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Gly71Arg UGT1A1 polymorphism is associated with breast cancer susceptibility in Han Chinese women

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ABSTRACT. Breast cancer is among the most common causes of cancer-related death in women worldwide. Previous studies have demonstrated an association between prolonged estrogen exposure and increased risk of breast cancer. Uridine 5'-diphospho-glucuronosyltransferase 1-1 (UGT1A1) plays a significant role in the detoxification of estrogens. Two major genetic polymorphisms have been identified in the *UGT1A1* locus. *UGT1A1*28* has been previously linked to increased risk of breast cancer. The aim of this study was to elucidate the possible correlation between *UGT1A1*6*, a single nucleotide polymorphism causing a Gly71Arg substitution, and breast cancer susceptibility. Forty-six women diagnosed with breast cancer, 15 patients with gastrointestinal cancer, and 13 healthy women were recruited to this study. The genotype in the polymorphic *UGT1A1* locus was determined by DNA

sequencing. The frequency of each genotype was compared among the three groups. The frequency of the *UGT1A1*6* allele was significantly higher in breast cancer and gastrointestinal cancer patients than that in healthy females (both $P < 0.05$). No significant associations were observed between the *UGT1A1*6* polymorphism and estrogen receptor, progesterone receptor, HER-2 expression status, menstrual status, or metastasis (all $P > 0.05$). Therefore, the *UGT1A1*6* polymorphism was deduced to be a risk factor for breast cancer in women of Han Chinese ethnicity. UGT1A1 may serve as a therapeutic target for the prevention and treatment of breast cancer and other estrogen-related diseases.

Key words: Breast cancer; Estrogen metabolism; UGT1A1; Genetic polymorphism

INTRODUCTION

Breast cancer is among the most commonly diagnosed types of human malignancies, and the most common cause of cancer-related death in women worldwide. Although the incidence rates of breast cancer vary around the world and among different groups of people, there has been an increase in the overall rate over the past 30 years (Jemal et al., 2010). The advances in cancer diagnosis and implementation of easily accessible screening programs for early detection have resulted in a decrease in the mortality rate of breast cancer in China; however, the incidence rate has increased rapidly in recent years (Fan et al., 2014). Therefore, there is an urgent need for the identification of simple and effective biomarkers or gene signatures for the prediction of breast cancer risk and survival.

Tumor biomarkers are widely used to diagnose and determine the prognosis of tumors, and to monitor for cancer recurrence and metastasis. Biomarkers that are currently in use (or emerging biomarkers) for the early detection and prognosis of breast cancer include CA 15-3, tissue polypeptide-specific antigen (TPS), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER-2), nm23 metastasis suppressor gene (*NME*), proliferating cell nuclear antigen (PCNA), human mammaglobin (hMAM), vascular endothelial growth factor (VEGF), Bcl-2, and p53 (Weigel and Dowsett, 2010; Koh et al., 2014). There are several risk factors that pose an increased risk of breast cancer, including age, diet, lifestyle, and genetics. The study of the relationship between the genetic predisposition to cancer and risk of cancer has attracted an increased amount of attention over the past few years. For example, individuals carrying mutations in the *BRCA1*, *BRCA2*, *PTEN*, or *p53* genes are at a higher risk of developing breast cancer (Ademuyiwa and Olopade, 2003; Nelson et al., 2012). Furthermore, genetic variations in genes involved in estrogen synthesis and metabolism have been suggested to play important roles in carcinogenesis (Yager and Davidson, 2006; Germain, 2011). This hypothesis has been tested in multiple epidemiological studies focusing on genetic polymorphisms affecting various enzymatic pathways, including polymorphisms in the genes encoding the cytochrome P450 family of enzymes, catechol-*O*-methyltransferase (*COMT*), glutathione S-transferase, N-acetyltransferase, and uridine 5'-diphospho-glucuronosyltransferase (UGT) (Park et al., 2006; Singh et al., 2007; Bugano et al., 2008; Huo et al., 2008; Dumas and Diorio, 2011). In this study, we have focused our investigations on the *UGT1A1* gene.

UGT1A1 is a member of the UGT superfamily that catalyzes the glucuronidation reaction in human bodies. It plays a significant role in the detoxification of a diverse range of molecules, including steroid hormones such as estrogens and their metabolites, catechol estrogens (Radominska-Pandya et al., 1999). UGT1A1 overexpression in the breast cancer cell line MCF-7 led to a decrease in cell proliferation (Leung et al., 2007), indicating that UGT1A1 protects against breast cancer via detoxification, and may serve as a potential target for cancer prevention. A few major genetic polymorphisms have been described in the *UGT1A1* locus to date. The most common and well-characterized polymorphism is located in the TATA box of the *UGT1A1* promoter. The wild-type (WT) allele consists of six TA repeats (*UGT1A1*1*, or **1*), while the variant allele, associated with reduced gene expression, is characterized by seven TA repeats (*UGT1A1*28*, or **28*) (Iyer et al., 2002). A previous study involving 200 African American women with breast cancer and 200 healthy controls demonstrated a strong association between the incidence of breast cancer and the *UGT1A1*28* polymorphism, and a stronger association between this incidence and menopause and ER status (Guillemette et al., 2000). However, this association was not observed in a Chinese population (Adegoke et al., 2004; Yao et al., 2010). Further stratification by age showed a correlation between the *UGT1A1*28* risk allele and a slight elevation in breast cancer risk in Chinese women less than 40 years of age (Adegoke et al., 2004). However, the *UGT1A1*28* risk allele was not significantly associated with the status of ER or PR in these women (Adegoke et al., 2004). The second *UGT1A1* polymorphism of interest is *UGT1A1*6* (or **6*), which results in a nonsynonymous mutation at nucleotide 211 (G211A or Gly71Arg). *UGT1A1*6* occurs at high frequencies only in the Asian population; on the other hand, this mutation is rarely seen in populations of other ethnicities. Therefore, the association between *UGT1A1*6* and cancer risk has been investigated very infrequently (Akaba et al., 1998). A recent study in patients from Taiwan has linked the *UGT1A1*6* polymorphism to colorectal cancer risk (Tang et al., 2005). However, there are currently no reports on the association between the *UGT1A1*6* single nucleotide polymorphism (SNP) and the development of breast cancer.

In this study, genomic DNA was obtained from the peripheral blood of breast and gastrointestinal cancer patients, and healthy controls. The loci containing the *UGT1A1*28* and *UGT1A1*6* polymorphisms were sequenced and the genotype was determined. The genotype frequency distribution was compared among the cancer patients and healthy controls. Furthermore, the association of the *UGT1A1*6* genotype with the expression status of ER, PR, and HER-2, menstrual status, and metastasis was investigated in breast cancer patients.

MATERIAL AND METHODS

Patients and controls

Potential confounding factors such as previous medical conditions and treatment status were excluded by enrolling only recently diagnosed cancer patients with no history of any anti-cancer treatment to this study. Strict inclusion and exclusion criteria resulted in a greatly reduced sampling pool; that is, only 46 histopathologically identified breast cancer patients who attended the Fourth Hospital of Hebei Medical University between May 2010 and October 2012 were enrolled to this study. The stage of cancer in each patient was determined using anatomic staging and the tumor, node, and metastasis (TNM) classification system (Singletary et al., 2002). Fifteen patients with newly diagnosed gastrointestinal cancer and 13

healthy women were recruited to the control group. All study subjects were of Han Chinese ethnicity. The included healthy control women did not have a family history of malignant tumors, and were non-smokers and non-drinkers. All study participants provided a written consent in a form approved by the Institutional Review Board of Hebei Medical University. The study was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All study and experimental procedures were approved by the Institutional Ethics Committee of Hebei Medical University.

Genomic DNA extraction and *UGT1A1* genotyping

Peripheral blood DNA was extracted using the RelaxGene Blood DNA System according to the manufacturer instructions (Tiangen Biotech, Beijing, China). Briefly, 5 mL lysis buffer was added to 2 mL whole blood and mixed by inverting the tube five times. Mixed samples were centrifuged for 2 min at 8000 rpm and the supernatant was discarded. Lysis buffer (7.5 mL) was added to the pellet, and the mixed samples were centrifuged again for 2 min at 8000 rpm. The supernatant was discarded and the tubes were inverted on a clean sheet of absorbent paper for 2 min to allow the pellet to dry. Freshly prepared Buffer FG/ Proteinase K (25 mL) was added to the tubes and mixed by vortexing until the pellet was completely homogenized. The samples were incubated at 65°C for 15 min and subsequently mixed thoroughly with 25 mL isopropanol until a visible DNA precipitate was obtained. This mixture was centrifuged for 8 min at 8000 rpm; the supernatant was discarded and the pellet was left to air dry for 2 min. Seventy percent ethanol (25 mL) was added to the pellet and the tube was vortexed for 5 s, and centrifuged for 8 min at 8000 rpm. Subsequently, the supernatant was discarded. This step was repeated once before inverting the tubes (to let the pellet dry) for 8 min. Buffer TB (500 μL) was added to the pellet, and the tube was vortexed at low speed for 5 s. DNA was dissolved by incubating for 1 h at 65°C, and subsequently stored at -20°C for further use.

PCR primer design and *UGT1A1* genotyping

PCR primers for the amplification of the genomic region containing the *UGT1A1* polymorphisms were designed using the Primer Premier Design Software 5.0, and synthesized by SinoGenoMax (Beijing, China). The primer sequences for the *UGT1A1*1/*28* polymorphism are as follows: 5'-AGCCAGTTCAACTGTTGTTGC-3' and 5'-CTAGGACAACTATTTCATGTCC-3'. The primer sequences for the *UGT1A1*6* (G211A) SNP are 5'-AACCTCAGGCAGGAGCAAAGG-3' and 5'-CATGCAAGAAGAATACAGTGG-3'.

PCR was performed in a 25-μL reaction mix containing 10 to 20 ng genomic DNA template, 1X reaction buffer, 200 μM deoxynucleotide triphosphates (dNTP), 10 pmol primer (each), and 1 U Tap DNA polymerase. PCR was performed under the following reaction conditions: pre-denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C (for *UGT1A1*1/*28*) or 60°C (for *UGT1A1*6*) for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 5 min. The size of the amplified DNA was verified by electrophoresing aliquots of the PCR products on an agarose gel. The size-verified PCR products were sent to SinoGenoMax Chinese National Human Genome Center and sequenced on an ABI PRISM 310 DNA Sequencer (Life Technologies, Grand Island, NY, USA).

Immunohistochemistry (IHC)

IHC was performed on paraffin sections of breast cancer tissues removed from the 46 patients (except for the tissue sample obtained from one patient that was too small for IHC). Monoclonal antibodies against ER, PR, C-erbB-2 (HER-2), and other related IHC reagents were all purchased from Maxin-Bio (Fujian, China). The results of ER and PR staining were evaluated for the presence and intensity of positive nuclear reaction, according to the guidelines recommended by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) (Hammond et al., 2010): weakly positive (+), 10 to 25% weak positive staining; positive (++), 25 to 75% positive staining; strongly positive (+++), >75% strong positive staining, negative (-), no staining, or faint staining within 10%. This study considered the staining results (+), (++), and (+++) as ER- or PR-positive, and (-) as ER- or PR-negative. The HER-2 staining was evaluated according to the ASCO/CAP guideline update (Wolff et al., 2013): negative (0), no staining; weakly positive (+), faint and incomplete membrane staining in <10% tumor cells; positive (++), weak or moderate complete membrane staining in >10% tumor cells; strongly positive (+++), complete and strong membrane staining in >30% tumor cells. The staining results (0) and (+) were classified as HER2-negative, and (++) and (+++) as HER2-positive.

Statistical analysis

Data obtained from the experiments were analyzed using the Statistical Package for Social Sciences (SPSS) software (v.13.0; SPSS Inc., Chicago, IL, USA). The genotype frequency distribution of *UGT1A1*1/*28* and *UGT1A1*6* in the experimental and control groups was compared using the χ^2 test and the two-tailed Fisher exact test. A P value <0.05 was indicative of statistical significance.

RESULTS

Clinical characteristics of breast cancer patients

A total of 46 female patients with breast cancer were recruited to the present study. The clinical and pathological characteristics of included patients are listed in Table 1. The mean age of these patients was 51.7 ± 10.3 years, which showed no significant difference from that of healthy female volunteers (41.6 ± 17.8 years). Additionally, 15 patients with gastrointestinal cancer (10 males and 5 females; mean age: 64.5 ± 10.5 years; gastric and esophageal cancer: 3; colon cancer: 10; others: 2) were recruited as positive controls, based on the recent finding that the *UGT1A1*6* polymorphism was associated with colorectal cancer risk (Tang et al., 2005). All study subjects were of Han Chinese ethnicity. The included patients were diagnosed at various anatomic and TNM stages, and had different expression status of ER, PR, and HER-2. Approximately 1/3 of these patients had lymph node or distant metastases.

*UGT1A1*28* polymorphism was not associated with breast cancer risk

The genotype of breast cancer patients, gastrointestinal cancer patients, and healthy controls was determined by DNA sequencing in the *UGT1A1*1/*28* polymorphic loci.

Table 1. Basic demographic information and pathological characteristics of breast cancer patients included in this study.

Characteristics	Cases [N (%)]
Ethnicity	
Han Chinese	46 (100.0)
Age (mean ± SD)	51.7 ± 10.3
Menopausal status	
Premenopausal	25 (54.3)
Postmenopausal	21 (45.7)
Anatomic stage at diagnosis	
Stage IA	10 (21.7)
Stage IIA	17 (37.0)
Stage IIB	7 (15.2)
Stage IIIA	4 (8.7)
Stage IIIB	1 (2.2)
Stage IIIC	5 (10.9)
Unclassified	2 (4.3)
TNM stage	
TisN0M0	1 (2.2)
T0N1M0	1 (2.2)
TxN3M0	1 (2.2)
T1N0M0-T1N3M0	19 (41.3)
T2N0M0-T2N3M0	21 (45.7)
T3N0M0-T3N2M0	2 (4.3)
Unclassified	1 (2.2)
Metastases	
None	31 (67.4)
Lymph node	13 (28.3)
Distant	2 (4.3)
ER	
-	11 (23.9)
+	10 (21.7)
++	7 (15.2)
+++	18 (39.1)
PR	
-	15 (32.6)
+	9 (19.6)
++	17 (37.0)
+++	5 (10.9)
HER-2	
- and +	20 (43.5)
++	15 (32.6)
+++	11 (23.9)

The genotype frequency in each group was analyzed, compared among the groups, and the results summarized in Tables 2, 3, and 4. Both WT alleles (**1/*1)* were expressed by 61% (frequency distribution) of the healthy controls, 89% of the breast cancer patients, and 87% of the gastrointestinal cancer patients. Thirty-nine percent of the healthy controls expressed a heterozygous variation (**1/*28*), while only 11 and 13% of breast cancer and gastrointestinal cancer patients expressed this mutation, respectively (Tables 2 and 3). None of the included subjects expressed a **28/*28* homozygous variation (Tables 2 and 3). A comparison of the genotype distribution showed no statistical differences between the breast cancer patients and healthy controls (P = 0.055; Table 2), between the gastrointestinal cancer patients and healthy controls (P = 0.198; Table 3), or between the two cancer groups (P = 0.055; Table 4). The results indicated that the *UGT1A1*28* polymorphism was not associated with breast cancer risk in Han Chinese women.

Table 2. *UGT1A1*28* genotype distribution among breast cancer patients and healthy controls.

Genotype	Breast Cancer		Healthy		P
	Number	%	Number	%	
*UGT1A1*1/*1*	41	89	8	61	
*UGT1A1*1/*28*	5	11	5	39	0.055
*UGT1A1*28/*28*	0	0	0	0	

Table 3. *UGT1A1*28* genotype distribution among gastrointestinal (GI) cancer patients and healthy controls.

Genotype	GI cancer patients		Healthy controls		P
	Number	%	Number	%	
*UGT1A1*1/*1*	13	87	8	61	
*UGT1A1*1/*28*	2	13	5	39	0.198
*UGT1A1*28/*28*	0	0	0	0	

Table 4. *UGT1A1*28* genotype distribution among breast cancer patients and gastrointestinal (GI) cancer patients.

Genotype	Breast cancer patients		GI cancer patients		P
	Number	%	Number	%	
*UGT1A1*1/*1*	41	89	13	87	
*UGT1A1*1/*28*	5	11	2	13	0.055
*UGT1A1*28/*28*	0	0	0	0	

*UGT1A1*6* polymorphism was associated with breast cancer risk

A similar analysis of the *UGT1A1*6* (G211A) polymorphism is summarized in Tables 5, 6, and 7. All healthy controls (100%) carried both WT alleles (G/G). Twenty-six percent of the breast cancer patients carried the heterozygous variation (G/A) and 7% expressed the homozygous variation (A/A); that is, the variant **6* allele was distributed in 33% of the study subjects (Table 5). Compared to the absence of the **6* polymorphism in healthy individuals, the *UGT1A1* G211A SNP may exert a positive effect on the risk of developing breast cancer in Han Chinese women. We also observed a similar potential association between the **6* polymorphism and risk of gastrointestinal cancer, which is consistent with previous findings (P = 0.018; Table 6) (Tang et al., 2005). The **6* polymorphism might not be used to predict the risk of a specific cancer type as the variant allele frequency did not differ significantly between the breast cancer and gastrointestinal cancer groups (P = 1; Table 7).

Table 5. *UGT1A1*6* genotype distribution among breast cancer patients and healthy controls.

Genotype	Breast cancer patients		Healthy controls		χ^2	P
	Number	%	Number	%		
G/G	31	67	13	100		
G/A	12	26	0	0		
A/A	3	7	0	0		
G/A+ A/A	15	33	0	0	4.095	0.043

Table 6. *UGT1A1*6* genotype distribution among gastrointestinal (GI) cancer patients and healthy controls.

Genotype	GI cancer patients		Healthy controls		P
	Number	%	Number	%	
G/G	9	60	13	100	
G/A	6	40	0	0	0.018
A/A	0	0	0	0	

Table 7. *UGT1A1*6* genotype distribution among breast cancer patients and gastrointestinal (GI) cancer patients.

Genotype	Breast cancer patients		GI cancer patients		P
	Number	%	Number	%	
G/G	31	67	9	60	
G/A	12	26	6	40	
A/A	3	7	0	0	
G/A+ A/A	15	33	6	40	1

*UGT1A1*6* polymorphism was not associated with hormone receptor status and disease stage in breast cancer patients

Cancer tissue was obtained from breast cancer patients and stained for ER, PR, and HER-2 expression. The ER and PR status was determined for the *UGT1A1*6* genotype, as summarized in Table 8. The **6* polymorphism and ER or PR expression status was not significantly associated (P = 0.763 and P = 1, respectively). Similarly, the HER-2 expression status was not associated with the *UGT1A1*6* genotype distribution (P = 0.553; Table 9). Longer lifetime exposure to estrogen is a risk factor for breast cancer. We observed no significant association between the menopausal status of patients and breast cancer risk (P = 0.923; Table 10). Analyses of the relationship between disease stages and the *UGT1A1*6* genotype distribution investigated also revealed the lack of a significant association between the metastatic status and the *UGT1A1*6* genotype (P = 1; Table 11). These results indicated that the variant *UGT1A1*6* allele may not predict, or be used to monitor metastasis, in breast cancer.

Table 8. *UGT1A1*6* genotype distribution in breast cancer patients with differing estrogen receptor (ER) or progesterone receptor (PR) expression status.

Genotype	ER				PR			
	-	+	χ^2	P	-	+	χ^2	P
G/G	6	25			10	21		
G/A	3	8			4	7		
A/A	1	2			1	2		
G/A+ A/A	4	10	0.091	0.763	5	9	<0.001	1

Table 9. *UGT1A1*6* genotype distribution in breast cancer patients with different HER-2 expression status.

Genotype	HER-2		χ^2	P
	-	+		
G/G	14	17		
G/A	2	9		
A/A	3	0		
G/A+ A/A	5	9	0.353	0.553

Table 10. *UGT1A1*6* genotype distribution in breast cancer patients with differing menopausal status.

Genotype	Menopausal status		χ^2	P
	Premenopausal	Postmenopausal		
G/G	17	14		
G/A	7	5		
A/A	1	2		
G/A+ A/A	8	7	0.009	0.923

Table 11. *UGT1A1*6* genotype distribution in breast cancer patients with or without lymph node and distant metastases.

Genotype	Lymph node and distant metastases		χ^2	P
	Yes	No		
G/G	10	21		
G/A	4	8		
A/A	1	2		
G/A+ A/A	5	10	<0.001	1

DISCUSSION

Estrogen is a major risk factor for breast cancer (Yager and Davidson, 2006). Under normal circumstances, estrogen promotes the growth and development of breast tissues and maintains the health of the female reproductive system. However, alterations in estrogen signaling pathways, estrogen synthesis, or metabolism contribute to tumorigenesis of breast cancer (Germain, 2011). Estrogen binds to its intracellular receptor, ER, and the estrogen/ER complex translocates into the nucleus to activate target gene transcription and initiate the signaling cascade, including the production of secondary messengers such as cyclic adenosine monophosphate (AMP), transforming growth factor (TGF)-α and epidermal growth factor (EGF) (Moggs and Orphanides, 2001). Deregulated cell proliferation, due to excessive stimulation of estrogen, greatly increases the chance of errors in DNA replication, which subsequently leads to DNA damage and genetic mutations. When the mutations affect the key factors involved in damage repair or apoptosis, such as BRCA1, BRCA2, PTEN, or p53, the damages accumulate and malignant transformation is initiated (Caldon, 2014). The metabolites of estrogen could also damage the DNA and induce malignant transformation (Zhu and Conney, 1998; Russo et al., 2003). Among these metabolic pathways, UGTs catalyze the glucuronidation reaction, a major pathway in phase II (conjugative) drug metabolism. As a member of the UGT superfamily, UGT1A1 plays a significant role in the detoxification of estrogens and their metabolites (Radominska-Pandya et al., 1999; Lépine et al., 2004). Therefore, it is logical to reason that any dysfunction in the UGT1A1-mediated estrogen metabolism could contribute to increased breast cancer risk.

Individuals carrying the *UGT1A1*28* or *UGT1A1*6* polymorphism have been reported to express reduced *UGT1A1* gene expression or enzymatic activity, respectively (Yamamoto et al., 1998; Guillemette et al., 2000; Gagné et al., 2002; Tang et al., 2005). Reduced rate of estrogen metabolism, and the resulting accumulation of estrogen and its metabolic products, could increase the risk of breast cancer development. The role of *UGT1A1*28* polymorphism in breast cancer susceptibility has been demonstrated by multiple studies (Guillemette et al., 2000; Adegoke et al., 2004). However, the results from our study did not indicate a correlation

between the *UGT1A1*28* polymorphism and breast cancer risk. Such controversial results have also been reported by previous studies and are speculated to result from inter-ethnic and regional variations in the *UGT1A1*28* polymorphism (Kaniwa et al., 2005; Yao et al., 2010). For example, the frequency of homozygous and heterozygous *UGT1A1*28* genotypes is approximately 10-15 and 35-50%, respectively, in Caucasians (Beutler et al., 1998; Innocenti et al., 2002). However, the homozygote frequency is much lower, ranging from 0 to 5%, in individuals from China, Southeast Asia, and the Pacific Islands (Premawardhena et al., 2003). In this study, the breast cancer patients and healthy controls expressed no homozygous *UGT1A1*28*, which is consistent with previous reports. The frequency of heterozygous *UGT1A1*28* in healthy controls was 39%, which is slightly higher than that reported previously. We attribute this inconsistency to the smaller sample size in this study and the potential regional variation of genetic polymorphism in China. Studies with larger sample sizes will provide a more conclusive result to elucidate the correlation between the *UGT1A1*28* polymorphism and breast cancer risk.

The *UGT1A1*6* polymorphism, causing a G→A substitution in the first exon, is a common SNP in the Asian population, but is rare in the Caucasian and African populations (Huang et al., 2000). Our study demonstrated the potential association of the *UGT1A1*6* SNP with the development of breast cancer in a Chinese population for the first time. We hypothesized that the functional defect in UGT1A1, caused by the G211A mutation, delays the elimination of estrogen from the human body, and that the resultant excessive exposure to estrogen and its metabolic products increases the risk of breast cancer. This hypothesis is strongly supported by the results of our study.

Accumulation of estrogen and its metabolites due to defective UGT1A1 activity could increase the chance of cancer recurrence and distant metastasis. Therefore, one may argue that the frequency of the variant *UGT1A1*6* allele is higher in recurrent and metastatic breast cancer patients than in patients in remission. However, our study did not reveal any significant correlation between the distribution of the *UGT1A1*6* genotype and metastasis. Breast cancer has multiple risk factors, including lifestyle, environment, genetics, and other medical conditions. In addition, estrogen is metabolized by several enzymes besides UGT1A1, such as cytochrome P450 1A1 (*CYP1A1*), COMT, and sulfotransferase (Zhu and Conney, 1998). Therefore, an SNP in *UGT1A1* is a risk factor, but not a sufficient one for the development and progression of breast cancer. More biological and clinical studies are needed to assess the role of individual metabolic enzymes in tumorigenesis of breast cancer, and to understand the effect of their interactions on disease development. This knowledge is crucial for the early diagnosis and treatment of breast cancer.

The *UGT1A1*6* polymorphism induces a similar susceptibility to gastrointestinal cancer patients, as observed in this study. This result is consistent with those of previous studies (Tang et al., 2005). In addition to estrogen metabolism, UGT1A1 participates in the detoxification of various endogenous and exogenous carcinogens. For example, UGT1A1 metabolizes 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), a human carcinogen abundant in cooked meat, which increases with the temperature and duration of cooking (Zheng and Lee, 2009). *In vitro* and *in vivo* animal studies have linked PhIP exposure with risk of colorectal, prostate, and breast cancer (Norrish et al., 1999; Felton et al., 2002; Cross and Sinha, 2004). Therefore, the relevance of the *UGT1A1*6* polymorphism must be analyzed in susceptibility to other types of cancer.

One should note that this study was conducted in a limited sample population,

including only Han Chinese women from the Hebei Province of China. The association of the *UGT1A1*6* polymorphism with breast cancer risk is a novel study, and we are unclear about the impact of previous medical conditions and treatments on our study. In order to exclude these potential confounding factors, we only enrolled patients who were recently diagnosed with breast or gastrointestinal cancer, and did not undergo any treatment prior to the sample collection. The strict inclusion and exclusion criteria ensured the enrollment of a defined patient population, and established precision in our cohort. However, this unfortunately reduced the available sample pool. Additionally, participation in clinical studies has not been widely accepted in the Chinese population. This resulted in the small sample size of this study. In future investigations, required to validate these results, we intend to raise public awareness and encourage a greater number of Chinese women to participate in clinical studies. We believe that a larger sample size and increased coverage, including different regions and ethnic groups, could clarify the relationship between the *UGT1A1*6* polymorphism and breast cancer risk. Furthermore, the small sample size also limited us from performing association studies in different cancer staging groups to explore the role of UGT1A1 in cancer progression. The relationship between the *UGT1A1*6* polymorphism and clinicopathological parameters such as ER, PR, and HER-2 status, and lymph node and distant metastases should be addressed in future studies.

In conclusion, the results of our study indicated the *UGT1A1*6* polymorphism as a potential risk factor for breast cancer in a Han Chinese population cohort. Hormone therapy, which uses anti-estrogen drugs, has been a great success in the treatment of ER-positive breast cancer patients. Given the central role played by UGT1A1 in estrogen metabolism and cancer susceptibility, UGT1A1 may serve as a therapeutic target for the prevention and treatment of breast cancer and other estrogen-related diseases.

Conflicts of interest

The authors declare no conflict of interest.

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