RESEARCH ARTICLE

Associations of ABCB1 and XPC Genetic Polymorphisms with Susceptibility to Colorectal Cancer and Therapeutic Prognosis in a Chinese Population

Ai-Min Yue*, Zhen-Bin Xie, Hong-Feng Zhao, Shu-Ping Guo, Yu-Hou Shen, Hai-Pu Wang

Abstract

Associations between ABCB1 and XPC genetic polymorphisms and risk of developing colorectal cancer (CRC) as well as clinical outcomes in CRCs with chemotherapy were investigated. A case-control study was performed on the ABCB1 C3435T, G2677T/A and XPC Lys939Gln polymorphisms in 428 CRC cases and 450 hospital-based, age and sex frequency-matched controls using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays. We observed that the ABCB1 3435CT or CC+CT variants were significantly linked with increasing risk of developing CRC (adjusted OR (95% CI): 1.814 (1.237-2.660), P=0.0022; adjusted OR (95% CI): 1.605 (1.117-2.306), P=0.0102, respectively). Moreover, the distribution frequency of XPC AC genotype or AC+CC genotypes also showed a tendency towards increasing the susceptibility for CRC (P=0.0759 and P=0.0903, respectively). Kaplan-Meier curves showed that the ABCB1 C3435T variant was associated with a tendency toward longer progression-free survival (PFS) (n=343, Log-rank test: P=0.063), and the G2677T/A variant genotypes (GT+TT+GA+AA) with a tendency for longer OS in postoperative oxaliplatin-based patients (n=343, Log-rank test: P=0.082). However, no correlation of the XPC Lys939Gln polymorphism was found with PFS and OS in patients with postoperative oxaliplatin-based chemotherapy (n=343). Our study indicated that ABCB1 polymorphisms might be candidate pharmacogenomic factors for the prediction of CRC susceptibility, but not for prognosis with oxaliplatin chemosensitivity in CRC patients.

Keywords: ABCB1 - XPC - genetic polymorphisms - colorectal cancer - susceptibility - prognosis

Introduction

Colorectal cancer (CRC) is the third most common malignancies in the world. The incidence or mortality of CRC has been rapidly increasing due to the changing lifestyles, environmental exposure, and inherited genetic background (Pourhoseingholi, 2012). Aside from the traditional risk factors, a number of studies have provided evidence those germline polymorphisms in genes encoding xenobiotics and drug transporters or DNA repair pathway is considered as a potential factor that may influence the risk of developing CRC and its clinical outcome associated with the carcinogenesis(Gervasini et al., 2006; Andersen et al., 2009; Engin et al., 2010; Wu et al., 2011; Kang et al., 2013).

ATP-binding cassette (ABC) transporters, which mediate efflux of a wide variety of endogenous substrates, xenobiotics, and chemotherapeutic agents, are known to mediate multidrug resistance in many tumors (Gottesman et al., 2002; Leonessa and Clarke, 2003; Ferguson et al., 2005).

P-glycoprotein (P-gp), encoded by the ATP-binding cassette B1 (ABCB1) gene, is an efflux transporter located on the luminal side of intestinal epithelial cells, which protects the gut from endogenous and exogenous toxins. The most frequent two variant alleles of ABCB1 gene (C3435T and G2677T/A) have been shown to be associated with altered P-gp (Kim et al., 2001; Samanian et al., 2011). Kim et al. (2011) reported that C3435T TT genotype was associated with lowered intestinal P-glycoprotein expression. Meanwhile, Samanian et al. observed that the GG2677 genotype was correlated with highest while the AT 2677 genotype was associated with the lowest levels of P-gp expression in Iranian CRC patients. Furthermore, recent studies also demonstrated that ABCB1 polymorphisms contribute to the susceptibility of CRC in different populations, but these results were inconsistence. Khedri et al. found that the ABCB1 C3435T T allele and the TT genotype were significantly increased in patients with CRC compared with controls in an Iranian population. However, Andersen et al. reported that carriers of the variant allele of MDR1 C3435T had a lower risk of...
Finally, 428 patients were included in the study. The principal clinical characteristics were obtained from the interviewer-administered health risk questionnaires and medical records. Tumor differentiation or pathological grade of these CRC patients was performed according to the World Health Organization criteria and DUKE’s criteria, respectively. The patients received several chemotherapy regimens, including oxaliplatin-based chemotherapy (Folfox regimen: oxaliplatin, leucovorin plus 5-FU; Xelox regimen: oxaliplatin plus capecitabine), LV5-FU2 (leucovorin plus 5-FU), or FU alone (fluouracil).

We also included 450 unrelated ages- and sex-matched healthy controls. The population had no known medical illness or hereditary disorders, and was not taking any medications. Before its commencement, this study was approved by the Research Ethics Committee of Xinxiang Central Hospital, and informed consent was obtained from each participant.

### Genotyping Assay

Genomic DNA was isolated from a leukocyte cell pellet of each blood sample, using the TIANGEN DNA Blood Mini Kit (TIANGEN BIOTECH (Beijing) Co., LTD, Beijing, China) according to the manufacturer’s instructions. The ABCB1 and XPC genetic polymorphisms were genotyped by PCR–RFLP assay. PCR was performed using 100 ng of genomic DNA, 300 nM of each primer, 200 nM dNTPs, and 0.5 U Taq polymerase in PCR buffer (TaKaRa Biotechnology (Dalian) Co., Ltd, Dalian, China) in a total volume of 25 μl. For ABCB1 C3435T, the 244-base pair (bp) PCR products were digested with Mbo I (Promega Corporation, U.S.A.) at 37 °C overnight. The T allele was uncut, and the C allele was cut into 198-bp and 26-bp bands. For ABCB1 G2677T/A, the 220-bp PCR products were digested with Ban I (Promega Corporation, U.S.A.) at 37 °C overnight. The T allele was uncut, and the C allele was cut into 175-bp and 69-bp bands. For XPC Lys939Gln, the 224-bp PCR products were digested with Bsr I (Promega Corporation, U.S.A.) at 37 °C overnight. The G allele was cut into 198-bp and 26-bp bands, whereas the C allele was uncut.

### Materials and Methods

#### Subjects

This study included 480 patients with CRC who were admitted to the Oncological surgery of Xinxiang Central Hospital between 2008 and 2012. Approximately 89% of contacted patients consented to enrollment in the study.

### Table 1. Frequency Distribution of ABCB1 and XPC Genotypes and Their Associations with CRC Risk

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Cases No. (%)</th>
<th>Controls No. (%)</th>
<th>Adjusted OR (95% CI)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>428(100.0)</td>
<td>450(100.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABCB1 C3435T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>57(13.32)</td>
<td>89(19.78)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>230(53.74)</td>
<td>198(44.00)</td>
<td>1.814(1.237-2.660)</td>
<td>0.0022</td>
</tr>
<tr>
<td>CC</td>
<td>141(32.94)</td>
<td>163(36.22)</td>
<td>1.351(0.904-2.018)</td>
<td>0.1419</td>
</tr>
<tr>
<td>XPC Lys939Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT/AA</td>
<td>49(11.45)</td>
<td>40(8.89)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
<tr>
<td>CT/AA</td>
<td>211(49.30)</td>
<td>223(49.55)</td>
<td>1.295(0.819-2.047)</td>
<td>0.2684</td>
</tr>
<tr>
<td>CC/CT</td>
<td>190(44.30)</td>
<td>198(44.55)</td>
<td>1.065(1.117-2.306)</td>
<td>0.4102</td>
</tr>
<tr>
<td>ABCB1 G2677T/A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OG</td>
<td>49(11.45)</td>
<td>40(8.89)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
<tr>
<td>GT/GA</td>
<td>211(49.30)</td>
<td>223(49.55)</td>
<td>1.295(0.819-2.047)</td>
<td>0.2684</td>
</tr>
<tr>
<td>TT/AA</td>
<td>190(44.30)</td>
<td>198(44.55)</td>
<td>1.065(1.117-2.306)</td>
<td>0.4102</td>
</tr>
<tr>
<td>XPC Lys939Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>174(40.65)</td>
<td>142(31.55)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>212(49.53)</td>
<td>225(50.00)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>214(50.00)</td>
<td>225(50.00)</td>
<td>1.00[Ref]</td>
<td></td>
</tr>
</tbody>
</table>

OR, indicates odds ratio; CI, confidence interval; ABCB1, C3435T, Be14558e; G2677T/A, Ala89358r/Thr; XPC Lys939Gln, Nucleotide A to C; Ref, reference category; The significance levels are P < 0.05 for all the bold values; *Adjusted OR and 95% CI values were calculated by unconditional logistic regression adjusted for age, sex, smoking status; *P values were calculated from 2-sided chi-square tests for each genotype distribution or allele frequency.
was cut into 206-bp and 14-bp bands. PCR products of XPC Lys939Gln were digested with PvuII overnight at 37 °C. The variant C allele had a PvuII restriction site, which was cut into 206-bp and 14-bp bands. PCR products of ABCB1 C3435T were digested with PvuII restriction enzyme overnight at 37 °C. The variant T allele had a PvuII restriction site, which was cut into 206-bp and 14-bp bands. 

**Statistical analysis**

SPSS software package version 16.0 (SPSS Inc, Chicago, IL, U.S.A.) was used to perform statistical analyses. All statistical significance was set at $P < 0.05$ and all tests were 2-sided. The chi-square (Pearson $\chi^2$ test) or Fisher exact test was used to determine the differences in distributions of demographic, epidemiological, and clinical variables between groups. The population genetic analysis program SNPAlyze 2.2 (Dynacom Co. Ltd., Yokohama, Japan) based on the expectation-maximization procedures. Two researchers independently performed RFLP and reviewed all genotyping results.

**Results**

The 428 patients enrolled in this study which included 263 (61.4%) men and 165 (38.6%) women. The mean age at diagnosis (SD) was 58.1(12.2), and at the range of 25~80 years old. Among 428 patients, most of the patients were in grade 2 (G2, moderate, 83.9%), 39 patients were in grade 3 (G3, poor, 9.1%), and only 30 patients were in grade 1 (G1, Well, 7%) according to tumor differentiation
classification. 50 patients (11.7%) of the patients had the first-degree family history of CRC. The results of Duke's pathological grade for tumors were as follows: Dukes A for 109 (25.4%), Dukes B for 172 (40.2%), Dukes C for 124 (29.0%), Dukes D for 23 (5.4%) patients. 224 (52.3%) of the cases had lymph node metastases. The vast majority of CRC patients (80.1%) received oxaliplatin-based chemotherapy, whereas 9.8% of them underwent fluorouracil chemotherapy. The variables of age, sex and smoking status were adjusted for any residual confounding effects in later logistic regression analyses.

Table 1 summarizes the genotype and allele frequencies of the \(ABCB1\) C3435T, G2677T/A and \(XPC\) Lys939Gln polymorphisms between CRC patients and controls. The genotype distributions in the control group did not significantly differ from Hardy-Weinberg equilibrium. A significant increased in the frequency of the CT genotype of the C3435T polymorphism was observed in CRC patients compared with controls (CT vs TT: \(P=0.002\); adjusted OR=1.814, 95% CI: 1.237-2.660). There was a significant difference in the CC+CT genotypes of the C3435T between CRC patients and controls and appeared to be associated with a increased risk of CRC (\(P=0.0102\); adjusted OR=1.605, 95% CI: 1.117-2.306) (Table 1). However, there were no significant associations between \(ABCB1\) G2677T/A polymorphisms and the risk of CRCs (Table 1). For \(XPC\) Lys939Gln polymorphism, the heterozygous variant AC genotype and variant genotypes (AC+CC) showed a tendency for a higher risk of developing CRC compared with the GG genotype (adjusted OR=1.300, 95% CI: 0.973-1.739, \(P=0.0759\); adjusted OR=1.237, 95% CI: 0.963-1.674, \(P=0.0903\), respectively) (Table 1). Next, we further investigated the correlation of \(ABCB1\) C3435T, G2677T/A and \(XPC\) Lys939Gln polymorphisms with clinicopathological features of 428 CRC patients using \(\chi^2\) test (Table 2). We observed that the distribution frequency of the \(ABCB1\) C3435T polymorphisms was...
associated with age (≤60 or >60 years old). The frequency (9.1%) of C3435T TT genotype in the patients with younger patients (≤60 years old) was significantly lower than that (17.3%) in patients with more than 60 years old (P=0.013). (Table 3). Moreover, a higher frequency of the ABCB1 G2677T/A GG genotype was observed in female patients (13.3%) compared with patients with male patients (6.8%) (P=0.025) (Table 2). In addition, there was a tendency towards higher distribution frequency of the XPC Lys939Gln polymorphisms observed in the female CRCs than those male CRCs (P=0.051) (Table 3). However, no significant correlation of genotype distributions of ABCB1 C3435T, G2677T/A and XPC Lys939Gln polymorphisms were found with smoking status, first-degree family history of CRC, tumor differentiation, pathological grade, relapse situation, prime cancer (rectal cancer or colon cancer) (Table 3).

We further evaluated the correlation of ABCB1 C3435T, G2677T/A and XPC Lys939Gln polymorphisms with the prognosis of CRC patients with postoperative oxaliplatin-based chemotherapy. For patients with postoperative oxaliplatin-based chemotherapy (n=343), the ABCB1 C3435T CC+CT genotype was associated with a tendency toward a longer PFS compared with the TT genotype (Log-rank test: P=0.063; Figure 1 A1). The estimated median PFS was 41 months for patients with CC + CT genotype (95% CI=35.98-46.04) and 36 months for patients with TT genotype (95% CI=34.19-37.81). However, no correlation of ABCB1 C3435T polymorphism was found with OS (Log-rank test: P=0.523; Figure 1 A2) in patients with postoperative oxaliplatin oxaliplatin-based chemotherapy (n=343).

For the ABCB1 G2677T/A polymorphism, no significant association of the ABCB1 G2677T/A genotypes were observed with PFS in patients with postoperative oxaliplatin-based chemotherapy (n=343, Log-rank test: P=0.430; Figure 1 B1). However, the variant ABCB1 G2677T/A GT+TT+GA+AA genotypes showed a tendency towards longer OS in postoperative oxaliplatin treated patients (n=343, Log-rank test: P=0.082; Figure 1 B2). In addition, no correlation of the XPC Lys939Gln polymorphism was found with PFS (Log-rank test: P=0.867) and OS (Log-rank test: P=0.523) in patients with postoperative oxaliplatin-based chemotherapy (n=343). A descriptive Cox proportional hazard model was performed to estimate the independent impact of each variable on PFS and OS (Table 3). Multivariate Cox regression identified that the prime cancer (rectum vs colon), tumor size (≤ 4cm vs > 4cm), pathological grade (Dukes A/Dukes B vs Dukes C/ Dukes D) had independent influence on the PFS of the patients with oxaliplatin oxaliplatin/5-FU-based chemotherapy (n=343). Furthermore, Smoking status (ever) and pathological grade (Dukes C/ Dukes D) were a predictive prognostic factor for a shorter OS (OR=2.192, 95% CI: 1.074-4.477; P=0.031; OR=1.407, 95% CI: 1.019-1.944; P=0.038, respectively) in patients with oxaliplatin-based chemotherapy (n=343).

**Discussion**

P-gp, ABCB1 encoded transporter, is expressed not only in normal human tissues but also in multidrug-resistant cancer cells, which contributes to the absorption and distribution of xenobiotics, toxins and drugs. The polymorphisms of ABCB1 C3435T, G2677T/A have been shown involving in altered expression or transporter activity of P-gp (Kim et al., 2001; Fromm, 2002; Leschziner et al., 2007; Samanian et al., 2011), and maybe associated with the susceptibility for developing cancer, but their biological significance is not fully understood yet. In the present study investigating 428 CRC patients and 450 cancer-free controls, we found that heterogeneous CT genotype or combined variants CC+CT genotypes of C3435T in ABCB1 was associated with significantly increased risk of developing CRC. In agreement with our results, Andersen and co-workers found that carriers of C allele of the C3435T polymorphism had an increased risk of CRC in Norwegian Caucasian population (Andersen et al., 2009). On the contrary, in an Iran population investigation, T allele of the ABCB1 C3435T polymorphism showed significantly increased risk of CRC (Khedri et al., 2011), while others did not find any statistically significant associations (Ba et al., 2006; Samanian et al., 2011). Regarding ABCB1 G2677T/A genotypes and risk for CRC, we did not find any correlation between CRC risk and the investigated G2677T/A genotypes, likewise, De Iudicibus et al. found no relation between ABCB1 G2677T/A polymorphism and CRC patients (De Iudicibus et al., 2008). These data suggest that the role of the ABCB1 polymorphisms in cancer risk may vary with ethnicity, a possibility that warrants further investigation.

Risk factors contribute to CRC risk through multiple genetic alterations and DNA repair plays an important role in maintaining genome integrity. Moreover, recent year studies have established that the polymorphisms in DNA repair genes can either change protein coding or alter the levels of transcription or translation, consequently,
which can reduce DNA repair capacity and induce genetic instability or carcinogenesis. Few studies have investigated the associations between XPC Lys939Gln and risk of CRC, and these findings were inconsistent (Hansen et al., 2007; Engin et al., 2010; Wu et al., 2011; Liu et al., 2012). Overall, the XPC Lys939Gln was found to be associated with CRC risk in two case-control studies (Wu et al., 2011; Liu et al., 2012) while others did not find any statistically significant associations (Hansen et al., 2007; Engin et al., 2010). In this study, although there were no statistically significant association observed between XPC Lys939Gln polymorphism and CRC risk, it is worth to note that current data regarding XPC Lys939Gln polymorphism showed a tendency for a higher risk of developing CRC in heterozygous AC genotypes ($P=0.0759$) or AC+CC variants carriers ($P=0.0903$). Thus, it might be stated that the associated between XPC Lys939Gln polymorphism and cancer risk was varied in different ethnicity, and need further research to establish it.

CRC treatment strategies mainly include surgical excision and adjuvant chemotherapy according to different tumor stage of CRC patients. Oxaliplatin is the third-generation platinum derivative compound that has found a place in the routine treatment of CRC. Despite reducing recurrence after chemotherapy, multi-drug resistance to chemotherapeutic agents remains a big problem in the treatment of CRC patients (Robert et al., 2005; Kerb, 2006). Therefore, we further analyzed the relationship between ABCB1 and XPC polymorphisms and the prognosis of CRC patients with postoperative oxaliplatin-based chemotherapy. Likewise, Chai et al. and Liu et al. (2006) found no relation between ABCB1 C3435T, G2677T/A or XPC Lys939Gln polymorphisms and prognosis in Chinese people. We only observed that ABCB1 C3435T variants carriers were associated with a tendency toward longer PFS, meanwhile G2677T/A variants were observed in a tendency for longer OS in postoperative oxaliplatin-based patients. However, no statistically significant correlation of the XPC polymorphism was found with prognosis in patients with postoperative oxaliplatin-based chemotherapy.

Although numerous studies have investigated the relevance between the polymorphisms of the drug resistance-associated genes or DNA repair pathway genes and CRC risk and prognosis, the roles of these genes in carcinogenesis remain unclear and need to be further elucidated. The present study is one of studies to address the potential association between ABCB1 and XPC polymorphisms and CRC risk and prognosis in a larger and well characterized population. Nonetheless, several potential limitations should be considered in this study. In the case-control study, the cohort was included only in Chinese Han population in Northeast areas, and the results should be replicated in other ethnicities or geographic areas. Although relatively large sample size, our data might not have adequate statistical power to detect weak genetic-disease associations or gene-environment interactions. Further studies estimating the effect of gene-gene and gene-environment interactions may provide a comprehensive understanding of the roles of ABCB1 and XPC polymorphisms involved in the initiation of cancer. Therefore, functional studies in vitro or in vivo to identify the physiological and molecular mechanism are also warranted.

In conclusion, this hospital-based case-controlled study indicates that in the Chinese population, ABCB1 C3435T polymorphism was significantly associated with the susceptibility to CRC, but not found ABCB1 and XPC polymorphism were associated with chemotherapeutic prognosis of CRC patients with oxaliplatin-based chemotherapy. Additional investigations are warranted to confirm our findings in predicting the cancer risk and prognosis of CRC patients with chemotherapy.

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References


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