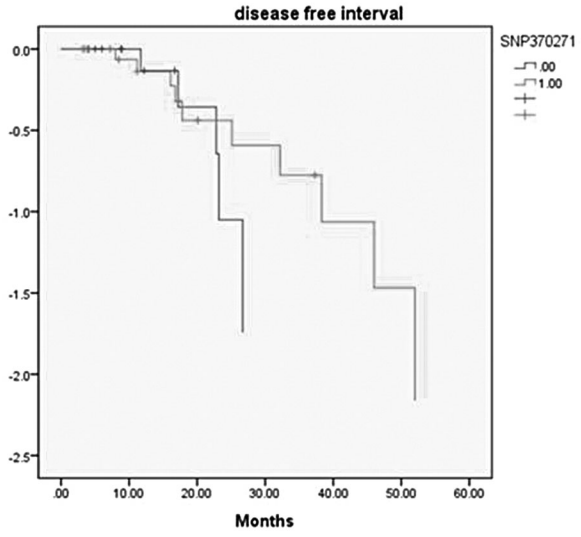


Figure 6. — Disease-free interval between the -370 & -271 mutation group and the -370 & -271 wild-type group with medians of 32.2 ± 9.1 months and 23.2 ± 3.3 months, respectively (log rank 0.30, $p$ = 0.582).

icosatrienoic acid [7, 18, 28-31].

Dai *et al.* [8, 32] recently reported that alleles *2 and *3 of CYP2C8 decreased CYP2C8 activity on paclitaxel metabolism, and Bahadur *et al.* [33] reported a similar association with allele *4. However, thus far, studies performed to date have failed to discover, much less identify allelic effects, in Asians. CYP2C8*5 is the only allele that has been described in Japanese, which is found at a very low frequency of 0.9% [34]. Discoveries and reports of CYP2C8 activity have been verified in vitro experiments only, frequently calling for additional in vivo studies to determine whether these findings are applicable in heterozygous individuals [35]. Although the frequency of tested polymorphisms varies individually, it was found that some polymorphisms are more prevalent in certain ethnic groups and races compared to others. In a study consisting of 306 healthy adults, Cho *et al.* [10] found 11 SNPs in CYP2C8 via two-dimensional gel scanning and sequencing of the gene's coding regions, including exon-intron junctions, and 2 kb of the 5′-flanking region. Of the SNPs identified, three were in the 5′-flanking region, two were found in introns, and six were located in exons. The most frequent SNP in Koreans was IVS2-13insT in intron 2, which has a frequency of 0.44, and three SNPs detected in the 5′-flanking region of CYP2C8 that have frequencies ≥ 0.1 [10]. Bahadur *et al.* [33] reported that expression of the CYP2C8 protein in individuals presenting the CYP2C8*1B (-271C>A) and CYP2C8*1C (-370T>G) alleles did not differ significantly from those that had the wild type allele. On the other hand, in a study on rosiglitazone (an antidiabetic drug), Cho *et al.* [10] reported that CYP2C8 expression was mildly reduced in individuals with the *1C allele. Based on an analysis of healthy Korean population, Cho *et al.* [10] found that the *1B and *1C allele frequencies differed between Korean and Caucasian populations. Dai *et al.* [8] reported the frequencies of these alleles in Caucasians to be 0.20 and 0.06, whereas the frequencies of these alleles in Koreans were 0.11 and 0.33, respectively [10].

Interracial differences in the types and frequencies of SNPs have been reported in multiple metabolic enzymes, and differences between Westerners and Koreans in CYP2C8 genotypes may reflect differences in metabolism of CYP2C8 substrate drugs, as well as in the pharmacokinetic properties of CYP2C8. In the present study, the authors compared and analyzed SNP frequencies between healthy individuals and individuals with epithelial ovarian cancer and did not find significant differences between allele frequencies of this patient cohort or in individuals with FIGO Stage III and IV cancer compared to those found in controls. Next, the authors hypothesized that other gene expression levels were associated with or linked to CYP2C8 metabolism and the development and progression of epithelial ovarian cancer. Based on the assumption that differences in CYP2C8 metabolism would be a result of genetic differences in SNPs, patients were divided into a chemotherapy response group and a non-response group to evaluate differences in allele frequencies of the three SNPs

in the 5′-flanking region of CYP2C8 that frequently occur in Koreans. However, the authors found that the two groups did not differ significantly in the frequencies of these polymorphisms. This finding may be expected because chemotherapy for ovarian cancer involves a combination of paclitaxel and another drug, but additional studies with appropriate study designs are required to evaluate this relationship. In addition, the authors found no significant differences between the CYP2C8*1D (-411T>C), CYP2C8*1B (-271C>A), and CYP2C8*1C (-370T>G) mutation groups and wild type groups in terms of haplotype pairs, with the exception of a slightly increased frequency of the -370 heterotype in the non-response group. In a comparison of different CYP2C8 SNP groups, the authors found an increased prevalence of hematological toxicity in the CYP2C8*1D (-411T>C) mutation group, CYP2C8*1C (-370T>G) wild type group to a statistically significant degree. In addition, they found that only the CYP2C8*1D (-411T>C) wild type group had a significantly longer disease-free interval compared to that found in the CYP2C8*1D (-411T>C) mutation group ($p = 0.020$), which indicate an association between prognosis and differences in pharmacokinetic properties of paclitaxel caused by the CYP2C8*1D polymorphism. The present study had limitations to be resolved in future studies. First, future studies should survey the frequency of the CYP2C8 mutation, which was found significantly higher in patients with epithelial ovarian cancer in this study, in a larger population, as such data would provide a broader understanding of the development and diagnosis of ovarian cancer. Second, the authors were unable to confirm the pharmacokinetic differences based solely on frequency differences according to clinical presentations of the patients. Therefore, prospective pharmacokinetic studies should be performed on individuals possessing alleles of different SNPs. Third, this study identified the differences in clinical presentation in relation to SNPs in the promoter region. However, further studies are warranted as SNPs located in the promoter region are likely in linkage disequilibrium with other variants or even nearby genes, which may be responsible for the pharmacokinetic changes that affect clinical presentation.

## Conclusion

This study was the first to examine differences in SNPs of CYP2C8 for evidence of an association with clinical presentation in patients with epithelial ovarian cancer. The authors found that among these three SNPs in the 5′-flanking region of CYP2C8 that occur frequently in Koreans (-411T>C, -370T>G, and -271C>A), specific alleles at these loci were similarly frequent in patient with ovarian cancer, of which the CYP2C8*1D (-411T>C) mutation was associated with poor prognosis of ovarian cancer. SNP-specific pharmacokinetic differences of paclitaxel, and differences in prognosis in relation to SNPs at other positions, should be further investigated in future studies. The present authors believe that this study provides useful data for researchers who undertake studies on the pharmacological and pharmacokinetic properties of cytochrome P450-related polymorphisms and anticancer drugs.

The present findings indicate that CYP2C8*1C may be an indicator for less severe side effects, as well as a good prognostic predictor of epithelial ovarian cancer, whereas CYP2C8*1D (-411T>C) may be a risk factor for toxicity and poor prognostic outcome. In addition, this study examined the differences in FIGO stages, prevalence of post-chemotherapy complications, and post-chemotherapy prognosis in relation to specific CYP2C8 allele. Such findings provide important information in predicting prognosis based on genetic polymorphisms and in determining the most effective drug (e.g., paclitaxel, belotecan, doxorubicin) for patients following recurrence. However, additional studies using larger populations are required to substantiate the present findings. In addition, as demonstrated in this study, because CYP2C8 genetic polymorphisms can vary significantly according to race and among individuals, additional studies are warranted to investigate the correlation between genetic polymorphisms and different chemotherapeutic strategies.

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