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Association between UDP-glucuronosyltransferase 2B7 tagSNPs and breast cancer risk in Chinese females

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Summary

This retrospective study was performed to evaluate the association between the *UGT2B7* tagSNPs (rs12233719, rs4356975, rs7435335 and rs7441774) and breast cancer in Chinese females. Blood samples were collected from 672 patients with breast cancer and 670 healthy controls for DNA extraction. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to analyze *UGT2B7* polymorphisms. Dual-luciferase reporter assays were further performed to investigate the regulatory function of *UGT2B7* tagSNPs. The frequency of rs7441774 G allele in the breast cancer cases was statistically significantly higher than in the controls (0.412 vs. 0.358, $P=0.006$; odds ratio (OR)=1.27, 95%CI=1.08-1.48). After adjusting for conventional risk factors, individuals with the GG genotype had a higher breast cancer risk than those with the AA genotype (adjusted OR=1.63, 95%CI=1.18-2.26; $P=0.008$). The GCGG haplotype of *UGT2B7* was also associated with breast cancer (OR=1.22, 95% CI=1.04-1.45; $P=0.027$). Meanwhile, the rs7441774 G allele could significantly decrease the transcriptional activity of the *UGT2B7* gene. This study indicates that *UGT2B7* polymorphisms may play a crucial role in the occurrence and development of breast cancer in the Han Chinese population.

KEY WORDS: UDP-glucuronosyltransferase (UGT) 2B7, polymorphism, breast cancer, sex hormones, susceptibility

1 INTRODUCTION

Breast cancer is the most commonly diagnosed malignancy among women worldwide. It has been reported that cumulative life-time estrogen exposure contributes to the development of breast cancer.^{1,2} Individuals with high estrogen levels (in the top 20-25%) have an approximately 2-fold higher risk of breast cancer compared to individuals with low levels (bottom 20-25%). Sex hormones may promote cancer via a number of mechanisms. However, the ways by which the sex hormones can be eliminated are limited. Sex hormones can be transformed to inactive compounds by glucuronidation.³⁻⁵

Glucuronidation is mainly catalyzed by UDP-glucuronosyltransferase (UGT) enzymes, which produces more hydrophilic compounds that can be secreted from the human body through urine or bile.⁴⁻⁶ Human UGTs are classified into four families based on amino acid sequence similarity: UGT1, UGT2, UGT3 and UGT8. UGT1A and UGT2B are the two major UGT subfamilies; UGT2B includes seven active members: UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17 and UGT2B28. UGT2B7, a major UGT2B isoform in humans,⁷ plays an important role in steroid hormone metabolism and is expressed at high levels in the human liver.

UGT2B7 glucuronidates a variety of endogenous compounds, including steroid hormones, bile acids and retinoids, as well as widely used drugs, such as zidovudine (AZT), morphine, mycophenolic acid, tamoxifen and oxcarbazepine.^{5,8-11,12} The *UGT2B7* gene is located on chromosome 4 and has six exons, with an overall length of approximately 16 kb.¹³ Polymorphisms in the *UGT2B7* gene have been reported to affect the pharmacokinetics and pharmacodynamics of drugs.⁸⁻¹¹

It is well-recognized that hormonal risk factors contribute to the development and progression of breast cancer through an individual's cumulative exposure to estrogen,

including age at menarche, age at menopause and age at first full-term pregnancy.¹⁴

Therefore, studying single nucleotide polymorphisms (SNPs) in genes encoding enzymes associated with estrogen metabolism may provide important data to help estimate breast cancer susceptibility.

The expression of UGT2B7 enzyme was significantly higher in estrogen receptor (ER) positive breast cancer compared to ER negative breast cancer. And ER negative breast cancer patients carrying UGT2B7 gene expression experienced a statistically significant decrease in overall survival. This research aimed to explore the association between four tagSNPs (rs12233719, rs4356975, rs7435335 and rs7441774) in the *UGT2B7* gene, which play a major role in the elimination of bioactive sex hormones from the body, and the risk of breast cancer in females of Chinese Han ethnicity.

2 RESULTS

2.1 Clinical characteristics of the breast cancer cases and controls

The characteristics of the study cohort are summarized in Table 1. Four polymorphisms in the *UGT2B7* gene were genotyped in 672 female patients with breast cancer and 670 female controls. The frequencies of postmenopausal status in the cases and controls were 40.1% and 38.8%, respectively. No significant differences in age, body mass index (BMI) or frequency of menopausal status were observed between the cases and controls. However, cases had a higher frequency of a familial history of cancer than controls (26.0% vs. 23.9%, $P<0.05$). Additional clinical pathological characteristics of the cases, including clinical stage according to the tumor node metastasis (TNM) system and hormone receptor status, are summarized in Table 1.

2.2 Association between UGT2B7 polymorphisms and breast cancer risk

The frequency distributions of the four *UGT2B7* polymorphisms/genotypes in cases and controls are shown in Table 2. All of the genotype frequencies observed was in consistent with Hardy-Weinberg equilibrium in both cases and controls. Of the four *UGT2B7* tagSNPs, the frequency of rs7441774 G allele in the breast cancer cases was statistically significantly higher than in the controls (0.412 vs. 0.358, $P=0.006$; OR=1.27, 95%CI=1.08-1.48). A significant difference of the GG genotype was also observed between the breast cancer cases and controls ($P=0.008$; OR=1.63, 95%CI=1.18-2.26) after assessing the adjusted odds ratios as shown in Table 2. There was no significant difference in the frequency of the rs12233719, rs4356975 or rs7435335 polymorphisms/ genotypes between breast cancer cases and controls after assessing the adjusted odds ratios. Furthermore, the GCGG haplotype showed an increased odds ratio in breast cancer cases ($P=0.027$; OR=1.22, 95%CI=1.04-1.45). There was no association of genotypes with breast cancer grades as shown in supplement Table 1.

2.3 Association between UGT2B7 tagSNPs and hormone receptor status in patients with breast cancer

The relationship between the four *UGT2B7* tagSNPs and hormone receptor status was analyzed in the patients with breast cancer. As shown in Table 3, no association was observed between the *UGT2B7* tagSNPs and estrogen receptor (ER) expression in patients with breast cancer. However, the TTGA haplotype was significantly associated with positive progesterone receptor (PR) expression in the patients with breast cancer ($P=0.017$; OR=1.58, 95%CI=1.04-2.40).

2.4 Identification of the activity of the rs7441774 A>G polymorphism

To determine the effect of the rs7441774 A>G polymorphism in intron 2 on the activity of the UGT2B7 enzyme, allele-specific constructs, pGL3-promoter A (wild-type allele) and pGL3-promoter G (variant allele), were generated and transiently expressed in HepG2 cells. The luciferase activity in cell extracts was analyzed 48 hours after transfection and was standardized against internal control Renilla activity. The results indicated that the rs7441774 G construct showed lower luciferase activity than the rs7441774 A construct ($P=0.005$, Figure 1).

3 DISCUSSION

Recently, a number of SNPs have been reported in the *UGT2B7* gene. Moreover, large differences in the distribution of *UGT2B7* polymorphisms have been observed between different populations and ethnicities.^{15,16} For example, the frequency of the rs7439366 C allele is much higher in a healthy Japanese population (0.756) than in a matched Caucasian population (0.463),^{15,17} in complete linkage disequilibrium with the rs7668258 T>C, rs7662029 A>G and rs7441774 A>G polymorphisms.^{15,16,18} Moreover, Lin et al. observed that *UGT2B7* rs7439366 C>T was associated with bladder cancer risk in benzidine-exposed workers in China.¹⁹

The impact of *UGT2B7* polymorphisms on the metabolism of drugs used to treat breast cancer has also been investigated. For example, the *UGT2B7* rs7668258 C>T polymorphism has been associated with the metabolism, efficacy and toxicity of epirubicin chemotherapy in breast cancer patients.²⁰⁻²¹ Moreover, *UGT2B7* 802Tyr has been suggested as a candidate biomarker for a good response to tamoxifen therapy in patients with breast cancer.¹¹

In the present study, the association between *UGT2B7* tagSNPs and the risk of breast cancer were assessed in females from the Chinese Han population. This study is the first attempt to evaluate the association between the rs12233719, rs4356975 and rs7435335 *UGT2B7* polymorphisms and breast cancer. The rs12233719 G>T, rs4356975C>T and rs7435335 G>A polymorphisms were not significantly associated with breast cancer in this population. Our results showed that the frequency of the rs7441774 G allele was 0.358 in controls, which was the same as that in our previous study of a healthy Chinese population (0.358 vs. 0.341).¹⁸ Interestingly, the frequency of the 2316 GG genotype was significantly higher in breast cancer cases than in controls after adjusting the odds ratios ($P = 0.008$; OR = 1.63, 95% CI = 1.18-2.26). Additionally, the GCGG haplotype of *UGT2B7* had a higher adjusted odds ratio and was significantly associated with breast cancer ($P = 0.027$; OR = 1.22, 95% CI = 1.04-1.45).

It is biologically plausible that *UGT2B7* SNPs have an impact on susceptibility to breast cancer. First, *UGT2B* polymorphisms are associated with the risk of various cancers. Lin et al. showed that the *UGT2B7* C802T polymorphism was associated with bladder cancer in benzidine-exposed workers in China.¹⁹ Moreover, the rs13129471 A>G polymorphism of *UGT2B4* was significantly associated with breast cancer after adjusting for ethnicity.²² Scherer et al. observed an association between a *UGT2B15* polymorphism and increased risk of colorectal cancer.²³ Vidal et al. reported African American men who carry the wild-type functional *UGT2B15* gene have an elevated risk of prostate cancer.²⁴ Second, estrogens (e.g. estradiol and estriol) and catecholestrogens (e.g. 16-OH-estrone) are substrates of *UGT2B7*.⁴⁻⁶ *UGT2B7* glucuronidates sex hormones to produce less active compounds⁶ that are more easily eliminated in bile and urine. Importantly, patients with breast cancer have a decreased expression of *UGT2B7* mRNA in the affected breast compared to the unaffected breast.²⁵ Finally, the *UGT2B7* rs7441774 A>G polymorphism, which was in complete

linkage disequilibrium with the rs7668258 T>C and rs7439366 C>T variants,^{15,16,18} may affect the enzymatic activity of UGT2B7. The *UGT2B7* -161 C>T and 802 C>T polymorphisms have been reported to (删除) affect the plasma concentrations of valproic acid in Chinese patients with epilepsy.²⁶ The *UGT2B7* -161C>T polymorphism was associated with altered lamotrigine pharmacokinetics in patients with epilepsy; patients with the CT and TT genotypes had significantly lower lamotrigine clearance rates than patients with the CC genotype.²⁷ Moreover, we examined the potential functional role of this polymorphism, and found that the A to G substitution at the 2316 allele may generate a decreased transcription of the *UGT2B7* gene. And decreased transcription of the *UGT2B7* gene may reflect in a reduced activity of the enzyme in vivo and this could impact hormone metabolism.

This study indicates that *UGT2B7* tagSNPs may influence individual susceptibility to breast cancer in the Chinese Han population. However, Sun et al. reported no association between *UGT2B7* polymorphisms and breast cancer risk in populations of African ancestry.²⁸ Moreover, no significant associations were observed between *UGT2B4* or *UGT2B7* genotypes and the plasma estradiol concentration.²⁹ These differences could be explained by the fact that the distribution frequencies of *UGT2B7* polymorphisms vary between Asian populations and other ethnic groups.¹⁵⁻¹⁸ Additional studies are required to confirm and further assess the association between *UGT2B7* polymorphisms and the risk of breast cancer.

Estrogen receptor-negative (ER-) and progesterone receptor-negative (PR-) breast cancer are associated with a poor prognosis.^{30, 31} Haddad et al. reported that the rs11571215 polymorphism of the progesterone receptor was strongly associated with ER- breast cancer in African American women.³² Moreover, *UGT1A1* polymorphisms are associated with the risk of ER- breast cancer.²⁹ However, the present study indicates that there is no association

between *UGT2B7* polymorphisms and ER status in breast cancer, consistent with the report that there is a decreased expression of *UGT2B7* mRNA in both ER+ and ER- breast cancer tissues.²⁴ However, the *UGT2B7* TTGA haplotype was significantly associated with PR status in patients with breast cancer in this study. It has been suggested that 6 α - and 21-hydroxyprogesterone could be used as selective “probes” for human liver microsomal *UGT2B7* activity,³³ which may explain the relationship between *UGT2B7* polymorphisms and the expression of the PR in breast cancer.

This study indicates that the GG genotype of the *UGT2B7* rs7441774 A>G and GCGG haplotype of *UGT2B7* are associated with increased breast cancer risk in the Chinese Han population.

4 METHODS

4.1 Study population

Six hundred and seventy-two non-smoking and non-alcohol consuming females diagnosed with pathologically confirmed primary breast cancer between January 2012 and October 2015 at the Affiliated Cancer Hospital of Zhengzhou University were enrolled. As population controls, six hundred and seventy non-smoking and non-alcohol consuming female outpatients were recruited over the same period; these controls were receiving regular physical examinations at the same hospital and had no known malignancy. Data on reproductive factors (age at menarche, age at first birth, age at menopause) and familial history of cancer were obtained through a questionnaire-based interview. Women were assumed to be postmenopausal if they had no periods for at least 12 months before the reference date or had undergone a bilateral oophorectomy. Tumors were categorized as

ER-positive and PR-positive if nuclear staining was observed in at least 10% of nuclei.

Tumor size, histological type and grade were obtained from each patient's pathology report.

Individuals with non-malignant breast lesions (benign breast tumors, mastitis, benign calcification, etc.), other benign or malignant tumors, chronic kidney or hepatic diseases, and/or diabetes mellitus (all of which may be related to metabolic enzyme activity) were excluded from this study. All subjects were from the Han ethnic group in China. This study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University, Zhengzhou, China and was performed in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants included in the study.

4.2 TagSNPs selection, haplotype and genotyping

Many SNPs show linkage disequilibrium (LD), or correlated genotypes, suggesting that only a subset of SNPs, known as tagSNPs, need to be genotyped for disease association studies.

TagSNPs in the *UGT2B7* gene were selected on the basis of the HapMap database and literature review. The Chinese Han population's SNP data of *UGT2B7* gene were downloaded from the HapMap database (<http://www.hapmap.org>). Then the tagSNPs were researched by the following criteria: 1) Detected by Haploview 4.2; 2) MAF (minor allele frequency) of Chinese Han database (CHB) ≥ 0.05 ; 3) A linkage disequilibrium value of $r^2 \geq 0.8$. Finally four candidate SNPs were selected according to these criteria: rs12233719, rs4356975, rs7435335 and rs7441774.

Haplotypes and their frequencies were estimated by using the PHASE program. We attempted to define the correlation coefficient between each SNP and a haplotype of tagging SNPs to be $r_s^2 > 0.8$.

Peripheral venous blood was drawn from each subject and genomic DNA was extracted using the TIANGEN DNA Blood kit (Tiangen Biotech Co., Ltd, Beijing, China). Genotyping UGT2B7 was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a Sequenom MassARRAY system (Sequenom Inc., San Diego, CA, USA). The manually designed primers used for genotyping were listed in supplement Table 2. Genotyping was performed using the iPLEX system (Sequenom Inc., San Diego, CA, USA), as previously described.³⁴ Genotype data analysis was performed using Sequenom real-time detection software (TYPER4.0 software).

4.3 Detecting the function of the rs7441774 A>G allele in the UGT2B7 gene

A dual-luciferase reporter assay was used to detect the effect of the rs7441774 allele on UGT2B7 activity.³⁵ The sequences of target introns with rs7441774A or G alleles were gene-synthesized and then cloned into XhoI and MluI restrictive sites of the pGL3 promoter plasmid (Promega, Madison, WI, USA). We amplified sequence by PCR from human samples carrying homozygous AA allele for pGL3 promoter-A plasmid and the primers were 5' - A A A C T C G A G T G G G A A A T G G - 3' (f o r w a r d) and 5' - A A A A C G C G T A C C T G T G T T T T G T G - 3' (reverse). The pGL3 promoter-A plasmid was prepared by overlap extension PCR. The normal primers were 5'-AAACTCGAGTGGGAAATGG-3' (forward) and 5'-AAAACGCGTACCTGTGTTTGTG-3' (reverse). The mutagenesis primers were 5'-AACTCATGTACACGTTAATACCA-3' (forward) and 5'-TGGTATTAAACGTGTACATGAGTT-3' (reverse). Plasmid constructs were verified by direct sequencing. Plasmid DNA was prepared using a plasmid mega kit (Qiagen, GmbH, Hilden, Germany) for further transfection.

These recombinant plasmids were cotransfected with pRL-SV40 (internal control) into HepG2 cells (hepatoma carcinoma cells) by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The pGL3-promoter plasmid was also co-transfected with pRL-SV40 as a negative control. After incubation for 48 h, cell lysis was performed and firefly luciferase and Renilla luciferase activities were measured with Glomax 20/20 luminometers using the standard protocols of Promega (Madison, WI, USA). Each construct was tested in triplicate and the transfection experiments were performed three times independently.

Immunohistochemistry

ER and PR status was assessed by immunohistochemistry (IHC) assay. The immunohistochemical analysis was conducted following the instructions inside the kit. The monoclonal rabbit ER (SP1) and PR (1E2) antibody used in this study were purchased from Spring Bioscience (Pleasanton, CA, USA).

4.4 Statistical analysis

Statistical analysis was performed using SPSS 23.0 software package (SPSS; IBM, New York, NY, USA) and STATA ver. 8.0 software package (STATA, College Station, TX, USA). Differences in continuous variables with a normal distribution (presented as the mean $\pm$ SD) between breast cancer cases and controls were assessed using the independent sample t-test. Differences in continuous variables with a non-normal distribution (even after transformation) were examined using the Mann-Whitney U-test. Allelic frequencies and Hardy-Weinberg equilibrium were assessed using the Pearson χ^2 -test. Odds ratio (OR) and 95% confidence intervals (95% CI) were calculated to evaluate the associations between

UGT2B7 tagSNPs and breast cancer. To explore the independent effect of *UGT2B7* tagSNPs on the development of breast cancer, we adjusted for conventional risk factors including lifestyle, reproductive factors and history of cancer, age at menarche, age at menopause and age at first full term pregnancy via multiple logistic regression analysis. The association between the *UGT2B7* tagSNPs and hormone receptor status in patients with breast cancer was examined using the Pearson χ^2 -test. The association of genotypes with grade of breast cancer was assessed using the Pearson χ^2 -test. The luciferase reporter assay data were evaluated using an independent-sample t test. Two-tailed P-values<0.05 were considered statistically significant.

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DISCLOSURES

The authors have no financial or commercial conflicts of interest.

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TABLE 1 Demographic features of the breast cancer cases and controls.

Characteristic	Cases (<i>n</i> = 672)	Controls (<i>n</i> = 670)	<i>P</i> -value
Age (years)	55.0 ± 6.53	54.0 ± 6.89	NS
BMI (kg/m ²)	23.5 ± 3.5	23.9 ± 2.6	NS
Postmenopausal, <i>n</i> (%)	270 (40.1)	260 (38.8)	NS
Family history of cancer, <i>n</i> (%)	175 (26.0)	160 (23.9)	<0.05
Age at first birth (years)	24.8 ± 3.9	25.2 ± 4.3	NS
Age of menopause (years)	45.4 ± 6.6	47.5 ± 5.9	<0.05
Age of menarche (years)	12.9 ± 1.50	13.4 ± 1.40	NS
OC use, <i>n</i> (%)	15.5	15.9	NS
HRT use, <i>n</i> (%)	4.6	3.9	NS
Grade of breast cancer, <i>n</i> (%)			
I	38 (5.6)		
II	364 (54.2)		
III	184 (27.4)		
IV	86 (12.8)		
Hormone receptor status, <i>n</i> (%)			
ER (+) PR (+)	405 (60.3)		
ER (+) PR (-)	52 (7.7)		
ER (-) PR (+)	34 (5.1)		
ER (-) PR (-)	181 (26.9)		

BMI, body mass index; NS, non-significant; ER: estrogen receptor; PR: progesterone receptor; OC, oral contraceptive; HRT, hormone replacement therapy. Age, age at first birth, age of menopause and age of menarche (expressed as the mean $\pm$ standard deviation) were abnormally distributed and analyzed using the Mann–Whitney *U*-test. BMI (expressed as the mean $\pm$ standard deviation) was normally distributed and analyzed using Student's *t*-test. Other data are expressed as frequencies and percentages and were evaluated using the χ^2 -test.

TABLE 2 The association between *UGT2B7* tagSNPs and breast cancer was estimated via multiple logistic regression analysis.

Polymorphism/Genotype	Cases (<i>n</i> = 672)	Controls (<i>n</i> = 670)	<i>P</i> -value	aOR (95% CI)
rs12233719 G>T				
G	1164 (86.6)	1129 (84.3)	0.091	0.83 (0.67-1.03)
T	180 (13.4)	211 (15.7)		
GG	500 (74.4)	477 (71.2)		
rs4356975C>T				
C	586 (43.6)	535 (39.9)	0.071	0.88(0.74-1.02)
T	758 (56.4)	805 (60.1)		
CC	138 (20.6)	112 (16.7)		
rs7435335 G>A				
G	1308 (97.3)	1293 (96.5)	0.353	0.76 (0.49-1.18)
A	36 (2.7)	47 (3.5)		
GG	636 (94.7)	625 (93.3)		
rs7441774 A>G				
A	788 (58.8)	860 (64.2)	0.006	1.27 (1.08-1.48)
G	556 (41.2)	480 (35.8)		
AA	237 (35.3)	277 (41.3)		

GTGA	575 (42.9)	603 (45.1)		
GCGG	550 (41.1)	472 (35.5)	0.027	1.22 (1.04-1.45)
GCAA	34 (2.6)	50 (3.8)	0.149	0.73 (0.45-1.13)

Abbreviations: OR = odds ratio; CI = confidence interval; aOR: adjusted for conventional risk factors.

TABLE 3 Association between *UGT2B7* tagSNPs and hormone receptor status in patients with breast cancer.

Polymorphism/Genotype	ER(+)/ER(-),	OR (95% CI)	PR (+)/PR(-),	OR (95% CI)
rs12233719 G>T				
G	777/353		746/132	
T	137/77	1.27 (0.81-1.98)	385/81	1.20 (0.89-1.68)
GG	331/145		317/159	
rs4356975 C>T				
C	391/172		381/497	
T	523/258	1.13 (0.78-1.54)	185/281	1.16 (0.92-1.46)
CC	87/37		87/39	
rs7435335 G>A				
G	870/411		834/42	
A	42/19	0.96 (0.55-1.67)	447/19	0.84 (0.48-1.49)
GG	416/196		398/214	
rs7441774 A>G				
A	560/281		537/341	
G	354/149	0.85 (0.66-1.08)	303/163	0.85 (0.67-1.07)
AA	175/90		167/98	

GCGA	224/99		213/108	
GCGG	127/49	0.86 (0.60-1.31)	128/51	0.79 (0.53-1.17)
GTGA	231/124	1.20 (0.89-1.68)	231/126	1.08 (0.78-1.50)
GTGG	154/65	0.96 (0.66-1.39)	134/84	1.24 (0.86-1.77)

ER = estrogen receptor; PR = progesterone receptor; * $P < 0.05$.

FIGURE 1 The effect of the rs7441774 A>G polymorphism on the transcriptional activity of the UGT2B7 gene. The plasmid with the G allele showed lower transcriptional activity than the A allele. * $P < 0.05$ compared with each of the constructs.