CYP2D6 genotype and the association between tamoxifen treatment and risk of contralateral breast cancer in the WECARE Study

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BACKGROUND

The most common malignancy in women who survive a first unilateral breast cancer (UBC) is the development of a second primary in the contralateral breast (CBC). UBC patients with estrogen receptor positive tumors are often treated with tamoxifen (TMX), which is metabolized into more active compounds by the Cytochrome P450 2D6 (CYP2D6) (Figure 1). Women who carry certain *CYPD26* gene variants have been shown to have reduced enzyme activity and poor response to TMX treatment

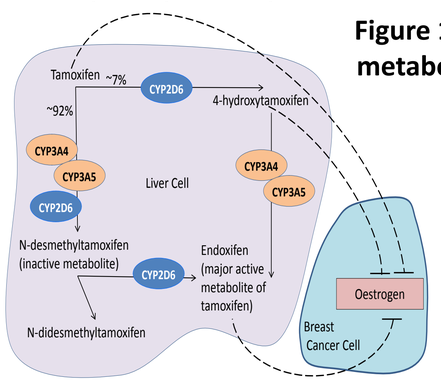


Figure 1. Tamoxifen metabolic pathway

Hypothesis: Women with poor metabolizing CYP2D6 phenotypes have a worse response to TMX and a subsequent increased risk of developing CBC compared to women not treated with TMX who are effective metabolizers

Goal: **(I)** To develop a specific assay for genotyping the *CYP2D6* full gene deletion and polymorphisms that were not previously captured by GWAS; **(II)** to investigate the risk of developing CBC according to TMX treatment and *CYP2D6* genotypes

Table 1. Patient characteristics

	CBC Cases (N=1,521)	UBC Controls (N=2,212)
	Median (range)	**Median (range)**
Age at first dx	46 yr (24-54)	46 yr (23-54)
Period at risk*	6.3 yrs (1-19.8)	5.5 yrs (1-19.8)
Menopausal status^	**N (%)**	**N (%)**
Premenopausal	1,124 (74)	1,676 (76)
Postmenopausal	389 (26)	522 (24)
Unknown	8 (1)	14 (1)
First-degree family history of breast cancer		
No	1,004 (66)	1,706 (77)
Yes	497 (33)	466 (21)
Unknown	20 (1)	40 (2)
Stage of first diagnosis		
Local	1,061 (70)	1,442 (65)
Regional	448 (29)	759 (34)
Unknown	12 (1)	11 (1)
Chemotherapy		
No	699 (46)	923 (42)
Yes	822 (54)	1,289 (58)
Radiation treatment		
No	641 (42)	522 (24)
Yes	880 (58)	1,689 (76)
Unknown	-	1 (0)
Hormone treatment		
No	964 (63)	1,270 (57)
Yes	557 (37)	940 (42)
Unknown	-	2 (0)
ER status at first diagnosis		
Positive	797 (52)	1,254 (57)
Negative	467 (31)	561 (25)
Other/unknown	257 (17)	397 (18)

ER, estrogen receptor; *Period refers to CBC diagnosis; ^menses or pregnancy within 2yrs of UBC dx

Table 2. Characteristics of studied CYP2D6 polymorphisms

RefSeq	Polymorphism	Effect	Allele nomenclature^	Metabolizing Phenotype
Reference	wt	wt	*1A	Extensive (EM)
rs16947; rs1135840	2850C>T, 4180G>C	R296C ; S486T	*2D	Extensive (EM)
rs35742686	2549delA	FS	*3A	Poor (PM)
rs3892097	1846G>A	Splicing	contributes to *4A, 4B-H, and 4J-P with other SNPs	Poor (PM)
	Full deletion	x	*5	Poor (PM)
rs5030655	1707delT	FS	*6A-B-C-D	Poor (PM)
rs5030656	2615_2617del AAG	K281del	*9	Intermediate (IM)
rs1065852; rs1135840	100C>T ; 4180G>C	P34S ; S486T	contributes to*10A-B-D with other SNPs	Intermediate (IM)
rs28371725	2988G>A	Splicing	contributes to *41 and *91 with other SNPs	Intermediate (IM)

RefSeq, reference sequence; FS, frameshift; x, no protein/abolished function; ^ Nomenclature as per database http://www.cypalleles.ki.se accessed 02/09/17

METHODS

- 1121 CBC 'cases' & 2212 UBC 'controls' from the Women's Environmental Cancer and Radiation Epidemiology (WECARE) Study
- Detailed epidemiologic and clinical data (Table 1)
- *CYP2D6,* located in a complex locus (Figure 2), was tested for full deletion using 3 primers in a long-range PCR (Figures 3 & 4)
- Smaller variants (Table 2) were typed with a modified MassArray assay (Agena Bioscience), in a two-stage assay (Figure 5)
- Conditional logistic regression was used to estimate risk ratios (RR) and 95% confidence intervals (CI) for the association between TMX and risk of CBC, stratified by *CYP2D6* genotype

Figure 2. CYP2D6 gene region

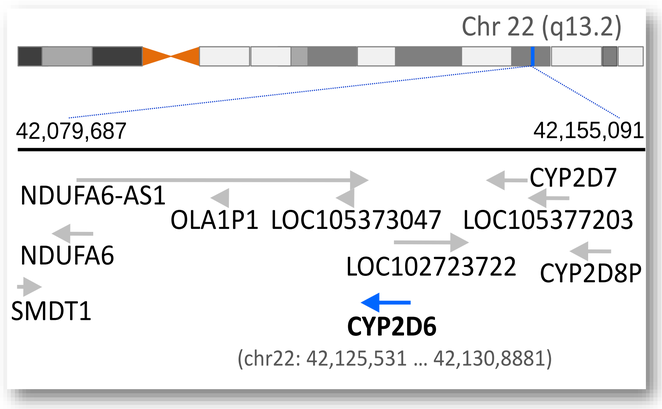
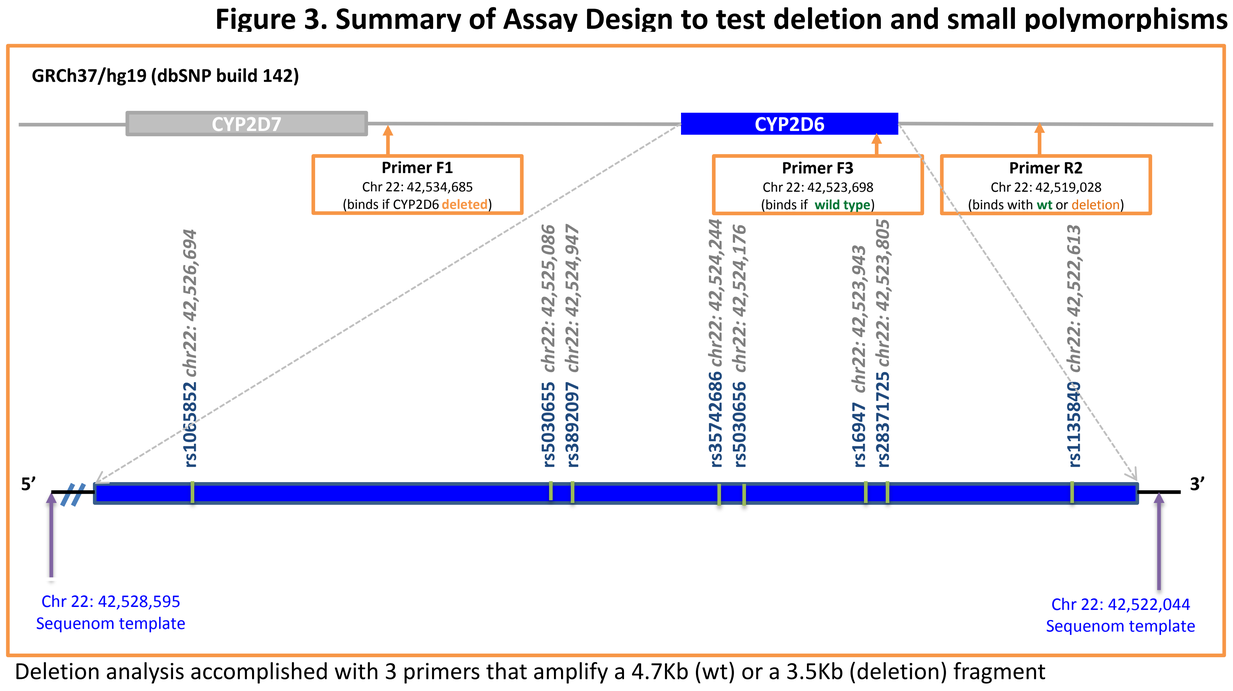


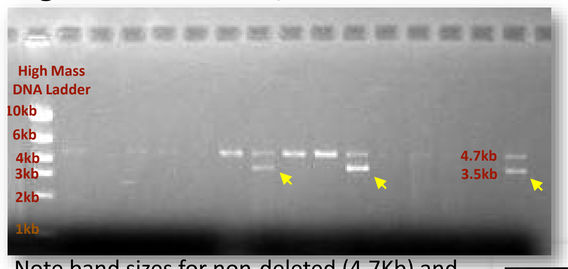
Figure 3. Summary of Assay Design to test deletion and small polymorphisms



Deletion analysis accomplished with 3 primers that amplify a 4.7Kb (wt) or a 3.5Kb (deletion) fragment

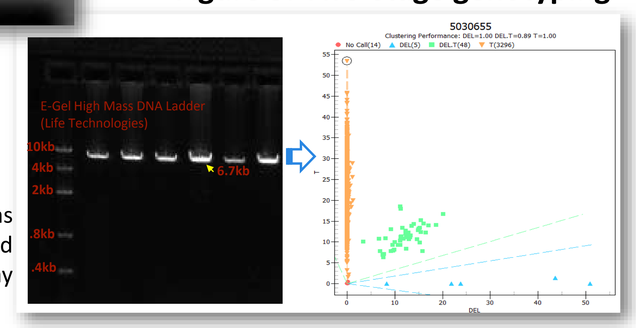
RESULTS

Figure 4. Insertion/deletion



Note band sizes for non-deleted (4.7Kb) and deleted (3.5Kb, yellow arrows) fragments

Figure 5. Two-stage genotyping



A specific 6.7Kb amplicon was amplified (Left panel) and used as template in the MassArray genotyping (Right panel)

Table 3. Association between tamoxifen and risk of CBC

RefSeq	Genotype & Metabolizing Phenotype		TMX(-) N (%) Cases	TMX(-) N (%) Controls	TMX (+) N (%) Cases	TMX (+) N (%) Controls	RR* (95% CI)*	p-het*
rs16947	CC		462(44)	655(47)	227(50)	353(45)	0.74 (0.58-0.95)	
	CT		441(42)	567(40)	163(35)	317(41)	0.56 (0.43-0.73)	
	TT	EM	140(13)	180(13)	67(15)	106(14)	0.67 (0.44-1.03)	0.28
rs1135840	GG		357(34)	477(34)	172(37)	265(34)	0.72 (0.55-0.94)	
	GC		494(47)	650(46)	187(40)	365(47)	**0.55 (0.43-0.70)**	
	CC	EM	195(19)	278(20)	105(23)	153(20)	0.82 (0.58-1.17)	0.11
rs1065852	CC		639(61)	844(60)	278(61)	480(62)	**0.65 (0.53-0.81)**	
	CT		343(33)	460(33)	139(30)	260(33)	**0.56 (0.42-0.74)**	
	TT	IM	61(6)	104(7)	39(9)	39(5)	1.54 (0.84-2.85)	**0.01**
rs3892097	GG		683(66)	893(63)	310(67)	525(67)	0.66 (0.54-0.81)	
	GA		316(30)	451(32)	135(29)	234(30)	0.61 (0.46-0.83)	
	AA	PM	42(4)	67(5)	17(4)	21(3)	1.30 (0.58-2.92)	0.24
rs5030655	T		1034(99)	1394(99)	459(99)	769(98)	0.67 (0.56-0.79)	
	deleted	IM	15(1)	20(1)	3(1)	15(2)	**0.17 (0.04-0.77)**	**0.06**
rs5030656	AAG		988(94)	1344(95)	444(95)	750(96)	0.67 (0.56-0.79)	
	deleted	IM	61(6)	73(5)	21(5)	35(4)	0.58 (0.28-1.18)	0.70
rs28371725	G		868(83)	1211(85)	393(85)	651(83)	0.69 (0.57-0.82)	
	GA		161(15)	188(13)	61(13)	115(15)	0.56 (0.37-0.85)	
	AA	IM	20(2)	18(1)	10(2)	19(2)	0.33 (0.11-0.97)	0.29
rs35742686	A		1020(97)	1365(96)	454(98)	763(97)	0.66 (0.55-0.78)	
	deleted		28(3)	50(4)	9(2)	20(3)	0.86 (0.31-2.39)	0.61
full gene	present		903(94)	1221(93)	391(93)	661(94)	0.63 (0.52-0.77)	
	deleted	PM	55(6)	85(7)	31(7)	43(6)	**1.43 (0.73-2.80)**	**0.02**

* RR, relative risk among treated compared to untreated group. Adjusted for age at first primary, age at menopause two years prior to first primary, histology & stage of first primary, family hx of breast cancer, chemotherapy at first primary, radiation at first primary, number of full term pregnancies at first primary, and age at menarche. Deleted includes heterozygotes and homozygotes

SUMMARY

- Patients carriers of 2 *CYP2D6* copies treated with TMX were at lower risk for CBC, while those carrying 0-1 copies were at higher risk for CBC compared to untreated individuals(p-heterogeneity=0.02)
- Women carriers of rs1065852-C/C (reference) treated with TMX were at lower CBC risk, while those with 2 variant alleles (T/T) were at increased risk for CBC (p-heterogeneity=0.01)

CONCLUSION

- *CYP2D6* polymorphisms modify response to TMX in relation to risk of CBC
- Further analyses in the context of other CYP2D6 polymorphisms (previously tested by GWAS) are warranted in order to categorize women into distinct TMX-response groups
- Knowledge on genetic modifiers such as CYP2D6 may contribute towards personalized breast cancer treatment

ACKNOWLEDGEMENTS

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