Pilot Study to Determine Prevalence of CYP2B6*6, CYP2C19*2 and CYP2C19*3 in Breast Cancer Patients Versus Normal Healthy Controls Among Three Major Ethnic Groups in Singapore

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Abstract

Background: Breast cancer is the top cancer suffered by women worldwide and has been identified to be the greatest killer for women living in Singapore. Unfortunately, most of breast cancer cases were detected only at later stage of disease development. This has crippled the effort of breast cancer therapy. As early detection of breast cancer could greatly improve the outcome of breast cancer therapy, it is of utmost importance to identify relevant biomarkers at the primitive stage of breast cancer development before the transformation of normal breast cells into cancerous cells. These biomarkers provide important clues leading to an efficient and targeted treatment approach in breast cancer preventive care.

Methods: 455 breast cancer patients were consented to join this study. Buccal swabs were collected for genotyping on CYP2B6*6, CYP2C19 *2 & CYP2C19*3. The genotyping data were then compared to data collected from healthy individuals. Clinical data were collected from patient notes and analysed. All the statistical analyses were done using SPSS statistical software, version 19.0. Chi-square or Fisher's Exact test were performed to examine the difference between subject's characteristics for categorical variables and 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: We reported that CYP2B6*6 could be a risk factor leading to earlier onset of breast cancer among Indian population with OR found to be 1.69 (95% C.I.= 0.549-5.191, p=0.359). In the case of CYP2C19*2, OR is 1.57 for Malay (95% C.I. = 0.696-3.522, p=0.278); 1.15 for Chinese population (95% C.I. =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) for Malay population. No CYP2C19*3 was detected in both cohorts of Indian patients and healthy controls.

Conclusions: CYP2B6*6 and CYP2C19*2 polymorphisms may confer a risk for breast cancer development in Singaporean breast cancer patients. This is an exploratory study to identify potential breast cancer susceptibility gene polymorphisms, a bigger sample size study could be done to corroborate these findings in future studies.

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 also 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 population 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 an early measure to detect 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 with respect to breast cancer due to their involvement in the metabolism of steroid hormones, chemical carcinogens, and other environment 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 whereby phase I metabolites, phase II exogenous or endogenous compounds are conjugated to a more polar molecule that produces inactive and water soluble compounds to be excreted 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 enzyme holds the potential to increase the exposure to carcinogenic compounds and consequently the risk of tumor formation. (8)

In a recent study done by Germany investigators, CYP2B6*6 variant had an increased breast cancer risk with OR of 1.1 ($p=0.027$) (9). In 2016, Joanne Siok 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 Chinese investigators in 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% confidence interval= 1.27-4.43) (11).

It has been known that elevated sex hormone levels (2fold increase) 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 also plays a part to 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 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 BRCA 2 were identified as two major breast cancer susceptibility genes (18). Harmful mutations in these two genes confer to 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 representing 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 level in breast tumor than normal breast. (8, 22)

A study also indicated that, estrogen receptor (ER) positive breast tumours show a higher CYP2B6 levels than ER-negative tumors (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, marital status, 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. Besides, the percentage of these SNPs in different treatments for breast cancer were also presented.

Methodology

In total, 455 breast cancer patients were recruited from Changi General Hospital Breast Centre Outpatient Clinic. This study had been approved by local ethics committee (CIRB 2014/371/B). Written informed consent were obtained from all patients. Inclusion criteria for selection of study subjects were subject must have been diagnosed of having breast cancer at any stages and must be descendant of similar ethnicity through 3 generations, either Chinese, Malay or Indian. Besides, subject must be able to do buccal swab at inner lining of mouth. Clinical data were collected from patient notes. Ethnically matched healthy control data was gathered from healthy volunteer's databank which was available from Singapore Breast Cancer Cohort Project. Buccal swab was obtained from each patient and genomic DNA was then extracted from the buccal swab by using E.Z.N.A. Blood DNA Mini Kit -buccal swabs protocol (Omega Bio-tek) based on manufacturer's guideline. 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 Step One Plus RT PCR Systems (Applied Biosystems). Details of SNPs are as indicated in Table 1.

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

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 were performed to examine the difference between subject's characteristics for categorical variables and 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 predefined as statistically significant.

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 & CYP2C19*3 homozygous mutant genotype 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. Please refer to Tables 4(a) to 4 (c) which indicate distribution of participants' ages for different variants studied:

BMI of participants ranged from $21.9(\pm 0.07)$ to $25.7 (\pm 0.83)$ kg/m². 81% of participants were post-menopausal. 74.7% were married women; 3.3% were divorced; 18.5% were single and 3.5% were widowed. 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 course of study. Table 2 shows the distribution of participants in the various breast cancer risk groups.

Table 2

showing 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
% post-menopausal	367	81
Use of Hormone Therapy		
Never use	419	92
>0<10 years	29	6
>10 years	5	1
Use of oral contraceptive		
Never use	353	78
0-5 years	78	17
5-10 years	12	3
>10 years	8	2
1st degree relative with breast or ovarian cancer		
Yes	102	22
No	351	77
Marital Status		
Married	340	74.7
Divorced	15	3.3
Single	84	18.5

Widowed	16	3.5
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
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 earlier onset of breast cancer among Indian population. A subgroup analysis with ethnicity was performed for genotyping CYP2B6*6, CYP219*2 and CYP2C19*3 with results as indicated in Table 3. It was discovered that Indian subjects with mutant allele “T” in CYP2B6*6 tend to have higher risk of getting breast cancer (OR: 1.69, 95%C.I.: 0.549- 5.191, P=0.359). Whereas Chinese and Malay subjects who have mutant allele in CYP2C19*2 are more likely to have breast cancer. (OR: 1.15, 95% C.I.:0.862-1.545, P=0.335; OR: 1.57, 95% C.I.:0.696-3.522, P=0.278 respectively)

Table 3

indicates 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

CYP2B6*6	Odds ratio	95% Confidence Interval		P-value
		Lower	Upper	
Chinese	0.749	0.555	1.010	0.058
Malay	0.770	0.347	1.708	0.52
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	N.A.	N.A.	N.A.	N.A.

We also analysed the distribution of CYP2B6*6, CYP2C19*2 and CYP2C19*3 variants based on the epidemiological risk factors (Tables 4(a) to 4(c)).

Table 4

(a), (b), (c) Association between subjects' characteristics and variants for genotype CYP2B6*6, CYP2C19*2 and CYP2C19*3

Table 4 (a) CYP2B6*6

	CYP2B6*6 (c.516 G>T) (n=455)			P value
	GG (n=275)	GT (n=151)	TT (n=29)	
Race				<0.001
Chinese	235(64%)	117(31.9%)	15(4.1%)	
Malay	22(51.2%)	14(32.6%)	7(16.3%)	
Indian	18(40%)	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	
Marital status				0.610
Married	210(61.8%)	109(32.1%)	21(6.2%)	
Widow/ Divorced	65(56.5%)	42(36.5%)	8(7.0%)	
Single				
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				
Yes				
	211(60.1%)	117(33.3%)	23(6.6%)	
	62(60.8%)	34(33.3%)	6(5.9%)	
Use of oral contraceptives				0.611
Never				
0 – 5 years	214(60.6%)	116(32.9%)	23(6.5%)	
5 – 10 years	49(62.8%)	26(33.3%)	3(3.8%)	

>10 years	6(50.0%)	4(33.3%)	2(16.7%)	
	4(50.0%)	4(50.0%)	0(0%)	
Use of hormone therapy				0.157
Never				
>0- <10 years	252(60.1%)	138(32.9%)	29(6.9%)	
>10 years	16(55.2%)	13(44.8%)	0(0%)	
	5(100%)	0(0%)	0(0%)	
Smoking status				0.870
Never	259(60.7%)	141(33%)	27(6.3%)	
Former/ Current	15(55.6%)	10(37.0%)	2(7.4%)	
Presence of Estrogen Receptor				0.961
Negative				
Positive	68(61.8%)	35(31.8%)	7(6.4%)	
	199(60.3%)	109(33%)	22(6.7%)	
Presence of Progesterone Receptor				0.285
Negative				
Positive				
	100(66.2%)	42(27.8%)	9(6.0%)	
		98(34.8%)	19(6.7%)	
	165(58.5%)			
ERBB2 status				0.776
Negative	151(60.6%)	79(31.7%)	19(7.6%)	
Positive	85(59%)	52(36.1%)	7(4.9%)	
Equivocal	11(64.7%)	5(29.4%)	1(5.9%)	
Histological tumour type				0.151
Ductal non-specific				
Lobular				
	11(62.2%)	110(32.4%)	18(5.3%)	
Others				
		10(52.6%)	1(5.3%)	
	8(42.1%)	31(32.3%)	10(10.4%)	

	55(57.3%)			
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				
1-3				
4+	170(60.5%)	96(34.2%)	15(5.3%)	
	54(60%)	30(33.3%)	6(6.7%)	
	38(62.3%)	20(32.8%)	3(4.9%)	

Table 4 (b) CYP2C19*2

	CYP2C19*2 (c.681G>A) (n=455)			P value
	AA (n=57)	GA (n=186)	GG (n=212)	
Race				0.076
Chinese	42(11.4%)	149(40.6%)	176(48%)	
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	
Marital status				0.463
Married	46(13.5%)	135(39.7%)	159(46.8%)	
Widow/divorced/ Single	11(9.6%)	51(44.3%)	53(46.1%)	
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				
Yes				
	43(12.3%)	139(39.6%)	169(48.1%)	
	14(13.7%)	45(44.1%)	43(42.2%)	
Use of oral contraceptives				0.38
Never				
0 – 5 years	49(13.9%)	144(40.8%)	160(45.3%)	
5 – 10 years	6(7.7%)	33(42.3%)	39(50%)	
>10 years	2(16.7%)	2(16.7%)	8(66.7%)	
	0(0%)	4(50%)	4(50%)	

Use of hormone therapy				0.471
Never				
>0- <10 years	51(12.2%)	167(39.9%)	201(48%)	
>10 years	5(17.2%)	14(48.3%)	10(34.5%)	
	1(20%)	3(60%)	1(20%)	
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				
Positive				
	16(14.5%)	45(40.9%)	49(44.5%)	
	39(11.8%)	136(41.2%)	155(47%)	
Presence of Progesteron Receptor				0.793
Negative				
Positive				
	21(13.9%)	61(40.4%)	69(45.7%)	
	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%)	
Histological tumour type				0.109
Ductal non-specific				
Lobular				
	38(11.2%)	136(40.1%)	165(48.7%)	
Others				
	4(21.1%)	4(21.1%)	11(57.9%)	
	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				
1-3				
4+	35(12.5%)	119(42.3%)	127(45.2%)	
	6(6.7%)	38(42.2%)		
	14(23%)	18(29.5%)	46(51.1%)	
			29(47.5%)	

Table 4 (c) CYP2C19*3

	CYP2C19*3 (c.636G>A) (n=454)			P value
	AA (n=2)	GA (n=45)	GG (n=407)	
Race				0.14
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	
Marital status				0.039
Married	2(0.6%)	27(7.9%)	311(91.5%)	
Widow/ Divorced/ Single	0(0%)	18(15.8%)	96(84.2%)	
BMI	21.9(±0.07)	24.5(±0.7)	24.5(±0.24)	0.743
Menopause status				0.343
Pre-menopausal	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				
Yes				
	2 (0.6%)	31 (8.8%)	318 (90.6%)	
	0(0%)	14(13.9%)	87(86.1%)	
Use of oral contraceptives				0.004
Never				
0 – 5 years	1(0.3%)	32 (9.1%)	319(90.6%)	
5 – 10 years	0(0%)	10(12.8%)	68(87.2%)	

>10 years	1(8.3%) 0(0%)	2(16.7%) 1(12.5%)	9(75%) 7(87.5%)	
Use of hormone therapy				0.867
Never				
>0- <10 years	2(0.5%)	40(9.6%)	377(90.0%)	
>10 years	0(0%) 0(0%)	4(13.8%) 1(20.0%)	25(86.2%) 4(80.0%)	
Smoking status				0.844
Never	2 (0.5%)	43(10.1%)	381(89.4%)	
Former/current	0 (0%)	2(7.4%)	25(92.6%)	
Presence of Estrogen Receptor				0.234
Negative				
Positive	0(0%) 2(0.6%)	7 (6.4%) 37 (11.2%)	103(93.6%) 290(88.1%)	
Presence of Progesteron Receptor				0.193
Negative				
Positive	0(0%) 2(0.7%)	11(7.3%) 33(11.7%)	140(92.7%) 246(87.5%)	
ERBB2 status				0.003
Negative	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%)	
Histological tumour type				0.948
Ductal non-specific				
Lobular	2(0.6%)	33(9.7%)	304(89.7%)	
Others	0(0%) 0(0%)	2(10.5%) 10(10.5%)	17(89.5%) 85(89.5%)	
Tumour size				0.160

<20.0mm	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%)	5(9.6%)	47(90.4%)	
Multifocal	1(2.7%)	6(16.2%)	30(81.1%)	
Tumour grade				0.438
I	0(0%)	8(11.9%)	59(88.1%)	
II	0(0%)	14(8.0%)	161(92%)	
III	2(1.1%)	20(10.6%)	166(88.3%)	
Number of involved axillary lymph nodes				0.277
None				
1-3				
4+	0(0%)	32(11.4%)	249(88.6%)	
	1(1.1%)	7(7.9%)	81(91%)	
	1(1.6%)	5(8.2%)	55(90.2%)	

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

Table 5

(a), (b), (c) indicating percentage of CYP2B6*6, CYP2C19*2 & CYP2C19*3 variants found in patients receiving treatments for breast cancer

5 (a) 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				0.2
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_				0.363
Cyclophosphamide				
No	220(62.1%)	113(31.9%)	21(5.9%)	
Yes	55(54.5%)	38(37.6%)	8(7.9%)	
Chemo_				0.892
Carboplatin				
No	269(60.6%)	147(33.1%)	28(6.3%)	
Yes	6(54.5%)	4(36.4%)	1(9.1%)	
Chemo_				0.365
Vinorelbine				
No	275(60.6%)	150(33%)	29(6.4%)	
Yes	0(0.0%)	1(100.0%)	0(0.0%)	
Chemo_				0.717
Capecitabine				
No	271(60.5%)	148(33.0%)	29(6.5%)	
Yes	4(57.1%)	3(42.9%)	0(0.0%)	
Chemo_ Doxil				N.A.
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_Gemcitabine				N.A.
No	275(60.4%)	151(33.2%)	29(6.4%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_				N.A.
Mitoxantrone				
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				N.A.
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%)	

5 (b) 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_				0.197

Cyclophosphamide				
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				N.A.
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_ Gemcitabine				N.A.
No	57(12.5%)	186(40.9%)	212(46.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_ Mitoxantrone				N.A.
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				N.A.
No	57(12.5%)	186(40.9%)	212(46.6%)	

Yes				
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%)	

5 (c) 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				0.92
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_				0.705

Cyclophosphamide				
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				N.A.
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_ Gemcitabine				N.A.
No	2(0.4%)	45(9.9%)	407(89.6%)	
Yes	0(0.0%)	0(0.0%)	0(0.0%)	
Chemo_ Mitoxantrone				N.A.
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				N.A.

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

	CYP2B6*6 (c.516 G>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%)	

	CYP2B6*6 (c.516 G>T) (n=455)			P value
	GG (n=275)	GT (n=151)	TT (n=29)	
Endocrine treatment				0.256
No	99(64.7%)	43(28.1%)	11(7.2%)	
Yes	176(58.3%)	108(35.8%)	18(6.0%)	

	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%)	

	CYP2C19*2(c.681G>A) (n=455)			P value
	AA (n=57)	GA (n=186)	GG (n=212)	
Endocrine treatment				0.448
No	20(13.1%)	68(44.4%)	65(42.5%)	
Yes	37(12.3%)	118(39.1%)	147(48.7%)	

	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%)	

	CYP2C19*3 (c.636G>A) (n=454)			P value
	AA (n=2)	GA (n=45)	GG (n=407)	
Endocrine treatment				0.598
No	0(0.0%)	15(9.8%)	138(90.2%)	
Yes	2(0.7%)	30(10.0%)	269(89.4%)	

Discussion

The conventional epidemiological breast cancer risk factors did not show significant association of risk factors to breast cancer. Although post-menopausal, age of diagnosis between 41 to 50 years, and first degree of relative having breast or ovarian cancer have obviously elevated the risk factor, the use of hormone therapy and smoking did not cause a significant increase in the breast cancer cases. Use of oral contraceptive between 0 to 5 years seemed to have an increase in percentage in breast cancer population studied. And most of the breast cancer patients were diagnosed between 40 to 50 years. This is in consistent with the National Cancer Registry which recorded the peak of breast cancer cases during this period of time in women's life (1). First degree relatives with breast cancer or ovarian cancer elevated the percentage of risk factor to get breast cancer in this cohort as well especially in the variant of CYP2C19*2 with 13.7% of the cohort showed homogzygous mutant in CYP2C19*2. It is well known that inheritance plays an important risk factor among environmental factors mentioned especially BRACA1 and BRACA 2 gene (26).

In this healthy case-control matched study, we found that Indian breast cancer patients with CYP2B6*6 are more likely to have breast cancer. This mutant was not significant in Chinese and Malay groups.

Germany investigators found that CYP2B6*6 was associated with breast cancer risk in patients of European ancestry. (9) However, this is not the case in majority Singaporean population except population of Indian descent in Singapore.

CYP2C19*2 appeared to be more frequent in Chinese and Malay breast cancer patients but it was found less in Indian breast cancer patients.

Nevertheless, CYP2C19*3 was found most frequently in Chinese breast cancer patients. . Although the p-value is not significant but this trend is in consistent to the finding reported by C.Q. Gan et al in their study that discovered association of CYP2C19*3 with onset of breast cancer in Chinese Han population (10). CYP2C19*3 was not found in this cohort study of Indian population either in patients or healthy control. CYP2C19*3 was found to be low in Caucasian population with 0.04% frequency compared to 5-11% in Asian population groups. (27) (28). Percentage of breast cancer patients with CYP2C19*3 homozygous

mutant receiving both chemotherapy and endocrine treatment are 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 either chemotherapy treatment or endocrine treatment, 10.1% and 12.3% respectively. Whereas 7.1% of breast cancer patients with CYP2B6*6 homozygous mutant receiving chemotherapy and 6.0% of them receiving endocrine treatment. This could be due to the fact that 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 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 & Indian groups. Similarly, mutant in CYP2C19*2 also recorded the highest percentage (13.7%) in breast cancer patients under risk factor with the family history of first degree relative with breast cancer or ovarian cancer. CYP2C19*2 homozygous mutant also recorded the highest percentage (13.9%) 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 Joanne Siok et al that patients with CYP2C19*2 polymorphism and the CYP2C19 H2 haplotype have significant lower plasma concentrations of NorEND and lower formation rates of NorEND (10). It was reported by Lu et al that NorEND inhibited recombinant human aromatase competitively, with a K_i 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, although its antagonism is reportedly 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 both aromatase and estrogen receptors, variability in its plasma concentration can potentially influence the therapeutic outcomes of tamoxifen therapy. Notwithstanding, Damkier et al showed no association of CYP2C19 *2 with association of breast cancer in larger group of patients (33). Early Breast Cancer Trialist Collaborative Group found that in oestrogen receptor (ER) positive patients, treatment with tamoxifen for 5 years had substantially reduced the recurrence rates throughout the first 10 years (34). A. B. Sanchez-Spitman 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).

Both CYP2B6*6 and CYP2C19*2 homozygous mutants seemed to exacerbate the spread of breast cancer in patients with higher percentage of patients found in bigger tumour size, tumour grade and

larger number of axillary lymph nodes involved. Although this finding was not significant but 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 have been found to be associated with prognostic factors though not significantly, resulting in potentially worsened prognoses for carriers of those polymorphisms. However, this is an exploratory study, 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 maybe examined in future studies.

Declarations

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

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 – Supported the study with research grant

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