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The effect of 14-3-3 ζ expression on Tamoxifen Resistance and Breast Cancer Recurrence: A Danish population-based study

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Abstract

Purpose—Overexpression of 14-3-3 ζ has been linked to breast cancer recurrence in several studies, including studies assessing its effect on tamoxifen resistance. The study was performed to estimate the effect of 14-3-3 ζ and differentiate potential prognostic or predictive utility.

Methods—A case-control study, nested in a population of 11,251 females residing on the Jutland Peninsula of Denmark. Participants were aged 35–69, diagnosed with stage I, II, or III breast cancer between 1985 and 2001, and registered with the Danish Breast Cancer Cooperative Group. We identified 541 recurrent breast cancer cases with estrogen receptor-positive disease treated with tamoxifen for at least 1 year (ER+/TAM+) and 300 cases with estrogen receptor-negative disease never treated with tamoxifen (ER–/TAM–). We matched cases to controls on ER/TAM status, date of surgery, menopausal status, stage, and county. 14-3-3 ζ expression was assessed using immunohistochemistry on tissue microarrays. We computed the odds ratio (OR) associating 14-3-3 ζ expression with breast cancer recurrence adjusting for confounding using logistic regression. A quantitative bias analysis was performed to account for bias due to expression assay methods.

Results—Associations for cytoplasmic and nuclear 14-3-3 ζ staining above the 50th percentile were near null in both ER+/TAM+ and ER–/TAM– patients. When examining combined 14-3-3 ζ staining, the association increased in the ER+/TAM+ group (adjusted OR = 1.44, 95% confidence interval (CI): 1.05, 1.99). A nearly two-fold increase in odds of recurrence was observed in above the 75th percentile staining of combined 14-3-3 ζ , both for ER+/TAM+ patients (adjusted OR = 1.93, 95% CI: 1.15, 3.24) and ER–/TAM– patients (adjusted OR = 1.93, 95% CI: 1.03, 3.62), indicating potential prognostic utility.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

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

Informed Consent

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

Conclusion—Evidence is lacking to conclude that 14-3-3 ζ is a useful marker of tamoxifen resistance, however, 14-3-3 ζ expression is a potentially useful prognostic marker of breast cancer recurrence. Independent utility beyond established prognostic markers needs to be determined.

Keywords

14-3-3 ζ ; tamoxifen; breast cancer recurrence; prognostic marker; predictive marker

Introduction

Treatment options for breast cancer include surgery, radiation therapy, systemic therapies, or a combination [1]. Although many patients benefit from initial treatments, approximately 30% of early-stage breast cancer patients develop a recurrence [2]. About 75% of breast cancers express estrogen receptor (ER) α . Its expression in the presence of estrogen is associated with highly distinct gene expression patterns for genes involved extensively in cell cycle regulation [3].

Patients whose tumors express ER α are candidates for long-term adjuvant therapies targeting growth stimulated by estrogen. Two primary classes of drugs are used to treat ER α -positive breast cancer patients: aromatase inhibitors and selective ER modulators (SERMs). Because of the success of these treatments, breast cancer patients whose tumors express ER α are at reduced risk of recurrence and cancer-specific mortality, independent of age, ethnicity, and tumor characteristics (stage, grade, and histology) [4, 5]. For example, five years of tamoxifen treatment halves the risk of recurrence in ER α -positive women [6]. Current guidelines of the National Comprehensive Cancer Network (NCCN) recommend tamoxifen for ER α -positive premenopausal women. They recommend either tamoxifen, aromatase inhibitors, or courses combining these therapies for postmenopausal women. As well, they recommend tamoxifen for postmenopausal women with contraindications to aromatase inhibitors [1]. Therefore, tamoxifen remains a cornerstone of breast cancer treatment for ER α -positive women.

In spite of extensive research, status of ER α expression remains the sole clinical predictor of an individual patient's response to tamoxifen treatment [7]. Patients at high risk of adverse effects when treated with tamoxifen may be better suited for treatment with aromatase inhibitors, as these drugs are sometimes better tolerated and show efficacy comparable to or greater than tamoxifen [8].

The 14-3-3 proteins comprise a highly conserved group of molecules that serve as scaffolds to integrate signaling proteins with targets involved in biological processes, including cell cycle regulation [9]; they are widely expressed in human tissue [10]. Overexpression of 14-3-3 ζ mRNA has been linked to recurrence of breast cancer [11–17]. Only two studies directly examined the effect of 14-3-3 ζ on resistance to tamoxifen [11, 13]. Studies using immunohistochemistry (IHC) also have demonstrated an increased risk of recurrence among patients overexpressing 14-3-3 ζ [16, 17]. These studies were limited by small sample size and insufficient control of confounding, with control limited to tumor characteristics. To address limitations of earlier research and to obtain a precise estimate of the effect of the 14-3-3 proteins on tamoxifen sensitivity in a large, well-defined cohort, we aimed to

measure 14-3-3 ζ expression and to estimate its association with breast cancer recurrence in a population-based registry of breast cancer patients in Denmark, allowing sufficient control for demographics, treatment information, and tumor characteristics.

Materials and Methods

Study Population

The Danish Breast Cancer Group (DBCG) maintains a population-based clinical registry that has collected data on nearly all breast cancer patients in Denmark since 1977. We used the DBCG registry to collect information on 11,251 female residents of the Jutland Peninsula with the following characteristics: aged 35–69; diagnosed with stage I, II, or III breast cancer as defined by the Union for International Cancer Control (UICC); and diagnosed between 1985 and 2001 [18]. All patients registered in the DBCG registry adhere to the same 10-year follow-up protocol [19]. Follow-up time in our study began 1 year from the diagnosis date and continued until the date of the first recurrence, death from any cause, loss to follow-up, 10 years after follow-up, or September 1, 2006. Datasets were linked using Danish Civil Personal Registration (CPR) numbers, a unique identifier assigned to each resident of Denmark [20].

The source population was divided into two groups defined by combined ER α and tamoxifen treatment (ER/TAM) status: (1) patients whose tumor expressed ER α and were treated with tamoxifen for at least one year (ER+/TAM+) and (2) patients whose tumor did not express ER α , were not treated with tamoxifen, and who survived at least one year (ER–/TAM–). All cohort members not in these two groups were excluded from our analysis (Figure 1). Patients also were excluded if their level of tumor 14-3-3 ζ staining could not be accurately determined using tissue microarray (TMA) IHC. Tumor cores were deemed unsatisfactory under the following conditions: if insufficient readable material remained after TMA construction, specimen processing, staining, and digital imaging; if staining and imaging artifacts precluded scoring of tumor cells; if minimal invasive cancer cells were seen in the core (<20 cells); if tumor cells were from an *in situ* carcinoma; or if the core contained predominantly benign epithelial cells.

Cases were patients diagnosed with a local or distant breast cancer recurrence or a contralateral breast cancer occurrence during follow-up. For each case patient, one control was selected without replacement from the source population. Controls had to be alive and have no recurrence or contralateral breast cancer after the same amount of follow-up time. Controls were matched to cases based on group membership (ER+/TAM+ or ER–/TAM–), menopausal status at diagnosis (premenopausal or postmenopausal), date of breast cancer surgery (caliper matched ± 12 months), county of residence at the time of diagnosis, and cancer stage at diagnosis (UICC stage I, II, or II).

Data Collection from the DBCG Registry

The DBCG registry was used to collect information on demographic characteristics (age, menopausal status, and hospital of diagnosis), tumor information (UICC stage at diagnosis,

histologic grade, and ER α status), local therapy (surgical management and radiation therapy), and systemic therapy (receipt of chemotherapy and tamoxifen).

Data Collection from Archived Tissue Samples

Laboratory personnel were blinded to all clinical information, including case or control status, ER α status, and receipt of tamoxifen therapy.

Tissue Microarray Construction and Immunohistochemistry—Formalin-fixed paraffin-embedded primary tumor tissue blocks from cases and controls were collected from the archives of the pathology departments of treating hospitals, where they had been stored routinely since being removed during diagnostic surgical procedures. The location of specimen blocks was identified using the records of the Danish Pathology Data Bank, a unique database linked to patient CPR number, that contains information on all pathology analyses performed in Denmark [21], supplemented by the DBCG registry and the electronic databases of the individual pathology departments. Given that paraffin blocks from diagnostic specimens are routinely stored permanently in Denmark, we were able to obtain primary tumor tissues from nearly all study cases.

TMA were constructed using standard techniques [22]. Cylindrical core samples were taken from each patient tumor donor block and re-embedded into a new recipient paraffin block. If there was sufficient material, three representative 1 mm diameter tumor cores and one marginal tissue core were sampled using the TMA Master from 3DHISTECH, Budapest, Hungary. Liver and placental cores were added to each microarray to serve as a positive and negative control, respectively.

Immunohistochemical stains were performed on 4 μ m TMA tissue sections according to standard protocols, optimized in house for use with the Ventana BenchMark XT automated staining system (Ventana Medical Systems, Tucson, AZ, USA). 14-3-3 ζ expression was assessed using the mouse anti-human 14-3-3 ζ monoclonal antibody (catalog # MAB2669) from R&D Systems (R&D Systems, Abingdon, UK). Briefly, sections were deparaffinized before antigen retrieval was achieved by incubation in Cell Conditioning 1 (CC1, pH 8.5; Ventana) at 100°C. Sections were incubated with the primary antibody diluted at 1:50 for 32 min at 37°C. Positive reactions were detected with the OptiView 3,3'-diaminobenzidine (DAB) IHC Detection Kit (Ventana) before counterstaining with hematoxylin. Specific staining was characterized by positivity localized to nuclei and/or the cytoplasm (and cell membrane).

Stained TMA sections were scanned at 40 $\times$ magnification with the Hamamatsu Nanozoomer 2.0HT (Hamamatsu Phototonics, Ballerup, Denmark) in.ndpi format. Slides were converted to conform to the 3DHISTECH software and uploaded to Panoramic Viewer TMA Module software (3DHISTECH).

TMA Core Scoring—Levels of 14-3-3 ζ staining were determined using the zoom feature in Panoramic Viewer to visualize and score invasive carcinoma cells and to determine the subcellular location of staining (Figure 2). Scoring was assessed separately as a cytoplasmic and a nuclear component since staining reactions differed in the two cellular compartments.

Cytoplasmic scoring used a semi-quantitative system (H-score) combining staining intensity in cells with the relative proportion of positive tumor cells [23]. To calculate H-scores, cytoplasmic staining intensity was divided into four categories: 0 (no staining), 1 (light staining), 2 (moderate staining), 3 (heavy staining). The staining intensity was then multiplied by the proportion of cancer cells exhibiting that staining intensity in a given core. Thus, H-scores could range from 0 (for 100% absent staining) to 300 (for 100% heavy staining). Nuclear staining was scored on an ordinal scale for the entire core in a simplified metric reflective of staining intensity and proportion: 0 (no staining), 1 (light staining), 2 (moderate staining), 3 (heavy staining). Between 1 and 4 cores were sampled per patient tumor; the final nuclear and cytoplasmic scores were the averages of the assessable cores for a given patient.

All cores were evaluated by at least one observer. Cytoplasmic and nuclear scores could be determined for 3,904 of 5,280 stained cores. The remaining cores were deemed to be unsatisfactory (usually because of absence of the core section in the TMA) and were excluded from further analysis. To assess the reliability of 14-3-3 ζ staining level determination, cores were evaluated independently by a second reader. Final tumor cytoplasmic and nuclear 14-3-3 ζ scores could be determined for 476 and 475 patients, respectively. Agreement was generally good for both cytoplasmic and nuclear scores (Table 1).

Statistical Analysis

Analytic Variables

14-3-3 ζ Staining: Cytoplasmic and nuclear staining were categorized in two ways: (1) as a dichotomous variable of low staining versus high staining, for which high staining was defined as a final H-score above the 50th percentile and low staining as a final H-score at or below the 50th percentile; and (2) as a four-category variable of quartiles to assess dose-response. Tumors above the 50th percentile for both nuclear and cytoplasmic 14-3-3 ζ staining were classified as having combined 14-3-3 ζ staining, compared with tumors at or below the 50th percentile for cytoplasmic staining, nuclear staining, or both. Tumors were classified into four categories of combined 14-3-3 ζ staining: 1) tumors above the 75th percentile for cytoplasmic and nuclear staining; 2) tumors with values above the 50th percentile for both cytoplasmic and nuclear staining but not above the 75th percentile for both; 3) tumors with values above the 25th percentile for cytoplasmic and nuclear staining but not above the 50th percentile for both; 4) any tumor with cytoplasmic or nuclear staining at or below the 25th percentile. When scoring cores, it was observed that sections from the different TMAs used in the study showed variation in staining intensity between slides. To address concerns of non-differential misclassification of 14-3-3 ζ staining level because of variable staining intensity, patients were categorized based on their percentile in the section with their tumor cores, not across all tumor cores.

Recurrence: Information on recurrences was obtained from the DBCG. The DBCG defines a recurrence as any breast cancer or distant metastasis diagnosed after the initial course of therapy. In our study population, cases were defined as having a recurrence occurring within 1 to 10 years after the initial diagnosis.

Covariates: Covariates were UICC stage, grade, menopausal status at diagnosis, receipt of chemotherapy, receipt of radiotherapy, surgery type (mastectomy versus breast-conserving surgery), diagnosis year, age at diagnosis, and county of residence.

Conventional Analysis—All analyses were stratified by ER/TAM grouping to evaluate whether the association between 14-3-3 ζ expression and breast cancer recurrence is predictive of tamoxifen resistance, prognostic of breast cancer recurrence, or neither [24]. The frequency and proportion of cases and controls were calculated within categories of 14-3-3 ζ cytoplasmic, nuclear, and combined expression and within all categories of covariates. Conditional logistic regression was used to estimate the measure of association between breast cancer recurrence, the outcome, and 14-3-3 ζ staining level, conditioned on the matched factors. Measures of association also were estimated using logistic regression with adjustment for UICC stage, grade, menopausal status, receipt of chemotherapy, receipt of radiotherapy, surgery type (mastectomy versus breast-conserving surgery), diagnosis year, age at diagnosis, and county of residence. All analyses were performed using SAS 9.3 (SAS Institute Inc., Cary, NC). The study was approved by the Danish Data Protection Agency (record number 2012-41-4979) and the by the Danish Ethical Committee (record no 1-10-72-16-15).

Estrogen Receptor Re-assay—DBCG recommendations for assays evolved during the years covered by the study [25], as IHC methods for ER α status determination improved. To account for improvements in ER α determination and potential variability across diagnosing hospitals, ER α status was centrally re-assayed from the original tumor of all patients, as described in a previous study [26].

Validation Substudy and Quantitative Bias Analysis—To test the validity of using TMA IHC instead of whole section IHC, we scored randomly sampled whole sections, estimated sensitivity and specificity values for cytoplasmic, nuclear, and combined 14-3-3 ζ staining level, and performed a quantitative bias analysis (Supplementary Materials and Methods).

Results

Descriptive Statistics

The majority of patients in the study population had Stage II (48%) or Stage III (48%) disease at diagnosis (Table 2), this being consequence of the DBCG criteria for tamoxifen therapy during the period of diagnosis of the study population [19]. Among ER+/TAM+ patients, the majority were aged 55–65 years (52%), whereas the most common age in ER–/TAM– patients was 45–54 years (39%). 85% of the study population underwent mastectomy and 39% had radiation therapy. 12% of ER+/TAM+ patients received chemotherapy, compared with 63% of the ER–/TAM– patients, consistent with indications for breast cancer treatment in these groups. Our study population consisted of patient diagnoses from 1985 to 2001; 40% of diagnoses occurred between 1985 and 1993, 23% occurred between 1994 and 1996, and 37% occurred between 1997 and 2001. Among ER+/TAM+ patients, 47% were assigned a 1-year tamoxifen protocol, 18% had a 2-year

protocol, and 35% had a protocol lasting 5 or more years. Medical records indicated that many patients initially assigned to a 1- or 2-year tamoxifen had longer durations of therapy [27].

Conventional Results

Among ER+/TAM+ patients, the association between breast cancer recurrence and cytoplasmic 14-3-3 ζ staining above the 50th percentile, compared with below, was near null (adjusted OR = 1.22, 95% CI: 0.94, 1.60; Table 3). For the same comparison, a near null association was also seen among ER-/TAM- patients (adjusted OR = 1.09, 95% CI: 0.74, 1.62). Regarding the association between breast cancer recurrence and 14-3-3 ζ nuclear staining above the 50th percentile, compared with below, results were comparable with the association for cytoplasmic staining in ER+/TAM+ patients (adjusted OR = 1.21, 95% CI: 0.92, 1.60). However, the OR was higher in ER-/TAM- patients (adjusted OR = 1.33, 95% CI: 0.90, 1.96). For patients with combined cytoplasmic and nuclear 14-3-3 ζ tumor staining above the 50th percentile, there was an association in the ER+/TAM+ group (adjusted OR = 1.44, 95% CI: 1.05, 1.99), but an association nearer to null in the ER-/TAM- group (adjusted OR = 1.22, 95% CI: 0.82, 1.82).

In our dose-response analysis, cytoplasmic, nuclear, and combined 14-3-3 ζ staining generally showed near null associations for recurrence above the 25th percentile up to the 50th percentile and above the 50th percentile up to the 75th percentile. However, there was an increased odds of recurrence for staining above the 75th percentile (Table 4). A nearly two-fold increase in odds of recurrence was observed in above 75th percentile staining of combined 14-3-3 ζ , both for ER+/TAM+ patients (adjusted OR = 1.93, 95% CI: 1.15, 3.24) and ER-/TAM- patients (adjusted OR = 1.93, 95% CI: 1.03, 3.62). In the dose-response analysis all estimated measures of association had confidence intervals that overlapped substantially between ER+/TAM+ patients and ER-/TAM- patients. All results were similar when the ER/TAM grouping was based on the results of the ER α re-assay. The results of the quantitative bias analysis indicate that use of TMA IHC instead of whole section IHC does not completely explain any non-null association with breast cancer recurrence (Supplementary Results; Supplementary Table 1).

Discussion

In this population-based study, associations between breast cancer recurrence and tumor cytoplasmic or nuclear 14-3-3 ζ staining were near null. When we examined patients who had tumors with cytoplasmic and nuclear 14-3-3 ζ staining, the adjusted association between combined 14-3-3 ζ expression and recurrence increased in ER+/TAM+ patients, but the confidence intervals widely overlapped those of corresponding estimates in ER-/TAM- patients. These results caution against any interpretation of 14-3-3 ζ tumor expression as a predictive marker of tamoxifen resistance, because any association would be limited to the ER+/TAM+ group. In a dose-response analysis, the confidence intervals between the ER+/TAM+ group and the ER-/TAM- group also widely overlapped in all categories of 14-3-3 ζ staining, further suggesting a lack of association between 14-3-3 ζ and an ER α -dependent pathway.

A nearly two-fold increase in odds of recurrence was observed for combined 14-3-3 ζ tumor staining above the 75th percentile in both ER+/TAM+ and ER-/TAM- patients, indicating its possible utility as a prognostic marker of breast cancer recurrence [24]. In the study population, 5.8% of ER+/TAM+ patients and 13.0% of ER-/TAM- patients had this level of tumor 14-3-3 ζ expression.

This study is the largest to date to examine the relation of 14-3-3 ζ tumor staining and breast cancer recurrence. In order to look for possible differential effects of 14-3-3 ζ localization, staining in both the nucleus and cytoplasm was analyzed. Analyses were stratified by ER/TAM status to differentiate 14-3-3 ζ as a marker predictive of tamoxifen resistance as opposed to one prognostic of breast cancer recurrence. Bias resulting from the use of TMA IHC compared with whole section IHC was accounted for in an analysis controlling for both misclassification and confounding, using internal data from a validation substudy. Concordance between ER α status determined at diagnosis and during central re-assay was good and results were similar when ER/TAM grouping was determined by the results of the re-assay. The use of a population-based registry, containing nearly all cases under age 70 at diagnosis and linked to tumor archives, provides results likely devoid of selection bias.

Inter-rater agreement was good for determination of both cytoplasmic and nuclear 14-3-3 ζ staining level. Recurrences were confirmed previously in a validation study using medical record review, thus eliminating bias because of misclassification of the outcome [28]. All covariates showed perfect agreement, with the exception of menopausal status in a single patient. Review of medical records did identify discrepancies regarding the duration of tamoxifen therapy, with the DBCG often indicating shorter durations of therapy expected at diagnosis compared with the medical record of actual duration, probably because patients and providers switched to longer protocols during the study period as evidence grew favoring a 5-year protocol. However, this strengthens our results, as patients receiving tamoxifen therapy longer than indicated by DBCG are less likely to have a recurrence due to lack of therapy.

A limitation of this study is ascertaining a cutoff for positivity, as staining will be observed in all samples when examining ubiquitous genes such as 14-3-3 ζ . Also, the level of staining that showed the strongest odds of recurrence had the smallest sample sizes, resulting in poorer precision compared with other estimates of association. Another limitation of this study was the variable staining intensity observed in sections from different TMA blocks used for IHC. To allow for this, percentile boundaries were determined within a patient's TMA section as opposed to the entire cohort, but in doing so, percentile boundaries were based on a smaller number of samples.

This study also lacks information on adherence to tamoxifen therapy. In a previous study, 20 ER+/TAM+ patients from this cohort underwent medical record review and six were found to have not completed their intended duration of tamoxifen therapy, two because of a recurrent breast cancer [27].

The results of our study serve to estimate precisely the association between 14-3-3 ζ tumor expression and breast cancer recurrence, and to differentiate potential predictive and

prognostic utility. In spite of findings from previous studies, evidence is lacking to conclude that 14-3-3 ζ is a useful marker of tamoxifen resistance. We found high levels of combined 14-3-3 ζ tumor staining to be a potentially useful prognostic marker of breast cancer recurrence in both ER+/TAM+ and ER-/TAM- patients. Research is needed to determine if 14-3-3 ζ has independent utility in a clinical setting beyond established prognostic markers of breast cancer recurrence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Design used to select the study sample and determine grouping based on the inclusion criteria. The source population consisted of 11,251 female residents of the Jutland Peninsula in Denmark aged 35–69 years who were diagnosed with stage I, II, or III breast cancer between 1985 and 2001. Most of the women (n = 4363) excluded because of unknown protocol had stage I breast cancer treated without a guideline protocol from the Danish Breast Cancer Cooperative Group. 14-3-3 ζ results were missing if tissue was not available or if tumor core was unsatisfactory after processing, staining and imaging. ER α re-assay results were missing if tissue was not available or if assay results were indeterminate. ER α = estrogen receptor α .

Figure 2.

Determination of 14-3-3 ζ expression level by scanning at 40 $\times$ magnification in Panoramic Viewer to evaluate invasive epithelial cells and differentiate subcellular location of staining. This core was scored as 55% light staining and 45% moderate staining for the cytoplasm and light staining for the nucleus.

Table 1

Inter-rater agreement of cytoplasmic and nuclear 14-3-3ζ expression above the 50th percentile.*

		Observer 1			
		Cytoplasmic 14-3-3ζ expression		Nuclear 14-3-3ζ expression	
Observer 2	Expression level	50 th percentile or lower	Above the 50 th percentile	50 th percentile or lower	Above the 50 th percentile
	50 th percentile or lower	184	32	221	60
	Above the 50 th percentile	55	205	37	157

* Scores were determined independently by two observers and compared. Cytoplasmic and nuclear 14-3-3ζ expression levels were determined for 476 and 475 patients, respectively.

Table 2

Characteristics of breast cancer recurrence cases and controls by ER/TAM group.*

Patient characteristic	ER+/TAM+, No. (%)		ER-/TAM-, No. (%)	
	Case patients	Control subjects	Case patients	Control subjects
Cytoplasmic 14-3-3 ζ expression				
50 th percentile or lower	246 (55)	266 (60)	99 (39)	102 (40)
Above the 50 th percentile	202 (45)	179 (40)	155 (61)	151 (60)
Missing ^f	93	96	46	47
Nuclear 14-3-3 ζ expression				
50 th percentile or lower	273 (61)	294 (66)	117 (46)	130 (52)
Above the 50 th percentile	175 (39)	151 (34)	140 (54)	120 (48)
Missing ^f	93	96	46	47
Combined 14-3-3 ζ expression				
50 th percentile or lower	327 (73)	353 (79)	156 (61)	161 (64)
Above the 50 th percentile	121 (27)	92 (21)	101 (39)	89 (36)
Missing ^f	93	96	46	47
Diagnosis year				
1985 – 1993	235 (43)	234 (43)	107 (36)	100 (33)
1994 – 1996	113 (21)	112 (21)	81 (27)	83 (28)
1997 – 2001	193 (36)	195 (36)	112 (37)	117 (39)
Age category at diagnosis, y				
35 – 44	16 (3.0)	13 (2.4)	68 (23)	58 (19)
45 – 54	116 (21)	111 (21)	120 (40)	113 (38)
55 – 64	286 (53)	281 (52)	82 (27)	86 (29)
65 – 69	123 (23)	136 (25)	30 (10)	43 (14)
Menopausal Status at diagnosis ^g				
Premenopausal	34 (6.3)	34 (6.3)	121 (40)	121 (40)
Postmenopausal	507 (94)	507 (94)	179 (60)	179 (60)
UICC tumor stage at diagnosis ^g				
I	9 (1.7)	9 (1.7)	25 (8.3)	25 (8.3)

Patient characteristic	ER+/TAM+, No. (%)		ER-/TAM-, No. (%)	
	Case patients	Control subjects	Case patients	Control subjects
II	250 (46)	250 (46)	153 (51)	153 (51)
III	282 (52)	282 (52)	122 (41)	122 (41)
Histological grade				
I	108 (25)	144 (35)	27 (11)	23 (10)
II	234 (54)	215 (52)	125 (49)	98 (43)
III	92 (21)	57 (14)	103 (40)	106 (47)
Missing	107	125	45	73
Surgery type				
Breast-conserving surgery	58 (11)	71 (13)	47 (16)	56 (19)
Mastectomy	483 (89)	470 (87)	252 (84)	244 (81)
Missing	0	0	1	0
Radiation therapy				
Yes	183 (34)	191 (35)	128 (47)	123 (47)
No	358 (66)	350 (65)	166 (56)	137 (53)
Missing	0	0	6	40
Tamoxifen protocol, years				
1	257 (48)	261 (48)		
2	98 (18)	92 (17)		
5	186 (34)	188 (34)		
Systemic adjuvant chemotherapy				
Yes	70 (13)	65 (12)	248 (83)	188 (63)
No	471 (87)	476 (88)	52 (17)	112 (37)
Current ER expression				
Positive	451 (92)	474 (96)	72 (26)	70 (25)
Negative	37 (7.6)	19 (3.9)	204 (74)	205 (75)
Not available	53	48	24	25

* The source population consisted of 11,251 female residents of the Jutland Peninsula in Denmark aged 35–69 years who were diagnosed with stage I, II, or III breast cancer between 1985 and 2001. Subjects were estrogen receptor (ER) α positive and received at least 1 year of tamoxifen therapy (ER+/TAM+) or were ER α negative, and never received tamoxifen therapy and survived at least 1 year after diagnosis (ER-/TAM-).

UICC = Union for International Cancer Control

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No tissue available or tissue material unsatisfactory after processing, staining, and imaging.
^f
^g Variable included in risk set sampling to match control subjects to case patients.

Table 3

Associations between 14-3-3 ζ expression and breast cancer recurrence by ER/TAM group.*

14-3-3 ζ expression	ER+/TAM+				ER-/TAM-			
	Case patients	Control subjects	Matched OR (95% CI) ^f	Adjusted OR (95% CI) ^g	Case patients	Control subjects	Matched OR (95% CI) ^f	Adjusted OR (95% CI) ^g
Cytoplasmic 14-3-3 ζ expression								
50 th percentile or lower	246	266			99	102		
Above the 50 th percentile	202	179	1.27 (0.95, 1.70)	1.22 (0.94, 1.60)	155	151	0.95 (0.65, 1.37)	1.09 (0.74, 1.62)
Nuclear 14-3-3 ζ expression								
50 th percentile or lower	273	294			117	130		
Above the 50 th percentile	175	151	1.21 (0.89, 1.63)	1.24 (0.95, 1.64)	140	120	1.38 (0.95, 2.01)	1.31 (0.89, 1.92)
Combined 14-3-3 ζ expression								
50 th percentile or lower	327	353			156	161		
Above the 50 th percentile	121	92	1.51 (1.08, 2.11)	1.44 (1.05, 1.98)	101	89	1.22 (0.82, 1.80)	1.22 (0.82, 1.82)

* The source population consisted of 11,251 female residents of the Jutland Peninsula in Denmark aged 35–69 years who were diagnosed with stage I, II, or III breast cancer between 1985 and 2001.

Subjects were estrogen receptor (ER) α positive and received at least 1 year of tamoxifen therapy (ER+/TAM+) or were ER α negative, and never received tamoxifen therapy and survived at least 1 year after diagnosis (ER-/TAM-).

OR = odds ratio; CI = confidence interval; UICC = Union for International Cancer Control

^f Estimated using conditional logistic regression with conditioning on the matched factors (time to recurrence or control selection, county of residence, menopausal status, and stage).^g Estimated using logistic regression with adjustment for UICC stage, menopausal status, receipt of chemotherapy, receipt of radiotherapy, surgery type, diagnosis year, age at diagnosis, and county of residence.

Table 4

Analysis of a dose-response between 14-3-3 ζ expression and breast cancer recurrence by ER/TAM group.*

14-3-3 ζ expression	ER+/TAM+				ER-/TAM-			
	Case patients	Control subjects	Matched OR (95% CI) [†]	Adjusted OR (95% CI) [‡]	Case patients	Control subjects	Matched OR (95% CI) [†]	Adjusted OR (95% CI) [‡]
Cytoplasmic 14-3-3 ζ expression								
25 th percentile or lower	136	142			54	51		
Above the 25 th percentile up to the 50 th percentile	110	124	0.80 (0.54, 1.20)	0.95 (0.67, 1.35)	48	48	1.03 (0.55, 1.94)	0.98 (0.54, 1.79)
Above the 50 th percentile up to the 75 th percentile	104	107	0.99 (0.67, 1.47)	1.04 (0.72, 1.49)	57	67	0.93 (0.53, 1.62)	0.88 (0.49, 1.56)
Above the 75 th percentile	98	72	1.43 (0.93, 2.21)	1.43 (0.97, 2.11)	98	84	0.98 (0.59, 1.65)	1.26 (0.74, 1.56)
Nuclear 14-3-3 ζ expression								
25 th percentile or lower	169	172			77	83		
Above the 25 th percentile up to the 50 th percentile	104	122	0.64 (0.43, 0.95)	0.87 (0.62, 1.23)	40	47	0.99 (0.54, 1.82)	0.80 (0.45, 1.42)
Above the 50 th percentile up to the 75 th percentile	74	84	0.79 (0.52, 1.21)	0.88 (0.60, 1.29)	60	64	1.10 (0.66, 1.83)	0.94 (0.56, 1.56)
Above the 75 th percentile	101	67	1.29 (0.85, 1.94)	1.57 (1.07, 2.30)	80	56	1.72 (1.03, 2.87)	1.57 (0.93, 2.63)
Combined 14-3-3 ζ expression								
25 th percentile or lower	221	224			97	102		
Above the 25 th percentile up to the 50 th percentile	106	129	0.71 (0.50, 1.00)	0.84 (0.61, 1.17)	59	59	1.15 (0.69, 1.89)	1.05 (0.64, 1.61)
Above the 50 th percentile up to the 75 th percentile	73	66	1.17 (0.78, 1.77)	1.14 (0.77, 1.68)	55	57	1.06 (0.63, 1.77)	0.98 (0.59, 1.61)
Above the 75 th percentile	48	26	1.77 (1.01, 3.11)	1.93 (1.15, 3.24)	46	32	1.64 (0.92, 2.94)	1.93 (1.03, 3.62)

* The source population consisted of 11,251 female residents of the Jutland Peninsula in Denmark aged 35–69 years who were diagnosed with stage I, II, or III breast cancer between 1985 and 2001. Subjects were estrogen receptor (ER) α positive and received at least 1 year of tamoxifen therapy (ER+/TAM+) or were ER α negative, and never received tamoxifen therapy and survived at least 1 year after diagnosis (ER-/TAM-).

OR = odds ratio; CI = confidence interval; UICC = Union for International Cancer Control.

[†] Estimated using conditional logistic regression with conditioning on the matched factors (time to recurrence or control selection, county, menopausal status, and stage).

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Estimated using logistic regression with adjustment for UICC stage, menopausal status, receipt of chemotherapy, receipt of radiotherapy, surgery type, diagnosis year, age at diagnosis, and county of residence.