








17 β -Hydroxysteroid dehydrogenase 1:2 and breast cancer recurrence: a Danish population-based study

Lindsay J. Collin^a , Sinna P. Ulrichsen^b, Thomas P. Ahern^c, Michael Goodman^a, Lauren E. McCullough^a, Lance A. Waller^d , Kristina Bang Christensen^e, Per Damkier^{f,g} , Stephen Hamilton-Dutoit^e , Kristina L. Lauridsen^e, Rami Yacoub^a, Peer M. Christiansen^h, Bent Ejlersen^{i,j}, Henrik Toft Sørensen^{b,k} , Deirdre P. Cronin-Fenton^b  and Timothy L. Lash^{a,b} 

^aDepartment of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA, USA; ^bDepartment of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark; ^cDepartment of Surgery, The Robert Larner, M.D. College of Medicine, The University of Vermont, Burlington, VT, USA; ^dDepartment of Biostatistics and Bioinformatics, Rollins School of Public Health, Emory University, Atlanta, GA, USA; ^eInstitute of Pathology, Aarhus University Hospital, Aarhus, Denmark; ^fDepartment of Clinical Biochemistry and Pharmacology, Odense University Hospital, Odense, Denmark; ^gDepartment of Clinical Research, University of Southern Denmark, Odense, Denmark; ^hBreast Unit, Aarhus University Hospital/Randers Regional Hospital, Aarhus, Denmark; ⁱDanish Breast Cancer Group, Copenhagen, Denmark; ^jRigshospitalet, Copenhagen, Denmark; ^kDepartment of Health Research and Policy (Epidemiology), Stanford University, Stanford, CA, USA

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Introduction

Breast cancer is the most commonly diagnosed malignancy among women [1] and, despite advances in targeted therapies, the risk of recurrence remains as high as 40% over twenty years of follow-up [2]. Approximately 75% of all breast malignancies express the oestrogen receptor (ER), and are dependent on oestrogen and its metabolites for growth and proliferation [3,4]. Conversion of oestrogen to its active form, oestradiol, occurs at the local tissue level. The final conversion is regulated by two enzymes: 17 β -hydroxysteroid dehydrogenase 1 and 2 (HSD17B1 and HSD17B2 respectively). This reaction occurs within the target cells where the oestrogenic effect is exerted via the ER.

Women diagnosed with non-metastatic ER+ breast cancer typically receive adjuvant therapy with tamoxifen or aromatase inhibitors, which are prescribed to prevent recurrence [5,6]. Tamoxifen is a selective ER modulator (SERM), and its metabolites have strong affinity for the ER. Therefore, they compete with metabolites of oestrogen (most notably oestradiol) at the receptor binding site [7]. The current clinical guideline for tamoxifen use is among premenopausal women with ER+ disease or postmenopausal women with contraindications for aromatase inhibitors [8].

Breast carcinomas with increased expression of HSD17B1 compared with HSD17B2 have increased intratumoral concentrations of oestradiol, which may reduce tamoxifen's effectiveness [9–12]. The ratio of the two enzymes, HSD17B1:HSD17B2, captures the balance between oestradiol synthesis and inactivation, which may predict treatment failure among women receiving tamoxifen therapy [13,14]. To

better understand tamoxifen resistance, we evaluated whether the HSD17B1:HSD17B2 ratio was associated with recurrence among women diagnosed with breast cancer.

Methods

Study population

This was a population-based case-control study of Danish women diagnosed with a first primary stage I–III breast cancer between 1985 and 2001 [15]. Women were included if their diagnosis occurred between 35 and 69 years of age and if they were recorded in the Danish Breast Cancer Group (DBCG) clinical database, which registers nearly all Danish breast cancer patients [16,17]. Eligible patients were divided into two strata: women whose tumours expressed ER and who were treated with tamoxifen for at least one year (ER+/TAM+, $n = 1826$), and those whose tumours did not express ER, who were not treated with tamoxifen, and who survived at least one year (ER-/TAM-, $n = 1808$). Stratifying by ER and TAM status allowed for separation of HSD17B1 and HSD17B2 prognostic effects, which would be observed in both strata, and predictive tamoxifen resistance effects, which would be observed in only the ER+/TAM+ stratum. Follow-up time was calculated from one year after breast cancer diagnosis until the first of (1) breast cancer recurrence, (2) death from any cause, (3) loss to follow-up, (4) completion of 10-years of follow-up, or (5) 1 September 2006.

Cases were defined as women with a diagnosis of a local, regional, or distant recurrence during follow-up. Controls were selected from members of the source population who

were not diagnosed with breast cancer recurrence or with contralateral breast cancer at the follow-up time of the matched cases' recurrence. Controls were matched to cases on stratum (ER+/TAM+ or ER-/TAM-), menopausal status at diagnosis, date of breast cancer surgery (calliper matched +/- 12 months), county of residence at the time of diagnosis, and UICC cancer stage at diagnosis. In the ER+/TAM+ stratum, 541 cases were identified; all were included in the analysis. In the ER-/TAM- stratum, 300 cases were randomly selected and frequency matched according to the distribution of stage and calendar period of diagnosis among the ER+/TAM+ case patients. We collected patient demographics, tumour and treatment information for the DBCG registry. A complete description of our methodology is outlined in the Supplemental digital content and described below in brief.

Tissue microarray construction and immunohistochemistry

For each case and control, formalin-fixed, paraffin-embedded (FFPE) primary tumour tissue blocks were retrieved from the pathology archives of treating hospitals. Tissue microarrays (TMAs) were constructed using standard techniques [18]. Core samples (1 mm diameter) were removed from each tumour donor block and re-embedded in a new recipient paraffin TMA block (3DHISTECH, Budapest, Hungary). If sufficient material was available, representative tumour ($n=3$) and marginal tissue ($n=1$) cores were sampled. Liver and placental cores were included in each TMA to facilitate orientation.

Immunohistochemistry (IHC) was performed on TMA tissue sections according to standard protocols. Expression of HSD17B1 was assessed using a rabbit monoclonal antibody at a concentration of 1:150 (Abcam, Cambridge, UK; catalogue no. EP1682Y). Expression of HSD17B2 was assessed using a rabbit polyclonal antibody at a concentration of 1:300 (Proteintech, Rosemont, IL; catalogue no. 10978-1-AP). For each biomarker, sections were incubated with the primary antibody for 30 minutes. Slides were digitalised using a whole slide image scanner (Panoramic SCAN 150; 3DHISTECH).

TMA core scoring

Expression of HSD17B1 and HSD17B2 was quantified using an H-score that incorporated staining intensity and percentage of positively stained tumour cells [19]. Staining intensity was a weighted scale ranging from 0 for no intensity to 3 for high staining intensity. Percent positivity ranged from 0 to 100% based on percentage of positively stained tumour cells. Therefore, the H-score has a plausible range from 0 to 300 (observed range = 0–294). There were 1–3 tumour cores available for each specimen and we used the average score.

Analytic variables

Expression of HSD17B1 and HSD17B2

The exposure of interest for this study was cytoplasmic expression of HSD17B1 and HSD17B2. To address our primary study aim, we considered the ratio of HSD17B1 and HSD17B2 H-scores as a dichotomous variable (>1 vs. ≤ 1). In a sensitivity analysis, we restricted to more extreme values of the HSD17B1:HSD17B2 ratio and compared the dichotomised H-scores (>1.25 vs. <0.75). To examine whether the independent expression of the markers was related to recurrence, we operationalised cytoplasmic expression of each biomarker as quartiles of its H-score.

Breast cancer recurrence

The study followed the DBCG definition of breast cancer recurrence, i.e., any contralateral or ipsilateral breast cancer occurring locally, regionally, or distally, after breast cancer diagnosis [16].

Covariates

In each analysis we adjusted for UICC stage (I, II, III), grade (I, II, III), menopausal status at diagnosis (premenopausal/postmenopausal), receipt of chemotherapy (yes/no), receipt of radiotherapy (yes/no), surgery type (mastectomy/breast-conserving surgery), year of diagnosis, age at diagnosis, and county of residence.

Statistical analysis

All analyses were stratified by the ER/TAM grouping to evaluate whether expression of HSD17B1 and HSD17B2 was predictive or prognostic of breast cancer recurrence. We first calculated descriptive statistics to characterise the distributions of covariates within ER/TAM strata. We then characterised the distribution of the HSD17B1:HSD17B2 ratio, and cytoplasmic expression of HSD17B1 and HSD17B2 within each stratum.

In the principal analysis, we used logistic regression to estimate the associations between HSD17B1 and HSD17B2 with breast cancer recurrence (yes/no). We considered the HSD17B1:HSD17B2 ratio and the expression of each enzyme independently. To avoid discarding matched sets due to missing tumour core samples, we used unconditional multivariable logistic regression to compute the odds ratios (ORs) and 95% confidence intervals (CIs) reflecting the association of expression with recurrence, adjusting for year of diagnosis, age at diagnosis, prescribed time of tamoxifen therapy (in the ER+/TAM+ stratum), menopausal status at diagnosis, stage, grade, region of residence, and receipt of chemotherapy and radiation therapy. To account for potential selection bias due to lack of availability of tumour cores, we used inverse probability of participant weighting (IPPW) to reweight the study population of women with complete expression data to the population we would have observed

had all patients been included [20,21]. All analyses were carried out using SAS v9.4 (Cary, NC).

The study was approved by the Danish Data Protection Agency (record number 2015-57-0002 and Aarhus University journal number 2016-051-000001, running number 458), the Danish Ethical Committee (record number 1-10-72-16-15), and the Emory University Institutional Review Board (record number 00071301).

Results

The vast majority (96%) of participants were initially diagnosed with stage II or III breast cancer (Supplemental Table 1). Most women were postmenopausal at diagnosis (81%), although this differed across ER+ and ER- strata (93 vs. 60%, respectively). Expression of HSD17B1 and HSD17B2 was present in more than 80% of the tumour cores. Approximately, 31% of cases and 31% of controls had an HSD17B1:HSD17B2 ratio >1 in the ER+/TAM+ stratum. Similarly, 30% of cases and 25% of controls in the ER-/TAM- strata had an HSD17B1:HSD17B2 ratio >1.

Cytoplasmic ratio of HSD17B1:HSD17B2 expression

In the ER+/TAM+ stratum, there was no notable association between the ratio of HSD17B1:HSD17B2 and breast cancer recurrence (OR = 1.03, 95% CI: 0.78, 1.40) (Table 1). Similarly, in the ER-/TAM- stratum, we observed a near-null association between the ratio of the two enzymes and breast cancer recurrence (OR = 1.02, 95% CI: 0.77, 1.19).

In analyses examining the independent association of each biomarker with breast cancer recurrence, we observed a slight increase in the odds of breast cancer recurrence with increased expression of each biomarker, although the estimates were imprecise. In the ER+/TAM+ stratum, the odds ratio for breast cancer recurrence comparing the top quartile of HSD17B1 expression with the bottom quartile was OR = 1.20 (95% CI: 0.82, 1.75). We obtained a similar estimate in the ER-/TAM- stratum (OR = 1.18, 95% CI: 0.69, 2.04). In the ER+/TAM+ stratum, the odds ratio for breast cancer recurrence comparing the top quartile of HSD17B2 expression with the bottom quartile was OR = 1.39 (95% CI: 0.89, 2.19). In the ER-/TAM- stratum, the estimate of association

was OR = 1.16 (95% CI: 0.66, 2.06). There was little evidence of a monotonic dose-response pattern across the quartiles of all strata.

Quantitative bias analysis

In our descriptive assessment of possible selection bias due to missing FFPE blocks or tumour cores, only surgery type seemed to vary between those with and without a tumour sample available for analysis. Women who received breast-conserving surgery were less likely to have FFPE blocks available compared with those who had a mastectomy, a pattern consistent in cases, controls and across ER/TAM strata. In addition, we observed variability in receipt of cores based on county of residence (data not shown). IPPW estimates of association were similar to the corresponding results from the conventional analysis, indicating that the availability of FFPE tumour blocks and cores did not substantially bias study results (Table 2).

Discussion

This is the first study to examine the predictive and prognostic associations between the HSD17B1:HSD17B2 ratio and breast cancer recurrence. We found no evidence to support

Table 2. IPPW analysis conducted to account for possible baseline selection bias of tumour core availability for the association between expression of cytoplasmic HSD17B1, HSD17B2, and the HSD17B1:HSD17B2 ratio and breast cancer recurrence among subjects from a Danish population-based case control study.

	ER+/TAM+ Adjusted OR (95%CI)	ER-/TAM- Adjusted OR (95%CI)
Cytoplasmic HSD17B1 Expression		
≥75th %tile	1.19 (0.81, 1.76)	1.19 (0.66, 2.17)
50th-<75th %tile	1.35 (0.90, 2.03)	0.92 (0.52, 1.63)
25th-<50th %tile	1.23 (0.83, 1.83)	1.17 (0.66, 2.09)
<25th %tile	Reference	Reference
Cytoplasmic HSD17B2 Expression		
≥75th %tile	1.41 (0.88, 2.45)	1.22 (0.67, 2.22)
50th-<75th %tile	1.07 (0.73, 1.57)	1.14 (0.66, 1.97)
25th-<50th %tile	1.28 (0.87, 1.90)	1.25 (0.71, 2.21)
<25th %tile	Reference	Reference
HSD17B1:HSD17B2 Expression		
> 1	1.04 (0.76, 1.42)	1.03 (0.76, 1.41)
≤ 1	Reference	Reference

Table 1. Association between cytoplasmic HSD17B1 expression, HSD17B2 expression, HSD17B1:HSD17B2 ratio, and breast cancer recurrence among 1312 subjects from a Danish population-based case control study.

	ER+/TAM+			ER-/TAM-		
	Case	Control	Adjusted OR (95%CI)	Case	Control	Adjusted OR (95%CI)
Cytoplasmic HSD17B1 Expression						
≥75th %tile	121	121	1.20 (0.82, 1.75)	66	54	1.18 (0.69, 2.04)
50th-<75th %tile	106	93	1.35 (0.91, 2.00)	68	77	0.92 (0.54, 1.57)
25th-<50th %tile	115	110	1.24 (0.84, 1.82)	67	58	1.18 (0.69, 2.04)
<25th %tile	104	124	Reference	61	60	Reference
Cytoplasmic HSD17B2 Expression						
≥75th %tile	73	58	1.39 (0.89, 2.19)	64	59	1.16 (0.66, 2.06)
50th-<75th %tile	142	154	1.04 (0.72, 1.51)	84	82	1.10 (0.65, 1.86)
25th-<50th %tile	124	115	1.23 (0.84, 1.80)	59	54	1.25 (0.71, 2.19)
<25th %tile	102	115	Reference	57	60	Reference
HSD17B1:HSD17B2 Expression						
>1	131	128	1.03 (0.78, 1.40)	75	60	1.02 (0.77, 1.19)
≤1	296	291	Reference	177	179	Reference

the use of the HSD17B1:HSD17B2 ratio as a predictive marker for tamoxifen resistance, or as a prognostic indicator for breast cancer recurrence. Increasing expression of each biomarker independently was suggestive of a slight increase in the odds of breast cancer recurrence in both strata of ER/TAM. The results for the ratio of expression demonstrated no association, which argues against an effect of this enzyme system on breast cancer recurrence.

Previous studies showed that specific enzymes of the hydroxysteroid dehydrogenase family might serve as novel therapeutic targets for breast cancer patients who do not respond to tamoxifen therapy. In previous population-based studies, HSD17B1 mRNA expression was associated with poorer survival, but this was not observed for HSD17B2 [22]. A study of postmenopausal patients reported that lower HSD17B2:HSD17B1 ratios (inverse ratio of what was used in this study) were associated with a higher rate of recurrence [23]. Similarly, a study nested in a Swedish clinical trial reported that an HSD17B1:HSD17B2 ratio greater than 1 decreased tamoxifen's effectiveness compared with tumours that expressed an HSD17B1:HSD17B2 ratio less than 1 [13]. Our study did not substantiate these findings.

A limitation of this study is that it was restricted to breast cancer diagnoses between 1985 and 2001. During this period, tamoxifen represented guideline-concordant care for postmenopausal, and later premenopausal women, but is now first line adjuvant hormone therapy only for premenopausal women. Regardless, postmenopausal women for whom aromatase inhibitors are contraindicated still receive tamoxifen; our results are still applicable to that target population [5].

In conclusion, in this population-based case-control study, we found no evidence of an association between the ratio of cytoplasmic expression of HSD17B1 to HSD17B2 and breast cancer recurrence. Future studies may be strengthened by examination of these enzymes in premenopausal breast cancer patients, for whom tamoxifen remains the guideline therapy.







Disclosure statement

The authors have no conflict of interest to declare.

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ORCID

Lindsay J. Collin  <http://orcid.org/0000-0003-0170-2592>
 Lance A. Waller  <http://orcid.org/0000-0001-5002-8886>
 Per Damkier  <http://orcid.org/0000-0003-0591-7187>
 Stephen Hamilton-Dutoit  <http://orcid.org/0000-0003-2158-3885>
 Henrik Toft Sørensen  <http://orcid.org/0000-0003-4299-7040>
 Deirdre P. Cronin-Fenton  <http://orcid.org/0000-0001-9738-2284>
 Timothy L. Lash  <http://orcid.org/0000-0002-5240-5195>

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