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Original Article

Variants in tamoxifen metabolizing genes: a case-control study of contralateral breast cancer risk in the WECARE study

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Abstract: Tamoxifen has been shown to greatly reduce risk of recurrence and contralateral breast cancer (CBC). Still, second primary contralateral breast cancer is the most common malignancy to follow a first primary breast cancer. Genetic variants in *CYP2D6* and other drug-metabolizing enzymes that alter the metabolism of tamoxifen may be associated with CBC risk in women who receive the drug. This is the first study to investigate the impact of this variation on risk of CBC in women who receive tamoxifen. From the population-based Women's Environment Cancer and Radiation Epidemiology (WECARE) Study, we included 624 Caucasian women with CBC (cases) and 1,199 women with unilateral breast cancer (controls) with complete information on tumor characteristics and treatment. Conditional logistic regression was used to assess the risk of CBC associated with 112 single nucleotide polymorphisms (SNPs) in 8 genes involved in the metabolism of tamoxifen among tamoxifen users and non-users. After adjustment for multiple testing, no significant association was observed between any of the genotyped variants and CBC risk in either tamoxifen users or non-users. These results suggest that when using a tagSNP approach, common variants in selected genes involved in the metabolism of tamoxifen are not associated with risk of CBC among women treated with the drug.

Keywords: Contralateral breast cancer, tamoxifen, single nucleotide polymorphisms

Introduction

The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) provided key evidence suggesting that for women younger than age 50 years with estrogen-receptor (ER)-positive(+) or ER-unknown breast cancer, administration of tamoxifen for a median of 5 years reduced the risk of contralateral breast cancer (CBC) compared to no tamoxifen treatment (HR=0.61, 95% CI 0.50, 0.73) [1]. These results are supported by findings from observational studies [2-4], including our own [5].

Despite the clear therapeutic benefit of tamoxifen, clinical response varies widely. Genetic variation in tamoxifen metabolizing enzymes and transporters can alter the metabolism, activity and distribution of tamoxifen and its metabolites, potentially influencing treatment efficacy [6]. How genotype may account for some of the variation in treatment response and impact clinical outcome remains an active

area of research and has been the subject of several reviews [6-8].

To date, the results of pharmacogenetic studies of genes involved in tamoxifen metabolism and the risk of recurrence and disease-free survival, namely, *CYP2D6* [9-20], *CYP3A5* [16, 21], and *SULT1A1* [9, 16, 22-24] among others [6, 8, 25] have been inconsistent and in the case of *CYP2D6*, somewhat controversial [26-28]. To our knowledge, no study has focused specifically on the impact of genetic variation in tamoxifen metabolizing genes on risk of CBC. In this study, we examined the impact of polymorphisms in genes that code for proteins that are centrally involved in tamoxifen metabolism; *CYP2B6, CYP2C9, CYP2C19, CYP2D6*, *CYP3A4, CYP3A5, SULT1A1*, *UGT2B15* [6], on CBC risk (among women treated with tamoxifen) in the Women's Environment Cancer and Radiation Epidemiology (WECARE) Study, a population-based case-control study of women with CBC (cases) and unilateral breast cancer (UBC) (controls).

Methods

Study population

The WECARE Study Population and the details of CBC case and UBC control eligibility have been described previously [29]. Briefly, cases were women diagnosed prior to age 55 years, from 1985 to 2000 with invasive breast cancer that had not spread beyond regional lymph nodes. This was followed by a second *in situ* or invasive breast cancer diagnosed in the contralateral breast at least one year later. The "at-risk" interval was defined as starting one year after the first diagnosis and ending at reference date: i.e., date of the second breast cancer diagnosis in cases (reference date) or the corresponding date in matched controls. Controls were diagnosed with a single invasive breast cancer, with no other intervening cancers, and were individually matched to each case on year of birth (in 5-year strata), year of diagnosis (in 4-year strata), registry region, and race/ethnicity. All women had to be alive at the time of contact, able to complete a telephone interview and donate a blood sample. Counter-matching based on registry-reported radiation treatment status was used to improve the statistical efficiency of the study design. Thus, for each exposed case, one exposed and one unexposed control were selected from the relevant stratum and for each unexposed case, two exposed controls were selected [29].

Across the five cancer registries, a total of 998 women with CBC and 2,112 women with UBC were identified as being eligible for the study as cases and controls, respectively. Of these, 708 cases (71%) and 1,399 controls (66%) completed the study interview and provided a blood sample. Reasons for non-participation have been published previously [30]. Of the 2,107 WECARE Study participants, four individuals were excluded because they did not consent to genotyping beyond the initial *ATM*, *BRCA1* and *BRCA2* mutation screening. To minimize the potential influence of ancestral differences in genotype frequencies, all analyses were restricted to Caucasian women (N=1,933). Further exclusions were made after genotyping (see below).

Data collection

The data collection protocol was approved by the institutional review board at each of the participating centers. Each woman provided written informed consent. At entry, all participants were interviewed by telephone using the same pre-tested, structured questionnaire administered by a trained interviewer at each data collection site between January 2000 and July 2004. For both CBC cases and UBC controls, questions focused on events occurring prior to the diagnosis of the first primary as well as during the at-risk period. Characteristics of the first breast tumors (including estrogen and progesterone receptor status) were extracted from tumor registry records, and from hospital and physician medical records. Medical records, pathology reports, and hospital charts, in addition to self-reported data, were used to collect detailed treatment information (chemotherapy, hormonal therapy, radiation therapy) on the first primary breast cancer as well as during the at-risk period. Information collected on chemotherapy and hormonal therapy included dates of administration, reason for treatment (e.g. primary disease, recurrence), and type of drug.

Genotyping

DNA was prepared from blood samples by red cell lysis and standard methods of phenol/chlo-

roform extraction. Samples were genotyped with Illumina's HumanOmni1-Quad BeadChip (Illumina Inc., San Diego) and the SNP data for the relevant genes abstracted. A series of quality control steps were applied to this genome-wide association study (GWAS) data, leading to further subject exclusions: 1) Women with SNP call rates <95% were excluded (n=22); 2) Population stratification was investigated using EIGENSTRAT [31]; using the first two principal components, 9 outliers with significant African or Chinese ancestry were identified for exclusion; and 3) 14 additional participants were excluded due to incomplete matched sets. Identity by descent was examined using PLINK [32] identifying 3 pairs of sisters, including one pair of identical twins. These women were not excluded from the analysis.

Additional genotyping in the selected genes was performed to improve gene coverage, beyond that of the HumanOmni1-Quad BeadChip. Multiplex SNP genotyping was carried out using the Illumina Golden Gate™ assay on custom BeadChips (Illumina Inc., San Diego). SNP selection, laboratory methods and sample control measures have been described previously [33]. The *CYP2D6*4* (rs3892097) variant was genotyped using MGB Eclipse probe assay (Epoch Biosciences, ELITech Group, Paris, France). Primers and conditions provided by Epoch Biosciences were modified in order to avoid pseudogenes (details available upon request). An additional 28 subjects were excluded because they had >5% missing genotypes on the SNP BeadChips, and 37 subjects were excluded due to missing information on tamoxifen use. Analyses are based on the remaining 1,823 participants (624 CBC cases and 1,199 UBC controls) with genotype data from both the Omni1-Quad and custom BeadChip platforms. Secondary analyses assessed associations after exclusion of carriers of deleterious mutations in the *BRCA1* and *BRCA2* genes (109 women with a *BRCA1* mutation and 72 with a *BRCA2* mutation).

Within the selected genes of interest, 246 SNPs were genotyped on the OMNI platform, 27 SNPs on the custom SNP BeadChip and rs3892097 (*CYP2D6*4*) on a modified MGB Eclipse probe assay, for a total of 273 genotyped SNPs. SNPs with >10% missing (n=20), those that were monomorphic (n=70) and those with a MAF <0.01 (n=66) were excluded. Although Hardy-Weinberg Equilibrium (HWE) may not strictly apply to this analysis since all participants in the study were affected with breast cancer, 5 SNPs deviating from HWE (p <0.001) were also excluded. This left 112 SNPs in 8 genes to be included in the analyses: 6 in *CYP2B6,* 4 in *CYP2C9,* 9 in *CYP2D6*, 7 in *CYP3A4,* 17 in *CYP3A5,* 62 in *CYP2C19*, 3 in *SULT1A1*, and 4 in *UGT2B15*.

Statistical analysis

Rate ratios (RR) and 95% confidence intervals (CI) were estimated using conditional logistic regression to assess the association between individual polymorphisms (using a log-additive model), tamoxifen use and risk of CBC. For each individual variant the potential interaction with tamoxifen treatment was examined using: 1) analysis stratified by tamoxifen treatment (yes/no) using the log-additive model (estimating the per allele RR) and 2) an interaction model that included parameters for the individual effects of the SNP (log-additive coding), tamoxifen, and a SNP x tamoxifen interaction term. Models were run adjusting for age at first breast cancer diagnosis and included an "offset term" (i.e., log weight 'covariate' in the model where the coefficient of this log weight is fixed at one [29]), taking into account the sampling probabilities of the counter-matching, and then again also adjusting for chemotherapy.

A prior published study by our group found that tamoxifen was associated with significantly lower CBC risk in the WECARE Study population [5]. Age- and multivariate-adjusted analyses (adjusting for age at first breast cancer diagnosis, family history of breast cancer, stage and histology of first primary breast cancer and treatment (chemotherapy and radiation) were run to confirm the association between tamoxifen and CBC risk in the subgroup of women included in the current analyses (N=1,823 (87%) of the 2,107 women in the WECARE Study, i.e., Caucasian women with available genotype and treatment data).

A conservative Bonferroni correction was used to determine the multiple comparison cut-point (α=0.0004, obtained from (0.05/112 SNPs)) at which results were considered statistically significant. All analyses were conducted using SAS 9.2 (SAS Institute Inc., Cary NC).

Table 1. Characteristics of selected cases (CBC) and controls (UBC) from the WECARE Study population[1]

Variable	Median (Range)	Cases (CBC) Median (Range)	Controls (UBC) Median (Range)
Age at first Diagnosis (years)	46 (23-55)	46 (24-55)	46 (23-55)
Age at reference date (years)	51 (27-71)	50 (27-71)	51 (27-69)
Length of at-risk period[2] (years)	4 (1-16)	4 (1-16)	4 (1-16)

Variable	Level	Cases (CBC) N	Cases (CBC) %	Controls (UBC) N	Controls (UBC) %
Center	Iowa	106	17	204	17
	UC Irvine	101	16	189	16
	Los Angeles	148	24	282	24
	Seattle	93	15	185	15
	Denmark	176	28	339	28
Year of first diagnosis	1985-88	217	35	415	35
	1989-92	209	33	406	34
	1993-96	157	25	300	25
	1997+	41	7	78	7
Chemotherapy	No	347	56	548	46
	Yes	277	44	651	54
Tamoxifen treatment	No	485	78	861	72
	Yes	139	22	338	28
Other hormonal treatment[3]	No	607	97	1150	96
	Yes	17	3	48	4
	Unknown	0	0	1	0.1
Radiation treatment[4]	Never	313	50	232	19
	Ever	311	50	967	81
Histology of first breast cancer	Lobular	81	13	118	10
	Other	543	87	1081	90
Stage of first breast cancer	Localized	447	72	776	65
	Regional	177	28	423	35
ER Status of first breast cancer[5]	Positive	296	47	635	53
	Negative	162	26	287	24
	Other	166	27	277	23
PR Status of first breast cancer[5]	Positive	248	40	519	43
	Negative	141	23	267	22
	Other	235	38	413	34
Menopausal status/age at menopause at first diagnosis	Premenopausal	460	74	905	75
	Postmenopausal age <45	83	13	175	15
	Postmenopausal age ≥45	80	13	115	10
	Unknown	1	0.2	4	0.3
Family history of breast cancer	None	415	67	935	78
	≥1 first-degree relative	198	32	240	20
	Adopted	11	2	24	2

Abbreviations: CBC=asynchronous contralateral breast cancer; UBC=unilateral breast cancer; ER=estrogen receptor, PR=progesterone receptor. [1]Includes Caucasian women with SNP call rates ≥95%, without significant African or Asian ancestry with complete information on tamoxifen treatment and genotype data from both the Omni1-Quad and custom BeadChip platforms (624 CBC cases and 1,199 UBC controls). [2]Beginning one year after first diagnosis extending to the reference date (date of second diagnosis in cases). [3]Other hormone therapies include raloxifene, toremifene citrate, anastrozole, letrozole, exemestane, aminoglutethimide, goserelin acetate, leuprorelin, fulvestrant and megestrol acetate. [4]CBC cases and UBC controls were counter-matched on registry-reported radiation treatment status. For each radiation exposed case, one exposed and one unexposed control were selected from the relevant stratum, and for each unexposed case, two unexposed controls were selected. This is reflected in the percentages of cases and controls who underwent radiation treatment and was taken into account in all analyses. [5]Refers to receptor status of the first primary breast cancer. The 'Other' category consists of women for whom no lab test was given, the test was given and the results are unknown or the test was given and the results were borderline.

Results

Selected characteristics of the eligible WECARE Study population are shown in **Table 1**. Cases and controls were similar for all matching characteristics. 296 (47%) of CBC cases and 635 (53%) of UBC controls had ER+ first primary breast cancer diagnoses. Of these women, 36% of ER+ cases and 45% of ER+ controls received tamoxifen as part of their breast cancer treatment. Of the 162 cases and 287 controls with ER-negative first primaries, 16 cases and 33 controls received tamoxifen treatment respectively. A relatively small proportion of ER+ women also received chemotherapy (5% of cases and 15% of controls). In multivariate-adjusted models, tamoxifen was associated with a significant reduction in CBC risk among all women (RR=0.8, 95% CI 0.6, 1.0, p=0.04), with a greater reduction seen among women with ER+ disease (RR=0.6, 95% CI 0.4, 1.0, p=0.04), consistent with our prior publication [5].

Overall, no significant associations between the genotyped variants and risk of CBC were seen in women who received tamoxifen (**Table 2**, results from the interaction model were similar and not shown). Results also did not differ when the co-dominant model of inheritance was used, when analyses were stratified by ER-status, when *BRCA* mutation carriers were excluded or when analyses were adjusted for chemotherapy (results not shown).

Specifically, among women who received tamoxifen as part of their treatment for a first primary breast cancer, the variant rs1057910 in *CYP2C9* (*CYP2C9*3*), known to be associated with reduced enzyme activity [34] was not associated with risk of CBC (RR=1.3, 95% CI 0.7, 2.4). Similarly, although *CYP2C19*2* (rs4244285) results in no enzyme activity [35], in the current analysis it was not associated with risk of CBC (RR=1.1, 95% CI 0.7, 1.7). The variants *CYP2D6*41* (rs28371725) and *CYP2D6*4* (rs3892097), associated with reduced and no enzyme activity respectively were also not associated with CBC risk (RR=0.8, 95% CI 0.5, 1.5 and RR=1.2, 95% CI 0.8, 1.7 respectively). Finally, *CYP3A5*3* (rs776746), a variant associated with low enzyme activity [36] was not associated with risk of CBC in women who received tamoxifen (RR=0.8, 95% CI 0.4, 1.6).

Discussion

Tamoxifen is widely used throughout the world and its efficacy in the treatment of ER+ breast cancer is well-established with reported risk reductions for CBC of 40-70% [2-5, 37], including results from a recent meta-analysis by the EBCTCG [38]. Despite its success in reducing CBC risk, the clinical response to tamoxifen is highly variable and a number of women will experience adverse outcomes including CBC. Inherited variation in genes involved in the metabolism of tamoxifen has been hypothesized to account for some of the variation in tamoxifen response. In this study we found no significant associations between any of the genotyped variants and CBC risk among women who received tamoxifen.

Tamoxifen is a selective estrogen receptor modulator (SERM) that exerts an anti-estrogenic effect on breast tissue by competitively inhibiting the binding of estradiol to the ERs, preventing the receptor from binding to estrogen-response elements on DNA [39] and resulting in a reduction in the cellular response to estrogen. Tamoxifen undergoes extensive biotransformation via CYP450 enzymes into active and inactive metabolites [40]. The major metabolite, N-desmethyltamoxifen, produced primarily by CYP3A4/5 (but also CYP2A6, CYP2C9, CYP2C19 and CYP2D6) has low affinity for the ER. Production of two active metabolites, 4-hydroxytamoxifen (4-OH-TAM) and 4-hydroxy-N-desmethyl tamoxifen (endoxifen), is predominantly catalyzed by CYP2D6 (but also CYP2B6, CYP3A5, CYP2C19, and CYP2C9), from tamoxifen and N-desmethyltamoxifen respectively. These metabolites have over one hundred-fold higher affinity for the ER and 30- to 100-fold greater potency in suppressing estrogen-dependent tumor cell growth compared to tamoxifen [41, 42]. Prior to excretion, active metabolites are further metabolized by phase II enzymes to inactive metabolites by sulfation (catalyzed by SULT1A1) or glucuronidation (catalyzed by the UDP-glucuronosyltransferases (UGTs) [6].

CYP2D6 is a key enzyme in tamoxifen metabolism [9, 10], and low-activity polymorphisms have been shown to reduce levels of the active metabolite endoxifen [43, 44]. Variation in *CYP2D6* has been central to the pharmacogenetic investigation of tamoxifen treatment

Table 2. Association between variation in genes involved in tamoxifen metabolism and risk of contralateral breast cancer stratified by tamoxifen treatment status

								Tamoxifen			No Tamoxifen	
Gene	SNP	chr	coordinate	alleles	MAF	HWE[1]	RR[2]	95% CI	un-corr *P* value	RR[2]	95% CI	un-corr *P* value
CYP2B6	rs2279342	19	46201967	A>T	0.09	0.85	1.4	0.8, 2.3	0.23	1.2	0.9, 1.6	0.24
CYP2B6	rs7250745	19	46195300	C>T	0.26	0.39	1.0	0.7, 1.5	0.96	1.1	0.9, 1.3	0.63
CYP2B6	rs2113103	19	46220507	G>A	0.15	0.87	0.8	0.5, 1.3	0.40	0.8	0.7, 1.1	0.16
CYP2B6	rs2306606	19	46208022	C>T	0.26	0.75	1.1	0.8, 1.6	0.54	1.0	0.8, 1.2	0.98
CYP2B6	rs1808682	19	46181288	G>A	0.23	0.48	0.9	0.6, 1.4	0.69	0.9	0.7, 1.1	0.35
CYP2B6	rs7255904	19	46220860	G>A	0.45	0.42	1.4	1.0, 1.9	0.07	1.1	0.9, 1.3	0.59
CYP2C9	rs1057910 (CYP2C9*3)	10	96731043	A>C	0.07	0.76	1.3	0.7, 2.4	0.37	1.1	0.8, 1.6	0.54
CYP2C9	rs1505	10	96740749	G>C	0.36	0.24	1.2	0.9, 1.6	0.40	0.9	0.8, 1.1	0.40
CYP2C9	rs12772884	10	96690620	T>A	0.44	0.16	0.9	0.7, 1.2	0.55	1.1	1.0, 1.4	0.15
CYP2C9	rs9332197	10	96730898	T>C	0.06	0.11	0.6	0.3, 1.1	0.10	1.1	0.7, 1.6	0.75
CYP2C19	NA	10	96522535	A>C	0.42	0.88	1.2	0.8, 1.6	0.38	1.0	0.8, 1.2	0.96
CYP2C19	NA	10	96524033	T>A	0.07	0.73	1.4	0.8, 2.6	0.23	1.1	0.8, 1.6	0.60
CYP2C19	rs6583954	10	96524253	A>G	0.15	0.37	1.1	0.7, 1.7	0.77	1.1	0.8, 1.4	0.64
CYP2C19	NA	10	96524465	C>A	0.07	0.73	1.4	0.8, 2.6	0.23	1.1	0.8, 1.6	0.54
CYP2C19	rs7916649	10	96524574	A>G	0.42	0.56	1.1	0.8, 1.5	0.48	1.0	0.8, 1.2	0.99
CYP2C19	rs17878459	10	96524912	C>G	0.03	0.32	1.7	0.7, 4.6	0.27	1.4	0.8, 2.2	0.23
CYP2C19	NA	10	96525114	G>A	0.15	0.42	1.1	0.7, 1.7	0.77	1.0	0.8, 1.3	0.76
CYP2C19	rs4388808	10	96526046	G>A	0.18	0.40	1.0	0.6, 1.5	0.86	1.3	1.0, 1.6	0.07
CYP2C19	NA	10	96526217	A>T	0.15	0.08	1.2	0.8, 2.0	0.36	1.1	0.8, 1.4	0.67
CYP2C19	rs7068577	10	96526698	A>G	0.20	0.22	0.9	0.6, 1.3	0.55	0.9	0.7, 1.1	0.43
CYP2C19	rs17878673	10	96529134	G>A	0.07	0.97	1.4	0.8, 2.6	0.23	1.1	0.8, 1.6	0.52
CYP2C19	rs4304697	10	96530879	A>G	0.07	0.66	1.5	0.8, 2.6	0.21	1.1	0.8, 1.6	0.51
CYP2C19	rs7088784	10	96531363	G>A	0.07	0.85	1.7	0.9, 2.9	0.09	1.2	0.8, 1.7	0.34
CYP2C19	rs4244285 (CYP2C19*2)	10	96531606	A>G	0.16	0.34	1.1	0.7, 1.7	0.76	1.1	0.8, 1.4	0.66
CYP2C19	rs12571421	10	96531972	G>A	0.16	0.19	1.1	0.7, 1.7	0.76	1.1	0.8, 1.4	0.66
CYP2C19	rs35390752	10	96533813	C>A	0.14	0.03	1.1	0.7, 1.8	0.73	1.0	0.8, 1.3	0.78
CYP2C19	NA	10	96534717	C>G	0.20	0.23	0.9	0.6, 1.3	0.55	0.9	0.7, 1.1	0.42

CYP2C19	NA	10	96534805	G>A	0.07	0.89	1.4	0.8, 2.5	0.27	1.1	0.8, 1.6	0.55
CYP2C19	NA	10	96534970	A>G	0.07	0.80	1.7	0.9, 3.0	0.08	1.2	0.8, 1.7	0.36
CYP2C19	NA	10	96535053	C>A	0.04	0.86	1.2	0.6, 2.2	0.65	1.2	0.8, 1.9	0.43
CYP2C19	NA	10	96535465	A>G	0.07	0.71	1.4	0.8, 2.5	0.30	1.1	0.8, 1.6	0.52
CYP2C19	NA	10	96535962	A>G	0.12	0.91	1.0	0.6, 1.6	0.83	0.7	0.6, 1.0	0.04
CYP2C19	NA	10	96536236	A>C	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.53
CYP2C19	NA	10	96536687	C>A	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.53
CYP2C19	rs12767583	10	96537453	A>G	0.16	0.34	1.1	0.7, 1.7	0.76	1.1	0.8, 1.3	0.69
CYP2C19	rs4494250	10	96553747	A>G	0.36	0.55	0.8	0.6, 1.2	0.31	0.9	0.7, 1.0	0.10
CYP2C19	NA	10	96555141	C>A	0.07	0.81	1.7	0.9, 2.9	0.09	1.2	0.8, 1.6	0.40
CYP2C19	NA	10	96556602	A>G	0.04	0.84	1.3	0.7, 2.4	0.50	1.2	0.8, 1.9	0.44
CYP2C19	NA	10	96556769	C>A	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.51
CYP2C19	rs12772672	10	96556879	G>A	0.16	0.39	1.1	0.7, 1.7	0.77	1.1	0.8, 1.3	0.72
CYP2C19	rs4641393	10	96557376	A>G	0.16	0.35	1.1	0.7, 1.7	0.74	1.1	0.8, 1.3	0.70
CYP2C19	rs1853205	10	96565059	C>G	0.16	0.32	1.1	0.7, 1.7	0.76	1.1	0.8, 1.3	0.69
CYP2C19	rs1322179	10	96565232	A>G	0.16	0.34	1.1	0.7, 1.7	0.76	1.1	0.8, 1.3	0.69
CYP2C19	NA	10	96565270	G>A	0.07	0.80	1.7	0.9, 3.0	0.08	1.2	0.8, 1.6	0.41
CYP2C19	rs10509678	10	96566180	G>A	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.53
CYP2C19	rs10786172	10	96571084	G>A	0.36	0.57	0.9	0.6, 1.2	0.34	0.9	0.7, 1.0	0.12
CYP2C19	NA	10	96572904	A>G	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.53
CYP2C19	NA	10	96587741	C>A	0.18	0.08	0.9	0.6, 1.4	0.58	0.9	0.7, 1.1	0.33
CYP2C19	NA	10	96588429	C>A	0.19	0.59	1.0	0.6, 1.5	0.82	1.2	1.0, 1.6	0.07
CYP2C19	NA	10	96591784	G>A	0.23	0.39	1.3	0.9, 1.8	0.19	1.1	0.9, 1.4	0.46
CYP2C19	NA	10	96591910	A>G	0.03	0.25	0.8	0.3, 2.2	0.59	0.7	0.4, 1.2	0.19
CYP2C19	rs28399513	10	96592388	A>T	0.16	0.33	1.1	0.7, 1.7	0.75	1.1	0.8, 1.4	0.66
CYP2C19	NA	10	96593081	A>T	0.07	0.60	1.4	0.8, 2.6	0.29	1.1	0.8, 1.6	0.46
CYP2C19	rs11592737	10	96593404	G>A	0.20	0.21	0.9	0.6, 1.3	0.46	0.9	0.7, 1.1	0.37
CYP2C19	NA	10	96593735	G>A	0.07	0.69	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.50
CYP2C19	NA	10	96595317	G>C	0.42	0.49	1.1	0.8, 1.5	0.55	1.0	0.8, 1.2	0.88
CYP2C19	rs1322181	10	96599054	A>G	0.42	0.69	1.1	0.8, 1.5	0.57	1.0	0.8, 1.2	0.96
CYP2C19	NA	10	96599463	A>G	0.04	0.83	1.2	0.6, 2.3	0.61	1.2	0.8, 1.9	0.44

CYP2C19	rs4917623	10	96599558	A>G	0.46	0.34	1.2	0.9, 1.6	0.27	1.0	0.9, 1.2	0.75
CYP2C19	rs17878382	10	96600621	G>A	0.07	0.71	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.52
CYP2C19	NA	10	96601618	G>A	0.20	0.17	0.9	0.6, 1.3	0.47	0.9	0.7, 1.1	0.29
CYP2C19	NA	10	96601824	A>G	0.42	0.43	1.1	0.8, 1.5	0.54	1.0	0.8, 1.2	0.98
CYP2C19	rs12268020	10	96602361	A>G	0.20	0.09	0.9	0.6, 1.3	0.47	0.9	0.7, 1.1	0.38
CYP2C19	rs35709381	10	96604715	A>C	0.16	0.34	1.1	0.7, 1.7	0.76	1.1	0.8, 1.3	0.69
CYP2C19	NA	10	96608493	G>A	0.04	0.86	1.2	0.6, 2.3	0.65	1.2	0.8, 1.9	0.44
CYP2C19	NA	10	96608992	A>G	0.07	0.68	1.4	0.8, 2.6	0.23	1.1	0.8, 1.6	0.50
CYP2C19	rs3862009	10	96609015	A>G	0.07	0.68	1.4	0.8, 2.5	0.29	1.1	0.8, 1.6	0.50
CYP2C19	rs733115	10	96609076	A>C	0.07	0.71	1.4	0.8, 2.6	0.23	1.1	0.8, 1.6	0.55
CYP2C19	NA	10	96609221	A>G	0.20	0.18	0.9	0.6, 1.3	0.51	0.9	0.7, 1.1	0.39
CYP2C19	NA	10	96610294	C>A	0.07	0.89	1.7	0.9, 2.9	0.09	1.2	0.8, 1.7	0.33
CYP2C19	NA	10	96611093	G>A	0.17	0.86	1.1	0.7, 1.7	0.85	1.0	0.8, 1.3	0.84
CYP2C19	rs12359148	10	96612303	G>A	0.03	0.18	1.5	0.6, 3.5	0.36	0.6	0.4, 1.1	0.10
CYP2D6	NA	22	40847933	G>C	0.44	0.59	1.1	0.8, 1.5	0.58	1.0	0.9, 1.2	0.78
CYP2D6	rs11090076	22	40844136	A>G	0.33	0.25	0.8	0.6, 1.1	0.16	0.9	0.8, 1.1	0.46
CYP2D6	rs28371717	22	40854254	C>A	0.01	0.76	0.0			2.1	0.9, 5.0	0.08
CYP2D6	rs28371725 (CYP2D6*41)	22	40853749	G>A	0.08	0.003	0.8	0.5, 1.5	0.50	1.1	0.8, 1.6	0.64
CYP2D6	rs5751221	22	40846312	G>A	0.23	0.61	1.2	0.8, 1.7	0.45	1.1	0.9, 1.3	0.66
CYP2D6	rs5758589	22	40848326	G>A	0.44	0.72	1.1	0.8, 1.5	0.52	1.0	0.9, 1.2	0.72
CYP2D6	rs6002623	22	40843707	G>A	0.33	0.25	0.8	0.6, 1.1	0.16	0.9	0.8, 1.1	0.46
CYP2D6	rs764481	22	40848370	G>A	0.33	0.23	0.8	0.6, 1.1	0.16	0.9	0.8, 1.1	0.46
CYP2D6	rs3892097 (CYP2D6*4)	22	40854891	G>A	0.22	0.01	1.2	0.8, 1.7	0.31	1.1	0.9, 1.3	0.63
CYP3A4	rs2242480	7	99199402	C>T	0.11	0.18	0.8	0.4, 1.3	0.32	0.9	0.7, 1.2	0.48
CYP3A4	rs11773597	7	99220387	G>C	0.07	0.54	1.2	0.7, 2.3	0.54	1.1	0.8, 1.6	0.57
CYP3A4	rs1851426	7	99220872	G>A	0.04	0.56	0.8	0.4, 1.8	0.61	1.1	0.7, 1.7	0.78
CYP3A4	rs2246709	7	99203655	A>G	0.27	0.32	0.9	0.6, 1.3	0.58	1.0	0.8, 1.2	0.87
CYP3A4	rs2404955	7	99191215	G>A	0.1	0.02	0.8	0.5, 1.5	0.51	0.9	0.6, 1.2	0.42
CYP3A4	rs2740574	7	99220032	A>G	0.04	0.56	0.8	0.4, 1.8	0.61	1.1	0.7, 1.7	0.78
CYP3A4	rs3735451	7	99193911	A>G	0.12	0.6	0.9	0.6, 1.5	0.72	1.0	0.7, 1.3	0.93
CYP3A5	rs776746 (CYP3A5*3)	7	99108475	G>A	0.07	0.13	0.8	0.4, 1.6	0.49	0.7	0.5, 1.0	0.05

CYP3A5	rs10242455	7	99078115	A>G	0.07	0.23	0.7	0.4, 1.4	0.38	0.7	0.5, 1.0	0.04
CYP3A5	rs1419745	7	99098028	A>G	0.03	0.53	1.0	0.4, 2.7	0.98	0.5	0.3, 0.9	0.02
CYP3A5	rs15524	7	99083850	A>G	0.08	0.15	0.8	0.4, 1.6	0.56	0.7	0.5, 1.0	0.04
CYP3A5	rs17161780	7	99076077	G>A	0.03	0.43	0.9	0.3, 2.7	0.89	0.5	0.3, 0.9	0.02
CYP3A5	rs17161783	7	99076278	A>G	0.03	0.43	0.9	0.3, 2.7	0.89	0.5	0.3, 0.9	0.02
CYP3A5	rs28365067	7	99110246	G>A	0.06	0.36	0.6	0.3, 1.3	0.23	1.0	0.7, 1.5	0.94
CYP3A5	rs28365083	7	99088172	C>A	0.01	0.001	1.3	0.3, 5.0	0.75	2.0	0.9, 4.7	0.10
CYP3A5	rs28365094	7	99088411	A>G	0.1	0.97	1.8	1.1, 3.1	0.02	1.2	0.9, 1.6	0.24
CYP3A5	rs28371764	7	99115529	G>A	0.04	0.49	0.8	0.4, 1.9	0.63	1.0	0.6, 1.6	0.87
CYP3A5	rs4646446	7	99113019	G>A	0.03	0.43	0.9	0.3, 2.7	0.89	0.5	0.3, 0.9	0.02
CYP3A5	rs4646447	7	99106326	G>A	0.03	0.43	0.9	0.3, 2.7	0.88	0.5	0.3, 1.0	0.04
CYP3A5	rs4646450	7	99104254	G>A	0.17	0.26	0.9	0.6, 1.4	0.75	1.0	0.8, 1.2	0.75
CYP3A5	rs4646456	7	99083211	A>G	0.03	0.43	0.9	0.3, 2.7	0.88	0.5	0.3, 0.9	0.02
CYP3A5	rs4646457	7	99083016	A>C	0.08	0.24	0.8	0.4, 1.5	0.49	0.7	0.5, 1.0	0.04
CYP3A5	rs4646458	7	99082949	A>C	0.03	0.53	1.0	0.4, 2.7	0.98	0.5	0.3, 0.9	0.02
CYP3A5	rs6956305	7	99079246	A>G	0.04	0.09	0.7	0.3, 1.7	0.42	0.9	0.6, 1.3	0.49
SULT1A1	rs2411453	16	28539522	C>A	0.38	0.45	1.1	0.8, 1.5	0.74	0.8	0.7, 1.0	0.07
SULT1A1	rs1968752	16	28539086	C>A	0.35	0.15	1.1	0.8, 1.5	0.77	0.9	0.7, 1.0	0.11
SULT1A1	rs2077412	16	28528812	G>A	0.3	0.01	1.0	0.7, 1.4	0.85	1.0	0.9, 1.3	0.74
UGT2B15	rs1377872	4	69588127	G>A	0.13	0.83	1.2	0.8, 1.9	0.42	1.3	1.0, 1.7	0.04
UGT2B15	rs3100	4	69547273	A>G	0.36	0.66	0.7	0.5, 0.9	0.02	0.9	0.7, 1.0	0.09
UGT2B15	rs4148271	4	69547255	T>A	0.02	0.57	0.8	0.2, 2.9	0.76	1.5	0.7, 3.4	0.29
UGT2B15	rs7696472	4	69572785	G>A	0.46	0.31	1.0	0.8, 1.4	0.82	1.1	0.9, 1.3	0.38

Abbreviations: SNP=single nucleotide polymorphism; CHR=chromosome; MAF=minor allele frequency; HWE=Hardy-Weinberg equilibrium; RR=relative risk; 95% CI=95% confidence interval; NA=Not applicable. [1]HWE in UBC controls, $p<0.001$. [2]Per allele RR (log-additive model) adjusting for age at diagnosis and the counter-matching offset term.

response, though results have been mixed [9-13, 15-19, 23, 45, 46] and now the center of significant controversy [26-28]. Variants in CYP2C9 (*CYP2C9*2* and *CYP2C9*3*) have been associated with lower plasma concentrations of active tamoxifen metabolites [47], though no association between these variants and tamoxifen outcome has been observed [10]. In contrast, although some variants in *CYP2C19* have been associated with reduced enzyme activity [35], but not with treatment outcome, others have been implicated in increased enzyme activity and improved tamoxifen outcome [10]. Variation in *CYP3A5* has been associated with altered circulating concentrations of tamoxifen metabolites in some [48] but not all [47, 49] studies, and results of studies showing the impact of this variation on clinical outcome have been mixed [10, 16, 21]. Similarly, studies of *SULT1A1* have found variants associated with altered enzyme activity [50, 51] that do not influence serum concentrations of tamoxifen or its metabolites [44, 48] and have a variable impact on treatment outcome [9, 16, 22-24]. Variation in another phase II enzyme, *UGT2B15*, has been associated with increased enzyme activity [52], but not with circulating concentrations of tamoxifen metabolites [47] or with clinical outcome [9, 16, 53]. Our study examined 112 SNPs in 8 genes hypothesized to influence risk of CBC through altered tamoxifen metabolism, including SNPs in the genes listed above, and none was associated with risk of CBC.

A unique strength of this study is our ability to investigate the impact of genetic variation in tamoxifen metabolizing genes specifically on risk of CBC. This is made possible through the multi-center population-based design, allowing for the inclusion of a large number of women with CBC, detailed questionnaire data, including detailed information on treatments received for first primary breast cancers, and confirmation of interview data, where possible, by medical records. Although we were able to confirm that tamoxifen use was associated with a reduction in CBC risk in the sub-group of the WECARE population included in this analysis, a limitation was our inability to assess adherence to prescribed tamoxifen intake. Additionally, because information regarding use of other medications was not collected, we were not able to account for drugs sometimes shown to affect the efficacy of tamoxifen (e.g., SSRIs), although any effect is likely to be small [8]. A limitation of the tagSNP approach is that it does not account for variation that is not in LD with the genotyped tagSNP, including rare variants, copy number variations or epigenetic modifications that could impact tamoxifen metabolism and efficacy. This approach also limited our ability to classify women by *CYP2D6* phenotype [54] and to achieve complete gene coverage for genes with poor coverage in HapMap (e.g., *SULT1A1*, *UGT2B15*). Further, the complex gene structure of some of the CYP genes (e.g., *CYP2D6*) restricted the use of high-throughput genotyping methods, requiring alternate genotyping strategies and assay development.

The current analysis addresses the question of whether variation in genes involved in tamoxifen metabolism is associated with CBC risk among women who receive the drug. Another important and clinically relevant question is how genotype modifies the association between tamoxifen and risk of CBC, i.e., the association between tamoxifen and risk of CBC conditional on genotype. This analysis is confounded by the strong relationship between ER-status and tamoxifen treatment and could be addressed by restricting the analysis to women with ER+ first primaries. When this is done however there are too few women with ER+ first primaries who did not receive tamoxifen to provide a stable reference group for statistical comparisons. Our inability to fully address this research question in all genotyped variants is a further limitation of the current study, one that deserves future consideration.

This is the first study to address the role of germline genetic variation in genes that code for enzymes involved in the metabolism of tamoxifen and the impact on risk of CBC in women who receive the drug. Tamoxifen has been shown to significantly reduce the risk of second primary breast cancers and the results of this study suggest that variation in these genes is not associated with risk of CBC in women who receive tamoxifen. Of note, many women with ER-positive first breast cancers did not receive tamoxifen as part of their treatment. This is likely because 35% of women included in this study were diagnosed with a first primary breast cancer prior to 1989. It was only after a report by the National Cancer

Institute in 1988 recommending tamoxifen treatment for women with lymph-node negative breast cancer that tamoxifen use increased rapidly [55] and not until a report by the EBCTCG ten years later that the full clinical benefit of tamoxifen was recognized [56].

Conclusion

Using a tagSNP approach, germline genetic variation in genes associated with tamoxifen metabolism is not associated with risk of CBC in women who take tamoxifen and does not explain the occurrence of CBC in some women who receive this treatment. This does not preclude a role of germline variation in influencing treatment response with respect to tamoxifen and risk of CBC, but rather provides further incentive for expansion to a systematic whole-genome approach.

Conflict of interest statement

Jonine Bernstein is a member of the editorial board at the International *Journal of Molecular Epidemiology and Genetics*. All other authors have no conflicts of interest to disclose.

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