Original Article

Polymorphisms in UGT2B4 and susceptibility to pancreatic cancer

Xu Che1, Dianke Yu2, Zongyong Wu2, Jianwei Zhang3, Yintai Chen1, Yaling Han2, Chenfeng Wang1, Jun Qi2

1Department of Abdominal Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China; 2State Key Laboratory of Molecular Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

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Abstract: As an important enzyme in the conjugation phase of drug clearance, UGT2B4 helps metabolize various endogenous and exogenous substances, and polymorphisms in the corresponding gene can influence enzyme activity. This study investigated the association between polymorphisms in UGT2B4 and the risk of developing pancreatic cancer in Han Chinese individuals. A hospital-based case-control study was conducted with 1579 healthy controls and 406 pancreatic cancer patients from China. Genomic DNA was obtained from peripheral blood lymphocytes. Time-of-flight mass spectrometry was used to genotype polymorphic loci in UGT2B4, and the association between these polymorphisms and susceptibility to pancreatic cancer was expressed as odds ratios (ORs) with 95% confidence intervals (CIs), as calculated using multivariable logistic regression analysis. The rs1131878C > T polymorphism (NT_016354.20: g.10558805C > T) in UGT2B4 was associated with an increased pancreatic cancer risk. Compared to the C/C genotype, the C/T genotype conferred 1.39 times higher the pancreatic cancer risk (95% CI = 1.09-1.77; P = 0.007), and the T/T genotype conferred 2.97 times higher the pancreatic cancer risk (95% CI = 1.24-7.08; P = 0.014). In contrast, compared with the A/A genotype, the A/C genotype at the rs3822179 locus in UGT2B4 (NT_016354.20: g.10569096C > A) bestowed a 20% risk reduction (OR = 0.80, 95% CI = 0.67-0.95; P = 0.011). However, the risk was not significantly reduced with the C/C genotype (OR = 0.77, 95% CI = 0.52-1.14, P = 0.191). Polymorphisms in UGT2B4 affect the risk of pancreatic cancer occurrence in Han Chinese individuals.

Keywords: UGT2B4, polymorphism, pancreatic cancer, hereditary susceptibility

Introduction

Pancreatic cancer has a poor prognosis, with a median survival time of only about 6 months and a five-year survival rate of less than 5% [1]. Over the past few years, the incidence of pancreatic cancer in China has been on the rise, and certain relatively more developed regions have an incidence rate close to that observed in western countries [2, 3]. Unfortunately, most patients who go to the hospital for treatment are already in the advanced or late stages of the disease, and their cancer has usually metastasized, so the best time for the resection operation has passed. Therefore, reliable biological indicators are needed to identify and assess the population susceptible to the development of pancreatic cancer. Prevention and diagnosis in the early stages of cancer development are critical for improving the prognosis of pancreatic cancer, but are also the very areas that currently require the most research and clinical attention.

UGT2B4, a member of the UGT2B family [4], is expressed mainly in the liver. Studies on its function are contradictory. Some publications indicate that the UGT2B4 enzyme is able to catalyze hydesoxycholic acid specifically but is unable to catalyze steroids or 5-hydroxytryptamine, while other publications suggest that steroids, phenols, and monoterpenoids can be catalyzed by UGT2B4 but not by hydesoxycholic acid. As implied by the reporting of such contradictory results, UGT2B4 catalytic activity is apparently quite complex and not fully understood. Although UGT2B4 catalyzes all of this substrates given sufficient time, according to
accumulating evidence, it predominantly takes part in the metabolism of sex hormones, and may even play a role in the occurrence and development of breast cancer and other malignant neoplasms. However, so far, reports concerning the association between UGT2B4 and cancer susceptibility are rare.

The occurrence of malignant neoplasm might be influenced by multiple factors and is often the result of environmental and genetic interactions. Sequence variation at loci within genes may not only affect individual sensitivity to environmental factors, but also influence the occurrence and development of malignant neoplasm [5]. As a conservative estimate, at least 0.2% genetic variations observed between individual genomes, of which approximately 0.08% arises from variation in DNA sequence, and the remaining 0.12% arises from variation in chromosomal structure such as insertions, deletions, and inversions [6]. Single nucleotide polymorphism (SNP) is a type of DNA sequence variation. There are extensive studies on the mechanisms by which different SNPs influence predisposition to the development of a number of malignant neoplasm. For example, susceptibility to pancreatic cancer occurrence is affected by SNP in genes encoding the cell cycle regulators p21 and p27 [7], the DNA repair proteins RecQ1 [8], RAD54L, and ERCC1 [9], and the pro-apoptosis factors FasL and CASP8 [10]. Therefore, genetic variation is an important molecular basis for individual differences in the risk of developing pancreatic cancer.

As a hospital-based case-control study, this work preliminarily investigates the association between polymorphisms in UGT2B4 and susceptibility to pancreatic cancer. It is hoped that the identification of loci relevant to pancreatic cancer risk by using methods that permit rapid molecular screening of populations will provide a means for early diagnosis and treatment.

**Material and methods**

**Subjects of study**

The study included 406 patients with pancreatic cancer who received treatment at the Cancer Institute and Hospital, Chinese Academy of Medical Sciences, from July 1998 to December 2011, as well as 1579 healthy controls. Pathology reports were generated for all patients in the course of treatment. Patients and healthy controls were all ethnically Han Chinese and were residents of China. Study participants did not include any lineal relatives. The subjects with pancreatic cancer were chosen from patients who suffered from pancreatic ductal adenocarcinoma, as diagnosed based on the corroboration of cytology and histopathology, without considering gender or age. The pathologic staging of pancreatic cancer followed the tumor-node-metastasis (TNM) classification system developed by the Union for International Cancer Control (UICC).

Healthy controls were selected from among the participants in a screening program for early diagnosis of cancer, conducted in various communities in Beijing. Healthy controls lacked any history of cancer, of the 406 patients with pancreatic cancer enrolled in the study, 257 (63.30%) were male and 149 (36.69%) were female. The character of the subjects is showed in Table 1.

**Experimental methods**

**Genomic DNA extraction:** DNA was obtained from peripheral white blood cells of patients and healthy controls. Extraction of DNA followed the commonly used phenol-chloroform extraction procedure, as described, briefly, below.

1. Whole blood (without anticoagulants) or isolated lymphocytes were thawed and homogenized; homogenates were centrifuged for 15 minutes at 5,000 x g to remove debris and insoluble material.

2. The supernatant was transferred to a fresh tube and 300 μl was set aside. The supernatant was mixed after addition of 500 μl of DNA lysis buffer and incubated at 37°C for 1 hour.
Table 2. Comparison of demographicsof study participants

<table>
<thead>
<tr>
<th></th>
<th>Pancreatic cancer patients (n = 406)</th>
<th>Healthy controls (n = 1579)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>%</td>
<td>Number of cases</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>257</td>
<td>63.30</td>
<td>965</td>
</tr>
<tr>
<td>Female</td>
<td>149</td>
<td>36.69</td>
<td>614</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 40 years old</td>
<td>20</td>
<td>4.93</td>
<td>84</td>
</tr>
<tr>
<td>41-60 years old</td>
<td>168</td>
<td>41.38</td>
<td>658</td>
</tr>
<tr>
<td>&gt; 60 years old</td>
<td>218</td>
<td>53.69</td>
<td>837</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>210</td>
<td>51.72</td>
<td>736</td>
</tr>
<tr>
<td>Smokers</td>
<td>125</td>
<td>30.79</td>
<td>449</td>
</tr>
<tr>
<td>Unknown</td>
<td>71</td>
<td>17.49</td>
<td>394</td>
</tr>
</tbody>
</table>

*P values determined using a bilateral Pearson $X^2$ test.

3. Protease K was mixed in to a final concentration of 100 mg/ml. The mixture was incubated overnight at 37°C.

4. An equal volume of Tris-HCl saturated phenol (pH 7.8) was added, and the suspension was mixed and then centrifuged for 15 minutes at 5,000 × g to remove particulates.

5. The supernatant was collected and transferred to another centrifuge tube. Equal volumes of phenol and chloroform (at a 1:1 ratio) were added, and, after mixing, the suspension was centrifuged for 5 minutes at 8,000 × g.

6. The aqueous layer was collected and transferred to another centrifuge tube. A volume of 10 M ammonium acetate equivalent to 10% of the aqueous volume was mixed in. A volume of absolute ethyl alcohol equivalent to twice the volume of the aqueous layer was added, and the suspension was gently mixed. The DNA was allowed to precipitate overnight.

7. After centrifugation, the supernatant was decanted, and the precipitated DNA was washed twice with 75% ethanol. The DNA was then dissolved in a defined volume of buffer (pH 8.0).

8. The concentration and purity of the DNA samples were measured by the absorbance at 260 nm and the ratio of absorbance at 260 nm versus that at 280 nm, respectively.

$D'$ value reflected the difference between the actual distribution frequency of the haplotype and the theoretical distribution frequency with linkage equilibrium: $D' = 0$ indicated no linkage, $D' = 1$ indicated complete linkage, and $D' < 1$ indicated linkage disequilibrium.

Genotyping of polymorphisms in UGT2B4 was conducted by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS). MALDI-TOF MS requires the co-crystallization of the biological analyte with a low molecular weight, acidic, polar, organic polymer with strong optical absorption. The matrix of organic polymers absorbs the energy of a laser directed at the matrix, and the polymers become desorbed and ionized. The ionized polymers transfer protons to (or from) the molecules of biological analyte. Thus, the biological molecules are ionized indirectly, via the polymer matrix, which is why the technique is called matrix-assisted laser desorption/ionization, or MALDI. The time-of-flight mass spectrometry (TOF MS) involves acceleration of the ionized biological molecule through a tube by an electric field, and the time required for the ion to reach the detector is measured. The mass to charge ratio (M/Z) of the biological ion is directly proportional to its flight duration, such that the time of flight can be used to determine the nature of the biological molecule. Since this method is high sensitive to the quality and nature of the biological analyte, we employed it to distinguish between
two genetic sequences that differed only at one position, using the different in mass between the two possible bases at that position. We also used allele specific primer extension to determine the nucleotide base at specific SNP loci.

rs1131878
Forward primer: 5’-ACGTTGGATGGAGGAAGAGACTTTCTATAC-3’.
Reverse primer: 5’-ACGTTGGATGCCATTTCATGCAGGATTGTG3’.

rs11249442
Forward primer: 5’-ACGTTGGATGGGTGTTAGCCTAGGGCTAAG-3’.
Reverse primer: 5’-ACGTTGGATGGTATCAACGTAAGGTTGTG3’.

rs1826690
Forward primer: 5’-ACGTTGGATGGTCTCTGAAGAACAAATACTG-3’.
Reverse primer: 5’-ACGTTGGATGTGTTCAGTAACTTCTAT-3’.

rs3822179
Forward primer: 5’-ACGTTGGATGTGCCAGAACTTTCTGCAGAG-3’.
Reverse primer: 5’-ACGTTGGATGCCAGCCAAGTACATCTTCAC-3’.

Figure 1. UGT2B4 haplotype domain mapping using genotype frequencies of polymorphic loci. A haplotype domain map was constructed using single nucleotide polymorphisms (SNPs) within UGT2B4, based on SNP frequency data for Han Chinese individuals residing in Beijing, available from the International Hap Map Project [11]. Red arrows point to rs11249442, rs1826690, and rs3822179, located in the promoter region, and to rs1131878, located in the 3’ untranslated region.
Table 3. UGT2B4 SNP genotype distributions and risk of pancreatic cancer

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pancreatic cancer patients (n = 406)</th>
<th>Healthy controls (n = 1579)</th>
<th>OR* (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of cases</td>
<td>%</td>
<td>Number of cases†</td>
<td>%</td>
</tr>
<tr>
<td>rs1131878</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>309</td>
<td>76.1</td>
<td>1283</td>
<td>81.3</td>
</tr>
<tr>
<td>C/T</td>
<td>87</td>
<td>21.4</td>
<td>284</td>
<td>18.0</td>
</tr>
<tr>
<td>T/T</td>
<td>10</td>
<td>2.5</td>
<td>12</td>
<td>0.7</td>
</tr>
<tr>
<td>C/T + T/T</td>
<td>97</td>
<td>23.9</td>
<td>296</td>
<td>18.7</td>
</tr>
<tr>
<td>rs11249442</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>122</td>
<td>30.0</td>
<td>456</td>
<td>28.9</td>
</tr>
<tr>
<td>A/G</td>
<td>193</td>
<td>47.5</td>
<td>752</td>
<td>47.7</td>
</tr>
<tr>
<td>G/G</td>
<td>91</td>
<td>22.5</td>
<td>368</td>
<td>23.4</td>
</tr>
<tr>
<td>A/G + G/G</td>
<td>284</td>
<td>70.0</td>
<td>1120</td>
<td>71.1</td>
</tr>
<tr>
<td>rs1826690</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/G</td>
<td>135</td>
<td>33.3</td>
<td>513</td>
<td>32.5</td>
</tr>
<tr>
<td>G/A</td>
<td>210</td>
<td>51.7</td>
<td>777</td>
<td>49.2</td>
</tr>
<tr>
<td>A/A</td>
<td>61</td>
<td>15.0</td>
<td>289</td>
<td>18.3</td>
</tr>
<tr>
<td>G/A + A/A</td>
<td>271</td>
<td>66.7</td>
<td>1066</td>
<td>67.5</td>
</tr>
<tr>
<td>rs3822179</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>210</td>
<td>51.7</td>
<td>714</td>
<td>45.5</td>
</tr>
<tr>
<td>A/C</td>
<td>161</td>
<td>39.7</td>
<td>687</td>
<td>43.8</td>
</tr>
<tr>
<td>C/C</td>
<td>35</td>
<td>8.6</td>
<td>168</td>
<td>10.7</td>
</tr>
<tr>
<td>A/C + C/C</td>
<td>196</td>
<td>48.3</td>
<td>855</td>
<td>54.5</td>
</tr>
</tbody>
</table>

*OR, odds ratio, was calculated using unconditional logistic regression, adjusted according to gender, age, and smoking history. †Some SNP genotyping reactions of samples from healthy controls (three for rs11249442 and 10 for rs3822179) failed to yield classifiable results.

Statistical analysis: The chi-squared test was employed to compare the differences among and between various genotype distributions. We calculated the odds ratios (ORs) and 95% confidence intervals (CIs) by using unconditional logistic regression and evaluated the association between various genotypes and vulnerability to pancreatic cancer.

Results

Demographics of study subjects

Subjects who took part in the study were classified by gender, age, and smoking status (Table 2). Among the patients with pancreatic cancer, 63.30% were male and 36.69% were female, while, among the healthy controls, 61.11% were male and 38.88% were female. Therefore, as expected based on the study design, there was no significant difference in the gender distribution between pancreatic cancer patients and healthy controls (P = 0.334; Table 2).

Among healthy controls, 84 (5.32%) were less than 40 years old, while 658 (41.67%) were between 41 and 60 years old, and 837 (53.01%) healthy controls were more than 60 years old. Among pancreatic cancer patients, 20 (4.93%) were less than 40 years old, while 168 (41.38%) were between 41 and 60 years old, and 218 (53.69%) were more than 60 years old. As observed with the gender distributions of the two groups, due to careful study execution, there was no significant difference between the age distribution of the healthy controls and that of the pancreatic cancer patients (P = 0.937; Table 2).

In regards to smoking history, subjects were defined as smokers if they had smoked for over one year prior to being enrolled into the study or receiving the study questionnaire. Among healthy controls, 449 (28.44%) were smokers, 736 (46.61%) were nonsmokers, and the smoking history of the remaining 394 (24.95%) was unknown. Among pancreatic cancer patients, 125 (30.79%) were smokers, 210 (51.72%) were nonsmokers, and the smoking history of the remaining 71 (17.49%) was unknown. Compared to the healthy controls, subjects with pancreatic cancer did not demonstrate a significant difference in smoking status or history (P = 0.848; Table 2).

Determination of SNP genotype frequencies and UGT2B4 haplotype construction

Utilizing SNP frequency data for Han Chinese individuals residing in Beijing, available from the International Hap Map Project [11], we con-
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Structured a local haplotype domain with the use of the HaploView4.0 software. Known SNPs were selected based on the criteria that the haplotype correlation coefficient, in the context of development of pancreatic cancer, was greater than 0.8 ($R^2 > 0.8$). Finally, four single nucleotide polymorphisms were chosen from 10 genetic variants having a minor allele frequency greater than 0.05 in the population. These four SNPs were rs11249442, rs1826690, and rs3822179, located in the promoter region, and rs1131878, located in the part of the gene encoding the 3' untranslated region (3'UTR) of the corresponding mRNA (Figure 1).

These four SNPs were genotyped in order to assay for an association between specific alleles at each locus and the risk of developing pancreatic cancer. In the population of healthy controls, the genotype distributions for all four SNPs were reasonably close to Hardy-Weinberg equilibrium. We found that, for the rs1131878 locus in the UGT2B4 gene, the rs1131878C > T allele was associated with increased risk of pancreatic cancer (Table 3). This was indicated by the fact that the C/C, C/T, and T/T genotypes were present in 76.1%, 21.4%, and 2.5%, respectively, of patients with pancreatic cancer, but in 81.3%, 18.0%, and 0.7%, respectively, of healthy controls; hence, there was a significant difference in the genotype distribution between these two populations ($P = 0.003$; Table 3). Upon further analysis utilizing unconditional logistic regression, it was revealed that, compared to those with the C/C genotype at thers1131878 locus, subjects with the C/T genotype had 1.39 times the pancreatic cancer risk (95% CI = 1.09-1.77; $P = 0.007$), and individuals with the T/T genotype had 2.97 times the risk (95% CI = 1.24-7.08; $P = 0.014$). In combination, the C/T and T/T genotypes were associated with 1.36 times the risk of developing pancreatic cancer (95% CI = 1.04-1.76, $P = 0.024$) (Table 3).

Regarding thers3822179 locus, the A/A, A/C, and C/C genotypes were present in 51.7%, 39.7%, and 8.6%, respectively, of patients with pancreatic cancer, but in 45.5%, 43.8%, and 10.7%, respectively, of healthy controls; therefore, for this SNP, a marginally significant difference in the genotype distribution was observed between the two groups ($P = 0.070$; Table 3). Further analysis utilizing unconditional logistic regression revealed that subjects with the A/C genotype had a 20% reduced risk of pancreatic cancer occurrence, compared to those with the A/A genotype (OR = 0.80; 95% CI = 0.67-0.95; $P = 0.011$). However, there was no significant reduction of risk in patients with the C/C genotype (OR = 0.77; 95% CI = 0.52-1.14; $P = 0.191$). In combination, the A/C and C/C genotypes conferred a significantly reduced risk of pancreatic cancer (OR = 0.74; 95% CI = 0.59-0.94; $P = 0.011$) (Table 3).

In contrast, neither the rs11249442 locus nor the rs1826690 locus exhibited any statistically significant differences in the genotype distributions in pancreatic cancer patients versus healthy controls ($P = 0.877$ and 0.296, respectively; Table 3). Likewise, the data was subdivided to determine whether specific genotypes of any of these SNPs in UGT2B4 were associated with differences in pancreatic cancer risk among subjects of different genders, ages, or smoking histories. No significant correlations were observed (results not shown).

Discussion

Pancreatic cancer is one of the most the common malignant neoplasms of the digestive system. As radiotherapy and chemotherapy are not currently effective against this type of cancer, the tumor must be removed by surgically resection. Unfortunately, by the time most patients are diagnosed and admitted to a hospital, the cancer has metastasized, making complete resection impossible. As a result, pancreatic cancer has a poor prognosis, with approximately 6-month median survival time and less than 5% five-year survival rate [1]. The pancreatic cancer incidence in China has been rising over the last few years, and the incidence rates in some of the more developed regions of the country are now close to that of western nations [2]. It is therefore critical to uncover reliable biological indicators, including genotype-phenotype correlations that may allow clinical follow-up of susceptible individuals and diagnosis in the early stages of pancreatic cancer, especially in populations with an increasing rate of incidence, such as the Han Chinese residents of Beijing and other cities in China.

Although the pathologic causes of pancreatic cancer are not yet fully understood, there have been reports demonstrating that its development is often related to certain environmental
Glucuronyl transferases (UGT) are some of the most important metabolic enzymes in the second phase of drug metabolism in the liver. They catalyze reactions between glucuronic acid (UDPGA) and a multitude of chemical substances such as alcohols, phenols, and mercaptans. The conjugation of UDPGA onto these chemicals increases their water solubility and facilitates their excretion into urine and bile, permitting clearance of these compounds from the body. Substrates of UGT enzymes include endogenous substances such as bilirubin and steroidal compounds as well as exogenous substances such as phenols, non-steroidal anti-inflammatory drugs (NSAIDs), mycophenolic acid, and antipsychotic drugs. To date, more than 35 kinds of glucuronyl transferases have been discovered in different species, and at least 19 genes encode different UGT enzymes in humans.

UGT enzymes are usually localized to the endoplasmic reticulum and to the nuclear membrane and are expressed in the kidneys, gastrointestinal tract, skin, and brain. Since UGT enzymes are localized to the endoplasmic reticulum antrum, in vitro assays for UGT activity require the presence of channel proteins. In contrast, a family of enzymes required for the first phase of drug metabolism, cytochromes P450 (CYP450s), which are also localized to the endoplasmic reticulum, are oriented with the catalytic domain facing the cytoplasm, and thus do not require other proteins in order to function in in vitro assays. Therefore, scientific awareness of the physiological functions of UGT enzymes lags behind that of CYP450 family members. As knowledge regarding the activity of UGT enzymes increases, more and more medications that take advantage of UGT-mediated metabolism have been developed. Similarly, significant individual and ethnic differences in the sequences of genes encoding UGT enzymes have been detected, making possible investigations into the relevant environmental and hereditary factors that may influence disease onset, such as those that are the focus of the present research.

The UGT gene family includes the UGT1 and UGT2 subfamilies, of which the UGT2 subfamily can be further divided into the UGT2A and UGT2B gene subfamilies. Although all genes in the UGT2 family span 6-exons, unlike in the UGT1 genes, exons of the UGT2 genes cannot be interchanged by splicing. Nevertheless, all members of the UGT2 gene subfamily share greater than 60% homology. Located on chromosome 4q13, the cluster of genes in the human UGT2 gene family includes three members of the UGT2A subfamily and 12 members of the UGT2B subfamily [3]. Of the latter dozen, 7 genes are known to be actively transcribed, namely UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28.

Despite contradictory reports of which types of compounds are catalyzed by the UGT2B4 enzyme, increasing evidence suggests that it takes part in the metabolism of sex hormones, and therefore may have a bearing on the occurrence and development of breast cancer and other malignant neoplasms. There has not been much research into the relationship between UGT2B4 and cancer susceptibility. Nonetheless, a polymorphism in UGT2B4 has been shown to affect the risk of developing esophageal squamous cell carcinoma [12], and the rs13129471 polymorphic locus has been...
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found to be a factor in hereditary predisposition to breast cancer [13].

Our findings demonstrate that the rs1131878 SNP in the 3'UTR of UGT2B4 influences the risk of developing pancreatic cancer. Unconditional logistic regression analysis of the revealed that, compared to the C/C genotype at the rs1131878 locus, the C/T genotype conferred 1.39 times the pancreatic cancer risk (95% CI = 1.09-1.77; \( P = 0.007 \)), and the T/T genotype conferred 2.97 times the pancreatic cancer risk (95% CI = 1.24-7.08; \( P = 0.014 \)). In combination, the C/T and T/T genotypes conferred 1.36 times the risk of pancreatic cancer relative to the C/C genotype (95% CI = 1.04-1.76; \( P = 0.024 \)).

It is possible that this site affects the binding of a micro RNA (miRNA) that may regulate expression of the gene, and, thereby, the susceptibility to pancreatic cancer. AmiRNA is a non-coding single stranded RNA, approximately 20 nucleotides in length, which can control aspects of cellular proliferation, differentiation, and apoptosis by modulating post-transcriptional expression of a gene and translation of the corresponding protein. The target genes of miRNAs are often ontogenesis or tumor suppressor genes; hence, mutation, deletion, or abnormal expression of specific miRNAs can play a critical role in the occurrence and development of a tumor. Furthermore, miRNA can affect the stability and translation of mRNAs by binding to the 3'UTR of the mRNA. As a result, an SNP in the 3'UTR of a gene is able to influence gene expression by altering the interaction with amRNA. In this case, if the rs1131878C > T allele of the SNP affects binding of an miRNA to the mRNA encoded by the UGT2B4 gene, then, by affecting expression of the UGT2B4 enzyme, it could alter the susceptibility to tumor formation, the prognosis, and the degree of drug resistance in cases of pancreatic cancer. In support of this idea, an SNP in the binding site of the let-7 miRNA in the KRAS 3'UTR can regulate KRAS expression, and thus potentially increase susceptibility to non-small-cell lung cancer. Similarly, another SNP is associated with the prognosis of oral cancers. The correlation between SNP alleles and miRNA binding in the context of human multigenic disease, including malignant neoplasm, needs to be explored further.

Regarding the rs3822179 polymorphic locus in UGT2B4, there were statistically marginal differences between the distributions of A/A, A/C, and C/C genotypes in patients with pancreatic cancer versus healthy controls (\( P = 0.070 \)). Unconditional logistic regression revealed that the A/C genotype at this locus bestowed a 20% reduction in the risk of pancreatic cancer compared to the A/A genotype (OR = 0.80; 95% CI = 0.67-0.95; \( P = 0.011 \)), but the C/C genotype did not significantly affect risk (OR = 0.77; 95% CI = 0.52-1.14; \( P = 0.191 \)). In combination, the A/C and C/C genotypes significantly decreased the risk of pancreatic cancer (OR = 0.74; 95% CI = 0.59-0.94; \( P = 0.011 \)). It was surprising that the A/C heterozygous genotype at this SNP locus significantly decreased the risk of developing pancreatic cancer, while C/C homozygous variant genotype had little impact on the occurrence and development of disease. Based on these results, it appears that the concept of heterozygote advantage, in which an individual with a heterozygous genotype is biologically more fit than individuals who are homozygous for either allele, may apply to SNPs and cancer predisposition, although further study, with a larger population, is required to confirm this result.

In summary, this study suggests UGT2B4 polymorphisms might be associated with the risk of pancreatic cancer occurrence in Han Chinese individuals.

Disclosure of conflict of interest

None.

Address correspondence to: Chenfeng Wang, Department of Abdominal Surgery, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China. Tel: +86-010-67781331; Fax: +86-010-67781331; E-mail: cheng1fengwang@163.com

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