SciForschen
Open HUB for Scientific Research

International Journal of Cancer Research and Molecular Mechanisms

ISSN 2381-3318 | Open Access

RESEARCH ARTICLE

Volume 7 - Issue 1

Pilot Study to Determine the Prevalence of CYP2B6*6(C.516G>T), CYP2C19*2 (C.681G>A) and CYP2C19*3 (C.636G>A) in Breast Cancer Patients *versus* Normal Healthy Controls among Three Major Ethnic Groups in Singapore

Gaik-Hong Soon[1,*], Seok Hwee Koo[1], Pei Ting Tan[1], Lawrence Soon-U Lee[2], Chee Kian Tham[3], Mikael Hartman[4], and Su Ming Tan[5]

[1]Clinical Trials and Research Unit, Changi General Hospital, Singapore
[2]Tan Tock Seng Hospital, Singapore
[3]Department of Surgery, Changi General Hospital, Singapore
[4]Saw Swee Hock School of Public Health, National University of Singapore, Singapore
[5]Breast Centre, Changi General Hospital, Singapore

***Corresponding author:** Gaik-Hong Soon, Clinical Trials and Research Unit, Changi General Hospital, No. 2 Simei Street 3, 529889, Singapore, Tel: 68504911; E-mail: gaik_hong_soon@cgh.com.sg; ghsoonum@hotmail.com

Received: 10 Dec, 2021 | **Accepted:** 20 Jan, 2022 | **Published:** 27 Jan, 2022

Citation: Soon GH, Koo SH, Tan PT, Lee LS, Tham CK, et al. (2022) Pilot Study to Determine the Prevalence of CYP2B6*6(C.516G>T), CYP2C19*2 (C.681G>A) and CYP2C19*3 (C.636G>A) in Breast Cancer Patients versus Normal Healthy Controls among Three Major Ethnic Groups in Singapore. Int J Cancer Res Mol Mech 7(1): dx.doi.org/10.16966/2381-3318.154

Abstract

Background: Breast cancer is the top cancer suffered by women worldwide and is the leading cause of cancer mortality for women living in Singapore. Unfortunately, most of breast cancer cases are detected at a later stage of disease development and cripple the outcome of the therapy. This is a study to identify potential breast cancer susceptibility gene polymorphisms.

Methods: 455 breast cancer patients were consented to join this study. Genotyping on CYP2B6*6, CYP2C19*2 & CYP2C19*3 was done and normalised to healthy individuals' data. Clinical data were collected and analysed. All the statistical analyses were done using SPSS statistical software. Chi-square or Fisher's Exact Test was performed to examine the difference between subjects' characteristics for categorical variables. One-Way Anova was performed to assess age difference across alleles of CYP2B6*6, CYP2C19*2 and CYP2C19*3. Binary logistics regression was performed to identify demographic factors associated with breast cancer.

Results: CYP2B6*6 could be a risk factor leading to earlier onset of breast cancer among Indian population with OR equals 1.69 (95% CI=0.549-5.191, p=0.359). In the case of CYP2C19*2, OR is 1.57 for Malay (95% CI=0.696-3.522, p=0.278); 1.15 for Chinese population (95% CI=0.862-1.545, p=0.335) and 1.03 for Indian (95% C.I=0.301-3.496, p=0.968). CYP2C19*3 OR in Chinese population is 1.34 (95% C.I=0.830-2.155, p=0.231) and 0.77 (95% C.I=0.172-3.394, p=0.724) in Malay population. No CYP2C19*3 was detected in both cohorts of Indian patients and healthy controls.

Conclusions: CYP2B6*6 and CYP2C19*2 could be risk factors for Singaporean breast cancer patients; a bigger sample size could be studied to corroborate these findings.

Keywords: Single nucleotide polymorphisms; Chemotherapy; Breast cancer; Biomarkers; Aromatase inhibitor

Introduction

According to statistics from the Singapore Cancer Registry Annual Report 2018, breast cancer was the most common cancer (29.3%) among Singaporean women followed by colon-rectal (13.3%) and lung cancers (7.5%) between 2014 and 2018. Besides, breast cancer was reported to be the leading cause of death in cancers affecting women in Singapore during the same period. The age-standardized mortality rate for breast cancer has increased from 5.7 per 100,000 in 1968-1972 to 12.6 per 100,000 populations in 2014-2018 in tandem with increasing incidence rate.

Early detection of cancers plays an important role in reducing the mortality rate of cancers. However, breast cancer is usually detected at later stages of diseases [1]. The reliability of existing breast cancer biomarkers as an efficient means of detection needs to be reviewed. There is continuous effort to search for efficient breast cancer biomarkers as tools for early detection of breast cancer. At the same time, the biomarkers could serve as an important indicator for prognosis in breast cancer treatment.

CYP2B6 enzyme and CYP2C19 enzyme belong to cytochrome P450 (CYP450) metabolic enzymes which are categorized under phase I superfamily metabolic enzymes. Phase I and II enzymes are of

particular interest concerning breast cancer due to their involvement in the metabolism of steroid hormones, chemical carcinogens, and other environmental toxicants [2,3]. During phase I metabolism reaction, substrates usually undergo reduction, oxidation or hydroxylation to become more polar metabolites. CYP450 enzymes are the predominant mediators [4]. Usually, phase I metabolism is followed by phase II conjugation reactions. During the later stage, phase I metabolites, phase II exogenous or endogenous compounds are conjugated to a more polar molecule that produces inactive and water-soluble compounds for excretion by urine or bile [5,6]. The combined phase I and phase II metabolism is mainly a detoxification and elimination process, however, both phases bear the risk of formation of toxic and highly reactive compounds which can induce or promote serious health problems such as cancer [5,7]. Therefore, altered activity of metabolic enzymes holds the potential to increase the exposure to carcinogenic compounds and consequently the risk of tumour formation [8].

In a recent study done by Justenhoven C, et al. from Germany, CYP2B6*6 variant had an increased breast cancer risk with an OR of 1.1 (p=0.027) [9]. In 2016, Liu Lim JS et al. discovered that CYP2C19*2, loss of function polymorphism, as well as the CYP2C19 H2 haplotype were found to be significantly associated with lower plasma concentrations of NorEND and lower formation rates of NorEND [10]. NorEND is an active metabolite of tamoxifen that inhibits both aromatase and estrogen receptors, variability in its plasma concentration can potentially influence the therapeutic outcomes of tamoxifen therapy. These data suggest that CYP2C19 may potentially serve as a complementary biomarker for the identification of patients who may or may not benefit from tamoxifen treatment. Another group of researchers from China has discovered a possible association of gene polymorphism of CYP2C19*3 with breast cancer in Chinese Han population. The OR for carriers of AG+AA genotype for breast cancer was 2.31 (95% CI=1.27-4.43) [11].

It has been known that elevated sex hormone levels were found in women with breast cancer [12]. This effect was attributed to estrogen-induced gene expression of factors involved in cell growth and division [13] as well as genotoxic action of metabolic compounds such as 4-hydroxy-catechol estrogens and estrogen-3, 4-quinones [14]. Besides, progesterone plays a part in hormone-induced carcinogenesis by promotion of estrogen synthesis, expression of estrogen receptor and cell proliferation [15,16]. Aside from hormonal factors, environmental carcinogenic factors like tobacco smoke, and genetic factors such as mutation and polymorphisms all contribute to breast cancer susceptibility. A family study on the genetic basis of breast cancer indicated 2-fold increased risk in the first-degree relatives of women with the disease [17]. In 1990, BRCA1 and BRCA2 were identified as two major breast cancer susceptibility genes [18]. Harmful mutations in these two genes confer a cumulative disease risk by age 70 years of 65% and 45%, respectively [19]. Recent genome-wide association studies revealed strong evidence for more than 18 common breast cancer susceptibility alleles including FGFR2, CCND1, TNRC9, MAP3K1 and LSP1 [20]. Most of these genes are related to DNA repair, cell cycle control, apoptosis, cell growth and division. These processes represent the most important pathways for the protection of cells against carcinogenic processes. Low coverage of genes coding for phase I and phase II enzymes in commercial genotyping arrays and lack of well-designed studies have downplayed the roles of phase I and phase II enzymes play in conferring breast cancer risk [21].

Although CYP2B6 contributes about 2-5% of the total liver cytochrome content, it is also expressed in extra-hepatic tissues such as the breast; notably with higher expression levels in breast tumours than normal breast [8,22].

A study also indicated that Estrogen Receptor (ER) positive breast tumours show a higher CYP2B6 level than ER-negative tumours [23,24]. Impaired CYP2B6 activity had increased the level of both estradiol and testosterone whereby testosterone conferred a stronger influence on breast cancer risk than estradiol in postmenopausal women [25].

In this pilot study, we investigated the percentage of CYP2B6*6, CYP2C19*2 and CYP2C19*3 in our breast cancer patients against the healthy population in Singapore. At the same time, we examined the prevalence of these SNPs in different demographic groups for instance ethnicity, family history, use of hormone therapy, amongst others. Furthermore, characteristics of breast cancer were studied to unravel the distribution of these SNPs in different stages of breast cancer. The percentages of these SNPs in different treatments for breast cancer were also presented.

Methodology

In this study, a total of 455 breast cancer patients were recruited from Changi General Hospital Breast Centre Outpatient Clinic. This study had been approved by the local ethics committee (CIRB 2014/371/B). Informed consent had been obtained from study subject prior to getting the buccal swab and information from them. The study subjects were enrolled into this study based on inclusion criteria that required the subject to be a descendant from similar ethnicity throughout 3 generations namely, parents and grandparents must be of similar ethnicity. And, study subjects had been diagnosed of having breast cancer of any stage. Clinical data were collected from patients' casenotes. Ethnically-matched healthy control data was gathered from healthy volunteers' databank which was available from Singapore Breast Cancer Cohort Project. The buccal swab was obtained from each patient and genomic DNA was then extracted from the buccal swab using E.Z.N.A. Blood DNA Mini Kit -buccal swabs protocol (Omega Bio-tek) according to manufacturer's guidelines. Laboratory genotyping analysis was performed on all samples for SNPs on CYP2B6*6, CYP2C19*2, CYP2C19*3 using Taqman SNP assay kit and Taqman GT express Master Mix (Life Technologies). RT PCR was run on StepOnePlus RT PCR Systems (Applied Biosystems). Details of SNPs are as indicated in table 1.

Statistics

All the statistical analyses were done using SPSS statistical software, version 19.0 (IBM Corp. Armonk, NY). Chi-square or Fisher's Exact Test was performed to examine the difference between subject's characteristics for categorical variables. One-Way Anova was performed to assess age difference across alleles of CYP2B6*6, CYP2C19*2 and CYP2C19*3. Binary logistics regression was performed to identify demographic factors associated with breast cancer. A two-tailed, p-value <0.05 was defined as statistically significant.

Table 1: SNP investigated in this study.

Gene	Ref SNP ID	Genotype	Mutation
CYP2B6*6	rs3745274	G516T	Gln172His
CYP2C19*2	rs4244285	G681A	Splice variant
CYP2C19*3	rs4986893	G636A	Trp212Ter

Results

Study subjects' demographic profile and medical history

A total of 455 breast cancer patients participated in this study. Among them, 365 are of Chinese origin, 45 Malays and 45 Indians. Participants aged from 20 to 89 years old with an average age of 51.5 years. 48% of them aged from 20 to 50 years old. It was found that the lowest age of breast cancer diagnosis for both CYP2B6*6 and CYP2C19*3 homozygous mutant genotypes was around 41 years old as compared to a younger age at diagnosis for homozygous mutant CYP2C19*2 which was reported at 27 years old. Tables 4a-4c indicates the distribution of participants' ages for different variants studied.

BMI of participants ranged from 21.9(± 0.07) to 25.7 (± 0.83) kg/m^2. 81% of participants were post-menopausal. The majority of participants (94.1%) had never smoked before; 3.7% were former smokers and 2.2% are current smokers. 92% of participants had never used hormone therapy and only 1% used hormone therapy. Similarly, 78% of participants had never used oral contraceptives in contrast to 1.8% who used oral contraceptives for more than 10 years. Most of the participants (77%) did not have first degree relatives with breast or ovarian cancer compared to 22% of participants who had first degree relatives with breast or ovarian cancer during the study. Table 2 shows the distribution of participants in the various breast cancer risk groups.

Table 2: Distribution of subjects under different epidemiological breast cancer risk factors.

Factors	Number	Percentage
Smoking Status		
Never smoke	428	94.1
Former smoker	17	3.7
Current smoker	10	2.2
Status of Menopause		
% pre-menopausal	88	19.0
% post-menopausal	367	81.0
Use of Hormone Therapy		
Never use	419	92.0
>0-<10 years	29	6.0
>10 years	5	1.0
Use of oral contraceptive		
Never use	353	78.0
0-5 years	78	17.0
5-10 years	12	3.0
>10 years	8	2.0
1st degree relative with breast or ovarian cancer		
Yes	102	22.0
No	351	77.0
Age at diagnosis		
20-30 years old	7	1.5
31-40 years old	49	10.8
41-50 years old	162	35.7
51-60 years old	153	33.7
61-70 years old	63	13.9
71-80 years old	18	4.0
81-90 years old	2	0.5

Genotyping results

The data analysis of the genotyping results revealed that CYP2B6*6 could be a risk factor leading to the earlier onset of breast cancer among the Indian population. Subgroup analysis with ethnicity was performed for CYP2B6*6, CYP219*2 and CYP2C19*3 genotypes with results as indicated in table 3. It was discovered that Indian subjects with mutant allele "T" in CYP2B6*6 tend to have a higher risk of getting breast cancer (OR: 1.69, 95% CI: 0.549-5.191, P=0.359). Whereas Chinese and Malay subjects who have mutant alleles in CYP2C19*2 are more likely to have breast cancer (OR: 1.15, 95% CI: 0.862-1.545, P=0.335; OR: 1.57, 95% CI: 0.696-3.522, P=0.278 respectively). However, it is of note that the observed trends were not statistically significant (p>0.05). We also analysed the distribution of CYP2B6*6, CYP2C19*2 and CYP2C19*3 variants based on the epidemiological risk factors tables 4a-4c.

Breast cancer patients receiving different cancer treatments were categorized according to the variants CYP2B6*6, CYP2C19*2 and CYP2C19*3, as shown in tables 5a-5c. Summary of variants found in patients receiving either chemotherapy or endocrine therapy were recorded in tables 6a-6c.

Discussion

The conventional epidemiological breast cancer risk factors did not show a significant association of risk factors to breast cancer. Although post-menopausal, age of diagnosis between 41 to 50 years, and first degree relative having breast or ovarian cancer have elevated the risk factor, the use of hormone therapy and smoking did not cause a significant increase in the breast cancer cases. In this cohort studied, use of oral contraception between 0-5 years recorded the highest occurrence of breast cancer as compared to other longer durations of consumption. And most of the breast cancer patients were diagnosed between 40 to 50 years. This is consistent with the National Cancer Registry which recorded the peak of breast cancer cases during this period in women's life [1]. First degree relatives with breast cancer or ovarian cancer elevated the percentage of a risk factor to get breast cancer in this cohort as well especially in the variant CYP2C19*2 with 13.7% of the cohort showed homozygous mutant in CYP2C19*2. It is

Table 3: Odds ratio, 95% confidence interval and p-value for mutants CYP2B6*6, CYP2C19*2, CYP2C19*3 in case-control study among 3 main races in Singapore.

	Odds ratio	95% Confidence Interval		P-value
		Lower	Upper	
CYP2B6*6				
Chinese	0.749	0.555	1.010	0.058
Malay	0.770	0.347	1.708	0.520
Indian	1.688	0.549	5.191	0.359
CYP2C19*2				
Chinese	1.154	0.862	1.545	0.335
Malay	1.565	0.696	3.522	0.278
Indian	1.026	0.301	3.496	0.968
CYP2C19*3				
Chinese	1.337	0.830	2.155	0.231
Malay	0.765	0.172	3.394	0.724
Indian	NA	NA	NA	NA

Table 4: Association between subjects' characteristics and variants for genotype CYP2B6*6, CYP2C19*2 and CYP2C19*3.

Table 4a: CYP2B6*6.

	CYP2B6*6 (c.516 G>T) (n=455)			P value
	GG (n=275)	GT (n=151)	TT (n=29)	
Ethnicity				<0.001
Chinese	235(64.0%)	117(31.9%)	15(4.1%)	
Malay	22(51.2%)	14(32.6%)	7(16.3%)	
Indian	18(40.0%)	20(44.4%)	7(15.6%)	
Age of onset	51.71(± 0.63)	51.0 (± 0.88)	52.0 (± 1.33)	0.771
Min-Max age	21-81	20-89	41-77	
BMI	24.4(± 0.28)	24.5(± 0.41)	25.7(± 0.83)	0.375
Menopause status				0.670
Pre-menopausal	50(56.8%)	31(35.2%)	7(8.0%)	
Post-menopausal	225(61.3%)	120(32.7%)	22(6.0%)	
Family history of at least one 1st degree relative with Breast or Ovarian Cancer				0.970
No	211(60.1%)	117(33.3%)	23(6.6%)	
Yes	62(60.8%)	34(33.3%)	6(5.9%)	
Use of oral contraceptives				0.611
Never	214(60.6%)	116(32.9%)	23(6.5%)	
0-5 years	49(62.8%)	26(33.3%)	3(3.8%)	
5-10 years	6(50.0%)	4(33.3%)	2(16.7%)	
>10 years	4(50.0%)	4(50.0%)	0(0.0%)	
Use of hormone therapy				0.157
Never use	252(60.1%)	138(32.9%)	29(6.9%)	
>0-<10 years	16(55.2%)	13(44.8%)	0(0.0%)	
>10 years	5(100.0%)	0(0.0%)	0(0.0%)	
Smoking status				0.870
Never	259(60.7%)	141(33.0%)	27(6.3%)	
Former/Current	15(55.6%)	10(37.0%)	2(7.4%)	
Presence of Estrogen Receptor				0.961
Negative	68(61.8%)	35(31.8%)	7(6.4%)	
Positive	199(60.3%)	109(33.0%)	22(6.7%)	
Presence of Progesterone Receptor				0.285
Negative	100(66.2%)	42(27.8%)	9(6.0%)	
Positive	165(58.5%)	98(34.8%)	19(6.7%)	
ERBB2 status				0.776
Negative	151(60.6%)	79(31.7%)	19(7.6%)	
Positive	85(59.0%)	52(36.1%)	7(4.9%)	
Equivocal	11(64.7%)	5(29.4%)	1(5.9%)	
Histology type				0.151
Ductal non-specific	11(62.2%)	110(32.4%)	18(5.3%)	
Lobular	8(42.1%)	10(52.6%)	1(5.3%)	
Others	55(57.3%)	31(32.3%)	10(10.4%)	
Tumour size				0.291
<20.0mm	91(64.1%)	40(28.2%)	11(7.7%)	
20.0-49.9mm	123(58.0%)	79(37.3%)	10(4.7%)	
50.0+mm	31(59.6%)	20(38.5%)	1(1.9%)	
Multifocal	25(67.6%)	9(24.3%)	3(8.1%)	
Tumour grade				0.283
I	42(62.7%)	22(32.8%)	3(4.5%)	
II	105(59.7%)	64(36.4%)	7(4.0%)	
III	113(60.1%)	58(30.9%)	17(9.0%)	
Number of involved axillary lymph nodes				0.987
None	170(60.5%)	96(34.2%)	15(5.3%)	
1-3	54(60.0%)	30(33.3%)	6(6.7%)	
4+	38(62.3%)	20(32.8%)	3(4.9%)	

Table 4b: CYP2C19*2.

	CYP2C19*2 (c.681G>A) (n=455)			P value
	AA (n=57)	GA (n=186)	GG (n=212)	
Ethnicity				0.076
Chinese	42(11.4%)	149(40.6%)	176(48.0%)	
Malay	5(11.6%)	15(34.9%)	23(53.5%)	
Indian	10(22.2%)	22(48.9%)	13(28.9%)	
Age of onset	53.2(± 1.31)	51.7(± 0.79)	50.86(± 0.69)	0.311
Min-Max age	27-81	26-89	20-77	
BMI	25.4(± 0.64)	24.5(± 0.37)	24.3(± 0.32)	0.296
Menopause status				0.636
Pre-menopausal	10(11.4%)	33(37.5%)	45(51.1%)	
Post-menopausal	47(12.8%)	153(41.7%)	167(45.5%)	
Family history of at least one 1st degree relative with Breast or Ovarian Cancer				0.566
No	43(12.3%)	139(39.6%)	169(48.1%)	
Yes	14(13.7%)	45(44.1%)	43(42.2%)	
Use of oral contraceptives				0.380
Never	49(13.9%)	144(40.8%)	160(45.3%)	
0-5 years	6(7.7%)	33(42.3%)	39(50.0%)	
5-10 years	2(16.7%)	2(16.7%)	8(66.7%)	
>10 years	0(0.0%)	4(50.0%)	4(50.0%)	
Use of hormone therapy				0.471
Never use	51(12.2%)	167(39.9%)	201(48.0%)	
>0-<10 years	5(17.2%)	14(48.3%)	10(34.5%)	
>10 years	1(20.0%)	3(60.0%)	1(20.0%)	
Smoking status				0.929
Never	53(12.4%)	174(40.7%)	200(46.8%)	
Former/Current	4(14.8%)	11(40.7%)	12(44.4%)	
Presence of Estrogen Receptor				0.742
Negative	16(14.5%)	45(40.9%)	49(44.5%)	
Positive	39(11.8%)	136(41.2%)	155(47.0%)	
Presence of Progesterone Receptor				0.793
Negative	21(13.9%)	61(40.4%)	69(45.7%)	
Positive	33(11.7%)	119(42.2%)	130(46.1%)	
ERBB2 status				0.823
Negative	33(13.3%)	105(42.2%)	111(44.6%)	
Positive	19(13.2%)	55(38.2%)	70(48.6%)	
Equivocal	1(5.9%)	7(41.2%)	9(52.9%)	
Histology type				0.109
Ductal non-specific	38(11.2%)	136(40.1%)	165(48.7%)	
Lobular	4(21.1%)	4(21.1%)	11(57.9%)	
Others	15(15.6%)	45(46.9%)	36(37.5%)	
Tumour size				0.189
<20.0mm	16(11.3%)	60(42.3%)	66(46.5%)	
20.0-49.9mm	27(12.7%)	93(43.9%)	92(43.4%)	
50.0+mm	10(19.2%)	12(23.1%)	30(57.7%)	
Multifocal	4(10.8%)	14(37.8%)	19(51.4%)	
Tumour grade				0.514
I	7(10.4%)	31(46.3%)	29(43.3%)	
II	26(14.8%)	71(40.3%)	79(44.9%)	
III	19(10.1%)	74(39.4%)	95(50.5%)	
Number of involved axillary lymph nodes				0.036
None	35(12.5%)	119(42.3%)	127(45.2%)	
1-3	6(6.7%)	38(42.2%)	46(51.1%)	
4+	14(23.0%)	18(29.5%)	29(47.5%)	

Table 4c: CYP2C19*3.

	CYP2C19*3 (c.636G>A) (n=454)			P value
	AA (n=2)	GA (n=45)	GG (n=407)	
Ethnicity				0.129
Chinese	2(0.5%)	42(11.5%)	322(88.0%)	
Malay	0(0.0%)	3(7.0%)	40(93.0%)	
Indian	0(0.0%)	0(0.0%)	45(100.0%)	
Age of onset	52.5(± 12.5)	50.6(± 1.63)	51.6(± 0.51)	0.813
Min-Max age	40-65	30-75	20-89	
BMI	21.9(± 0.07)	24.5(± 0.7)	24.5(± 0.24)	0.743
Menopause status				0.343
Pre-menopausal	0(0.0%)	12(13.6%)	76(86.4%)	
Post-menopausal	2(0.5%)	33(9.0%)	331(90.4%)	
Family history of at least one 1st degree relative with Breast or Ovarian Cancer				0.254
No	2(0.6%)	31(8.8%)	318(90.6%)	
Yes	0(0.0%)	14(13.9%)	87(86.1%)	
Use of oral contraceptives				0.004
Never	1(0.3%)	32(9.1%)	319(90.6%)	
0-5 years	0(0.0%)	10(12.8%)	68(87.2%)	
5-10 years	1(8.3%)	2(16.7%)	9(75.0%)	
>10 years	0(0.0%)	1(12.5%)	7(87.5%)	
Use of hormone therapy				0.867
Never use	2(0.5%)	40(9.6%)	377(90.0%)	
>0-<10 years	0(0.0%)	4(13.8%)	25(86.2%)	
>10 years	0(0.0%)	1(20.0%)	4(80.0%)	
Smoking status				0.844
Never	2(0.5%)	43(10.1%)	381(89.4%)	
Former/Current	0(0.0%)	2(7.4%)	25(92.6%)	
Presence of Estrogen Receptor				0.234
Negative	0(0.0%)	7(6.4%)	103(93.6%)	
Positive	2(0.6%)	37(11.2%)	290(88.1%)	
Presence of Progesterone Receptor				0.193
Negative	0(0.0%)	11(7.3%)	140(92.7%)	
Positive	2(0.7%)	33(11.7%)	246(87.5%)	
ERBB2 status				0.003
Negative	0(0.0%)	21(8.5%)	227(91.5%)	
Positive	1(0.7%)	16(11.1%)	127(88.2%)	
Equivocal	1(5.9%)	4(23.5%)	12(70.6%)	
Histology type				0.948
Ductal non-specific	2(0.6%)	33(9.7%)	304(89.7%)	
Lobular	0(0.0%)	2(10.5%)	17(89.5%)	
Others	0(0.0%)	10(10.5%)	85(89.5%)	
Tumour size				0.160
<20.0mm	0(0.0%)	18(12.8%)	123(87.2%)	
20.0-49.9mm	1(0.5%)	16(7.5%)	195(92%)	
50.0+mm	0(0.0%)	5(9.6%)	47(90.4%)	
Multifocal	1(2.7%)	6(16.2%)	30(81.1%)	
Tumour grade				0.438
I	0(0.0%)	8(11.9%)	59(88.1%)	
II	0(0.0%)	14(8.0%)	161(92.0%)	
III	2(1.1%)	20(10.6%)	166(88.3%)	
Number of involved axillary lymph nodes				0.277
None	0(0.0%)	32(11.4%)	249(88.6%)	
1-3	1(1.1%)	7(7.9%)	81(91.0%)	
4+	1(1.6%)	5(8.2%)	55(90.2%)	

well known that inheritance acts as an important risk factor among environmental factors mentioned, especially BRCA1 and BRCA2 genes [26].

In this healthy case-control matched study, we found that Indians with CYP2B6*6 are more likely to have breast cancer. This mutant was not significant in Chinese and Malay groups. The group of German investigators found that CYP2B6*6 was associated with breast cancer risk in patients of European ancestry [9]. However, this is not the case in the majority of Singaporean population except the population of Indian descent in Singapore.

CYP2C19*2 appeared to be more frequent in Chinese and Malay breast cancer patients as compared to Indian breast cancer patients.

Nevertheless, CYP2C19*3 was found most frequently in Chinese breast cancer patients. Although the p-value is not significant, this trend is consistent with the finding reported by Gan CQ, et al. in their study that discovered an association of CYP2C19*3 with the onset of breast cancer in the Chinese Han population [11]. CYP2C19*3 was not found in this cohort study of the Indian population either in patients or healthy controls. CYP2C19*3 was found to be low in the Caucasian population with 0.04% frequency compared to 5-11% in asian population groups [27,28]. The percentage of breast cancer patients with CYP2C19*3 homozygous mutant receiving both chemotherapy and endocrine treatment is the lowest compared to the other two homozygous mutants studied. Among the 3 mutants studied, CYP2C19*2 homozygous mutant recorded the highest percentage among breast cancer patients receiving chemotherapy treatment or endocrine treatment, 10.1% and 12.3% respectively. Whereas 7.1% of breast cancer patients with CYP2B6*6 homozygous mutant received chemotherapy and 6.0% of them received endocrine treatment. This could be because CYP2C19*2 is found in approximately 23-39% of Asians, 10-20% of Caucasians, and 15% of Africans [29,30].

Patient's characteristics classified by SNP

This study showed a significant difference in breast cancer patients with CYP2B6*6, p-value <0.001 in all 3 major races in Singapore; the mutant homozygous TT appears in higher proportion in both Malay and Indian groups. Homozygous mutant CYP2C19*2 recorded the highest percentage (13.7%) *versus* homozygous mutant CYP2B6*6 (5.9%) in breast cancer patients under risk factor with the family history of first-degree relative with breast cancer or ovarian cancer. Similarly, CYP2C19*2 homozygous mutant recorded the highest percentage (13.9%) as compared to homozygous mutant CYP2B6*6 (6.5%) and homozygous mutant CYP2C19*3 (0.3%) in the group of breast cancer patients who have never used any oral contraceptives. Homozygous mutant in CYP2C19*2 was found to be prevalent in various characteristics of breast cancer studied, for instance, usage of hormone therapy, smoking status, presence of estrogen receptor, progesterone receptor, ERBB2 status, histological tumour type (ductal non-specific, lobular or others), various tumour size and grade, number of involved axillary lymph nodes (from none, 1-3 to 4+).

It was shown by Liu Lim JS, et al. that patients with CYP2C19*2 polymorphism and the CYP2C19 H2 haplotype had significantly lower plasma concentrations of NorEND and lower formation rates of NorEND [10]. It was reported by Lu WJ, et al. that NorEND inhibited recombinant human aromatase competitively, with a Ki of 35 nm, and these effects were shown to be comparable with that of the commonly used aromatase inhibitor letrozole [31]. NorEND has been previously shown to antagonize the activity of estrogen receptors in breast tissues. Though, NorEND antagonism is reportedly

Table 5: Percentage of CYP2B6*6, CYP2C19*2 & CYP2C19*3 variants found in patients receiving treatments for breast cancer.

Table 5a: CYP2B6*6.

	CYP2B6*6 (c.516 G>T) (n=455)			P value
	GG (n=275)	GT (n=151)	TT (n=29)	
Adjuvant treatment R				0.255
No	32(65.3%)	12(24.5%)	5(10.2%)	
Yes	243(59.9%)	139(34.2%)	24(5.9%)	
Adjuvant Chemo				0.806
No	140(61.9%)	72(31.9%)	14(6.2%)	
Yes	135(59.0%)	79(34.5%)	15(6.6%)	
Adjuvant Radiotherapy				0.374
No	157(62.8%)	76(30.4%)	17(6.8%)	
Yes	118(57.6%)	75(36.6%)	12(5.9%)	
Adjuvant endocrine				0.200
No	99(65.1%)	42(27.6%)	11(7.2%)	
Yes	176(58.1%)	109(36.0%)	18(5.9%)	
Adjuvant Herceptin				0.996
No	238(60.4%)	131(33.2%)	25(6.3%)	
Yes	37(60.7%)	20(32.8%)	4(6.6%)	
Chemo Anthracyclines				0.813
No	212(60.9%)	113(32.5%)	23(6.6%)	
Yes	63(58.9%)	38(35.5%)	6(5.6%)	
Chemo Taxanes				0.075
No	206(62.2%)	109(32.9%)	16(4.8%)	
Yes	69(55.6%)	42(33.9%)	13(10.5%)	
Chemo 5- FU				0.480
No	266(60.0%)	148(33.4%)	29(6.5%)	
Yes	9(75.0%)	3(25.0%)	0(0.0%)	
Chemo Cyclophosphamide				0.363
No	220(62.1%)	113(31.9%)	21(5.9%)	
Yes	55(54.5%)	38(37.6%)	8(7.9%)	
Chemo Carboplatin				0.892
No	269(60.6%)	147(33.1%)	28(6.3%)	
Yes	6(54.5%)	4(36.4%)	1(9.1%)	
Chemo Vinorelbine				0.365
No	275(60.6%)	150(33.0%)	29(6.4%)	
Yes	0(0.0%)	1(100.0%)	0(0.0%)	
Chemo Capecitabine				0.717
No	271(60.5%)	148(33.0%)	29(6.5%)	
Yes	4(57.1%)	3(42.9%)	0(0.0%)	
Chemo Doxil				NA
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Gemcitabine				NA
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Mitoxantrone				NA
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Abraxane				0.461
No	271(60.4%)	150(33.4%)	28(6.2%)	
Yes	4(66.7%)	1(16.7%)	1(16.7%)	
Chemo Halaven				NA
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Endocrine Tamoxifen				0.143
No	145(62.8%)	68(29.4%)	18(7.8%)	
Yes	130(58.0%)	83(37.1%)	11(4.9%)	
Endocrine Anastrozole				0.946
No	253(60.5%)	138(33.0%)	27(6.5%)	
Yes	22(59.5%)	13(35.1%)	2(5.4%)	
Endocrine Exemestane				0.886
No	269(60.4%)	148(33.3%)	28(6.3%)	
Yes	6(60.0%)	3(30.0%)	1(10.0%)	
Endocrine Letrozole				0.844
No	202(60.5%)	112(33.5%)	20(6.0%)	
Yes	73(60.3%)	39(32.2%)	9(7.4%)	

Table 5b: CYP2C19*2.

	CYP2C19*2 (c.681G>A) (n=455)			P value
	AA (n=57)	GA (n=186)	GG (n=212)	
Adjuvant treatment R				0.161
No	9(18.4%)	23(46.9%)	17(34.7%)	
Yes	48(11.8%)	163(40.1%)	195(48.0%)	
Adjuvant Chemo				0.900
No	29(12.8%)	90(39.8%)	107(47.3%)	
Yes	28(12.2%)	96(41.9%)	105(45.9%)	
Adjuvant Radiotherapy				0.534
No	34(13.6%)	105(42.0%)	111(44.4%)	
Yes	23(11.2%)	81(39.5%)	101(49.3%)	
Adjuvant endocrine				0.389
No	20(13.2%)	68(44.7%)	64(42.1%)	
Yes	37(12.2%)	118(38.9%)	148(48.8%)	
Adjuvant Herceptin				0.736
No	51(12.9%)	159(40.4%)	184(46.7%)	
Yes	6(9.8%)	27(44.3%)	28(45.9%)	
Chemo Anthracyclines				0.724
No	46(13.2%)	141(40.5%)	161(46.3%)	
Yes	11(10.3%)	45(42.1%)	51(47.7%)	
Chemo Taxanes				0.766
No	43(13.0%)	137(41.4%)	151(45.6%)	
Yes	14(11.3%)	49(39.5%)	61(49.2%)	
Chemo 5- FU				0.459
No	56(12.6%)	179(40.4%)	208(47.0%)	
Yes	1(8.3%)	7(58.3%)	4(33.3%)	
Chemo Cyclophosphamide				0.197
No	49(13.8%)	139(39.3%)	166(46.9%)	
Yes	8(7.9%)	47(46.5%)	46(45.5%)	
Chemo Carboplatin				0.300
No	55(12.4%)	184(41.4%)	205(46.2%)	
Yes	2(18.2%)	2(18.2%)	7(63.6%)	
Chemo Vinorelbine				0.563
No	57(12.6%)	186(41.0%)	211(46.5%)	
Yes	0(0.0%)	0(0.0%)	1(100.0%)	
Chemo Capecitabine				0.798
No	56(12.5%)	184(41.1%)	208(46.4%)	
Yes	1(14.3%)	2(28.6%)	4(57.1%)	
Chemo Doxil				NA
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Gemcitabine				NA
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Mitoxantrone				NA
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Abraxane				0.637
No	57(12.7%)	183(40.8%)	209(46.5%)	
Yes	0(0.0%)	3(50.0%)	3(50.0%)	
Chemo Halaven				NA
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(50.0%)	0(0.0%)	
Endocrine Tamoxifen				0.655
No	31(13.4%)	97(42.0%)	103(44.6%)	
Yes	26(11.6%)	89(39.7%)	109(48.7%)	
Endocrine Anastrozole				0.912
No	52(12.4%)	170(40.7%)	196(46.9%)	
Yes	5(13.5%)	16(43.2%)	16(43.2%)	
Endocrine Exemestane				0.165
No	54(12.1%)	184(41.3%)	207(46.5%)	
Yes	3(30.0%)	2(20.0%)	5(50.0%)	
Endocrine Letrozole				0.179
No	41(12.3%)	145(43.3%)	148(44.3%)	
Yes	16(13.2%)	41(33.9%)	64(52.9%)	

Table 5c: CYP2C19*3.

	CYP2C19*3 (c.636G>A) (n=454)			P value
	AA (n=2)	GA (n=45)	GG (n=407)	
Adjuvant treatment R				0.254
No	0(0.0%)	8(16.3%)	41(83.7%)	
Yes	2(0.5%)	37(9.1%)	366(90.4%)	
Adjuvant Chemo				0.213
No	1(0.4%)	28(12.4%)	197(87.2%)	
Yes	1(0.4%)	17(7.5%)	210(92.1%)	
Adjuvant Radiotherapy				0.920
No	1(0.4%)	26(10.4%)	223(89.2%)	
Yes	1(0.5%)	19(9.3%)	184(90.2%)	
Adjuvant endocrine				0.603
No	0(0.0%)	15(9.9%)	137(90.1%)	
Yes	2(0.7%)	30(9.9%)	270(89.4%)	
Adjuvant Herceptin				0.757
No	2(0.5%)	40(10.2%)	351(89.3%)	
Yes	0(0.0%)	5(8.2%)	56(91.8%)	
Chemo Anthracyclines				0.658
No	1(0.3%)	34(9.8%)	313(89.9%)	
Yes	1(0.9%)	11(10.4%)	94(88.7%)	
Chemo Taxanes				0.502
No	2(0.6%)	35(10.6%)	294(88.8%)	
Yes	0(0.0%)	10(8.1%)	113(91.9%)	
Chemo 5-FU				0.713
No	2(0.5%)	43(9.7%)	397(89.8%)	
Yes	0(0.0%)	2(16.7%)	10(83.3%)	
Chemo Cyclophosphamide				0.705
No	2(0.6%)	36(10.2%)	316(89.3%)	
Yes	0(0.0%)	9(9.0%)	91(91.0%)	
Chemo Carboplatin				0.971
No	2(0.5%)	44(9.9%)	397(89.6%)	
Yes	0(0.0%)	1(9.1%)	10(90.9%)	
Chemo Vinorelbine				0.944
No	2(0.4%)	45(9.9%)	406(89.6%)	
Yes	0(0.0%)	0(0.0%)	1(100.0%)	
Chemo Capecitabine				0.663
No	2(0.4%)	45(10.1%)	400(89.5%)	
Yes	0(0.0%)	0(0.0%)	7(100.0%)	
Chemo Doxil				NA
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Gemcitabine				NA
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Mitoxantrone				NA
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo Abraxane				0.704
No	2(0.4%)	45(10.0%)	401(89.5%)	
Yes	0(0.0%)	0(0.0%)	6(100.0%)	
Chemo Halaven				NA
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Endocrine Tamoxifen				0.837
No	1(0.4%)	21(9.1%)	209(90.5%)	
Yes	1(0.4%)	24(10.8%)	198(88.8%)	
Endocrine Anastrozole				0.847
No	2(0.5%)	42(10.1%)	373(89.4%)	
Yes	0(0.0%)	3(8.1%)	34(91.9%)	
Endocrine Exemestane				0.978
No	2(0.5%)	44(9.9%)	398(89.6%)	
Yes	0(0.0%)	1(10.0%)	9(90.0%)	
Endocrine Letrozole				0.756
No	1(0.3%)	33(9.9%)	299(89.8%)	
Yes	1(0.8%)	12(9.9%)	108(89.3%)	

Table 6: Summary of CYP2B6*6, CYP2C19*2 and CYP2C19*3 variants found in patients under chemotherapy or endocrine treatment respectively.

Table 6a: CYP2B6*6.

	CYP2B6*6 (c.516G>T) (n=455)			P value
	GG (n=275)	GT (n=151)	TT (n=29)	
Chemo treatment				0.538
No	161(62.6%)	81(31.5%)	15(5.8%)	
Yes	114(57.6%)	70(35.4%)	14(7.1%)	
Endocrine treatment				0.256
No	99(64.7%)	43(28.1%)	11(7.2%)	
Yes	176(58.3%)	108(35.8%)	18(6.0%)	

Table 6b: CYP2C19*2.

	CYP2C19*2(c.681G>A) (n=455)			P value
	AA (n=57)	GA (n=186)	GG (n=212)	
Chemo treatment				0.364
No	37(14.4%)	101(39.3%)	119(46.3%)	
Yes	20(10.1%)	85(42.9%)	93(47.0%)	
Endocrine treatment				0.448
No	20(13.1%)	68(44.4%)	65(42.5%)	
Yes	37(12.3%)	118(39.1%)	147(48.7%)	

Table 6c: CYP2C19*3.

	CYP2C19*3 (c.636G>A) (n=454)			P value
	AA (n=2)	GA (n=45)	GG (n=407)	
Chemo treatment				0.213
No	1(0.4%)	31(12.1%)	225(87.5%)	
Yes	1(0.5%)	14(7.1%)	182(92.4%)	
Endocrine treatment				0.598
No	0(0.0%)	15(9.8%)	138(90.2%)	
Yes	2(0.7%)	30(10.0%)	269(89.4%)	

weaker than those observed with (Z)-4-OHT and endoxifen, which are other well-characterized active metabolites of tamoxifen [32]. As NorEND is an active metabolite of tamoxifen that inhibits aromatase and estrogen receptors, variability in its plasma concentration can potentially influence the therapeutic outcomes of tamoxifen therapy. Notwithstanding, Damkier P, et al. showed no association of CYP2C19*2 with breast cancer in a larger group of patients [33]. Early Breast Cancer Trialist Collaborative Group found that in estrogen receptor (ER) positive breast cancer patients, treatment with tamoxifen for 5 years substantially reduced the recurrence rates throughout the first 10 years [34]. Sanchez-Spitman AB, et al. demonstrated that CYP2C19 polymorphisms have no or little impact on concentration levels and metabolic rate of tamoxifen, endoxifen, 4-hydroxy-tamoxifen and NDM-tamoxifen, or clinical outcomes in breast cancer patients [35].

In this study, CYP2B6*6 and CYP2C19*2 homozygous mutants seemed to exacerbate the spread of breast cancer in patients. This is shown from the data that CYP2B6*6 and CYP2C19*2 were associated

with a relatively higher percentage of patients found with bigger tumour size, higher tumour grade and a larger number of axillary lymph nodes involved. Although this finding was not significant, the trend shown in this study could serve as a factor for consideration in the treatment regimen of breast cancer patients.

Conclusion

In conclusion, the findings of this study suggest that CYP2B6*6 and CYP2C19*2 polymorphisms may confer a risk for breast cancer development in Singaporean breast cancer patients. Moreover, polymorphisms in these 2 genes are associated with prognostic factors though not significantly, resulting in potentially worsened prognoses for carriers of those polymorphisms. However, this represents a pilot study to determine the prevalence of three CYP SNPs in our breast cancer patients. By identifying potential breast cancer susceptibility gene polymorphisms, a bigger sample size study could be done to corroborate these findings in future studies. In addition, the impact of SNPs found in metabolic enzymes (for instance CYP2C19) or transporters on pharmacokinetics and pharmacodynamics of anticancer drug metabolism may be examined in future studies.

Ethical Approval & Consent to participate

This study has been approved by Singhealth Centralised Institutional Review Board (CIRB) with CIRB reference number 2014/371/B.

Every participant had signed patient informed consent form (PICF) to join this study. PICF copies are available for inspection upon request.

Consent for publication

Yes.

Availability of supporting data

Yes, data are available upon request.

Competing interests

The authors declare that they have no competing interests.

Funding

This project was funded by Changi General Hospital Research Grant (Grant Ref. No: CHF 2013.14-P).

Authors' contributions

Gaik-Hong Soon- Designed, executed the study, analysed and wrote the manuscript.

Seok Hwee Koo- Designed, as a back-up to run the study when required, review the manuscript.

Pei Ting Tan- Analysed the data by using the SPSS software.

Lawrence Soon-U Lee- Provided idea and advice.

Chee Kian Tham- Provided idea and advice.

Mikael Hartman- Provided the normal healthy control data.

Su Ming Tan- Supervised and supported the study with research grant.

Acknowledgements

We thank Ms. Hong Chui Sim and Clinical Trials & Research Unit for coordinating this study. This study was funded by Changi General Hospital Research Grant (CHF 2013.14-P) awarded to Prof. Su Ming Tan

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. National Registry of Diseases Office (2021) Cancer Registry Annual Report 2018.
2. Bray J, Sludden J, Griffin MJ, Cole M, Verrill M, et al. (2010) Influence of pharmacogenetics on response and toxicity in breast cancer patients treated with doxorubicin and cyclophosphamide. Br J Cancer 102: 1003-1009.
3. Aminkeng F, Ross CJD, Rassekh SR, Brunham LR, Sistonen J, et al. (2014) Higher frequency of genetic variants confering increased risk for adverse drug responses for commonly used drugs treating cancer, AIDS and tuberculosis in persons of African descent. Pharmacogenomics J 14: 160-170.
4. Thompson PA, Ambrosone C (2000) Molecular epidemiology of genetic polymorphisms in estrogen metabolizing enzymes in human breast cancer. J Natl Cancer Inst Monogr 125-134.
5. Reszka E, Wasowicz W, Gromadzinska J (2006) Genetic polymorphism of xenobiotic metabolising enzymes, diet, and cancer susceptibility. Br J Nutr 96: 609-619.
6. Guengerich FP (1999) CytochromeP-450 3A4: regulation and role in drug metabolism. Annu Rev Pharmacol Toxicol 39: 1-17.
7. Smith CA, Smith G, Wolf CR (1994) Genetic polymorphisms in xenobiotic metabolism. Eur J Cancer 30: 1921-1935.
8. Brockstedt U, Krajinovic M, Richer C, Mathonnet G, Sinnett D, et al. (2002) Analyses of bulky DNA adduct levels in human breast tissue and genetic polymorphisms of cytochrome P450 (CYPs), myeloperoxidase (MPO), quinone oxidoreductase (NQO1), and glutathione S-transferases (GSTs). Mutat Res 516: 41-47.
9. Justenhoven C, Pentimalli D, Rabstein S, Harth V, Lotz A, et al. (2013) CYP2B6*6 is associated with increased breast cancer risk. Int J Cancer 134: 426-430.
10. Liu Lim JS, Sutiman N, Muerdter TE, Singh O, Cheung YB, et al. (2016) Association of CYP2C19*2 and associated haplotypes with lower norendoxifen concentrations in tamoxifen-treated Asian breast cancer patients. Br J Clin Pharmaco 81: 1142-1152.
11. Gan CQ, Wang XY, Cao YD, Ye WX, Liu H, et al. (2011) Association of CYP2C19*3 gene polymorphism with breast cancer in Chinese Women. Genet Mol Res 10: 3514-3519.
12. Justenhoven C (2012) Polymorphisms of phase I and phase II enzymes and breast cancer risk. Front Genet 3: 258.
13. Liu S, Lin YC (2004) Transformation of MCF-10A human breast epithelial cells by zeranol and estradiol-17 beta. Breast J 10: 514-521.
14. Yager JD, Davidson NE (2006) Estrogen carcinogenesis in breast cancer. N Eng J Med 354: 270-282.
15. Poutanen M, Isomaa V, Peltoketo H, Vihko R (1995) Role of 17 beta-hydroxysteroid dehydrogenase type 1 in endocrine and intracrine

estradioal biosysnthesis. J Steroid Biochem Mol Biol 55: 525-532.

16. Shyamala G, Chou YC, Louie SG, Guzman RC, Smith GH, et al. (2002) Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones, development and aging. J Steroid Biochem Mol Biol 80: 137-148.

17. Collaborative group on hormonal factors in breast cancer (2001) Familial breast cancer: collaborative reanalysis of individual data from 52 epidemiological studies including 58,209 women with breast cancer and 101,986 women without the disease. Lancet 358: 1389-1399.

18. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71.

19. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, et al. (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case Series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72: 1117-1130.

20. Cox A, Dunning AM, Garcia-Closas M, Balasubramanian S, Reed MW, et al. (2007) A common coding variant in CASP8 is associated with breast cancer risk. Nat Genet 39: 352-358.

21. Gamazon ER, Skol AD, Perera MA (2012) The limits of genome-wide methods for pharmacogenomic testing. Pharmacogenet Genomics 22: 261-272.

22. El-Rayes BF, Ali S, Heilbrun LK, Lababidi S, Bouwman D, et al. (2003) Cytochrome p450 and glutathione transferase expression in human breast cancer. Clin Cancer Res 9: 1705-1709.

23. Iscan M, Klaavuniemi T, Coban T, Kapucuoglu N, Pelkonen O, et al. (2001) The expression of cytochrome P450 enzymes in human breast tumours and normal breast tissue. Breast Cancer Res Treat 70: 47-54.

24. Tozlu S, Girault I, Vacher S, Vendrell J, Andrieu C, et al. (2006) Identification of novel genes that co-cluster with estrogen receptor alpha in breast tumor biopsy specimens, using a large-scale real-time reverse transcription-PCR approach. Endocr Relat Cancer 13: 1109-1120.

25. Lo R, Burgoon L, Macpherson L, Ahmed S, Matthews J (2010) Estrogen receptor-dependent regulation of CYP2B6 in human breast cancer cells. Biochim Biophys Acta 1799: 469-479.

26. King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group (2003) Breast and Ovarian cancer risks due to inherited mutations in BRACA1 and BRACA2. Science 302: 643-646.

27. Tamminga WJ, Wemer J, Oosterhuis B, de Zeeuw RA, de Leij LF, et al. (2001) The prevalence of CYP2D6 and CYP2C19 genotypes in a population of healthy Dutch volunteers. Eur J Clin Pharmacol 57: 717-722.

28. Sugimoto K, Uno T, Yamazaki H, Tateishi T (2008) Limited frequency of the CYP2C19*17 allele and its minor role in a Japanese population. Br J Clin Pharmacol 65: 437-439.

29. Kiyotani K, Mushiroda T, Nakamura Y, Zembutsu H (2012) Pharmacogenomics of tamoxifen: roles of drug metabolizing enzymes and transporters. Drug Metab Pharmacokinet 27: 122-131.

30. Scott SA, Sangkuhl K, Gardner EE, Stein CM, Hulot JS, et al. (2011) Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 90: 328-332.

31. Lu WJ, Xu C, Pei Z, Mayhoub AS, Cushman M, et al. (2012) The tamoxifen metabolite norendoxifen is a potent and selective inhibitor of aromatase (CYP19) and a potential lead compound for novel therapeutic agents. Breast Cancer Res Treat 133: 99-109.

32. Lv W, Liu J, Lu D, Flockhart DA, Cushman M (2013) Synthesis of mixed (E,Z)-, (E)-, and (Z)- norendoxifen with dual aromatase inhibitory and estrogen receptor modulatory activities. J Med Chem 56: 4611-4618.

33. Damkier P, Kjærsgaard A, Barker KA, Cronin-Fenton D, Crawford A, et al. (2017) CYP2C19*2 and CYP2C19*17 variants and effect of tamoxifen on breast cancer recurrence: Analysis of the International Tamoxifen Pharmacogenomics Consortium dataset. Sci Rep 7: 7727.

34. Davies C, Godwin J, Gray R, Clarke M, Cutter D, et al. (2011) Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. Lancet 378: 771-784.

35. Sanchez-Spitman AB, Swen JJ, Dezentjé VO, Moes DJAR, Gelderblom H, et al. (2021) Effect of CYP2C19 genotypes on tamoxifen metabolism and early-breast cancer relapse. Sci Rep 11: 415.