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Impact of CYP2D6 Polymorphisms on the Efficacy of Tamoxifen in Iraqi Women With Breast Cancer

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Abstract

Objectives: The aim of the present study was to investigate the impact of the CYP2D6 genetic polymorphism on clinical outcome in Iraqi breast cancer patients who were candidates for Tamoxifen therapy.

Methods: Comprehensive CYP2D6 genotyping was performed in 140 Iraqi women with breast cancer who were women on adjuvant treatment with tamoxifen. Breast cancer patients recruited into the study were divided into two groups: seventy breast cancer women who had no history of recurrence at the time of sampling and had a long time on tamoxifen without recurrence and seventy breast cancer women who had recurrence at the time of sampling after one year of treatment with tamoxifen therapy. Recurrence free survival (RFS) was determined in the recruited patients.

Results: Multiple genetic variants of the gene encoding the CYP2D6 enzyme were detected with significant differences in their frequencies and percentages in both recurrent and non-recurrent groups of breast cancer patients. The findings of this study suggest that interindividual variation in clinical outcome may be related to genetic variation in CYP2D6 enzyme, which is characterized by variable RFS periods.

Conclusion: This study revealed that the CYP2D6 enzyme of breast cancer patients who participated in this study is highly polymorphic. The CYP2D6 gene of study participants exhibited different allelic combinations with variable frequencies. The multiple genetic variants (alleles) of the gene encoding the CYP2D6 enzyme exhibited significant differences in their frequencies and percentages in both recurrent and non-recurrent groups of breast cancer patients. The study revealed variable Recurrence free survival (RFS) with highly polymorphic gene. Our study quotes the presence of increased CYP2D6 enzyme polymorphism is associated with variable clinical response.

Keywords: Tamoxifen, CYP2D6, breast cancer, genetic polymorphism, Iraqi, variable, clinical outcome

Introduction

Breast cancer is one of the most common cancers and the second leading cause of death worldwide.[1] Steroid hormones (estrogen and progesterone) have been implicated in breast cancer pathogenesis.[2] Tumor expression of estrogen (ERs) and/or progesterone receptors (PRs) plays a central role in the choice of anticancer therapy,[3] Anti-estrogen therapy in ER-positive and/or PR-positive breast tumors has proven effectiveness in adjuvant and metastatic settings.[4] Tamoxifen remains the standard hormonal therapy for the treatment of women with estrogen receptor (ER)-positive and/or PR-positive breast cancer. More than a 50% reduction in recurrence and mortality is conveyed with 5 years of tamoxifen treatment administered in the adjuvant setting;[5] however, a large percentage of breast cancer patients do not achieve benefit from tamoxifen therapy. Although the reasons for tamoxifen therapy resistance are multifaceted, an important contributing factor may lie in the metabolic pathways of tamoxifen.[6] Tamoxifen is a prodrug that is extensively metabolized by hepatic cytochrome P450 (CYP2D6) into several metabolites.[7] The most active and abundant metabolite is endoxifen (4-hydroxy-N-desmethyl-tamoxifen), which binds to the ER with higher affinity than tamoxifen itself and demonstrates a 30-fold to 100-fold higher potency than the parent drug in the inhibition of estrogen-dependent cell proliferation.[8] Four subgroups have been identified among the CYP2D6 allelic variabilities based on the activity of CYP2D6: 1) functional alleles (e.g., CYP2D6*1, *2, *35); 2) reduced function alleles (e.g., CYP2D6*9, *10, *17, *41); 3) null functional alleles (e.g., CYP2D6*3, *4, *5, *6, *7, *8); and 4) ultra-functional alleles (e.g., CYP2D6*1xN, *2xN, *35xN).[9] The present study was designed to examine the great ethnic and interindividual variability in tamoxifen metabolism by the CYP2D6 enzyme that can be explained by genetic polymorphisms and how they may affect enzyme function.[10]

Aim of the Study

The aim of this study was to determine the genotype of the CYP2D6 enzyme, which is responsible for the activation of tamoxifen in Iraqi women with breast cancer recruited from Al-Amal National Hospital by sequencing the isolated gene. Additionally, the effect of CYP2D6 gene polymorphisms on clinical outcome was investigated by determining their correlation with recurrence-free survival (RFS).

Materials and Methods

Patients

The present study was performed at Al-Amal National Hospital for Oncology in Baghdad from February 2017 until the end of September 2018. The study protocol was approved by the ethical committee of Al-Nahrain Medical College, and informed signed consent was obtained from each participant. One hundred- forty women aged 45–56 with ER- and/or PR-positive early-stage ductal breast carcinoma was enrolled in this study.

Participating women were recruited by consultation with an oncologist according to the inclusion and exclusion criteria of the study.

All participants included in this study were starting 20 mg oral tamoxifen tablets once per day. Patients underwent surgery, radiation, and adjuvant chemotherapy. They were excluded from the study if they started tamoxifen concurrently with either adjuvant radiation therapy or adjuvant chemotherapy (or both) or if they were undergoing other endocrine therapies. Pregnant or lactating patients were excluded from the study. Women who had taken CYP2D6 inducer or inhibitor drugs within 28 days of the study were excluded. Patients with surgery or a previous history of GI disorders that may affect the absorption of tamoxifen were excluded from the study. The one-hundred forty women who participated in this study who were on tamoxifen therapy were divided into two groups:

I - Non-recurrent group: included seventy women with breast cancer who had no history of recurrence at the time of sampling, regardless of whether it was local, regional or had metastasized to a distant area.

II - Recurrent group: included seventy women with breast cancer who had recurrence at the time of sampling either locally, regionally or metastasis to a distant area that may occur after one year of treatment with tamoxifen therapy.

Clinical Data Collection

At the time blood sampling, each participant was questioned about use of any medication that may interfere with CYP2D6 metabolism. Additionally, patients were also questioned about whether they had taken any additional medications, supplements, herbs or vitamins/minerals for a duration of 3 months or more to avoid all potentially interacting medications.

Furthermore, the following additional clinical data were extracted from the medical records of consenting patients: type of cancer, date of diagnosis, histological grade, site (left breast, right breast, or bilateral), clinical stage, number of lymph nodes removed in surgery, number of lymph nodes involved, tumor markers in primary breast cancer tissue (estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER)-2), dose and date of tamoxifen therapy received, date of first recurrence, site of first recurrence, systemic treatment for metastatic disease, current status and date of last follow- up.

CT- scans, bone scans, mammograms, MRI and ultrasound were performed as routine follow-ups for each patient.

Sample Collection

Blood samples (2 ml) were obtained from eligible patients who signed the informed consent after approval by the Ethical Committee of Al-Nahrain Medical College. Venous blood was withdrawn from all participating women and then placed in EDTA tubes for genetic testing.

CYP2D6 Genotyping

Blood was collected for DNA extraction to assess CYP2D6 genotyping. Extraction of the DNA genome from blood samples was performed according to the protocol of the ReliaPrep™ Blood gDNA Miniprep System, Promega. Using a Quantus fluorimeter.

The DNA sample was quantified and checked for purity, and then it was stored at 4°C until use. Gene variant determination was performed in the ASCO learning center in Baghdad using polymerase chain reaction (PCR) and sequencing of PCR products.

For PCR amplification, the following four specific primers were used for the CYP2D6 gene with specific sequence and product size: Forward Primer P1 (CAGGAAACAGCTATGACCGTTCACTCACAGCAGAGGGCAA), Reverse Primer P1 (TGTAAAACGACGGCCAGTTCATGCCATGTATAAATGCCCTTCT, 499 bp); Forward Primer P2 (TG TA AAA CGACGGCCAGTATCTCTGACGTGG AT AGGAGGT), Reverse Primer P2 (CAGGAAACAGCTATGACCGCACCTGTGCTGTAAGCTCAGT, 1000 bp); Forward Primer P3 (TG TAAAA CGACGGCCAGTCGGGTGTCCCAGCAAAGTTCAT), Reverse Primer P3(CAGGAAACAGCTATGACCAGCTGCTAACTAGTTCACAGGAT, 600 bp), and Forward Primer P4 (ACTCCACCAACCTGATCCAGGAAACAGCTAT PCR products (P1, P2, P3, and P4) of the CYP2D6 gene from collected samples were fractionated by 1% agarose gel electrophoresis stained with ethidium bromide. For determination of alleles, PCR products of the CYP2D6 gene were sent for Sanger sequencing using an ABI3730XL automated DNA sequencer, by Microgen Corporation (Korea). Then, the results were received by email and analyzed using Geneious software.

End Point and Study Design

The defined end point in the present study was recurrence free survival (RFS), which was defined as the time from breast cancer diagnosis to documentation of a new breast cancer event, any loco regional, distant recurrence of breast cancer, contralateral breast cancer or any cancer of any organ in the body.

Statistical Analysis

The collected data were introduced into a computer system utilizing a database program, and the collected data are expressed as the mean and median ± SD. Chi square and Fisher's exact test were used to determine any significant difference between categorical data. A P-value of less than 0.05 was considered significant. Unpaired Student's t-test and Mann-Whitney test were used to examine differences in the means and medians of the RFS period in recurrent and nonrecurrent breast cancer patients. A *P*-value of less than 0.05 was considered significant.

ANOVA and Kruskal-Wallis tests were used to examine differences in the means and medians of RFS among CYP2D6 genotypes detected in breast cancer patients. $P < 0.05$ was considered significant.

Results

Genetic Analysis

CYP2D6 genotyping

PCR products of the CYP2D6 gene were sequenced, and the sequencing results were analyzed using Geneious software. The CYP2D6 genotype of each of our breast cancer patients in the present study was detected.

Frequency and percentage of detected alleles of the CYP2D6 gene in breast cancer patients

Table 1 shows the frequency and percentage of detected alleles of the CYP2D6 gene, the reference SNP (RS) and the

Table 1. **The frequency and percentage of detected alleles of the CYP2D6 gene in breast cancer patients**

Alleles name	Defining name/change	Rs#	Frequency	The supposed effect on tamoxifen metabolism	%
CYP2D6*1	Wild type		2	Normal enzymatic activity	0.65
CYP2D6*2	2850C>T 4180G>C	rs16947(A) rs1135840(C)	95	Normal enzymatic activity	30.94
CYP2D6*41	2988G>A	rs28371725	83	Decreased enzymatic activity	27.04
CYP2D6*39	1661G>C, 4180G>C	rs1135840	34	Normal enzymatic activity	11.07
CYP2D6*10	100C>T	rs1065852	32	Decreased enzymatic activity	10.42
CYP2D6*2XN	1661G>C, 2850C>T, 4180G>C with multiple copies	rs16947(A) rs1135840(C)	33	Increased enzymatic activity	10.75
CYP2D6*4	1846G>A	rs3892097(A)	24	No enzymatic activity	7.82
CYP2D6*3B	1749A>G, 2549delA	rs1135824(G) rs35742686(-)	3	No enzymatic activity	0.98
CYP2D6*7	2935A>C	rs5030867	1	No enzymatic activity	0.33
Total			307		100

supposed enzymatic activity of the breast cancer patients in the study. The most frequent allele in 140 women with breast cancer recruited in our study was CYP2D6*2, with normal functional activity and a frequency and percentage of 95 and 30.94%, respectively, while CYP2D6*1, which is the wild type allele, was detected at very low frequency and percentage (2 and 0.65%, respectively). Our study identified the CYP2D6 *39 allele, which has unknown functional activity, with a frequency and percentage of 34 and 11.07%, respectively. Additionally, CYP2D6*41 and CYP2D6*10 alleles with reduced enzymatic activity were detected with frequency and percentage for each of 83, 27.04%, 32, and 10.42%, respectively. Alleles of CYP2D6*4, CYP2D6*3B and CYP2D6*7 with null functional activity were identified in frequency and percentage of 24, 7.82%, 3, 0.98%, 1, and 0.33%, respectively.

We found gene duplication (CYP2D6 *2XN) at a frequency and percentage of 33 and 10.75% of the population analyzed in our study.

Frequency and percentage of detected alleles of the CYP2D6 gene in the recurrent versus nonrecurrent breast cancer patients

Table 2 shows that there were no significant differences in the percentage of CYP2D6*2 alleles in the recurrent versus nonrecurrent groups of breast cancer patients (50.53% and 49.47%, respectively); correspondingly, there was no significant difference in the percentage of CYP2D6*2 alleles in the recurrent versus nonrecurrent groups of breast cancer patients (28.91% and 33.33, respectively).

In contrast, in the recurrent group of breast cancer patients, the percentage of the CYP2D6*41 allele was significantly higher (68%) than in the nonrecurrent group (31.33%); similarly, the percentage of the CYP2D6 *41 allele (34.33%) was significantly higher than in the nonrecurrent group (18.43%) among recurrent and nonrecurrent breast cancer patients recruited in the present study.

In the study population, in the nonrecurrent group, women with the CYP2D6*39 allele comprised a significantly higher percentage (79.41%) than recurrent breast cancer patients (20.59%); likewise, the percentage of this allele in the nonrecurrent group was significantly higher (19.14%) than in the recurrent group (4.21%) among recurrent and nonrecurrent breast cancer patients recruited in the present study. Similarly, there were significant differences in the percentages of both CYP2D6*10 (78.13%) and CYP2D6*4 (83.33%) alleles in the recurrent versus nonrecurrent groups of breast cancer patients. Our data also revealed significant differences in the percentage of both CYP2D6*10 (15.06%) and CYP2D6*4 (12.04%) alleles in the recurrent group compared to the nonrecurrent group for this study. Moreover, in women with nonrecurrent breast cancer, there were significantly higher percentages of CYP2D6*2XN (78.79%), as well as CYP2D6*3B, CYP2D6*1, and CYP2D6*7 (66.67%), compared to the recurrent group. Correspondingly, these alleles showed significantly higher percentages (18.43% and 5.67%, respectively) in women with nonrecurrent breast cancer than in women with recurrent breast cancer.

The frequency and percentage of allelic combinations of the CYP2D6 gene in breast cancer patients

Table 3 shows the frequency and percentage of allelic combinations of the CYP2D6 gene in the entire study population. The present study detected allelic combinations of CYP2D6 *2/*41 at a percentage of 34.29%, the higher percentage of allelic combinations detected in the study population. Data also revealed allelic combinations (genotypes) of CYP2D6 *2XN/*39; 2/*2XN/39; *4/*10/*41; *2XN/*39/*41;*2/*10/*41; *2/*4/*10/*41; *2/*4/*10; *2/*2XN/*39/*41; *2XN/*39/*10/*4; *2/*3B; *2/*4/*41; *10/*41; *2/*2XN/*39/*4/*10; *10/*2/*41; *2XN/*39/*7; *2/*2XN/*39/*3B and *39/*41 at percentages of 9.29%, 5.71%, 5.71%, 5%, 5%, 3.57%, 2.14%, 1.43%, 1.43%, 1.43%, 0.71%, 0.71%, 0.71%, 0.71%, .71%, 0.71% and 0.71%, respectively. Concerning the CYP2D6*2 genotype, the present study detected this genotype with a frequency of 16 and percentage of 11.43%. For the CYP2D6*10 genotype, 2 of 140 breast cancer patients in the study population had this genotype, and the percentage of this genotype in the present study was 1.43%. The frequency and percentage of CYP2D6*4/*10

Table 2. **Detected alleles of the CYP2D6 gene and their frequency and percentage in the recurrent versus nonrecurrent groups of breast cancer patients**

CYP2D6 alleles	Recurrent (R) *N* = 70	Non-Recurrent (NR) *N* = 70	Total	
*2	48	47	95	*P*>0.05
% (R vs NR)	50.53	49.47	100	Chi-sq. *P*<0.05, Fisher exact test, *P*<0.05
*41	57	26	83	
% (R vs NR)	68.67	31.33	100	
*39	7	27	34	
% (R vs NR)	20.59	79.41	100	
*10	25	7	32	
% (R vs NR)	78.13	21.88	100	
*2XN	7	26	33	
% (R vs NR)	21.21	78.79	100	
*4	20	4	24	
% (R vs NR)	83.33	16.67	100	
Others (*3B, *1, *7)	2	4	6	
% (R vs NR)	33.33	66.67	100	
Total frequency	166	141	307	

Table 3. **Allelic combinations of the CYP2D6 gene and their frequency and percentage in breast cancer patients**

Allelic combinations of CYP2D6 gene	Frequency	Percentage (%)
*2/*41	48	34.29
*2	16	11.43
*2XN/*39	13	9.29
*2/*2XN/*39	8	5.71
*4/ *10/*41	8	5.71
*2XN/*39 /*41	7	5.00
*2/*10/*41	7	5.00
*2/*4/*10/* 41	5	3.57
*2/*4/*10	3	2.14
*4/*10	3	2.14
*41	3	2.14
*1	2	1.43
*2/*2XN/*39/*41	2	1.43
*10	2	1.43
*2XN, *39/*10, *4	2	1.43
*2/*10	2	1.43
*2/*3B	2	1.43
*2/*4/*41	1	0.71
*10/*41	1	0.71
*2/*2XN/*39/*4, *10	1	0.71
*10/*2/*41	1	0.71
*2XN/*39/*7	1	0.71
*2/*2XN/*39/*3B	1	0.71
*39/*41	1	0.71
Total	140	100

genotypes were 3 and 2.14%, respectively. Additionally, the CYP2D6*1 genotype was determined in the population at a frequency and percentage of 2 and 1.43%, respectively. Moreover, the present data detected CYP2D6 genotypes (*41 and *2/*10 with percentages of 2.41% and 1.43%, respectively).

Frequency and percentage of the allelic combinations of the CYP2D6 gene in the recurrent versus nonrecurrent groups of breast cancer patients

Table 4 shows the frequency and percentage of the allelic combinations of the CYP2D6 gene in the entire study population within recurrent and nonrecurrent breast cancer patients.The present study detected the allelic combination of CYP2D6*2/*41 with a percentage of 42.68% within the recurrent group. Which was significantly higher than its percentage within the nonrecurrent group (25.71%). The percentage of this genotype was high (62.5%) in the recurrent versus nonrecurrent group (37.5%). CYP2D6*2/*4/*41; *10/*41; *4/*10/*41; *41; *2/*4/*10/*41; *2/*4/*10 and *4/*10 showed significant differences in the percentages in recurrent group versus nonrecurrent groups of breast cancer patients (1.43% vs 0%), (1.43% vs 0%), (11.43% vs 0%), (2.86% vs 1.43%), (7.41% vs 0%), (2.86% vs 1.43%) and (4.29% vs 0%), respectively. Similarly, the percentage of these genotypes was high in recurrent versus nonrecurrent tumors (100% vs 0%, 100% vs 0%, 100% vs 0%, 100% vs 0%, 66.66% vs 33.33%, 100% vs 0%, 66.66% vs 33.33% and 100% vs 0%, respectively. Concerning allelic combinations of CYP2D6*1, CYP2D6*2, CYP2D6*2XN/*39, CYP2D6*2/*2XN/*39, CYP2D6*2/*2XN/*39/*41, CYP2D6*2 XN/*39/*10/*4, CYP2D6*2/*10 and CYP2D6* 2XN/*39/*7 exhibited significantly lower percentages in recurrent compared to nonrecurrent groups of breast cancer patients (0% vs 2.86%, 4.29% vs 18.57%, 0% vs 18.57%, 1.43% vs 10%, 0% vs 2.86%, 0% vs 2.86%, 0% vs 2.86% and 0% vs 1.43% respectively). Correspondingly, the percentage of these genotypes was low in recurrent versus nonrecurrent tumors (0% vs

Table 4. **Frequency and percentage of allelic combinations of the CYP2D6 gene in the recurrent versus nonrecurrent groups of breast cancer patients**

Allelic combinations	Frequency in recurrent group	Percentage within recurrent group (%)	Percentage in recurrent vs. non-recurrent (%)	Frequency in non-recurrent group	Percentage within non-recurrent group (%)	Percentage in non recurrent vs. recurrent (%)
*2/*41	30	42.86	62.50	18	25.71	37.5
*2	3	4.29	18.75	13	18.57	81.25
*2XN/*39	0	0.00	0.00	13	18.57	100
*2/*2XN/*39	1	1.43	12.50	7	10.00	87.5
*4/*10/*41	8	11.43	100.00	0	0.00	0
*2XN/*39 /*41	3	4.29	42.86	4	5.71	57.14
*2/*10/*41	4	5.71	57.14	3	4.29	42.85
*2/*4/*10/*41	5	7.14	100.00	0	0.00	0
*2/*4/*10	2	2.86	66.67	1	1.43	33.33
*4/*10	3	4.29	100.00	0	0.00	0
*41	2	2.86	66.67	1	1.43	33.33
*1	0	0.00	0.00	2	2.86	100
*2/*2XN/*39/*41	0	0.00	0.00	2	2.86	100
*10	2	2.86	100.00	0	0.00	0
*2XN, *39/*10/*4	0	0.00	0.00	2	2.86	100
*2/*10	0	0.00	0.00	2	2.86	100
*2/*3B	1	1.43	50.00	1	1.43	50
*2/*4/*41	1	1.43	100.00	0	0.00	0
*10/*41	1	1.43	100.00	0	0.00	0
*2/*2XN/*39/*4/*10	1	1.43	100.00	0	0.00	0
*10/*2/*41	1	1.43	100.00	0	0.00	0
*2XN/*39/*7	0	0.00	0.00	1	1.43	100
*2/*2XN/*39/*3B	1	1.43	100.00	0	0.00	0
*39/*41	1	1.43	100.00	0	0.00	0
Total	70	100		70	100	

100%, 18.75% vs 81.25%, 0% vs 100%, 12.5% vs 87.5%, 0% vs 100%, 0% vs 100%, 100 vs 0% and 0% vs 100%, respectively). The allelic combination of CYP2D6*2XN/*39/*41 and CYP2D6*2/*10/*41 showed significant differences in both groups, while the allelic combination of CYP2D6*2/*3B showed no significant differences in frequency or percentage in the recurrent group versus the nonrecurrent group of breast cancer patients.

Odds and risk ratios of the detected alleles of the CYP2D6 gene in the development of recurrence in breast cancer patients treated with tamoxifen

Table 5 shows that CYP2D6*41 has a significant effect on increasing recurrence in breast cancer patients (odds ratio 2.06, p<0.05) (risk ratio 1.34, p<0.05). Similarly, CYP2D6*10 and CYP2D6*4 had negative effects on recurrence development (odds ratio 3.3, p<0.05, risk ratio 1.52, p<0.05, odds ratio 6.53, p<0.05, risk ratio 1.72, p<0.05, respectively).

Concerning CYP2D6*2XN and CYP2D6*39 alleles Table 5 shows that they have a significant effect in attenuating the recurrence of breast cancer patients of current study (odds ratio 0.23, p<0.05; risk ratio 0.38, p<0.05; odds ratio 0.24, p<0.05; and risk ratio 0.396, p<0.05, respectively), while both CYP2D6*3B and CYP2D6*7 alleles had no effect on the development of breast cancer recurrence (odds ratio 1.96, p>0.05), (risk ratio 1.32, p>0.05) for CYP2D6*3B or CYP2D6*7 (odds ratio 0.32, p>0.05; (risk ratio 0.49, p>0.05, respectively).

The Odds and Risk ratios of the Allelic Combinations of CYP2D6 Gene in the Development of Recurrence in Breast Cancer Patients treated with Tamoxifen

Table 6 shows that allelic combinations of CYP2D6 *2/*41; CYP2D6*4/*10/*41; CYP2D6*2/*10/*41; CYP2D6 *2/*4/*10/*41; CYP2D6*2/*4/*10; CYP2D6*4/*10; CYP2D6 *41; CYP2D6*10; CYP2D6*2/*4/*41; CYP2D6*10/*41; CYP2 D6*2/*2XN/*39/*4/*10; CYP2D6* 10/*2/*41; CYP2D6 *2/*2XN/*39/*3B; and CYP2D6*39/*41 significantly promoted recurrence in breast cancer patients of the current study (odds ratio 8.3, *P*<0.05; risk ratio 3.75, *P*<0.05; odds ratio 75.28, *P*<0.005; risk ratio 6, *P*<0.05; odds ratio 6.66, *P*<0.05; risk ratio 3.42, *P*<0.05; odds ratio 48.71, *P*<0.05; risk ratio 6,

Table 5. **Odds and risk ratios of detected alleles of the CYP2D6 gene in the development of recurrence in breast cancer patients treated with tamoxifen**

Alleles	Odds ratio	CI-95	*P*	Risk ratio	CI-95	*P*	Significant effect
*1							
*2							
*41	2.06	1.1317–3.78	0.018	1.34	1.0523–1.7154	0.017	bad
*39	0.23	0.0948–0.590	0.002	0.38	0.1925–0.7698	0.007	good
*10	3.3	1.3693–7.989	0.007	1.52	1.1711–1.9921	0.0018	bad
*2XN	0.24	0.0979–0.613	0.002	0.396	0.1985–0.7901	0.0086	good
*4	6.53	1.8240–23.40	0.003	1.72	1.3393–2.213	<0.0001	bad
*3B	1.96	0.1721–22.321	0.58	1.32	0.5793–3.007	0.5	no effect
*7	0.32	0.0182–11.37	0.49	0.49	0.0445–5.502	0.56	no effect

Significant difference at $P < 0.05$.

Table 6. **Odds and risk ratios of allelic combinations of the CYP2D6 gene in development of recurrence in breast cancer patients treated with tamoxifen**

Allelic combinations	Odds ratio	CI-95	*P*-value	Risk ratio	CI-95	*P*-value	Significant effect
*2/*41	8.33	2.1166 – 32.8091	0.002	3.75	1.3044–10.7807	0.014	Bad
*2XN/*39	0.16	0.0078 – 3.469	0.24	0.19	0.0109–3.4597	0.26	No effect
*2/*2XN/*39	0.71	0.0626 – 8.150	0.7	0.75	0.0914–6.1517	0.78	No effect
*4/*10/*41	75.28	3.4633 – 1636.5545	0.0059	6	2.1356–16.8571	0.0007	Bad
*2XN/*39/*41	3.75	0.5370 – 26.1894	0.18	2.57	0.6725–9.8323	0.16	No effect
*2/*10/*41	6.66	0.9546 – 46.5589	0.055	3.42	1.0163–11.5671	0.047	Bad
*2/*4/*10/*41	48.71	2.1536 – 1101.9153	0.014	6	2.1356–16.8571	0.0007	Bad
*2/*4/*10	10	0.6709 – 149.0465	0.094	4	1.0829–14.7754	0.037	Bad
*4/*10	31	1.2864 – 747.0733	0.034	6	2.1356–16.8571	0.0007	Bad
*41	10	0.6709 – 149.0465	0.094	4	1.0829 to 14.7754	0.037	Bad
*2/*2XN/*39/*41	0.88	0.0343 – 22.8527	0.94	0.9	0.0607 to 13.4828	0.94	No effect
*10	22.14	0.8582 – 571.3165	0.061	6	2.1356 to 16.8571	0.0007	Bad
*2XN/*39, *10/*4	0.88	0.0343 – 22.8527	0.94	0.9	0.0607 to 13.4828	0.94	No effect
*2/*10	0.88	0.0343 – 22.8527	0.94	0.9	0.0607 to 13.4828	0.94	No effect
*2/*3B	5	0.2400 – 104.1526	0.29	3	0.5326 to 16.8976	0.21	No effect
*2/*4/*41	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad
*10/*41	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad
*2/*2XN/ *39/*4/*10	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad
*10/*2/*41	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad
*2XN/*39/*7	1.47	0.0491 – 44.4263	0.82	1.35	0.1028 to 17.9153	0.81	No effect
*2/*2XN/*39/*3B	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad
*39/*41	13.28	0.4415 – 399.8366	0.13	6	2.1356 to 16.8571	0.0007	Bad

P<0.05; odds ratio 10, *P*>0.05; risk ratio 4, *P*<0.05; odds ratio 31, *P*<0.05; risk ratio 6, *P*<0.05; odds ratio 10, *P*>0.05; risk ratio 4, *P*<0.05; odds ratio 22.14, *P*>0.05; risk ratio 6, *P*<0.05; odds ratio 13.28, *P*>0.05; risk ratio 6, *P*<0.05; odds ratio 13.28, *P*>0.05; risk ratio 6, *P*<0.05; odds ratio 13.28, *P*>0.05; risk ratio 6, *P*<0.05; odds ratio 13.28, *P*>0.05; risk ratio 6, *P*<0.05; odds ratio 13.28, *P*>0.05; risk ratio 6, *P*<0.05, respectively. In contrast, allelic combinations of CYP2D6*2XN/*39; CYP2D6*2/*2XN/*39; CYP2D6*2XN/*39/*41; CYP2D6*2/*2XN/*39/*41; CYP2D6 *2XN/*39/*10/*4; CYP2D6 *2/*10; CYP2D6 *2/*3B and *2XN/*39/*7 had no significant effect on the development of breast cancer recurrence (odds ratio 0.16, *P*>0.05; risk ratio 0.19, *P*>0.05; odds ratio 0.71, *P*>0.05; risk ratio 0.75, *P*>0.05; odds ratio 3.75, *P*>0.05; risk ratio 2.75, *P*>0.05; odds ratio 0.88, *P*>0.05; risk ratio 0.9, *P*>0.05; odds ratio 0.88, *P*>0.05;

risk ratio 0.9, *P*>0.05; odds ratio 0.05, *P*>0.05; odds ratio 0.05, *P*>0.05; odds ratio 0.05, *P*>0.05; odds ratio 0.05, *P*>0.05; odds ratio 0.05, *P*>0.05; and odds ratio 0.05, *P*>0.05, respectively.

Recurrence Free Survival (RFS)

Recurrence free survival (RFS) in recurrent versus nonrecurrent groups of breast cancer patients

Recurrence free survival (RFS) period in the recurrent group of breast cancer patients treated with tamoxifen showed a lower significant difference (mean 2.61, median 2.4 years) than that in the nonrecurrent group (mean 5.32, median 5.05 years) (*P*<0.0001), as shown in Figure 1.

Recurrence free survival (RFS) among different allelic combinations of the CYP2D6 gene detected in breast cancer patients

Figure 2 shows that there were significant differences in median and mean recurrence free survival (RFS) among different allelic combinations detected in breast cancer patients in the current study (ANOVA *P*<0.05; Kruskal-Wallis *P*<0.05).

Present study determined that patients with the CYP2D6*4/*10/*41 genotype had the shortest RFS (mean 2.01 years, median 1.9 years). Additionally, the study population demonstrated that breast cancer patients with allelic combinations of *2/*4/*10, *4/*10, *2/*4/*10/*41, *41, and *2/*10/*41 exhibited shorter RFS (mean 2.87 years, median 2.4 years; mean 2.37 years, median 2.6 years; mean 2.24 years, median 2.1 years; mean 2.83 years, median 2.2 years; and mean 2.94 years, median 2.3 years, respectively. In contrast, participants with *2XN/*39, *2/*2XN/*39 and *2 had significantly longer RFS (ANOVA *P*< 0.05; Kruskal-Wallis, *P*<0.0001; mean 6.07 years, median 5.95 years; mean 5.63 years, median 5.8 years; and mean 5.29 years, median 5.15 years, respectively. Patients with CYP2D6*2/*41 and CYP2D6*2XN/*39/*41 genotypes in the current study had RFS of mean 3.49 years, median 3.15 years and mean 4.6 years, median 4.85 years.

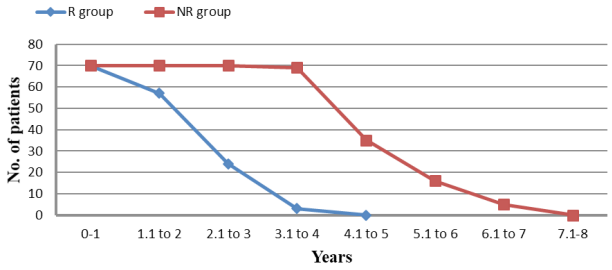

Fig. 1 **The number of patients in recurrent (R) and non-recurrent (NR) groups who are free of recurrence plotted with time in years after diagnosis.**

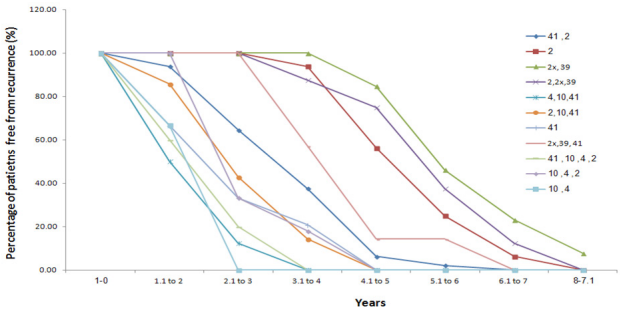

Fig 2. **The percentage of patients with different allelic combinations who are free of recurrence plotted with time relapsed after diagnosis.**

*Recurrence free survival (RFS) in breast cancer patients with and without CYP2D6 gene alleles *4, *10, and *41*

Breast cancer patients who had the CYP2D6 gene without alleles *4, *10, or *41 in the current study showed a significantly longer (mean 5.54, median 5.5) recurrence-free survival (RFS) than patients who had the CYP2D6 gene with alleles *4, *10, or *41 (mean 3.28, median 3.1) (*P* value< 0.0001), as shown in Figure 3.

Discussion

Tamoxifen, a selective estrogen receptor modulator, is generally used for the treatment and prevention of breast cancer.[11] The clinical response to tamoxifen is highly variable among patients,[12] and identification of factors that contribute to this variability is important, particularly in the era of personalized medicine. Tamoxifen undergoes extensive hepatic and gut metabolism mediated by many CYP450 enzymes.[13] The most important enzyme involved in the metabolism of tamoxifen, which is highly polymorphic, is CYP2D6, and polymorphisms in the gene encoding the CYP2D6 enzyme may influence the plasma level of tamoxifen and its metabolites, as well as the clinical outcome of breast cancer.[14]

To the best of our knowledge, this is the first study investigating a purely perimenopausal Iraqi breast cancer population for interindividual variability of the CYP2D6 gene, its involvement in tamoxifen metabolism and its influence on clinical outcome.

This study on breast cancer patients accounts for potential determinants of tamoxifen response, including tamoxifen metabolite, CYD2D6 genotype, and clinical outcome by measuring disease free survival from the diagnosis of breast cancer until the recurrence date.

CYP2D6 Genotyping

CYP2D6 is highly variable in the human population,[15] mostly as a result of polymorphisms in the CYP2D6 gene with more than 80 genetic variants.[16,17]

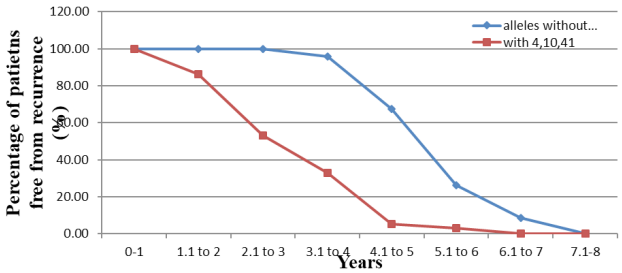

Fig. 3 **The percentage of patients with allelic *4, *10 and *41 versus those without *4, *10, and *41 who are free of recurrence plotted with time elapsed after diagnosis.**

An exhaustive genetic analysis of the CYP2D6 gene in breast cancer patients was performed by screening for different genetic variants. The DNA of 140 breast cancer patients participating in the present study was examined for the detection of CYP2D6 alleles. Genomic DNA was extracted from peripheral whole blood of each patient using a DNA extraction kit, and the CYP2D6 alleles were genotyped using polymerase chain reaction (PCR).

Amplicons of PCR were sequenced, and the results of sequencing were analyzed using Geneious software. The CYP2D6 genotype of each individual breast cancer patient in the present study was determined.

Prevalence of detected alleles of the CYP2D6 gene within breast cancer patients

Variation in the CYP2D6 gene is high among different populations and among individuals in the same population[18] Genotype testing determined the frequencies and percentages of CYP2D6 alleles within breast cancer patients in this study, as presented in Table 1.

The most frequent allele among all 140 women with breast cancer recruited in this study was CYP2D6*2, which contains a point mutation that does not affect the catalytic properties of the gene product, the frequency or the percentage of CYP2D6*2 (95, 30.94%), while CYP2D6*1, which is the wild type allele that codes for a fully functional enzyme, was detected at very low frequency and percentage.

Frequencies of 31% for the wild type allele and 40.47% for allele 2 were reported in the general Spanish population, whereas the Caucasian population showed frequencies of 33–37% for allele 1 and 22–33% for allele 2.[19,20] In African Americans and Asians, the frequencies of CYP2D6*1 were 28–50% and 23–42%, respectively, while frequencies of 11-78% and 9-20% were detected in African American and Asian breast cancer patients for allele 2, respectively.[21,22]

In contrast to other results that did not detect the presence of allele *39, which has normal functional activity,[23] this study identified the CYP2D6*39 allele with frequency and percentage of 34 and 11.07%, respectively.

Alleles with decreased enzymatic activity that were most frequently identified in the present study were CYP2D6*41 and CYP2D6*10, with frequency and percentage of each allele of 83, 27.04%, 32, and 10.42%, respectively. Results demonstrated an allelic frequency for CYP2D6*10 of 10.42%, which is higher than that of Mexican Americans at a frequency of 7.4%[24] and lower than that of the Mexican Mestizo population at a frequency of 12.45%[25] and in Colombia with a frequency of 17.5%.[26]

The CYP2D6*10 allele is the most common reduced function allele in Asian populations with a frequency of (38–70%).[20] The CYP2D6*10 allele has a high frequency of 50.7% in Chinese[27] and 38.1% in Japanese[28] populations, while in African American and Caucasian populations, the frequency of CYP2D6*10 was 1.9–8% and 3.1–8.6%[20] respectively.

The findings of this study showed that CYP2D6*41 is the most prevalent allele with reduced enzymatic activity frequency (27.04%), which is a higher percentage than in another study performed in Spanish patients who that detected the allele with a frequency of 6.43%.[29] The frequency of the CYP2D6*41 allele in Caucasians is 8%, while there is no registration of this allele in African American or Asian populations.[20]

Alleles that code for null functional activity that were identified in participating women included CYP2D6*4, CYP2D6*3B and CYP2D6*7. CYP2D6*4 showed the highest frequency and percentage of null functional alleles detected in the recruited population (24, 7.82%), while the CYP2D6*7 allele showed the lowest frequency and percentage (1, 0.33%), and the CYP2D6*3B null allele was detected with a frequency and percentage of 3 and 0.98%. The findings of the present study demonstrate that the CYP2D6*4 allele is the most prevalent null function allele at a frequency of 7.82%, similar to other studies that showed allele 4 with the highest frequency among absent functional alleles at 18.4%[30] and 15.4%.[31]

In the Caucasian population, the most common nonfunctional allele is CYP2D6*4 at a frequency of 20%, but the frequency of this allele is rare among Asian and Black African populations.[18]

CYP2D6*7 and CYP2D6*3B were detected in the current study with the lowest frequency of null functional alleles, in contrast to other results that did not detect these alleles,[29] while the CYP2D6*7 allele was detected in another study with a frequency of 0.5%.[30]

Gene amplification or duplication of functionally active CYP2D6 alleles is the genetic basis of the UM phenotype, resulting in increased enzymatic activity.[31] Gene duplication (CYP2D6 *2XN) was found in 10.75% of the population analyzed in this study, which is close to the range reported for Spaniards (7-10%)[32] and slightly lower than the frequency reported in the Mexican Mestizo population (12.76%). In Caucasians, the percentage of subjects having duplicated/multiple duplicated genes is 1–2% in Sweden,[25] 7–10% in Spain,[32] 3.6% in Germany[33] and 10% in Sicily.[34] Black Ethiopians have the highest percentage of duplicated alleles at 29%.[35]

Among European Caucasians, Spaniards and Mexican Mestizo distinguish themselves by having both the lowest percentage of null functional alleles (CYP2D6*4) and the highest percentage of duplicated alleles;[32] in this regard, the data of the current study for CYP2D6*4 and CYP2D6 *2XN did not follow this pattern.

The present data did not report any subject having null functional alleles (CYP2D6 *3, *5, *6), in contrast to other studies that reported allele frequencies of 1.4%, 1.9%, and 1.4%, respectively.[36]

The current study did not identify any individual carrying the low-frequency alleles (CYP2D6*8, *11, *14, *15, *18, * 19, *20, *25, *26, *30, *31, *35, *36 or *40). These findings were in agreement with the findings of other studies.[37]

These variants result in poor, intermediate, extensive or ultra-rapid metabolizers of the CYP2D6 phenotype; among Caucasians, approximately 7–10% of individuals are PM, 10–15% are IM, and up to 10–15% are UM.[38]

This study offers insight into the different alleles of CYP2D6 and highlights the possible clinical outcomes of treatment with tamoxifen in breast cancer patients with distinct genotypes. The current study is an important clinical study because genetic polymorphisms of CYP2D6 that impair or abolish enzyme function are common in all ethnic groups; in addition, allele frequencies differ among different ethnic groups.[39] For example, CYP2D6*2, *3, *4, *5, *6, *10 and *41 alleles are common in white subjects, and CYP2D6*17 is common in Africans and African Americans, with a percentage of 20–35%. Similarly, CYP2D6*2 is the most common in black subjects, CYP2D6*10 is most common in Asian subjects, with a percentage of more than 50%, and 3–9% is common in Africans;[40,41] furthermore a large number of CYP2D6 variants with

lower frequencies resulting in reduced activity contribute to extensive individual variation.[42]

The probable explanation of these conflicting results may be related to the relatively small sample sizes of these different studies, different patient populations,[43] and diverse methods of CYP2D6 genotyping.[44,45]

At present, there is controversy regarding the efficacy of tamoxifen in breast cancer patients who are carriers of CYP2D6 gene polymorphisms in terms of recurrence. Consequently, the aim of the present study was to investigate the association of CYP2D6 gene polymorphisms with breast cancer recurrence by determining the frequency and percentage of distinct alleles in recurrent versus nonrecurrent groups, as shown in Table 2.

In the present study, the most common allelic variant among the 140 patients assessed was the normal variant CYP2D6*2. Patients in the recurrent group and patients in the nonrecurrent group showed nearly the same percentage of the CYP2D6*2 allele (50.53% and 49.47%, respectively), while in another study, both recurrent and nonrecurrent patients showed the percentage of the CYP2D6*2 allele (14.2% and 17.9%, respectively).[36]

Concerning CYP2D6*1, the wild type allele was detected in two patients in the nonrecurrent group with no detection in the recurrent group, while another study showed a percentage of 65% and 45% in the recurrent and nonrecurrent groups, respectively.[1]

In the present study, CYP2D6*39 exhibited a significant difference in percentage between the recurrent and nonrecurrent groups (20.59% and 79.41%, respectively), in contrast to other studies that did not determine this allele in either group of patients.[30]

CYP2D6*4 is the most important null allelic variant responsible for abolishing enzymatic activity, and it is the most predominant null variant in the studied populations of the present study. There was a highly significant difference in percentage in the recurrent group compared to the noncurrent group, while another study showed no significant difference between the recurrent and nonrecurrent groups.[1,46]

The assay validated detection of the CYP2D6*41 variant allele as the most prevalent reduced function allele, which was significantly higher in the recurrent group (68.67%) than in the nonrecurrent group (31.33%). These findings contradict the findings reported by other authors who determined that there was no difference in percentages of this allele between recurrent and nonrecurrent groups (10.4% and 9.4%, respectively).[36]

The high frequency of allelic variant *10, which is predominant in Asians and relatively rare in Caucasians,[47] was assessed in the present study at a percentage of (10.42%), with a significantly higher percentage in recurrent (78.13%) versus nonrecurrent (21.88%) patients, in contrast to other studies, which showed the same percentage in both groups (1.9%).[36] In another study, the percentage in the recurrent group was (10%), while that in the nonrecurrent group was (7%).[1]

The CYP2D6*2XN variant is associated with increased enzymatic activity. In regard to the current data, there was a higher percentage of allele *2XN in the nonrecurrent group (78.79%) than in the recurrent group (21.21%), while another study showed the same percentage of this allele in the recurrent and nonrecurrent groups (0.9%).[1] Concerning the CYP2D6*3B allele that was detected in this study, this null function allele was observed in two patients in the recurrent group and one patient in the nonrecurrent group. Regarding the CYP2D6*7 allele, which has no enzymatic activity, the present results showed that only one subject in the nonrecurrent group had this allele, which is similar to another study.[1] As a consequence, the findings of the present study determined that CYP2D6*4, *10, and *41 were significantly associated within the recurrent group of breast cancer patients, which is comparable to some findings in other studies showing that tumors with CYP2D6*4, *10, and *17 are more aggressive and tamoxifen-resistant breast cancers of a recurrent nature,[48-50] in contrast to other studies that did not report any significant association of these CYP2D6 gene variants with breast cancer recurrence in patients receiving tamoxifen.[30,51] These studies are inconsistent due to variances in study design, sample size and tamoxifen adherence.[52]

The predicted CYP2D6 phenotype can be derived from patient CYP2D6 alleles and classified into 4 categories as previously mentioned: extensive metabolizer or normal activity (EM), which includes functional or wild type (wt) alleles (e.g., CYP2D6*1, *2, *35), intermediate metabolizer (IM), which include alleles with reduced enzymatic activity (e.g., CYP2D6*9, *10, *17, *41), poor metabolizer (PM), which includes null alleles having no activity (e.g., CYP2D6*3, *4, *5, *6, *7, *3B), and ultra-metabolizer (UM), which refers to a subset of patients having multiple copies of the wild type or normal CYP2D6 alleles and therefore high enzymatic activity (e.g. ,CYP2D6*1X N, *2XN, *35XN).[53]

Data obtained in the current study showed that the CYP2D6 gene of 140 Iraqi breast cancer patients was comprised of different allelic combinations with different frequencies and percentages , as shown in Table 3.

The most common allelic combination of the CYP2D6 gene in the entire population of the present study was (*2/*41) at percentage of 34.29%, which is the highest percentage of allelic combinations detected in recruited women. This finding was different from the finding of previous studies, which showed much lower percentages than reported in the present results (1.1%)[29] in a Spanish population, and a percentage of 0.6% was reported in a study of breast cancer patients recruited from Washington, Michigan and Indian cancer centers.[37]

This study identified allelic combinations *2Xn/*39, 2/*2Xn/39, *4/*10/*41, *2Xn/*39/*41, *2/*10/*41, 2/*4/*10/*41, *2/*4/*10, *2/*2Xn/*39/*41, *2Xn/*39/*10/*4, *2/3B, 2/*4/*41, *2/*2Xn/*39/*4/*10, *10/*2/*41, *2Xn/*39/*7, *2/*2Xn/*39/*3B, and *39/*41 with variable percentages. These findings conflict with findings reported by other studies as they showed completely different genotyping.[54,1,44]

Concerning the CYP2D6*2 genotype, the present study detected this genotype with a frequency of 16 and percentage of 11.43%. Certain studies reported the CYP2D6*2 genotype at a frequency of 2.47%[25] (Jorge et al., 1999), while others detected this genotype at percentages of 6.7%[29] and of 3.3%.[37]

Regarding the CYP2D6*10 genotype, 2 of 140 breast cancer patients in the recruited population had this genotype, and the percentage of this genotype in the present study was 1.43%, while another study showed this genotype at a frequency of 0.82%[25] and a frequency of 22.4%.[29]

The present study showed that the frequency and percentage of CYP2D6*4/*10 genotypes were 3 and 2.14%, respectively. However, another study showed a contradictory

result that did not detect the presence of this allelic combination[14,56] while another study showed the CYP2D6*4/*10 genotype at a frequency of 2.2%.[29]

The CYP2D6*1 genotype was determined in the study at a frequency and percentage of 2 and 1.43%, respectively, which is very low compared to other studies.[56,57]

The present data showed that the CYP2D6*41 genotype percentage was 2.14%, while other studies did not detect this genotype.[57]

Allelic combinations of CYP2D6*2/*10 and CYP2D6*10/*41 genotypes were detected with frequencies and percentages of 2, 1.43%, 1, and 0.71%, respectively, in contrast to other studies that showed no determination of these allelic combinations in their results.[14] In contrast, the CYP2D6*2/*10 genotype was detected in other studies at a frequency of 9.47%[25] or a frequency of 2.2%.[29] Although it is difficult to predict enzymatic activity of the detected allelic combinations for CYP2D6 in the study according to their four categories mentioned above, because many of the participated patients had more than two alleles, according to the CYP2D6-predicted phenotype, allelic combinations in the this study can be classified into different phenotypic groups as shown in Table 3.

The extensive metabolizer (EM)/intermediate metabolizer (IM) genotype group that was detected in the present study included three allelic combinations of CYP2D6*2/*10, CYP2D6*2/*41 and CYP2D6*39/*41. Additionally, the ultrarapid metabolizer (UM)/extensive metabolizer (EM) phenotype group was primarily composed of the *2XN/*39 genotype, and the EM/UM/EM phenotype included the *2/*2XN/39 genotype. Allelic combinations of *4/*10/*41, *2Xn/*39/*41, *2/*10/*41, 2/*4/*10/*41, *2/*4/*10, *2/*2XN/*39/*41, *2XN/*39/*10/*4, *2/3B, 2/*4/*41, *2/*2XN/*39/*4/*10, *10/*2/*41, *2XN/*39/*7 and *2/*2XN/*39/*3B can be classified phenotypically, respectively into the following: PM/IM/IM; UM/EM/IM; EM/IM/IM; EM/PM/IM/IM; EM/PM/IM; EM/UM/EM/IM; UM/EM/IM/PM; EM/PM; EM/PM/IM; EM/UM/EM/PM/IM; IM/EM/IM; UM/EM/PM and EM/UM/EM/PM.

The PM/IM group was composed of 4/*10 genotypes, and the study population had an IM/IM phenotype that was composed of *10/*41 genotypes. Furthermore, the present study detected the IM phenotype, which was composed of CYP2D6*10 and CYP2D6*41 genotypes, while the EM group was composed of CYP2D6*1 and CYP2D6*2 genotypes.

In contrast to the findings of the present study that detected these allelic combinations with variable predicted phenotypes and variable percentages, other results reported different CYP2D6 genotype with different predicted phenotypes and different percentages: PM/PM, 4.4%; PM/IM, 3.8%; IM/IM, 3.8%; EM/PM, 26.6%; EM/IM, 17.7%; EM/EM, 38% and UM/EM, 4.4%,[37] and different genotypes with different percentages were observed in another study as well: EM/UM (6.5%), EM/EM (31.2%), EM/IM (15.4%), EM/PM (34.8%), IM/IM (1.1%), IM/PM (3.9%) and PM/PM (7.2%).[23]

Among patients in this analysis, the allelic combinations of CYP2D6 that were detected in the current study exhibited significant variable percentages within the recurrent and nonrecurrent groups of breast cancer patients; additionally, these genotypes were detected in the current study with significantly different percentages within recurrent versus nonrecurrent tumors as shown in Table 4.

As a consequence, the present analysis postulates that these significant variabilities in the percentages of CYP2D6 genotypes (allelic combinations) within recurrent and nonrecurrent groups may affect the enzymatic activity of CYP2D6 and, consequently, tamoxifen efficacy.

The first clinical evidence correlating CYP2D6 activity with the tamoxifen response was reported by,[58] with inclusion of only the CYP2D6*4 and CYP2D6*6 alleles. The authors reported that the CYP2D6*4 allele was a predictor of recurrence risk.

The present analysis linking alleles of the CYP2D6 gene to the development of recurrence in Iraqi breast cancer patients treated with tamoxifen in terms of odds and risk ratios is shown in Table 5.

Although some studies have shown a significant effect of the CYP2D6 genotype on the development of breast cancer recurrence[59,60] others have not shown this correlation.[61,62] However, there is controversy between positive and negative findings regarding the effect of CYP2D6 polymorphisms on clinical outcomes.[63]

The findings of the current study demonstrated that women harboring the CYP2D6*41 allele had a significantly worse prognosis with respect to recurrence, which is consistent with other authors that genotyped for CYP2D6*41 and *10 alleles, and their study reported that patients who carried these alleles had significantly increased relapse compared to carriers of functional alleles.[49]

This study revealed that CYP2D6*4 and CYP2D6*10 alleles had a significant promoting effect on recurrence development. Another study showed no significant association between the CYP2D6*4 genotype and disease recurrencem,[64] while others showed a protective effect of the CYP2D6*4 allele.[59]

The present study showed that patients treated with tamoxifen who carried CYP2D6*39 and CYP2D6*2XN alleles had a significantly protective effect on recurrence development. Similarly, among patients with wild type CYP2D6, the outcome was normal and similar in both CYP2D6*1 and CYP2D6*2 alleles. Surprisingly, another study reported that women harboring wild type alleles showed similar outcomes between tamoxifen- and nontamoxifen-treated patients.[40]

In women in this study who carried CYP2D6*3B and CYP2D6*7 alleles, the outcome of disease recurrence was not affected.

Several investigators have addressed the relationship between CYP2D6 genotype and clinical outcome in women treated with tamoxifen in terms of hazard ratio, some of which have been consistent with Goetz's initial findings[63-65] while others failed to support their finding.[66] Remarkably, another study established the opposite effect.[40]

Additionally, the results considered in this study that were conducted in a sample of Iraqi women with breast cancer treated with tamoxifen related the genetic variant of CYP2D6 with the outcome of women treated with adjuvant tamoxifen by determining the odds and risk ratios of allelic combinations of the CYP2D6 gene in the development of breast cancer recurrence (Table 6).

Among patients in the present study, the allelic combination of CYP2D6*2/*41 had a significantly promoting effect on recurrence development. Similarly, the present findings revealed that the allelic combinations of *4/*10/*41, *10/*2/*41, *2/*4/*10/*41, *2/*4/*10, *4/*10, *41, *10, and

*2/*4/*41 had a worse significant effect on disease prognosis. However, the study determined that patients harboring allelic combinations of *2XN/*39, 2/*2XN/39, *2XN/*39/*41, *2/*2XN/*39/*41, *2XN/*39/*10/*4, *10/*2 and *2/3B had no significant effect on recurrence development.

The present study evaluated the association between gene polymorphisms and the clinical outcome of breast cancer patients by determining RFS.

The data obtained from the current study showed that women with recurrent breast cancer had reduced mean and median recurrence free survival (RFS) compared to nonrecurrent breast cancer patients ($P< 0.01$, Mann-Whitney test < 0.01) (Figure 1).

The findings of the present study determined the association of RFS among different CYP2D6 genotypes detected in participating women (Figure 2). This study determined that patients with the CYP2D6*4/*10/*41 genotype had worse RFS. Additionally, the study population revealed that breast cancer patients with allelic combinations of *2/*4/*10, *4/*10, *2/*4/*10/*41, *41, and *2/*10/*41 had poor clinical outcomes, as they had shorter times to recurrence (ANOVA $P< 0.01$; Kruskal-Wallis $P<0.01$).

In contrast, participants with *2XN/*39, *2/*2XN/*39 and *2 had the best clinical outcomes among our study population, as they had significantly higher RFS (ANOVA $P< 0.01$; Kruskal-Wallis $P<0.01$). Patients with CYP2D6*2/*41 and CYP2D6*2XN/*39/*41 genotypes in the study had RFS of mean 3.49 years, median 3.15 years and mean 4.6 years, median 4.85 years, respectively.

In addition to determining the association between different CYP2D6 genotypes and RFS in the studied population, the present clinical study found that participants harboring the CYP2D6 gene with alleles *4, *10, and *41 had poorer clinical outcomes and shorter times to recurrence than those without these alleles ($P< 0.01$, Mann-Whitney test < 0.01) (Figure 3).

Numerous studies regarding treatment and clinical outcomes in adjuvant and/or metastatic settings have reported that patients with impaired CYP2D6 enzymatic activity appeared to have a considerably higher recurrence rate of breast cancer than those with normal CYP2D6 enzyme activity[63,67] signifying that variants associated with reduced function alleles (*4, *10 and *41) are powerful predictors of tamoxifen 's anticancer efficacy.[40,68]

Overall, the findings of this study suggest that interindividual variation in the tamoxifen response and clinical outcome may be explained in part by genetic variation in CYP2D6 enzyme, which is characterized by variable RFS periods. However, the discordant findings may reflect the variability in dose and duration of tamoxifen therapy, concomitant medications, discrepancy in the inclusion and exclusion of events and diverse methods of CYP2D6 genotyping, which makes it difficult to compare results. Additionally, using variable endpoints as well as the inconsistent description of these endpoints confound the analysis of results and make comparisons variable and difficult.[43]

At the present time, there are not sufficient data to validate adoption of genotyping the CYP2D6 enzyme into clinical settings, so official recommendations on the incorporation CYP2D6 genotypes into treatment decisions must wait for further data from larger clinical trials.

Conclusion

The CYP2D6 enzyme in Iraqi breast cancer patients is highly polymorphic and widely expressed genetic variable with different allelic combinations that are associated with variable clinical outcomes. In summary, the interindividual variation in response to tamoxifen therapy that is related in part to genetic variability would improve the capability of physicians to select the optimal endocrine therapy with the optimal dose for breast cancer treatment. However, further evaluation of the role of CYP2D6 is required with much larger sample sizes from randomized trials.

Conflict of Interest

No conflicts of interest, It was self-limited.

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References

1. Del Re, M., Michelucci, A., Simi, P., & Danesi, R. (2012). Pharmacogenetics of antiestrogen treatment of breast cancer. Cancer treatment reviews, 38(5), 442–450.
2. International Agency for Cancer Research: Estimated Incidence, Mortality and Prevalence Worldwide in 2012. http://globocan. iarc.fr/old/Fact Sheets/ cancers / breast- new.asp. Accessed February 16, 2016.
3. Early Breast Cancer Trialists' Collaborative Group. (2011). Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomized trials.The lancet, 378(9793), 771–784.
4. Yager, J. D. (2000). Chapter 3: endogenous estrogens as carcinogens through metabolic activation. JNCI Monographs, 2000(27), 67–73.
5. Davies, C., Pan, H., Godwin, J., Gray, R., Arriagada, R., Raina, V., & Bradbury, J. (2013). Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of estrogen receptor-positive breast cancer: ATLAS, a randomized trial. The Lancet, 381(9869), 805–816.
6. Brauch, H., Mürdter, T. E., Eichelbaum, M., & Schwab, M. (2009). Pharmacogenomics of tamoxifen therapy. Clinical chemistry, 55(10), 1770–1782.
7. Ratliff, B., Dietze, E. C., Bean, G. R., Moore, C., Wanko, S., & Seewaldt, V. L. (2004). Re: Active tamoxifen metabolite plasma concentrations after co administration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. Journal of the National Cancer Institute, 96(11), 883–883.
8. Melo, M. D. A., Vasconcelos Valença, D., José, R., Neto, F. M., Borges, R. S., Costa Silva, D. R., & Da Silva, B. B. (2016). CYP2D6 gene polymorphisms in Brazilian patients with breast cancer treated with adjuvant tamoxifen and its association with disease recurrence. Biomedical reports, 5(5), 574–578.
9. Committee HCPCAN. Available at: http://www.cypalleles.ki.se/. 2008. Accessed November 22, 2010.
10. Del Re, M., Citi, V., Crucitta, S., Rofi, E., Belcari, F., Van Schaik, R. H., & Danesi, R. (2016). Pharmacogenetics of CYP2D6 and tamoxifen therapy: Light at the end of the tunnel?. Pharmacological research, 107, 398–406.

11. Antunes, Marina Venzon, et al. "Sensitive HPLC–PDA determination of tamoxifen and its metabolites N-desmethyl tamoxifen, 4- ydroxytamoxifen and endoxifen in human plasma." Journal of pharmaceutical and biomedical analysis 76 (2013): 13–20.
12. Lim, Y. C., Li, L., Desta, Z., Zhao, Q., Rae, J. M., Flockhart, D. A., & Skaar, T. C. (2006). Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells. Journal of Pharmacology and Experimental Therapeutics, 318(2), 503–512.
13. Desta, Z., Ward, B. A., Soukhova, N. V., & Flockhart, D. A. (2004). Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. Journal of Pharmacology and Experimental Therapeutics, 310(3), 1062–1075.
14. Kiyotani, K., Mushiroda, T., Sasa, M., Bando, Y., Sumitomo, I., Hosono, N., & Zembutsu, H. (2008). Impact of CYP2D6* 10 on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy. Cancer science, 99(5), 995–999.
15. Gaedigk, A., Sangkuhl, K., Whirl-Carrillo, M., Klein, T., & Leeder, J. S. (2017). Prediction of CYP2D6 phenotype from genotype across world populations. Genetics in Medicine, 19(1), 69.
16. Khan, B. A., Robinson, R., Fohner, A. E., Muzquiz, L. I., Schilling, B. D., Beans, J. A & Beatty, P. (2018). Cytochrome P450 genetic variation associated with tamoxifen biotransformation in American Indian and Alaska native people. Clinical and translational science, 11(3), 312–321.
17. Drögemöller, B. I., Wright, G. E., Shih, J., Monzon, J. G., Gelmon, K. A., Ross, C. J & Carleton, B. C. (2018). CYP2D6 as a treatment decision aid for ER-positive nonmetastatic breast cancer patients: a systematic review with accompanying clinical practice guidelines. Breast cancer research and treatment, 1–12.
18. Pietarinen, P., Tornio, A., & Niemi, M. (2016). High Frequency of CYP 2D6 Ultrarapid Metabolizer Genotype in the Finnish Population. Basic & clinical pharmacology & toxicology, 119(3), 291–296
19. Marez D, Legrand M, Sabbagh N, et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics. 1997; 7: 193–202.
20. Beverage, J. N., Sissung, T. M., Sion, A. M., Danesi, R., & Figg, W. D. (2007). CYP2D6 polymorphisms and the impact on tamoxifen therapy. Journal of pharmaceutical sciences, 96(9), 2224–2231.
21. Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K. H. & Blanchard, R. (2005). CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. Journal of the National Cancer Institute, 97(1), 30–39.
22. Borges, S., Desta, Z., Li, L., Skaar, T. C., Ward, B. A., Nguyen, A & Hillman, G. (2006). Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. Clinical Pharmacology & Therapeutics, 80(1), 61–74.
23. Hennig, E. E., Piatkowska, M., Karczmarski, J., Goryca, K., Brewczynska, E., Jazwiec, R & Ostrowski, J. (2015). Limited predictive value of achieving beneficial plasma (Z)-endoxifen threshold level by CYP2D6 genotyping in tamoxifen-treated Polish women with breast cancer. BMC cancer, 15(1), 570.
24. López, Marisol, et al. "CYP2D6 genotype and phenotype determination in a Mexican Mestizo population." European journal of clinical pharmacology 61.10 (2005): 749–754.
25. Jorge LF, Eichelbaum M, Griese EU, Inaba T, Arias TD (1999) Comparative evolutionary pharmacogenetics of CYP2D6 in Ngawbe and Embera Amerindians of Panama and Colombia: role of selection versus drift in world populations. Pharmacogenetics 9:217–228.
26. Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjoqvist F, Ingelman–Sundberg M (1994) Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol 46:452–459.
27. Nishida Y, Fukuda T, Yamamoto I, Azuma J (2000) CYP2D6 genotypes in a Japanese population: low frequencies of CYP2 D6 gene duplication but high frequency of CYP2D6*10. Pharmacogenetics 6:567–570.
28. De Dueñas, Eduardo Martinez, et al. "Adjusting the dose of tamoxifen in patients with early breast cancer and CYP2D6 poor metabolizer phenotype." The Breast 23.4 (2014): 400–406.
29. Zafra-Ceres, M., De Haro, T., Farez-Vidal, E., Blancas, I., Bandres, F., de Dueñas, E. M., & Gomez-Llorente, C. (2013). Influence of CYP2D6 polymorphisms on serum levels of tamoxifen metabolites in Spanish women with breast cancer. International journal of medical sciences, 10(7), 932.
30. Morrow, P. K., Serna, R., Broglio, K., Pusztai, L., Nikoloff, D. M., Hillman, G. R., & Gonzalez-Angulo, A. M. (2012). Effect of CYP2D6 polymorphisms on breast cancer recurrence. Cancer, 118(5), 1221–1227.
31. Okat, Z., Yaman, K., Çiftçi, K. U., Toplayıcı, S., Kurt, E., & Taga, Y. (2018). Determination of CYP2D6* 3 and* 4 allele frequency among Turkish population
32. Alcazar-González, G. A., Calderón-Garcidueñas, A. L., Garza-Rodríguez, M. L., Rubio-Hernández, G., Escorza-Treviño, S., Olano-Martin, E & Simon-Buela, L. (2013). Comparative study of polymorphism frequencies of the CYP2D6, CYP3A5, CYP2C8 and IL-10 genes in Mexican and Spanish women with breast cancer. Pharmacogenomics, 14(13), 1583–1592.
33. Sachse C, Brockmoller J, Bauer S, Roots I (1997) Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 60:284–295
34. Scordo MG, Spina E, Facciola G, Avenoso A, Johansson I, Dahl ML (1999) Cytochrome P450 2D6 genotype and steady state plasma levels of risperidone and 9–hydroxyrisperidone. Psychopharmacology (Berl) 147:300–305
35. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman–Sundberg M (1996) Frequent distribution of ultrarapid metabolizers of debrisoquine in an Ethiopian population carrying duplicated and multiduplicated functional CYP2D6 alleles. J Pharmacol Exp Ther 278:441–446.
36. Wigle, T., Jansen, L., Teft, W., & Kim, R. (2017). Pharmacogenomics guided-personalization of warfarin and tamoxifen. Journal of personalized medicine, 7(4), 20.
37. Mendoza R, Wan YJ, Poland RE, Smith M, Zheng Y, Berman N, Lin KM (2001) CYP2D6 polymorphism in a Mexican American population. Clin Pharmacol Ther 70:552–560.
38. Brauch, H., Mürdter, T. E., Eichelbaum, M., & Schwab, M. (2009). Pharmacogenomics of tamoxifen therapy. Clinical chemistry, 55(10), 1770–1782.
39. Yu, C. Y., Ang, G. Y., Subramaniam, V., Johari James, R., Ahmad, A., Abdul Rahman, T & Salleh, M. Z. (2017). Inference of the genetic polymorphisms of CYP2D6 in six subtribes of the malaysian orang asli from whole-genome sequencing data. Genetic testing and molecular biomarkers, 21(7), 409–415.
40. Wegman, P., Vainikka, L., Stål, O., Nordenskjöld, B., Skoog, L., Rutqvist, L. E., & Wingren, S. (2005). Genotype of metabolic enzymes and the benefit of tamoxifen in postmenopausal breast cancer patients. Breast Cancer Research, 7(3), R284.
41. Desta, Z., & Flockhart, D. A. (2017). Pharmacogenetics of Drug Metabolism. In Clinical and Translational Science (Second Edition) (pp. 327–345).
42. Wegman, P., Elingarami, S., Carstensen, J., Stål, O., Nordenskjöld, B., & Wingren, S. (2007). Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Research, 9(1), R7.
43. Ferraldeschi, R., & Newman, W. G. (2010). The impact of CYP2D6 genotyping on tamoxifen treatment. Pharmaceuticals, 3(4), 1122–1138.
44. Kiss, Á. F., Tóth, K., Juhász, C., Temesvári, M., Paulik, J., Hirka, G., & Monostory, K. (2018). Is CYP2D6 phenotype predictable from CYP2D6 genotype?. Microchemical Journal, 136, 209–214.
45. Cronin-Fenton, D. P., & Damkier, P. (2018). Tamoxifen and CYP2D6: A Controversy in Pharmacogenetics. In Advances in Pharmacology (Vol. 83, pp. 65–91). Academic Press.
46. Brooks, J. D., Comen, E. A., Reiner, A. S., Orlow, I., Leong, S. F., Liang, X & Bernstein, L. (2018). CYP2D6 phenotype, tamoxifen, and risk of contralateral breast cancer in the WECARE Study. Breast Cancer Research, 20(1), 149.
47. Ingelman-Sundberg, M. (2005). Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. The pharmacogenomics journal, 5(1), 6.
48. Goetz, M. P., Knox, S. K., Suman, V. J., Rae, J. M., Safgren, S. L., Ames, M. M & Weinshilboum, R. M. (2007). The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. Breast cancer research and treatment, 101(1), 113–121.
49. Schroth, W., Antoniadou, L., Fritz, P., Schwab, M., Muerdter, T., Zanger, U. M & Brauch, H. (2007). Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. Journal of Clinical Oncology, 25(33), 5187–5193.
50. Irvin Jr, W. J., Walko, C. M., Weck, K. E., Ibrahim, J. G., Chiu, W. K., Dees, E. C & Raab, R. E. (2011). Genotype-guided tamoxifen dosing increases active metabolite exposure in women with reduced CYP2D6 metabolism: a multicenter study. Journal of clinical oncology, 29(24), 3232.
51. Martins DM, Vidal FC, Souza RD, Brusaca SA and Brito LM. (2014). Determination of CYP2D6 *3, *4, and *10 frequency in women with breast

cancer in São Luís, Brazil, and its association with prognostic factors and disease-free survival. Braz J Med Biol Res 47, 1008–1015.
52. Okishiro, M., Taguchi, T., Kim, S. J., Shimazu, K., Tamaki, Y., & Noguchi, S. (2009). Genetic polymorphisms of CYP2D6* 10 and CYP2C19* 2,* 3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. Cancer, 115(5), 952–961.
53. Caraco Y. Genes and the response to drugs. N Engl J Med. 2004;351: 2867–2869
54. Lim, H. S., Ju Lee, H., Seok Lee, K., Sook Lee, E., Jang, I. J., & Ro, J. (2007). Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. Journal of Clinical Oncology, 25(25), 3837–3845.
55. Lim, J. S., Chen, X. A., Singh, O., Yap, Y. S., Ng, R. C., Wong, N. S & Chowbay, B. (2011). Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. British journal of clinical pharmacology, 71(5), 737–750.
56. Nazir, N., Waheed, A., Farhat, K., Ismail, M., & Mansoor, Q. (2016). Frequency of CYP2D6* 10 genotypes in Pakistani breast cancer patients taking adjuvant tamoxifen. JPMA. The Journal of the Pakistan Medical Association, 66(12), 1554–1558.
57. Del Tredici, A. L., Malhotra, A., Dedek, M., Espin, F., Roach, D., Zhu, G. D., & Moreno, T. A. (2018). Frequency of CYP2D6 Alleles Including Structural Variants in the United States. Frontiers in pharmacology, 9, 305.
58. Goetz, M. P., Rae, J. M., Suman, V. J., Safgren, S. L., Ames, M. M., Visscher, D. W & Desta, Z. (2005). Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. Journal of Clinical Oncology, 23(36), 9312–9318.
59. Zembutsu, H., Nakamura, S., Akashi-Tanaka, S., Kuwayama, T., Watanabe, C., Takamaru, T. & Hasegawa, Y. (2017). Significant effect of polymorphisms in CYP2D6 on response to tamoxifen therapy for breast cancer: a prospective multicenter study. Clinical Cancer Research, 23(8), 2019–2026.
60. Sim, S., Lövrot, J., Lindh, J. D., Bergh, J., & Xie, H. (2018). Effect of CYP2C19 and CYP2D6 genotype on tamoxifen treatment outcome indicates endogenous and exogenous interplay. Pharmacogenomics, 19(13), 1027–1037.
61. Kelly, C. M., Juurlink, D. N., Gomes, T., Duong-Hua, M., Pritchard, K. I., Austin, P. C., & Paszat, L. F. (2010). Selective serotonin reuptake inhibitors and breast cancer mortality in women receiving tamoxifen: a population based cohort study. Bmj, 340, c693.
62. Seruga, B., & Amir, E. (2010). Cytochrome P450 2D6 and outcomes of adjuvant tamoxifen therapy: results of a meta-analysis. Breast cancer research and treatment, 122(3), 609–617.
63. Schroth, W., Goetz, M. P., Hamann, U., Fasching, P. A., Schmidt, M., Winter, S & Safgren, S. L. (2009). Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. Jama, 302(13), 1429–1436.
64. Bijl, M. J., van Schaik, R. H., Lammers, L. A., Hofman, A., Vulto, A. G., van Gelder, T & Visser, L. E. (2009). The CYP2D6* 4 polymorphism affects breast cancer survival in tamoxifen users. Breast cancer research and treatment, 118(1), 125–130.
65. Gonzalez-Santiago, S., Zárate, R., Haba-Rodríguez, J., Gómez, A., Bandrés, E., Moreno, S & Aranda, E. (2007). CYP2D6* 4 polymorphism as blood predictive biomarker of breast cancer relapse in patients receiving adjuvant tamoxifen. Journal of Clinical Oncology, 25(18_suppl), 590–590.
66. Nowell, S. A., Ahn, J., Rae, J. M., Scheys, J. O., Trovato, A., Sweeney, C & Ambrosone, C. B. (2005). Association of genetic variation in tamoxifen-metabolizing enzymes with overall survival and recurrence of disease in breast cancer patients. Breast cancer research and treatment, 91(3), 249–258.
67. Ferna´ndez-Santander A, Gaibar M, Novillo A, Romero-Lorca A, Rubio M, Chicharro LM, et al.(2013). Relationship between genotypes Sult1a2 and Cyp2d6 and tamoxifen metabolism in breast cancer patients. PLoS One, 8:e70183.
68. Bonanni, B., Macis, D., Maisonneuve, P., Johansson, H. A., Gucciardo, G., Oliviero, P& Decensi, A. U. (2006). Polymorphism in the CYP2D6 tamoxifen-metabolizing gene influences clinical effect but not hot flashes: data from the Italian Tamoxifen Trial. J Clin Oncol, 24(22), 3708–3709.