Original Study

The Effect of Polymorphism in UGT1A4 on Clinical Outcomes of Adjuvant Tamoxifen Therapy for Patients With Breast Cancer in China

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Abstract

More markers are needed to guide adjuvant endocrine therapy decisions for patients with breast cancer. In this study, we found that patients with breast cancer with rs869283 variations (G/A or A/A) in the UGT1A4 gene, accounting for 21.3% of the population, received less benefit from adjuvant tamoxifen treatment. The efficacy of adjuvant aromatase inhibitors could not be influenced by this polymorphism.

Introduction: UGT1A4 is a major enzyme responsible for the glucuronidation of tamoxifen (TAM) and its metabolites. Genetic variations in the UGT1A4 gene could have a significant impact on the clinical efficacy of TAM. This study was performed to validate the association between UGT1A4 polymorphisms and the clinical outcomes for patients with breast cancer who received adjuvant TAM. **Patients and Methods:** A total of 773 patients with breast cancer who received adjuvant TAM (n = 321) or aromatase inhibitors (n = 452) at the National Cancer Center in China were analyzed. Through a series of screenings, the single nucleotide polymorphism rs869283 (c.-1180G>A) in the promoter region of the UGT1A4 gene was selected. The associations of rs869283 genotype with disease-free survival (DFS) and clinicopathologic characteristics were analyzed. **Results:** A total of 608 (78.7%) patients were wild-type G/G genotype, 154 (19.9%) patients were G/A genotype, and 11 (1.4%) patients were A/A genotype. In the TAM treatment group, patients with A/A or G/A genotype had a lower 5-year DFS rate than those with the wild-type G/G genotype (69.3% vs. 83.7%; $P = .031$). The rs869283 genotype remained an independent prognostic marker for DFS in multivariate analysis (hazard ratio, 1.74; $P = .014$). No association between the rs869283 genotype and DFS was found in patients who received AIs ($P = .772$). **Conclusions:** Our findings showed that patients with the UGT1A4 rs869283 G/A or A/A genotype received less benefit from adjuvant TAM treatment than those with the G/G genotype. Further studies are warranted to confirm our findings.

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Introduction

Tamoxifen (TAM) is the most widely used endocrine drug in premenopausal women with breast cancer. Adjuvant treatment with TAM reduces the recurrence and mortality rates of estrogen receptor (ER)-positive breast cancer by 50% and 30% respectively.[1] Currently, it is the standard adjuvant endocrine therapy recommended by the National Comprehensive Cancer Network (NCCN) guidelines for premenopausal patients with breast cancer.[2]

TAM is a prodrug that undergoes bioactivation in the liver mainly by cytochrome P450 2D6 (CYP2D6) and cytochrome P450 3A4 into several metabolites, including 4-hydroxytamoxifen

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(4-OH-TAM) and 4-hydroxy-N-desmethyl-TAM (endoxifen). These 2 metabolites are considered to be the main contributors to the activity of TAM in vivo.[3,4] In the meantime, the elimination of TAM and its metabolites is mainly through glucuronidation by the UDP-glucuronosyltransferases (UGTs). Glucuronidation catalyzed by the UGT enzymes is one of the major phase II drug-metabolizing pathways for a variety of chemicals.[5,6] This reaction increases the polarity of the target compounds and facilitates their excretion in bile or urine. TAM is conjugated to glucuronic acid through UGTs' catalyzation and then excreted predominantly through the bile. In the meantime, the glucuronide conjugates of TAM and its metabolites exhibited no antiestrogenic activity.[7] The UGT enzymes comprise a superfamily of proteins including 4 different families, namely UGT1, UGT2, UGT3, and UGT8. Among these, UGT1 and UGT2 (containing UGT2A and UGT2B) are considered to be the most important ones in drug glucuronidation. Currently, a total of 19 isoforms are known from the UGT1 and UGT2 subfamilies.[8]

One of the major UGTs involved in the glucuronidation of TAM and its metabolites is the UGT1A4 in the liver. In vitro studies have demonstrated that the UGT1A4 in the liver is the only enzyme responsible for the N-glucuronidation of TAM and 4-OH-TAM.[9,10] Several studies investigated the relationship between the genetic variations of UGT1A4 and the glucuronidation rates of TAM and its metabolites. One of these studies demonstrated that the UGT1A4^{48Val} variant exhibits increased N-glucuronidation activity against 4-OH-TAM compared with the wild-type enzyme.[11] Benoit-Biancamano et al found that several coding variants in the UGT1A4 gene significantly modified the enzyme kinetics for TAM and 4-OH-TAM.[12] In another research, the team of Greer et al demonstrated a significant impact of genetic variations in the UGT1A4 promoter region on the glucuronidation efficiency of TAM and 4-OH-TAM.[13]

However, less is known about the relationship between UGT1A4 polymorphisms and the clinical efficacy of TAM. There has been no such data reported so far. Through a series of screenings mentioned in the text below, the single nucleotide polymorphism (SNP) rs869283 (c.-1180G>A) in the promoter region of UGT1A4 gene was selected. This study was performed in Chinese women with breast cancer to validate the association between UGT1A4 polymorphisms and the outcomes of patients who received TAM. The patients treated with adjuvant aromatase inhibitors (AIs) were used as a control group. The results may help to optimize the individualized adjuvant endocrine treatments for patients with breast cancer.

Patients and Methods

Subjects

The study included 773 patients with breast cancer who received adjuvant TAM (n = 321) or AI (n = 452) treatment after completion of surgery at the National Cancer Center in China from June 1991 through March 2014. The inclusion criteria used were as follows: (1) diagnosed with invasive breast cancer; (2) ER- and/or progesterone receptor (PR)-positive on immunohistochemistry; and (3) TAM or AIs used as adjuvant treatment for 5 years, and therapy was stopped when a recurrence was identified. All patients were also included in our previous study discussing the relationship between the CYP2D6 *10 genotype and the clinical efficacy of TAM.[14]

The median follow-up was 75.5 months (range, 1.6-206.0 months). Disease-free survival (DFS) was defined as the time from surgery to recurrence or death. The following clinicopathologic data were collected: age at diagnosis; tumor grade; clinical stage (American Joint Committee on Cancer [AJCC], seventh edition); ER, PR, and human epidermal growth factor receptor 2 (HER2) status; year of diagnosis; adjuvant chemotherapy; and adjuvant radiotherapy. All patients underwent standard surgery and adjuvant treatments, such as chemotherapy, endocrine therapy, and radiotherapy, according to NCCN guidelines.

All the pathologic and immunohistochemistry assessments were performed according to typical standards and confirmed by 2 pathologists. Peripheral blood samples were used as a source of DNA for UGT1A4 genotyping.

Genotyping

Drug-response UGT1A4 SNPs that had been reported were selected from an online database (www.cypalleles.ki.se). The dbSNP database (www.ncbi.nlm.nih.gov) was used to find the potential functional SNPs in the promoter region. The 1000Genomes database (www.internationalgenome.org) was used to screen the candidate SNPs with minor allele frequencies of > 5% in the Chinese Han population. Haploview software 4.2 was used to analyze the linkage disequilibrium of candidate SNPs based on the Chinese Han population data of the 1000Genomes database, and SNPs of r^2 > 0.8 were abandoned. In the end, there was only 1 SNP that was selected: the rs869283 (c.-1180G>A) in the promoter region of the UGT1A4 gene.

DNA for testing was extracted from peripheral blood samples. Genotypes were detected using the MassArray system (Agena, San Diego, CA). Approximately 10 to 20 ng of genomic DNA was isolated from peripheral blood. Sample DNA was amplified using a multiplex polymerase chain reaction. Assay design 3.1 software (Agena) was used to design the primers. The following primers were used for rs869283: forward: 5'-ACGTTGGATGAGA-CATGGCCAGGCATGTAG-3' and reverse: 5'-ACGTTG-GATGCACTCACAATGGTCTCATGC-3'. The polymerase chain reaction products were used for a locus-specific single-base extension reaction. The unextension primer used was as follows: 5'-GCTTCCTTGTCCTGGT-3'. The resulting products were desalted and transferred to a 384-element SpectroCHIP array. The alleles were discriminated using mass spectrometry (Agena). The CYP2D6 *10 genotype was also detected in all the patients, and the primers used were described in our previous work.[14]

Statistical Analysis

Statistical analyses were performed using SPSS 22.0 software. Correlation between the rs869283 genotypes and baseline clinicopathologic characteristics was determined using the Pearson χ^2 test. Kaplan-Meier estimates and log-rank tests were used in univariate analysis of DFS. Cox proportional hazard model analysis was performed to identify the significant factors associated with DFS. All statistical tests were 2-sided, and P values < .05 were considered statistically significant.

Results

Frequencies of Genotypes

The UGT1A4 rs869283 genotype was assessed in all patients. A total of 608 (78.7%) patients were homozygous for the wild-type genotype (G/G), 11 (1.4%) patients were homozygous for the variant genotype (A/A), and 154 (19.9%) patients had a heterozygous genotype (G/A). The frequency of the rs869283 G>A allele in our study was 12.3%. A test for Hardy-Weinberg equilibrium was done and demonstrated that the rs869283 allele was within Hardy-Weinberg equilibrium ($P = .726$).

Association Between the rs869283 Genotype and DFS in Patients Who Received TAM

The study we conducted previously showed that Chinese patients with breast cancer with the CYP2D6 *10 T/T genotype had worse clinical outcomes when receiving adjuvant TAM treatment.[14] Therefore, the CYP2D6 *10 genotype was also included in this study. As there were too few patients with the A/A genotype, they were combined with G/A genotype patients into 1 group. No significant associations between the rs869283 genotype and age; grade; clinical stage; ER, PR, or HER2 status; year of diagnosis; adjuvant chemotherapy; adjuvant radiotherapy; and CYP2D6 *10 genotype were found in the 321 patients who received adjuvant TAM treatment (Table 1). Kaplan-Meier estimates indicated that the rs869283 genotypes were significantly associated with DFS. Patients with A/A or G/A genotypes had a lower 5-year DFS rate than those with the wild-type G/G genotype (69.3% vs. 83.7%; $P = .031$) (Figure 1). In univariate Cox proportional hazard analysis, age, clinical stage, HER2 status, adjuvant chemotherapy, and CYP2D6 *10 genotype were significant variables for DFS. The rs869283 genotype remained an independent prognostic marker of DFS in multivariate analysis (hazard ratio, 1.74; 95% confidence interval, 1.12-2.70; $P = .014$) after adjusting for these variables (Table 2).

Association Between the rs869283 Genotype and DFS in Patients Who Received AIs

In the control group, no association between the rs869283 genotype and DFS was found in patients who received AIs. The 5-year DFS rate of patients with A/A or G/A genotypes was similar to those with the G/G genotype (87.9% vs. 87.7%; $P = .772$) (Figure 2).

Table 1 Associations Between the rs869283 Genotypes and Patient Characteristics in the TAM Group

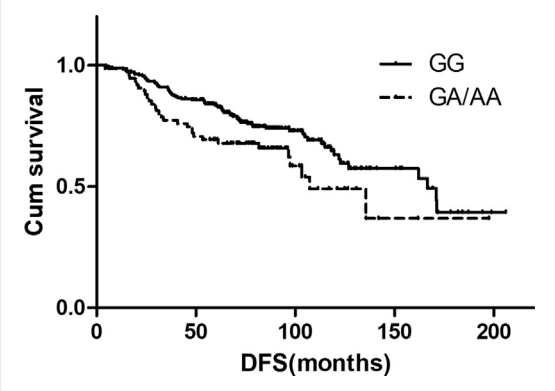
Characteristics	rs869283 Genotypes, N (%)		*P*
	G/G (n = 246)	G/A or A/A (n = 75)	
Age, y			.339
≥ 50	66 (26.8)	16 (21.3)	
< 50	180 (73.2)	59 (78.7)	
Clinical stage			.221
0-I	71 (28.9)	23 (30.7)	
II	110 (44.7)	32 (42.7)	
III	51 (20.7)	11 (14.7)	
Unknown	14 (5.7)	9 (12.0)	
Tumor grade			.837
I	16 (6.5)	3 (4.0)	
II	123 (50.0)	39 (52.0)	
III	44 (17.9)	15 (20.0)	
Unknown	63 (25.6)	18 (24.0)	
ER			.897
0	30 (12.2)	8 (10.7)	
1+	61 (24.8)	17 (22.7)	
2+	52 (21.1)	20 (26.7)	
3+	97 (39.4)	28 (37.3)	
Unknown	6 (2.4)	2 (2.7)	
PR			.721
0	24 (9.8)	8 (10.7)	
1+	90 (36.6)	21 (28.0)	
2+	47 (19.1)	18 (24.0)	
3+	79 (32.1)	26 (34.7)	
Unknown	6 (2.4)	2 (2.7)	
HER2			.417
Positive	32 (13.0)	6 (8.0)	
Negative	131 (53.3)	45 (60.0)	
Unknown	83 (33.7)	24 (32.0)	
Year of diagnosis			.932
≤ 1999	9 (3.7)	3 (4.0)	
2000-2009	156 (63.4)	49 (65.3)	
≥ 2000	81 (32.9)	23 (30.7)	
Adjuvant chemotherapy			.790
Yes	217 (88.2)	67 (89.3)	
No	29 (11.8)	8 (10.7)	
Adjuvant radiotherapy			.386
Yes	147 (59.8)	49 (65.3)	
No	99 (40.2)	26 (34.7)	
CYP2D6 *10 genotype			.896
C/C or C/T	192 (78.0)	58 (77.3)	
T/T	54 (22.0)	17 (22.7)	

Abbreviations: ER = estrogen receptor; HER2 = human epidermal growth factor receptor 2; PR = progesterone receptor; TAM = tamoxifen.

Discussion

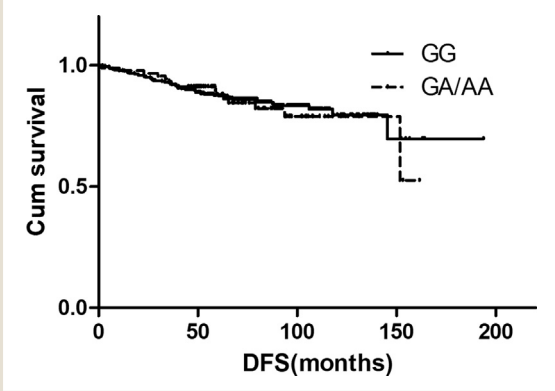
Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death among women worldwide and in China.[15,16] The National Cancer Center in China estimated that 268,600 Chinese women would be diagnosed with breast cancer and that 69,500 would die of the disease in 2015.[16] Postoperative treatment with TAM for at least 5 years is considered a standard method for adjuvant endocrine therapy for premenopausal ER-positive breast cancer patients. But according to the latest reports based on the TEXT (Tamoxifen and Exemestane Trial) and SOFT (Suppression of Ovarian Function Trial) trials, the addition of ovarian suppression to TAM resulted in significantly higher DFS and overall survival (OS) rates. The use of exemestane plus ovarian function suppression (OFS) resulted in an even higher DFS rate.[17] This makes it urgent to find more markers to help us with the individualization decisions of adjuvant endocrine treatment for patients with breast cancer.

Figure 1 Kaplan-Meier Estimates of Disease-free Survival for Different rs869283 Genotypes in 321 Patients With Breast Cancer Receiving Adjuvant Tamoxifen. Patients With the G/A or A/A Genotype Were Compared With Those With the G/G Genotype



Abbreviations: Cum = cumulative; DFS = disease-free survival.

Figure 2 Kaplan-Meier Estimates of Disease-free Survival for Different rs869283 Genotypes in 452 Patients With Breast Cancer Receiving Adjuvant Aromatase Inhibitors. Patients With the G/A or A/A Genotype Were Compared With Those With the G/G Genotype



Abbreviations: Cum = cumulative; DFS = disease-free survival.

The pharmacology of TAM is complex. It undergoes extensive metabolism through 2 major pathways: N-demethylation and 4-hydroxylation. CYP2D6 and CYP3A4 are the 2 main enzymes involved in this activation process.[3,4] Many studies, including ours, have proven that patients with breast cancer with the CYP2D6 *10 T/T genotype had worse clinical outcomes when receiving adjuvant TAM treatment.[14,18,19] However, the potential effect of genetic variations of other enzymes in the metabolic pathway on the pharmacologic activity and clinical efficacy of TAM is still not fully understood.

Table 2 Multivariate Analysis of Disease-free Survival in 321 Patients With Breast Cancer Receiving TAM Treatment

Variable	DFS HR (95% CI)	*P*
rs869283 genotype		
G/G	1.00	
G/A or A/A	1.74 (1.12-2.70)	.014
Clinical stage		
0-I	1.00	
II	1.51 (0.86-2.63)	.149
III	1.87 (1.01-3.47)	.048
Unknown	0.97 (0.38-2.49)	.954
HER2		
Negative	1.00	
Positive	0.54 (0.26-1.10)	.089
Unknown	0.49 (0.31-0.78)	.002
Adjuvant chemotherapy		
No	1.00	
Yes	2.85 (0.98-8.31)	.055
CYP2D6 *10 genotype		
C/C or C/T	1.00	
T/T	1.83 (1.20-2.80)	.005

Abbreviations: CI = confidence interval; DFS = disease-free survival; HER2 = human epidermal growth factor receptor 2; HR = hazard ratio; TAM = tamoxifen.

In the TAM metabolic pathway, UGT1A4 plays an important role in the glucuronidation of TAM and 4-OH-TAM. Our study found a strong relationship between the UGT1A4 variations and the clinical outcome of TAM treatment. Patients with breast cancer with the UGT1A4 rs869283 A/A or G/A genotype exhibited worse DFS than patients with the G/G genotype when receiving TAM treatment; the A/A or G/A genotypes remained an independent poor prognostic factor of DFS in multivariate analysis. This group accounted for 21.3% of the total patients. As we mentioned above, several studies demonstrated a significant impact of genetic variations in the UGT1A4 gene on the glucuronidation of TAM and its metabolites. But the present study is one of the first to clarify the potential role of UGT1A4 genetic polymorphisms on the clinical efficacy of TAM.

In the control group, we investigated the association between rs869283 genotype and DFS in patients who received AI treatment. The results showed that there was no effect of variations in rs869283 on clinical outcomes of adjuvant AI therapy, suggesting that rs869283 genotype was a predictive marker for the efficacy of TAM treatment, but not a prognosis marker for breast cancer.

The SNP rs869283 is located in the promoter region of the UGT1A4 gene. We predicted that rs869283 was located within a transcription factor binding site through an online tool (https://snpinfo.niehs.nih.gov/). Its polymorphisms might affect the binding of transcription factors to the gene, resulting in the change of gene expression and further affecting the biological function.[20] But there were still no reports on the function of this SNP.

There have also been several studies investigating the relationship between other UGT genes polymorphisms and the efficacy of TAM. It was reported by Nowell et al that TAM-treated patients with UGT2B15 high-activity genotypes had an increased risk of

recurrence and poorer survival, but with no statistical significance.[21] In another study conducted by Wegman et al, no association between the outcome of TAM treatment and the genotype of UGT2B15 was found.[22] Similarly, Rae et al found no association between UGT2B7 genotype and recurrence in TAM-treated patients.[23] Owing to the lack of prospective studies, the NCCN guidelines have not recommended UGT gene testing for patients receiving TAM treatment.

OFS plus AIs/TAM for 5 years have already been recommended by NCCN guidelines as adjuvant endocrine therapy options for premenopausal women with high risk of recurrence. Based on the results of our study, more data regarding the influence of polymorphisms of rs869283 affecting the function of the UGT1A4 enzyme and the serum concentration of TAM metabolites is needed. With further solid evidence to support our observations, OFS plus an AI might become a good option for the adjuvant endocrine therapy of premenopausal women in rs869283 A/A or G/A genotype patients, irrespective of recurrence risk.

We note several limitations in our study. First, we did not test the plasma concentrations of TAM metabolites. Second, this study was a retrospective study. We did not include the sample size estimation, because it was difficult to estimate the accurate sample size with hardly any data on the relationship between UGT1A4 polymorphisms and the clinical efficacy of TAM. Third, other drug-metabolizing enzymes such as CYP3A4 and sulphotransferases may also influence the metabolism of TAM. We did not determine the impact of these genetic variations. The frequency of these variants was quite rare, with unclear effects on the clinical efficacy of TAM in the studied Chinese populations. Fourth, all patients included in this study were Chinese. It is thus not known if the SNP frequency or relationship with outcomes would be observed in populations with other ethnic backgrounds. Therefore, our results should be carefully interpreted.

Conclusion

We found that 21.3% of Chinese patients with breast cancer had the UGT1A4 rs869283 variations (G/A or A/A). Women with these genotypes had a worse clinical outcome when receiving adjuvant TAM treatment. The efficacy of AIs could not be influenced by these variations. Further prospective studies are warranted to confirm our findings

Clinical Practice Points

- TAM for 5 years is still the standard adjuvant endocrine therapy for premenopausal patients with breast cancer. But according to the latest results of the TEXT and SOFT trials, OFS plus AIs/TAM for 5 years could result in a higher survival rate. This makes it urgent for us to find more markers to help with the adjuvant endocrine treatment decision.
- UGT1A4 is a major enzyme responsible for the glucuronidation of TAM and its metabolites. In our study, patients with rs869283 variations (G/A or A/A) in the UGT1A4 gene, accounting for 21.3% of the population, received less benefit from adjuvant TAM treatment.
- The results of our study suggest alternative treatments such as OFS plus AIs might be used for adjuvant endocrine treatment in this subgroup of premenopausal patients with breast cancer. But further solid data is needed before our observations could be used in clinical practice.

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Disclosure

The authors have stated that they have no conflicts of interest.

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