Increased trace amine-associated receptor 1 (TAAR1) expression is associated with a positive survival rate in patients with breast cancer

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Abstract

Purpose A correlation between breast cancer and thyroid disorders has been described in previous studies. Degraded thyroid hormones are referred to as trace amines. These endogenous amines have the ability to bind to the G-protein-coupled receptor TAAR1 (trace amine-associated receptor) and thereby activate it. TAAR1 is able to modulate the serotonergic and dopaminergic system in the brain and has so far been studied in the neurological field. The following study represents the first investigation of the regulation of TAAR1 in primary breast cancer (no metastases, M0).

Methods Immunohistochemical analyses were carried out to detect TAAR1 expression in formalin fixed paraffin embedded breast cancer samples. Survival times of primary breast cancer patients (M0) with and without TAAR1 expression in their tumours were compared by Kaplan–Meier curves, and correlations between ordinal variables were determined with Spearman's rank correlation coefficient.

Results The investigation showed a correlation between TAAR1 expression and tumour differentiation grade. A well differentiated tumour grade (G1) was associated with higher TAAR1 expression and HER2 and HER4 positivity predicted higher TAAR1 expression. A TAAR1 overexpression (IRS $\geq$ 6) was associated with significantly longer overall survival (OS) ($p = 0.02$) than that of reduced TAAR1 expression (IRS $<$ 6) during a maximum follow-up of 14 years, demonstrating that TAAR1 has a favourable effect on OS of early breast cancer patients.

Conclusions We conclude that TAAR1 seems to be an independent predictor for breast cancer survival. Modulation of TAAR1 may represent a novel targeting strategy for breast cancer prevention and therapy.

Keywords TAAR1 (trace amine-associated receptor 1) · Breast cancer · Trace amines · L-Dopa decarboxylase · HER2 · HER4

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Purpose

Breast cancer is the most common malignant tumour in female patients worldwide and one of the three most common cancers worldwide along with colon- and lung cancer (Harbeck and Gnant 2017). In 2012, about 1.7 million patients worldwide were diagnosed with breast cancer, and almost half a million people died from it (Torre et al. 2015; Ferlay et al. 2015). There is evidence that breast cancer and thyroid disorders correlate and patients with thyroid dysfunctions show higher incidences of breast cancer in comparison to healthy controls (Türken et al. 2003; Rasmusson et al. 1987; Kuijpens

et al. 2005). However, the detailed role of thyroidal hormones in breast cancer is still unclear, and future investigations are necessary (Heublein et al. 2015). In a recently published study, an increased risk of breast cancer in women with hyperthyroidism and a decreased risk in women with hypothyroidism could be shown, thereby indicating an association between thyroidal function and breast cancer risk (Sogaard et al. 2016). Furthermore, fT4 and TSH levels seem to be predictive for therapeutic response and prognosis of patients with recurrent breast cancer (Yokoe et al. 1996). In a prospective study, Ditsch et al. (2010) showed that blood levels of fT_3 and fT_4, and concentrations of antibodies against thyroidal peroxidase and TSH are significantly elevated at the time point of primary diagnosis of breast cancer.

Thyroid hormones bind to the thyroid hormone receptor (THR), which is a member of the nuclear receptor superfamily. Nuclear receptors play a role in numerous physiological processes, and they act via transcriptional cis-regulation of target genes (Pestka et al. 2013). Thyroid hormones regulate numerous genes that are involved in cell differentiation, proliferation and apoptosis (Perri et al. 2014; Chi et al. 2013). Ditsch et al. (2013) analysed THR expression in breast cancer patients. The isoform THRα2 was found to be significantly associated with prognostic histopathological parameters such as tumour size, axillary lymph node involvement, tumour grade and hormone receptor status (Ditsch et al. 2013). Via a multivariate analysis, a trend for THRα2 as an independent predictor of disease-free and overall survival could be shown (Ditsch et al. 2013). Hence, patients with a higher expression of THRα2 in their tumours have a better prognosis than patients with low THRα2 levels (Ditsch et al. 2013). In addition, THRβ is a positive prognostic factor for overall survival at 5 years and overall survival (OS), while THRα positivity predicts a reduced overall survival at 5 years (Heublein et al. 2015). Heublein et al. (2015) assume that THRα and THRβ represent interesting alternative targets for endocrine treatment of *BRCA1* associated triple negative breast cancer.

The degradation of thyroid hormones results in the formation of trace amines (TAs), which are endogenous amines (Harmeier et al. 2015). The enzyme L-dopa-decarboxylase, also known as aromatic amino acid decarboxylase, is involved in the transformation of thyroxine (T4) into 3-iodothyronamine (T1AM) (Sumi-Ichinose et al. 1992; Le Van Thai et al. 1993). During the transformation of thyroxine into 3-iodothyronamine, a deiodisation and a decarboxylation take place (Sumi-Ichinose et al. 1992; Le Van Thai et al. 1993). The enzyme L-dopa-decarboxylase is also involved in the synthesis of dopamine, serotonin, tryptamine, phenylethylamine and histamine (The Human Metabolome Database 2016). A deficiency in L-dopa-decarboxylase (AADC) is associated with severe developmental delay, oculogyric crises and autonomic dysfunction (Pons et al. 2004).

Classical thyroid hormones act rather epigenetically, while decarboxylated thyroid hormones act in the opposite direction through rapid actions, such as rapid lowering of body temperature and heart rate (Brix et al. 2011). Future investigations are necessary to further clarify the physiological role of 3-iodothyronamine-TAAR1 signalling (Wang et al. 2014). Up to now, no data exist in the current literature about the influence of thyroid hormone dérivâtes and their receptors on breast cancer outcome.

Thyronamines, the thyroid hormone dérivâtes, function via the activation of a G-protein coupled receptor, called trace amine-associated receptor 1 (TAAR1) (Brix et al. 2011). TAAR1 was first described in 2001 by Borowsky et al. (2001). Upon activation of adenylcyclase, the receptor induces a rise of intracellular cAMP level (Borowsky et al. 2001; Barak et al. 2008). TAAR1 is expressed by numerous organs such as the placenta, brain, spinal cord, stomach, pancreatic β-cells and in immune cells like macrophages, leukocytes and dendritic cells (Lam et al. 2015; Gozal et al. 2014; Babusyte et al. 2013). TAAR1 can be activated by different compounds, including dopaminergic, adrenergic and serotonergic classes (Sotnikova et al. 2009). A wide spectrum of agonists, such as trace amines, common biogenic amines, amphetamine-like stimulants and thyroid hormone dérivâtes like 3-iodothyronamine have the ability to activate TAAR1 (Scanlan et al. 2004; Bunzow et al. 2001).

So far, TAAR1 has been investigated in the neurological field as TAAR1 is able to modulate the serotonergic and dopaminergic system in the brain (Lam et al. 2015). As TAAR1 is a negative regulator of dopamine transmission, it represents a target for neuropsychiatrie disorders such as depression and Parkinson's disease (Lam et al. 2015). Furthermore, stimulation of TAAR1 has a beneficial effect on glucose- and obesity control in mice with type-2 diabetes mellitus (Raab et al. 2016).

In addition to thyroid hormones, ErbB receptors play an important role in the pathogenesis of breast cancer (Karamouzis et al. 2007). The proto-oncogene ErbB receptors are members of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (Naresh et al. 2006), including ERBB2/HER2/neu (ERBB2), ERBB3 and ERBB4. They can influence cell proliferation, survival and differentiation (Karamouzis et al. 2007; Zoi et al. 2016). ERBB2 is responsible for the progression of primary tumours, and its expression is associated with poor prognosis of breast cancer patients (Yarden and Sliwkowski 2001; Tan and Yu 2007). In recent years, especially ERBB2 has become an important biomarker and target in the treatment of breast cancer (Mitri et al. 2012; Witzel and Muller 2015). Treatment with the monoclonal antibody

Trastuzumab (Herceptin) is recommended in patients with HER2-positive early breast cancer for 1 year (Mates et al. 2015). In June 2012, the FDA approved the monoclonal antibody Pertuzumab, which inhibits the dimerization between HER2 and HER3 receptors, as a combination therapy together with Trastuzumab in metastatic breast cancer (USFaD Administration 2016).

The aim of our study was the analysis of TAAR1 expression in breast cancer tissue and the correlation of TAAR1 expression with survival of breast cancer patients. An additional aim was the assessment of the association of TAAR1 expression with the expression of ErbB receptors. As TAAR1 has not been described in breast cancer tissue before, this investigation represents the first analysis of TAAR1 regulation in breast cancer.

Table 1 Patients' characteristics

	n	%
Histology		
NST	131	57.7
Non NST	96	42.3
Tumour grade		
G1, G2	103	45.4
G3	53	23.3
NA (not available)	71	31.3
pT		
pT1	154	67.8
pT2–pT4	72	31.7
NA	1	0.5
pN		
pN0	122	53.7
pN1	93	41.0
NA	12	5.30
CIS		
No	107	47.1
Yes	120	52.9
ER		
Negative	30	13.2
Positive	175	77.1
NA	22	9.7
PR		
Negative	62	27.3
Positive	128	56.4
NA	37	16.3
HER2		
Negative	160	70.5
Positive	20	8.8
NA	47	20.7
HER4		
Negative	201	88.6
Positive	16	7.0
NA	10	4.4
TAAR1		
Negative	95	41.9
Positive	127	55.9
NA	5	2.2
Age		
≤55 years	90	39.6
>55 years	136	60.0
nNA	1	0.4

In total, 227 patients were included in the study. All patients are M0 (no metastases)

Methods

Patients' and specimens' characteristics

Formalin fixed paraffin embedded (FFPE) primary breast cancer samples of 227 patients (all M0) who had undergone surgery for a malignant breast tumour at the Department of Gynaecology and Obstetrics, Ludwig-Maximilians-University Munich, Germany, from 1998 until 2000 were included in the study. Women with benign tumours of the breast were excluded from the study. Histopathological tumour subtypes were assigned according to the WHO criteria, and tumour grade (G1, 2, 3) was determined according to the Elston and Ellis criteria (1993) by a gynaecological pathologist (Table 1). Tumour grade is a measure of the cell appearance and increases with the lack of cellular differentiation. The grading system includes G1: well differentiated, G2: moderately differentiated and G3: poorly differentiated. Clinical and follow-up data regarding patient age, overall survival and relapse free survival, lymph node status, presence of metastases, ER/PR results and HER2 and HER4 detection were retrieved from patients' charts and from the Munich Cancer Registry. HER2 positivity is clearly defined by the DAKO Scoring system (DAKO, *HER2 FISH pharmDx™ Kit*). As HER2 status was not determined routinely in Germany before 2001, it was retrospectively assessed for patients who had surgery before 2001. HER2 status was determined as recommended in the national guidelines, i.e., by DAKO Score and FISH analysis in cases of DAKO 2+.

Immunohistochemistry

Expression of the G-protein coupled receptor TAAR1 and the ErbB receptors HER2 and HER4 was identified immunohistochemically from the embedded breast cancer samples. Tissue samples were fixed in neutral-buffered formalin (3.7%) right after resection and they underwent standardised paraffin embedding. For immunohistochemistry, FFPE tissue sections (3 µm) were deparaffinised in xylol, rehydrated

in a descending ethanol gradient and prepared for epitope retrieval in a pressure cooker using sodium citrate buffer (pH 6.0). Next, sections were blocked with 3% H_2O_2 in methanol (20 min) at room temperature for the inactivation of the endogenous peroxidase. Blocking solution was applied for blocking of the non-specific binding of the primary antibodies. Sections were then incubated with the following primary antibodies consecutively: Anti-TAAR1 (polyclonal rabbit IgG, Abeam, Cambridge, UK), anti-HER2 (c-erbB-2 oncoprotein, polyclonal rabbit IgG, Dako, Santa Clara, USA), anti-HER4 (polyclonal rabbit IgG, Epitomics, Burlingame, USA) and phosphorylated anti-HER4 (polyclonal rabbit IgG, Epitomics, Burlingame, USA). Antibody reactivity was detected using the Vectastain Elite ABC-Kit (Vector Laboratories, Burlingame, USA) according to the manufacturer's protocol. In the following step, substrate and chromogen (3,3′-diaminobenzidine DAB; Dako, Glostrup, Denmark) were added to the slides, which were then counterstained with Mayer's acidic haematoxylin and cover slipped. Appropriate positive (samples from the sigma) and negative controls were included in each experiment (Fig. 1).

Breast cancer sections were examined by two independent observers using a Leitz Diaplan microscope (Leitz, Wetzlar, Germany). For each slide, tumour tissue was examined independently by applying the semiquantitative immunoreactive score (IRS) which optically estimates the intensity and distribution pattern of antigen expression (Remmele and Stegner 1987). The IRS is calculated by multiplying the percentage of positively stained cells (0: no staining; 1: ≤10% of the cells; 2: 11–50%; 3: 51–80%, 4: >80%) with the intensity of the cells' staining (0: none; 1: weak; 2: moderate; 3: strong). A receiver operating characteristic curve (ROC curve) was used to determine the cut-off level between TAAR1 overexpression and reduced TAAR1 expression. For identification of the cut-off level, the maximum difference between sensitivity and specificity was used. Images were taken with a CCD colour camera (JVC, Victor Company of Japan, Japan).

Statistical analysis

SPSS statistics 22 (Armonk, NY: IBM Corp.) was used for data analysis, p values lower than 0.05 were considered to be statistically significant. Survival times were compared by Kaplan–Meier analysis, and differences in the patients' overall survival times were tested for significance by Cox Mantel log-rank test. Group comparisons regarding ordinal analysis variables were tested with Mann–Whitney U tests or Kruskal–Wallis tests as appropriate. Correlations between ordinal variables were tested with Spearman's rank correlation coefficient. Cox-regression analysis was used to compare the risk of death in patients with and without TAAR1 expression when the effects of further factors were accounted for. Independent variables included in the Cox-regression model were TAAR1 expression (IRS > 6), histological subtype, tumour grade, tumour size, lymph node status, hormone receptor status (ER/PR), HER2

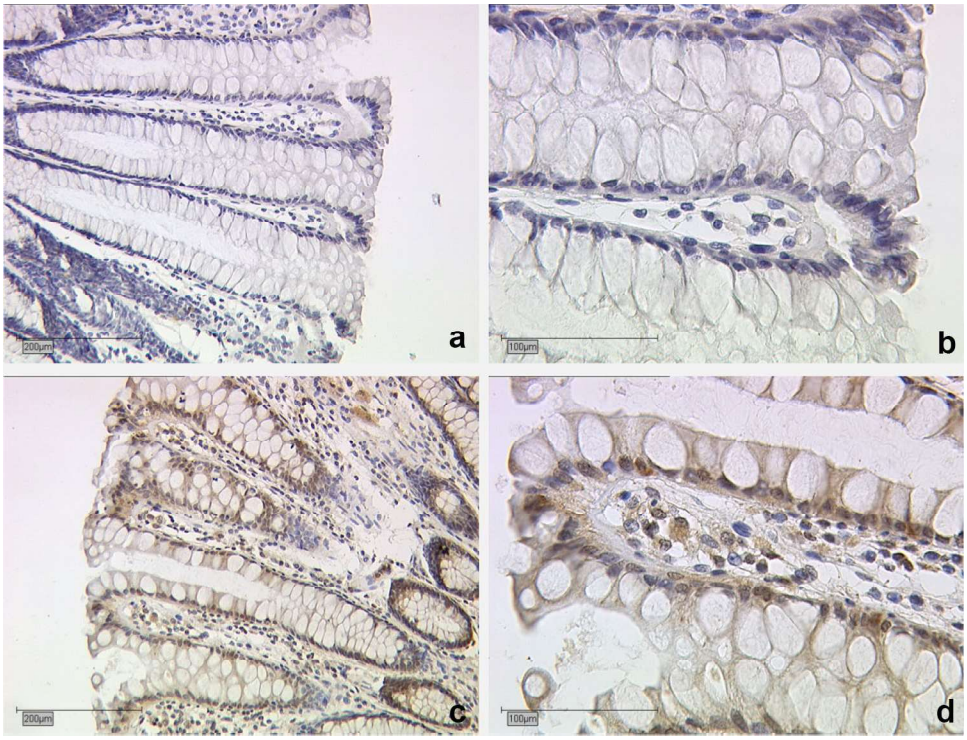

Fig. 1 Negative and positive controls of TAAR1 (Sigma). **a** Negative isotype control of TAAR1, sigma 1872-02; x10 magnification; **b** negative isotype control of TAAR1, sigma 1872-02; x25 magnification; **c** positive isotype control of TAAR1, sigma 1872-02; x10 magnification; **d** positive isotype control of TAAR1, sigma 1872-02; x25 magnification

status, HER4 and phosphorylated HER4 status and age at surgery.

Results

Clinical and histopathological data of the patient cohort

In total, 227 patients were included in the study. The clinical and histopathological data of the patient cohort are shown in Table 1. 90 patients (39.6%) were 55 years or younger, and 136 patients (60.0%) were older than 55. Most of the patients had a pT1 tumour size ($n = 154$; 67.8%) and the rest of the patients had a pT2–pT4 tumour ($n = 72$, 31.7%). Tumour size data of one patient was not available ($n = 1$, 0.5%). G1 or G2 tumour grade were found in 103 tumours (45.4%) and 53 tumours (23.3%) had a G3 differentiation grade. The tumour grade of lobular cancer was formerly not assessed; therefore, 71 tumours (31.3%) were not classified by tumour grade. 122 patients (53.7%) were lymph node negative in comparison to 93 patients (41.0%) with positive lymph nodes. The lymph node status of five patients (5.3%) was missing. With regard to ER/PR status, most patients had hormone receptor positive tumours (ER 77.1%, PR 56.4%). In total, 227 samples have been analysed according to the HER2-status. 23 samples were HER2-positive (10.1%), 181 samples (79.8%) were HER2-negative and 23 samples (10.1%) could not be assessed properly. The HER2-status was not assessed as part of the clinical routine; therefore, we analysed HER2 expression retrospectively in cooperation with the Institute of Pathology. 201 tumours were HER4-negative (88.6%), 16 tumours were HER4-positive (7.0%) and 10 tumours were not assessed (4.4%).

Expression of TAAR1 in different breast cancer subtypes and classification of TAAR1 expression by tumour grade

TAAR1 was detected in 127 cases (55.9%), and 95 cases (41.9%) showed no TAAR1 expression in their primary breast tumour. Five cases (2.2%) could not be assessed. In Table 2 the distribution of TAAR1 IRS score is shown. Figure 2 shows a boxplot analysis of TAAR1 expression (quantified by IRS) in different breast cancer subtypes of the collective. TAAR1 expression was most frequently found in breast cancer not otherwise specified (NST; $n = 126$, 55.6%), followed by invasive lobular cancer ($n = 47$, 20.7%), and medullary breast cancer ($n = 20$, 8.8%). This difference, however, was not significant. A median IRS of 4 of the TAAR1 expression was observed in invasive lobular and tubular cancer. TAAR1 expression with a higher median IRS of 8 was observed in the invasive-papillary and tubular-lobular breast cancer.

Table 2 Distribution of TAAR1 IRS-Scores

IRS	*n*	%
0	9	4.0
1	9	4.0
2	27	11.9
3	23	10.1
4	27	11.9
6	28	12.3
8	64	28.2
9	12	5.3
12	23	10.1
NA	5	2.2

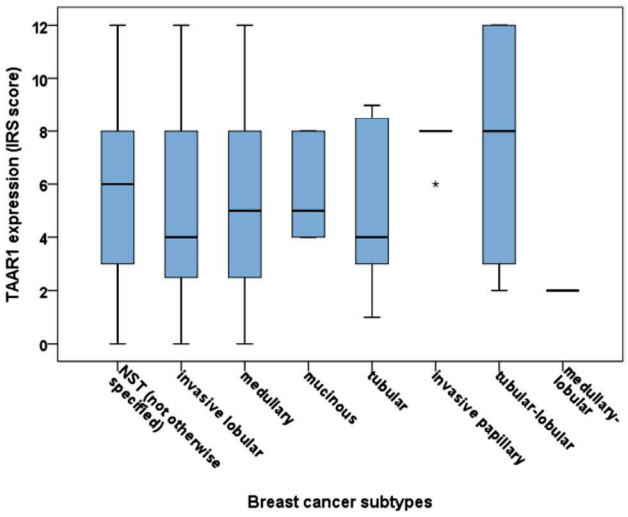

Fig. 2 Expression of TAAR1 in different breast cancer subtypes. NST, $n = 126$; invasive-lobular, $n = 47$; medullary, $n = 20$; mucinous, $n = 6$; tubular, $n = 7$; invasive-papillary, $n = 5$; tubular-lobular, $n = 10$; medullary-lobular, $n = 1$. Median TAAR1 expression is highest in NST (IRS 6) and the tubular-lobular subtype (IRS 8)

Furthermore, the association of TAAR1 expression and the tumour grade was tested. Seventeen (7.5%), 86 (37.9%) and 53 (23.3%) cases had a tumour grading of 1, 2 and 3, respectively (Fig. 3). TAAR1 expression correlated with tumour grade. Breast cancer samples with a tumour grade of 1 showed a median IRS TAAR1 staining of 8, whereas a median IRS TAAR1 staining of 6 was found in grade 2 as well as in grade 3 tumours.

Positive correlation of TAAR1 with HER2 and HER4 expression in breast cancer patients

For both HER receptors, a correlation with TAAR1 expression was identified. HER2-positive breast cancers

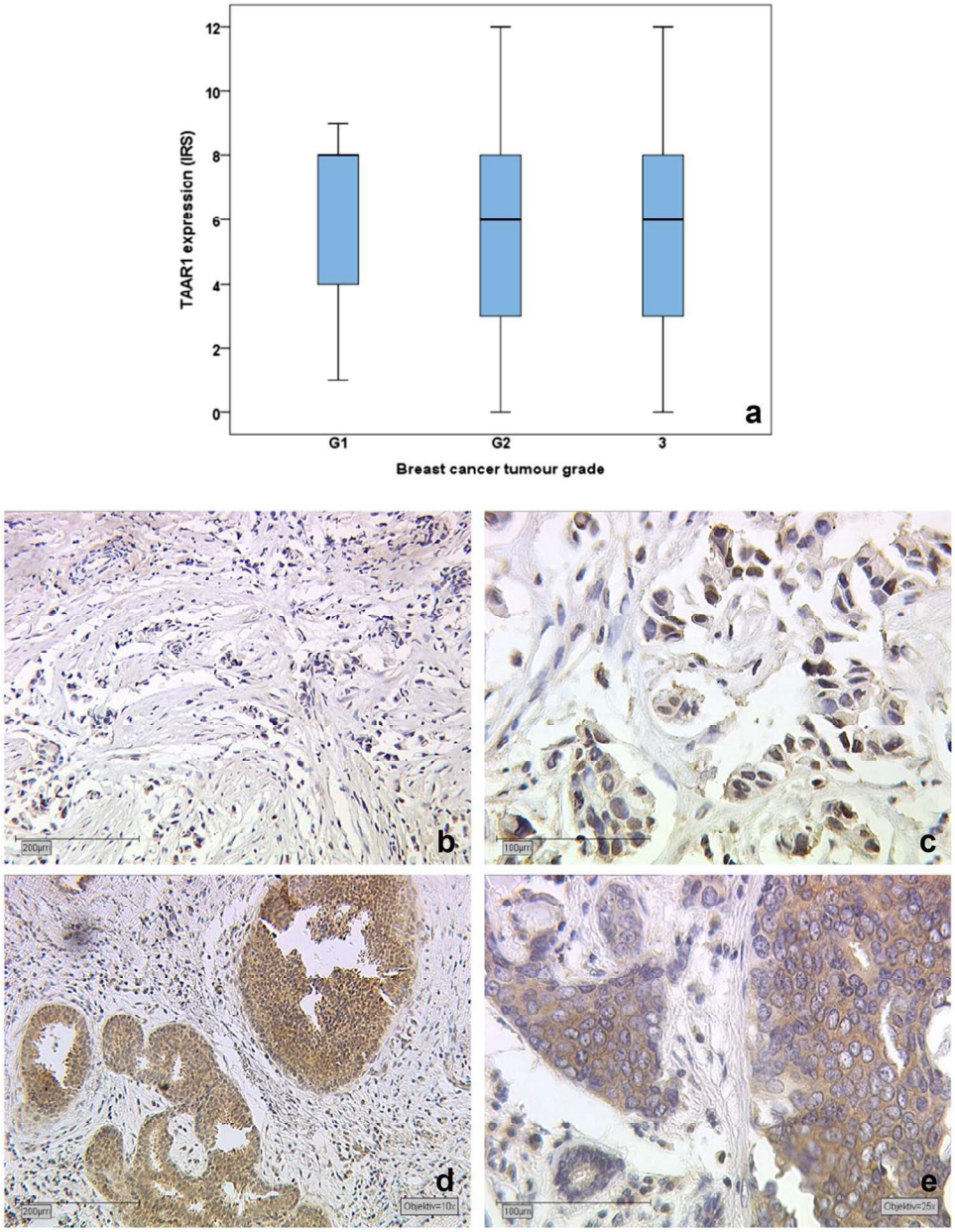

Fig. 3 **a** Expression of TAAR1 in different breast cancer types classified by tumour grade (1 ≙ G1, 2 ≙ G2, 3 ≙ G3); **b** TAAR1 nuclear expression in breast cancer, low expression, magnification x 10; **c** TAAR1 nuclear expression in breast cancer, low expression, magnification x25; **d** TAAR1 cytoplasmic expression in breast cancer, high expression, magnification x 10; **e** TAAR1 cytoplasmic expression in breast cancer, high expression, magnification x25

showed a significantly higher TAAR1 expression (median IRS 8; n ≙ 23) in comparison to HER2-negative tumours (median IRS 6; n ≙ 181; p ⩽ 0.001) (Fig. 4). Hence, HER2 positivity is associated with an increased expression of TAAR1.

In breast cancer patients with HER4-positive breast tumours, the same trend could be shown as demonstrated for HER2-positive tumours (Fig. 5). The median IRS TAAR1 was 4 in HER4-negative tumours (n = 108) and 8 in HER4-positive tumours (n ≙ 114) with a p value <0.001.

Association of TAAR1 expression (IRS ≥ 6) with a more favourable overall survival rate of breast cancer patients

Patients with a positive staining of TAAR1 in breast cancer tissue were compared to patients with a negative TAAR1 staining regarding their overall survival rate. Patients with tumours showing a TAAR1 IRS greater than or equal to 6 had a more favourable OS in comparison to patients with lower TAAR1 expression (p ≙ 0.04). The significant difference is shown in the Kaplan–Meier

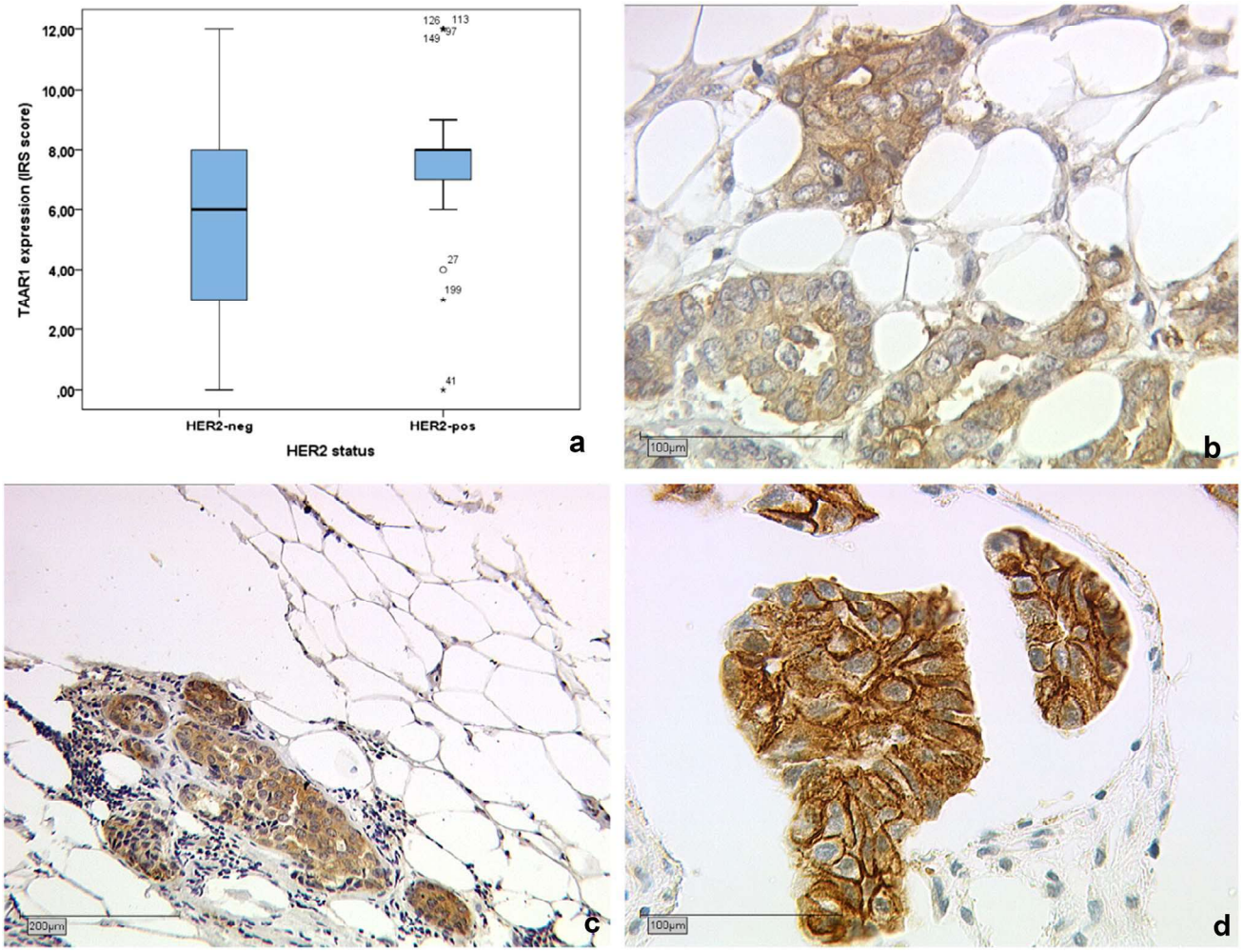

Fig. 4 Correlation of TAAR1 with HER2. **a** *Box plot* analysis of TAAR1 and HER2 correlation ($p < 0.001$); HER2-negative, $n = 181$; HER2-positive, $n = 23$. **b** TAAR1 expression, magnification x 10; **c** HER2 expression, magnification x 10; **d** HER2 expression, magnification x25

curve in Fig. 6. TAAR1 therefore seems to be a positive prognosticator for overall survival of breast cancer patients.

TAAR1 expression with an IRS ≤ 6 showed a median overall survival of 8 years with a box length between 4 and 13 years and TAAR1 IRS score ≥ 6 showed a median overall survival of 9 years with a box length between 7 and 13 years (Fig. 7).

According to the nodal status, a negative nodal status (pN0) showed a median overall survival of 10 years with a box length between 8 and 12 years and a positive nodal status (pN1) showed a median overall survival status showed a median overall survival of 8 years with a box length between 3 and 12 years (Fig. 8).

Cox-regression of histopathological variables and age on overall survival in breast cancer patients

Multivariate Cox-regression was performed to test which histopathological variables are independent prognosticators for the overall survival (OS) rate in the tested breast cancer collective. It could be shown that TAAR1 expression ($p = 0.021$), lymph node status (pN) ($p < 0.001$) and age at surgery ($p = 0.01$) are independent prognosticators for the overall survival (Table 3). Breast cancer tissue has also been assessed for ER and PR status and TAAR1 expression. For both ER ($p = 0.608$) and PR ($p = 0.597$) no significant correlation with TAAR1 expression could be detected. No effect could furthermore be demonstrated

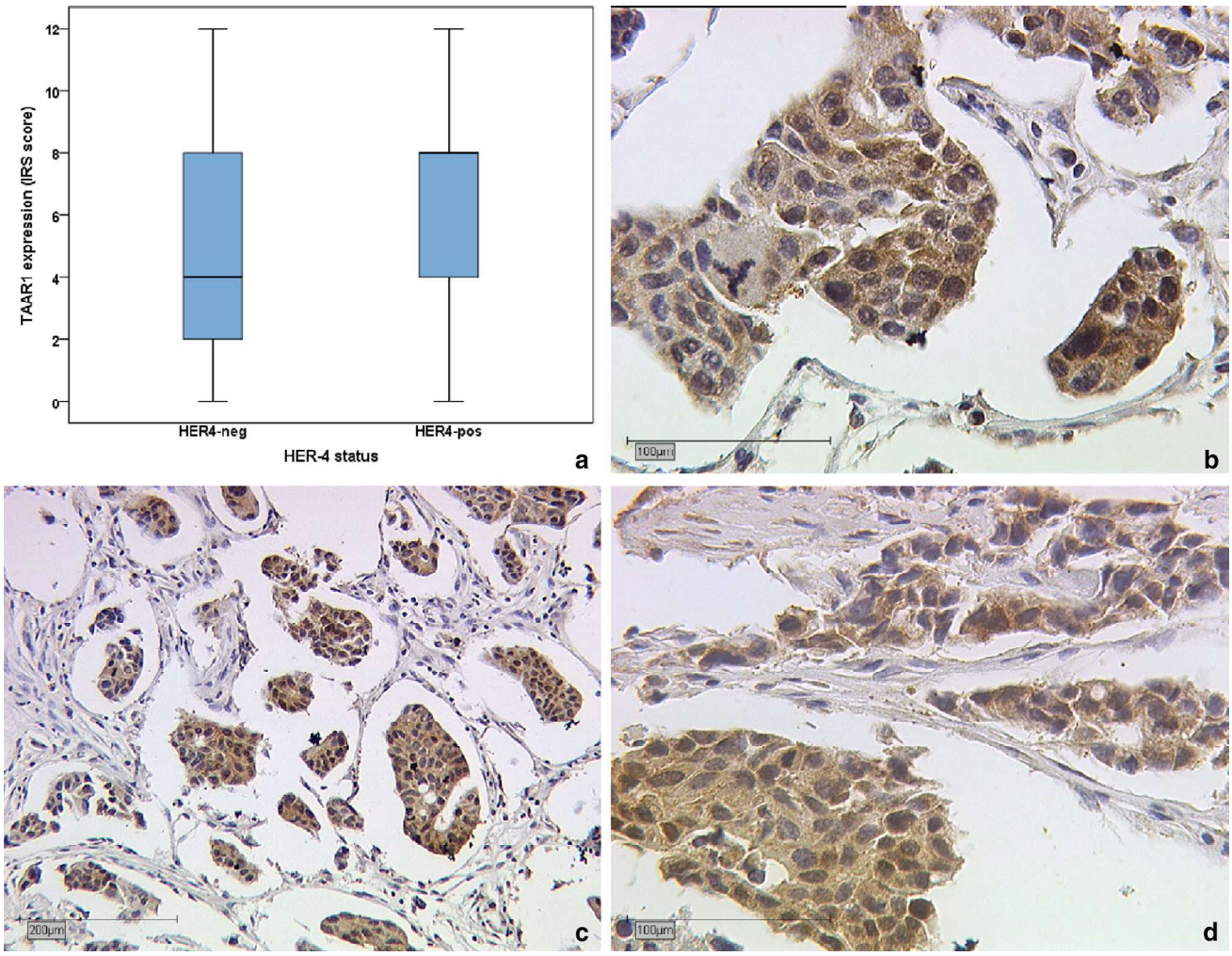

Fig. 5 Correlation of TAAR1 with HER4. **a** *Box plot* analysis of TAAR1 and HER4 correlation HER4-negative, $n = 108$; HER4-positive, $n = 114$. **b** TAAR1 expression, magnification x25; **c** HER4 expression, magnification x 10; **d** HER4 expression, magnification x25

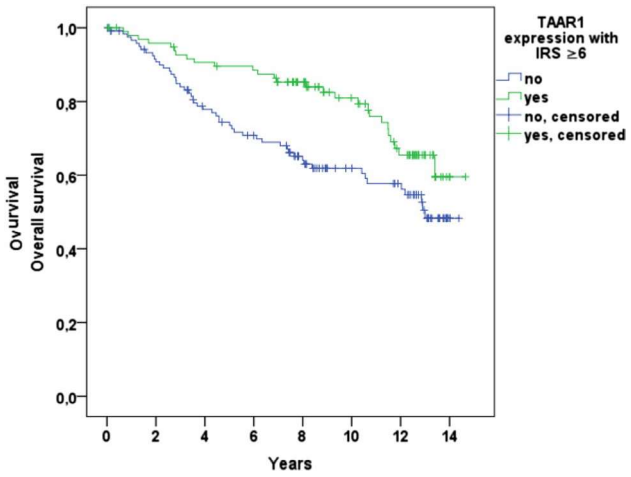

Fig. 6 Influence of TAAR1 expression on OS of patients with early breast cancer. Increased TAAR1 expression (IRS $\geq$ 6) is associated with a longer overall survival ($p = 0.04$)

for the other histopathological variables, such as histology, tumour grade, tumour size, ER and PR status, HER2-, HER4- and pHER4 status.

Conclusion

We examined TAAR1 expression in different histological subtypes of primary malignant breast cancer (MO). To our knowledge, this is the first time that associations of TAAR1 with other biological characteristics of breast cancer and the effect of TAAR1 overexpression on overall survival of breast cancer patients have been analysed.

A positive correlation between TAAR1 and HER2 status and HER4 status was present. Furthermore, a higher TAAR1 expression was associated with a positive HER2 status as well as staining of HER4 in primary breast cancers. Interactions between G-protein coupled receptors

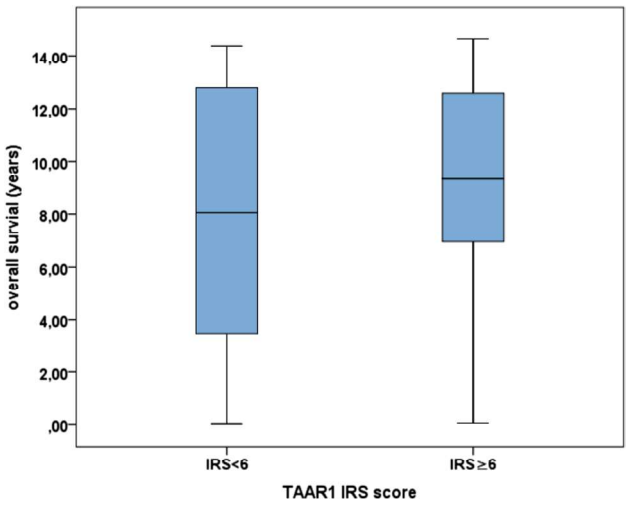

Fig. 7 Correlation of TAAR1 IRS score and OS of patients with early breast cancer. The *boxes* represent the range between the 25th and 75th percentiles with a *horizontal line* at the median. Hence, 50% of the cases lie within the *box*. The *bars* delineate the 5th and 95th percentiles

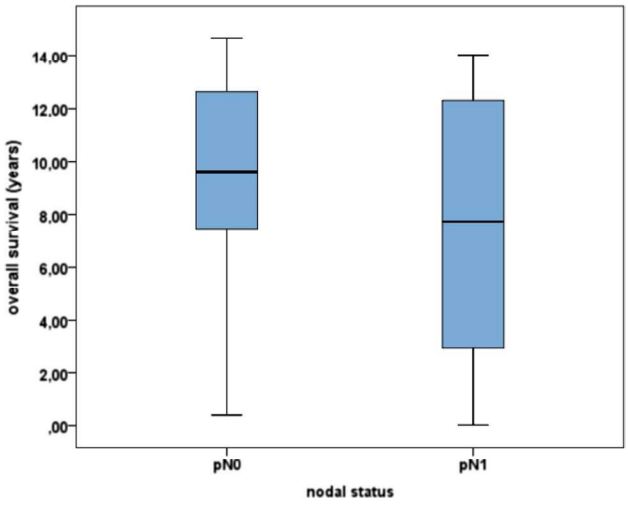

Fig. 8 Correlation of nodal status and OS of patients with early breast cancer. The *boxes* represent the range between the 25th and 75th percentiles with a *horizontal line* at the median. Hence, 50% of the cases lie within the *box*. The *bars* delineate the 5th and 95th percentiles

and ErbB receptors have also been investigated by Pan et al. (2011), who assume that ErbB4 (HER4) signalling interacts with GPCRs. HER2 is known for its negative effect on the outcome of breast cancer, in particular before the advent of modern targeted therapies (Yarden and Sliwkowski 2001; Tan and Yu 2007). In contrast, HER4 has the ability to suppress tumour cell proliferation, and it has been suggested that HER4 may weaken HER2 signalling activity, which leads to the assumption that an overexpression of HER4 can inhibit breast cancer progression (Karamouzis et al. 2007; Barnes et al. 2005; Vidal et al. 2005). Naresh et al. (2006) suggest that HER4 suppresses breast cancer cell growth through the activation of the intrinsic apoptotic pathway. The positive correlation of TAAR1 and HER4 expression, which has been detected in our study, is in line with the impact of HER4 on breast cancer cell growth which has been described in the literature and the influence of TAAR1 overexpression on overall survival demonstrated in this study.

An association between TAAR1 and tumour grade was found, showing that the levels of TAAR1 (quantified by IRS) inversely correlate with tumour grade. This is in line with the prognostic implications of higher TAAR1 expression and lower tumour grade, which are both associated with better survival. A limitation to those findings is that tumour grade of lobular cancer was formerly not assessed and hence 71 patients of the cohort (31.3%) were not classified by tumour grade and are, therefore, not included in the sub analysis.

We demonstrated for the first time that TAAR1 overexpression (IRS $\geq$ 6) has a favourable effect on overall survival of primary breast cancer patients. Increased TAAR1 expression correlates with a positive outcome of over-all survival of primary breast cancer patient. Upregulation of TAAR1 in breast cancer tissue can possibly induce an increased decarboxylisation of thyroid hormones, which may result in a positive effect on the outcome of breast cancer. One aim of future investigations should therefore be analysis of the enzyme L-dopa-decarboxylase, which is responsible for the transformation of thyroid hormones into differentiated thyroid hormones (trace amines). Upregulation of TAAR1 is possibly based on an increased synthesis of biological active amines. Further studies are required to investigate the impact of TAAR1 ligands on the expression of TAAR1, and it is necessary to identify which ligand can lead to an upregulation of the expression of TAAR1 and of L-dopa decarboxylase. Up to now, only little knowledge exists about the regulation of TAAR1 but latest research shows that TAAR1 can be upregulated through methamphetamine (Sriram et al. 2016). Sriram et al. (2016) could demonstrate an induction of TAAR1 mRNA expression in resting T-lymphocytes in response to methamphetamine. Treatment of the T-lymphocytes with methamphetamine for 6 h significantly increased TAAR1 mRNA expression ($p < 0.001$) and protein expression ($p < 0.01$) (Sriram et al. 2016). The research group suggests that TAAR1 is likely to play an important role in methamphetamine-mediated immune modulatory responses (Sriram et al. 2016).

The present work demonstrates that TAAR1 is an independent prognosticator for the overall survival in breast cancer patients. A positive TAAR1 expression (vs. negative expression) is associated with better survival in

Table 3 Cox-regression of histopathological variables in breast cancer regarding overall survival

	Significance	Hazard ratio (Exp(*B*))	Lower 95% Cl of Exp(*B*)	Upper 95% Cl of Exp(*B*)
TAAR1 with IRS > 6	**0.021**	0.35	0.573	1.530
Histology (NST)	0.243	1.06	0.938	1.055
Tumour grade	0.292	0.71	0.501	1.133
pT	0.622	1.18	0.771	1.955
pN	**0.000**	3.96	0.989	2.467
ER	0.872	1.00	0.988	1.002
PR	0.696	0.99	0.995	1.010
HER2	0.343	1.26	0.652	3.560
HER4	0.881	0.99	0.507	1.387
pHER4	0.358	1.07	0.838	2.203
Age at surgery	**0.012**	1.05	0.997	1.040

TAAR1 expression (IRS > 6), lymph node status and age at surgery are independent prognostic markers for overall survival in breast cancer patients

The significant *p*-values of the independent prognostic markers for overall survival in breast cancer patients are marked in bold

breast cancer patients. Positive lymph node status and age at surgery represent additional independent histopathological parameters which prognosticate survival in our breast cancer cohort. Those results are in line with our earlier findings that showed that THRα2 is an independent prognostic factor for overall survival (Ditsch et al. 2013). Heublein et al. (2015) compared 5 year survival in THRα and THRβ1 positive cases of sporadic breast cancer patients and BRCA1 carriers. That study demonstrated that THRα positivity was associated with a significantly reduced 5-year survival rate in *BRCA1* carriers, while THRα had no effect on patient survival in sporadic cancer cases. In contrast, THRβ1 positivity was associated with a significantly higher 5-year survival rate in *BRCA1*-associated cancers, while THRβ had no significant impact on survival of sporadic breast cancer (Heublein et al. 2015). Further studies are required to investigate protein and gene detection. Moreover, patient cohorts that are clinically well defined according to biological subtype and potentially confounding systemic therapy are needed in order to make definitive statements concerning TAAR1 as a prognostic factor in breast cancer.

In conclusion, TAAR1 is expressed by breast cancer tissue, and in our hypothesis-generating study, it could be identified as an independent predictor for breast cancer survival. Increased TAAR1 expression correlates with a favourable overall survival in patients with early breast cancer. In future, modulation of TAAR1 may represent an interesting and novel targeting strategy for breast cancer prevention and therapy. To permit valid conclusions, additional prospective studies with larger und clinically better defined patient cohorts are necessary to confirm the prognostic relevance of TAAR1 in breast cancer. Furthermore, studies are required to investigate the influence of TAAR1 ligands such as T¡AM on the expression of TAAR1 in breast cancer and to identify which ligand has the ability to increase TAAR1 expression in breast cancer.

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Compliance with ethical standards

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Conflict of interest We, the authors, declare that we 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. Prior to surgery, the patients gave their informed consent for their tissue to be used for research and future scientific work.

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