Pharmacogenetics as Personalized Medicine: Association Investigation of SOD2 rs4880, CYP2C19 rs4244285, and FCGR2A rs1801274 Polymorphisms in a Breast Cancer Population in Iraqi Women

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Abstract

This study was carried out in Al-Diwanyah Teaching Hospital in the city of Qadisiyah in southern Iraq. The study was caused by the large and dangerous prevalence of breast cancer among Iraqi women. Samples of breast cancer patients as well as samples from healthy individuals were collected to compare the 2 groups on the basis of mutations in genes that cause breast cancer. Our study suggests that SOD2 rs4880 and CYP2C19 rs4244285 polymorphisms play an important role in development of breast cancer in an Iraqi population, and no significant association between FCGR2A rs1801274 and breast cancer.

Background: Breast cancer is the most common cancer in women characterized by a high variable clinical outcome among individuals treated with targeted therapies. **Patients and Methods:** In this study, we performed a population-based approach intersecting high-throughput genotype data from Iraqi populations with publicly available pharmacogenomics information to estimate the frequency of genotypes correlated with responsiveness to breast cancer treatment thus improving the clinical management of this disease in an efficient and cost effective way. A total of 50 patients and 25 healthy controls were enrolled in our study. Genotyping of rs4880, rs4244285, and rs1801274 were examined in association with breast cancer in Iraqi women. **Results:** We found that individuals carrying the CT genotype of rs4880 manifested an increased risk of breast cancer compared with those carrying the TT genotype (odds ratio [OR], 0.171; 95% confidence interval [CI], 0.053-0.551; $P = .002$). In the dominant model, we observed that the CT and CC genotype of rs4880 showed an increased risk of breast cancer compared with the TT genotype (OR, 0.248; 95% CI, 0.089-0.690; $P = .006$). Moreover, subjects with the GA genotype of rs4244285 presented a higher risk of breast cancer than the GG genotype (OR, 0.256; 95% CI, 0.066-0.987; $P = .038$) and dominant models (OR, 0.025; 95% CI, 0.054-0.775; $P = .013$). **Conclusion:** The analysis revealed that rs1801274 showed linkage disequilibrium and decreased risk of breast cancer. In conclusion, our study suggests that rs4880 and rs4244285 polymorphisms play an important role in development of breast cancer in an Iraqi population, and no significant association was found between rs1801274 and the risk of breast cancer.

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Introduction

Breast cancer is the most frequent carcinoma in women and the second most common cause of cancer related mortality in women.[1] It is the most commonly diagnosed malignancy in women around the world, especially in the Western countries. It accounts for almost one-fifth of deaths caused by cancer.[2] Every year, 1 million new cases are reported worldwide, representing 18% of the total number of cancers in women. It has been established that 1 of 8 women (in the

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Table 1 Sequence of Primers Used in the Present Study

Gene Name	SNP Name	Sequence (5′−3′)	Tm, °C	Product Size
CYP2C19	rs4244285	F 5-TTACAACCAGAGCTTGGCAT-3	54	408
		R 5-TCCTTGACCTGTTAAACATCCGT-3	56	
SOD2	rs4880	F 5-GAGGGGACGCGGGGA-3	60	400
		R 5-CCCTGGGGTCGCCTCT-3	60	
FCGR2A	rs1801274	F 5-CTTGGCAGACTCCCCATACC- 3	57	420
		R 5-AGTTCTGTGAGTAACGTACCTCTG-3	55	

Abbreviations: F = forward; R = reverse; SNP = single nucleotide polymorphism; Tm = temperature.

United States) and 1 of 10 women (in the United Kingdom)[3] will develop breast cancer at some point in her life.[4] The death rate for breast cancer has been slowly declining over the past decade, and the incidence has remained level since1988 after increasing steadily for nearly 50 years. Twenty-five percent to 30% of women with invasive breast cancer will die of their disease.[5] However, this statistic, as grim as it is, also means that 70% to 75% of women with invasive breast cancer will die of some thing other than their breast cancer. Hence, a diagnosis of breast cancer, even invasive breast cancer, is not necessarily the "sentence of death" that many women imagine.

Mortality rates are highest in the very young (younger than age 35 years) and in the very old (older than age 75 years). It appears that the very young have more aggressive disease, and that the very old might not be treated aggressively or might have comorbid disease that increases breast cancer fatality[6] in Iraq, where the population was exposed to high levels of depleted uranium after the first and second gulf wars. Breast cancer is the most common type of malignancy among the Iraqi population in general; responsible for approximately one-third of the registered female cancers and almost one-quarter of female deaths from the disease. Within the past 2 decades, there has been an obvious increase in the incidence rates of breast cancer, which became one of the major threats to Iraqi female health.[7]

Pharmacogenetics has been defined as the science of pharmacological response and its modification by hereditary influence; the subjects of interest relate to efficacy (therapeutic effectiveness) as well as toxicity (production of side effects or unwanted effects).[8] Dealing with the genetic basis that underlies variable drug response in individual patients, results in an individualized approach to the therapy, where optimally effective drugs are matched to a patient's unique genetic profile.[9] Pharmacogenetics provides insight into the molecular level of drug function and, as a consequence, offers the potential of individualized drug therapy.[10] Thus it can help in optimizing drug efficacy and minimizing adverse drug reactions.[11]

The term, pharmacogenetic polymorphism, defines a monogenetic trait, caused by the presence of more than 1 allele at the same locus and more than 1 phenotype in the same population with regard to drug interaction with the organism, with the frequency of the rarest allele of >1%. DNA sequence variations might occur as insertions or deletions of nucleotides, differences in the copy number of repeated sequences or single nucleotide polymorphisms (SNPs).[12] If the mutations occur in proteins that are drug targets or drug-metabolizing enzymes, or in proteins that are involved in drug transport mechanisms, they can affect drug efficacy and safety.[13]

Anthracycline-based adjuvant regimens have become the standard of care for early-stage breast cancer in the United Kingdom. The regimen of AC (doxorubicin and cyclophosphamide) is one of a number of available choices, with widespread use in patients with an indication for chemotherapy, but a low to moderate risk of recurrence. The combination regimen AC was first tested by the National Surgical Adjuvant Breast and Bowel Project as a simple alternative regimen to replace CMF (cyclophosphamide, methotrexate, and 5-fluorouracil), which had been established as an effective adjuvant treatment.[14]

Cyclophosphamide is a prodrug activated by a number of different cytochrome P450 enzymes, including CYP2C19 rs4244285.[15] Each of these enzymes and transporter genes is known to exhibit a degree of genetic variation, characterized by SNPs. These SNPs are present at significant frequencies in a European population and their influence on the pharmacology of a number of different agents has been characterized.[16]

The aim of this study was to investigate SOD2 rs4880, CYP2C19 rs4244285, and FCGR2A rs1801274 polymorphisms in a breast cancer population in Iraqi women and examine the possible influence of SNPs on the tolerance, side effects, and overall clinical outcome of chemotherapy in patients with breast cancer.

Patients and Methods

Patients and Clinical Samples

The blood samples from 50 patients with different stages of breast cancer were provided by Tumor center/Al-Diwanyah Teaching Hospital/AL-Dewanyah during the period from May 2016 to December 2016. Twenty-five blood samples from healthy donors were used as a control in this study. We collected 2 mL of peripheral blood into an ethylenediaminetetraacetic acid (EDTA)-containing tube. The samples were stored at −20°C until further processing.

Measurement of DNA Concentration and Purity

Extracted DNA concentration and purity were measured using the nanodrop (Quawell/Hong Kong); 2 μL of DNA was loaded to the lens of the nanodrop and measured on 260/280-nm wavelength; the

Table 2 Polymerase Chain Reaction Components for Amplification of the 408-Base Pair Band

Component	Reaction Size
Template DNA	5 μL
Primers (10 pmol/mL)	1 μL F 1 μL R
Deionized water	13 μL

Abbreviations: F = forward; R = reverse.

Table 3 Clinical Characteristics and Breast Cancer Risk

Variable	Control (G1; n = 25), %	Patients (G2; n = 50), %	OR (95% CI)	*P*
Logging				
Urban	18	32	0.691 (0.243-1.969)	.488
Rural	7	18		
Smoking				
No smoker	23	42	2.190 (0.492-11.188)	.337
Smoker	2	8		
Mean age (±SE)	29.42 ± 10.21	51.29 ± 12.18		

Abbreviations: G1 = group 1; G2 = group 2.

result appeared on the laptop screen that attached to the nanodrop. The nanodrop lens was cleaned using distilled water and cotton swab after each sample; the other samples were measured consequently.

Primers

Primers used to identify and amplify the CYP2C19 rs4244285, SOD2 rs4880, and FCGR2A rs1801274 fragment are given in Table 1.

Polymerase Chain Reaction

The polymerase chain reaction (PCR) technique was used to identify and amplify the 408-base pair (bp) band of the *CYP2C19* gene, by using the primers designed by Alfa Gene (Ottawa, Canada; Table 2). The primers were lyophilized, they dissolved in a free ddH_2O to give a final concentration of 100 pmol/µL (as a stock solution) and kept as a stock in −20° C to prepare a 10-pmol/µL concentration as a work primer resuspended in 10 µL of the stock solution in 90 µL of free ddH_2O to reach a final volume of 100 µL (10 pmol). The total volume of PCR reaction was 20 µL; the reaction components are described in Table 2. The PCR product was then separated on 2% agarose gel at 50 V for 45 minutes in 0.5X tris/borate/EDTA buffer by taking 5 µL from each sample. Agarose gels were stained with ethidium bromide 0.5 mg/mL for 20 to 30 minutes. The DNA bands were visualized using

Figure 1 The Polymerase Chain Reaction Product Was Electrophoresed on 2.5% Agarose Gel at 50 V, for 45 Minutes. "M" Refers to a 25 to 1000-Base Pair Ladder. "A" Refers to rs4880 for *SOD2*, "B" Refers to rs4244285 for CYP2C19, and "C" Refer to rs1801274 for FCGR2A

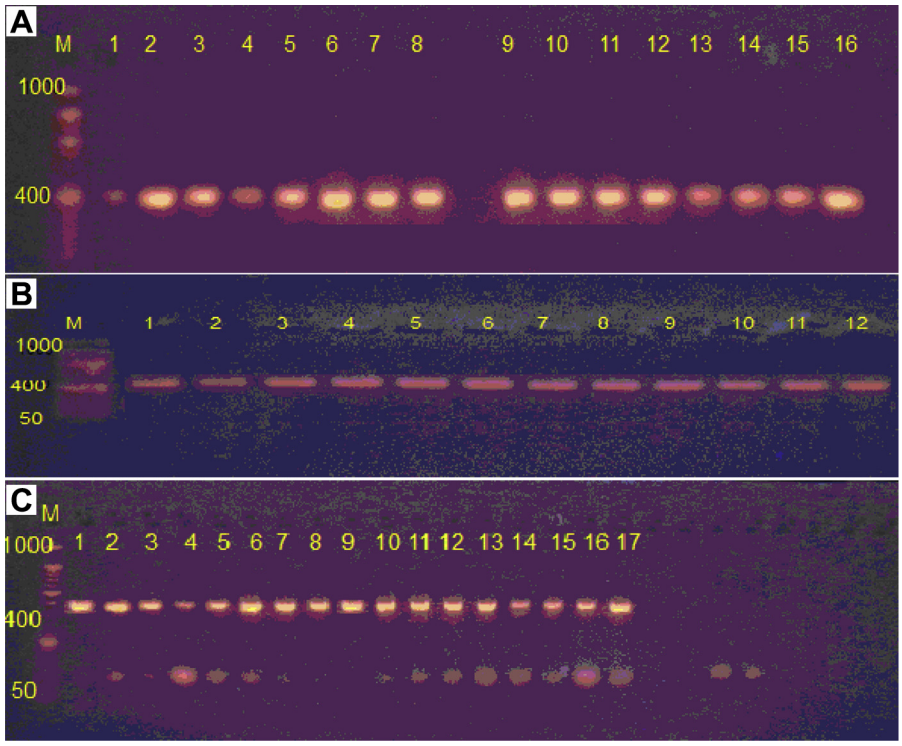

Table 4 The Genotypes and Allele Distribution of SOD2 Polymorphism in G1 and G2

Polymorphism SOD2 (C/T)	G1: Control (n = 25), %	G2: Patients (n = 50), %	χ^2	*P*	OR (95% CI)	*P*
TT	14 (56.0)	12 (24.0)	0.357	.008	1.0^{ref} (1.0^{ref})	.002
CT	6 (24.0)	30 (60.0)			0.171 (0.053-0.551)	.365
CC	5 (20.0)	8 (16.0)			0.536 (0.138-2.082)	
T Allele	34 (68.0)	54 (54.0)	2.694	.116	1.0^{ref} (1.0^{ref})	.101
C Allele	16 (32.0)	46 (46.0)			1.810 (0.888-3.691)	
TT	14 (56.0)	12 (24.0)	7.535	.01	1.0^{ref} (1.0^{ref})	.006
CT and CC	11 (44.0)	38 (76.0)			0.248 (0.089-0.690)	
CC	5 (20.0)	8 (16.0)	0.186	.750	1.0^{ref} (1.0^{ref})	.666
CT and TT	20 (80.0)	42 (84.0)			0.762 (0.221-2.627)	

Abbreviations: G1 = group 1; G2 = group 2; ref = reference.

electrophoresis and captured by a gel documentation system to document the observed bands.

Statistical Analysis

Genotype and allele carrier frequencies were defined as the percentage of individuals carrying the genotype and allele of the total number of individuals. The χ^2 and *P* value $< .05$, the odds ratio, and their 95% confidence interval tests of SPSS 23.0 for Windows (IBM Corp, Armonk, NY) were used to compare the frequency of discrete variables between patients with breast cancer and control individuals.

Results

The risk of breast cancer with some variables such as smoking and lodging were estimated according to the odds ratio and their confidence interval at $P < .05$; the matching in ages between cases and controls was indicated by the *P* value using the SPSS software as shown in Table 3. In our study and as obvious from the results described previously there is no association between lodging and smoking with risk of breast cancer (*P* value was $> .05$).[17]

Genomic DNA of sufficient quality and quantity was extracted from 50 blood samples from women with breast cancer and 25 healthy (control) women from Al-Diwanyah Teaching Hospital, in the south of Iraq. Amplification of the SOD2 rs4880, CYP2C19 rs4244285, and FCGR2A rs1801274 target sequence from these archival samples resulted in 400, 408, and 420 bp products, respectively. The amplified fragment, which yielded a single band of the desired product with a molecular weight of genes appeared sharp in agarose gel using a gel electrophoreses technique and loaded with a 25 to 1000 bp DNA ladder (Figure 1).

Seventy-five PCR product samples were sent for sequence analysis, 20 μL of PCR product for each sample was sent as well as 100 μL (10 pmol) from the forward primer. The samples were treated with an AB13730XL Applied Biosystems machine from NICM/USA Company, New York, NY. The result of the sequence analysis was analyzed using BLASTN in the National Center for Biotechnology Information (NCBI). The results were compared with data obtained from Gene Bank, which are available at the NCBI online.

To determine whether the rs4880 for SOD2 (T>C), rs4244285 for CYP2C19 (G>A), and rs1801274 for FCGR2A (T>C), were associated with susceptibility to breast cancer. In the Iraqi female population living in the south of Iraq, we analyzed these SNPs in 25 healthy individuals and 50 patients with breast cancer living in this region. Polymorphic analysis revealed that all 3 possible genotypes (TT, CT, and CC at the rs4880 site; GG, GA, AA at rs4244285; and TT, CT, and CC at rs1801274) could be detected for these SNPs. The TT genotype was the major genotype at the rs4880. The GG genotype was the major genotype at the rs4244285 and the TT genotype was the major genotype at the rs1801274 locus in the subjects studied.

Table 5 The Genotypes and Allele Distribution of CYP2C19 Polymorphism in G1 and G2

Polymorphisms CYP2C19 (G/A)	G1: Control (n = 25), %	G2: Patients (n = 50), %	χ^2	*P*	OR (95% CI)	*P*
GG	22 (88.0)	30 (60.0)	0.295	.032	1.0^{ref} (1.0^{ref})	.038
GA	3 (12.0)	16 (32.0)			0.256 (0.066-0.987)	.095
AA	0 (00.0)	4 (8.0)			1.733 (1.373-2.188)	
G Allele	47 (94.0)	76 (76.0)	7.317	.012	1.0^{ref} (1.0^{ref})	.007
A Allele	3 (6.0)	24 (24.0)			0.202 (0.058-0.708)	
GG	22 (88.0)	30 (60.0)	6.145	.017	1.0^{ref} (1.0^{ref})	.013
GA and AA	3 (12.0)	20 (40.0)			0.205 (0.054-0.775)	
AA	0 (00.0)	4 (8.0)	2.113	.294	1.0^{ref} (1.0^{ref})	.146
GA and GG	25 (100.0)	46 (92.0)			1-543 (1.300-1.832)	

Abbreviations: G1 = group 1; G2 = group 2; ref = reference.

Table 6 The Genotypes and Allele Distribution of FCGR2A Polymorphism in G1 and G2

Polymorphisms FCGR2A (T/C)	G1: Control (n = 25), %	G2: Patients (n = 50), %	χ^2	*P*	OR (95% CI)	*P*
TT	13 (52.0)	30 (60.0)	0.097	.735	1.0^{ref} (1.0^{ref})	.705
CT	8 (32.0)	15 (30.0)			1.231 (0.419-3.613)	.409
CC	4 (16.0)	5 (10.0)			1.846 (0.426-8.006)	
T Allele	34 (68.0)	75 (75.0)			1.0^{ref} (1.0^{ref})	
C Allele	16 (32.0)	25 (25.0)	0.822	.438	1.412 (0.669-2.980)	.364
TT	13 (56.0)	30 (60.0)			1.0^{ref} (1.0^{ref})	
CT and CC	12 (44.0)	20 (40.0)	0.436	.622	1.385 (0.526-3.643)	.509
CC	4 (16.0)	5 (10.0)			1.0^{ref} (1.0^{ref})	
CT and TT	21 (84.0)	45 (90.0)	0.568	.709	1-714 (0.417-7.044)	.451

Abbreviations: G1 = group 1; G2 = group 2; ref = reference.

The results revealed significant association between SOD2 rs4880 T,C-alleles and the risk of breast cancer where χ^2 is 0.357 and $P = .008$. This indicates that there was a significant relationship between this SNP and breast cancer. Also the results show that the frequencies were 56% for TT, 24% for CT, and 20% for CC in group 1 (G1; control). The frequencies were 24% for TT, 60% for CT, and 16% for CC in group 2 (G2; patients). There was a significant association in rs4880 polymorphism of SOD2 between G1 represents control (healthy) and G2 represents patients ($P < .05$) as shown in Table 4. In G1, T allele frequency was 68% and the C allele was 32% and in G2 the frequencies were 54% and 46% for T and C alleles, respectively. Descriptive statistical analyses revealed that the rs4880 polymorphism, CT and CC genotypes compared with the TT genotype was significant ($P = .01$, $< .05$), whereas the CT and TT genotypes compared with the CC genotype there was a significant difference. There was also no significant relationship between the C allele with the reference T allele ($P = .101$).

The results revealed a significant association between CYP2C19 rs4244285 A,G-alleles and the risk of breast cancer where χ^2 was 0.295 and $P = .032$, $< .05$. This indicates that there was a significant relationship between this SNP and breast cancer. Also the results show that the frequencies were 88% for GG, 12% for GA, and 0% for AA in G1 (control). The frequencies were 60% for GG, 32% for GA, and 8% for GG in G2 (patients). There was a significant association in the rs4244285 polymorphism of CYP2C19 between G1 healthy (control) and G2 patients ($P < .05$) as shown in Table 5. In G1, the G allele frequency was 94% and the A allele was 6%, and in G2 the frequencies were 76% and 24% for G and A alleles, respectively. Descriptive statistical analyses revealed that the rs4244285 polymorphism, GA and AA genotypes compared with the GG (dominant) genotype was significant ($P = .013$, $< .05$), whereas compared with GA and GG genotypes and AA (recessive) genotype we found there was no significant association ($P = .294$). There was also a significant association between the G allele and the reference A allele ($P = .007$). It was observed that the allele AA was not found in the healthy G1, but was found to be 8% in patients with breast cancer. In addition, the ratio of allele A to allele G was very small in the healthy G1 whereas the percentage of allele A in patients was increased.

The results revealed no significant association between FCGR2A rs1801274 T,C-alleles and the risk of breast cancer where $\chi^2$was 0.097 and $P = .735$, $> .05$, where it was noted that the *P* value was much greater than the .05 and that there was no significant association between the healthy group and patients with the alleles. This indicates that there was no significant relationship between this SNP and breast cancer. Also the results show that the frequencies were 52% for TT, 32% for CT, and 16% for CC in G1 (control). The frequencies were 60% for TT, 30% for CT, and 10% for CC in G2 (patients). The percentage of alleles was close in the healthy and patient groups so there was no significant association, as shown in Table 6. In G1, T allele frequency was 68% and the C allele was 32%, and in G2 the frequencies were 75% and 25% for T and C alleles, respectively. Descriptive statistical analyses revealed that for the rs1801274 polymorphism, CT and CC genotypes compared with the TT genotype, there was no significant ($P = .622$, $> .05$), and also when compared between CT and TT genotypes with CC genotype we found there was no significant difference. There was also no significant association between the C allele with the reference T allele ($P = .0438$).

Discussion

Pharmacogenetic Role of Polymorphisms

From the pharmGKB database (Table 7), these 3 polymorphisms are reported to be related to the efficacy or toxicity of breast cancer drugs, and statistical analyses revealed that rs4880 and rs4244285 were significantly different in frequency in our samples and these polymorphisms were related to cyclophosphamide and doxorubicin, whereas rs1801274 related to trastuzumab, doxorubicin, paclitaxel, and cyclophosphamide. Because Iraqi patients with breast cancer have significant association with rs4880 and rs4244285 as we show

Table 7 List of SNPs Reported as Associated With Specific Drugs in the PharmGKB Database

SNP (Gene)	Drugs (Reported as Related in PharmGKB Database)	Alleles
rs4880 (*SOD2*)	Cyclophosphamide	T/C
rs4244285 (*CYP2C19*)	Cyclophosphamide, doxorubicin	A/G
rs1801274 (*FCGR2A*)	Trastuzumab, doxorubicin paclitaxel, cyclophosphamide	T/C

in the previous statistical analysis, and no significant association with rs1801274, we suggest that the drugs cyclophosphamide and doxorubicin be used as treatment for Iraqi patients with breast cancer. All of these findings increase the overall knowledge on the prevalence of specific variants related to breast cancer treatment responsiveness in an Iraqi population and highlight the importance of assessing gene polymorphisms with cancer medication in isolated communities.

Doxorubicin and cyclophosphamide are the backbone of the chemotherapeutic regimens used for the treatment of breast cancer patients. Cyclophosphamide is a prodrug that needs to be oxidized to exert its cytotoxic effect. This step is catalyzed by a number of cytochrome P450 enzymes, including CYP2C19.[18]

With regard to CYP2C19, the rs4244285 variant has been reported to be associated with differential response to the cyclophosphamide and doxorubicin adjuvant regimen. With regard to the frequency of the G allele in the Iraqi cohorts, the GG genotype was the most frequent in Iraqi cohorts; individuals bearing the AA genotype show a trend of an increased risk of poorer outcome if treated with the cyclophosphamide and doxorubicin polychemotherapeutic regimen.[19] These results correspond to the results obtained by Cocca et al regarding this gene in an Italian population.[20]

Conclusion

Our study first suggests that SOD2 rs4880 and CYP2C19 rs4244285 polymorphisms play an important role in the development of breast cancer in an Iraqi population, so this study has provided more evidence to support the concept that that the functional polymorphism is significantly associated with breast cancer, in addition, that FCGR2A rs1801274 appears to have no significant association with breast cancer in Iraqi women. Our explorative study highlights the importance of assessing gene polymorphisms related to cancer medications in isolated populations. In particular, the finding that specific functional variants, strongly associated with toxicity or lack of efficacy are more prevalent in a specific community could lead to the development of regional targeted interventions aimed at a direct screening of such risk genes/variants, for example by using a targeted resequencing approach. This in turn could facilitate a more effective and rational usage of the health care economic resources, thus paving the way for personalized medicine. Overall, rs4880 and rs4244285 appeared to influence the tolerance and effectiveness of cyclophosphamide and doxorubicin chemotherapy in this group of breast cancer patients.

Disclosure

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

Clinical Practice Points

- We found that Iraqi patients with breast cancer have mutations in the SOD2 gene and CYP2C19 gene.
- The new finding that is that only 2 drugs benefit patients—cyclophosphamide and doxorubicin—with no other medication found to be helpful.
- In future, every patient should receive the suitable drugs.

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