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CLINICAL TRIAL

Prevalent breast cancer patients with a homozygous mutant status for CYP2D6*4: response and biomarkers in tamoxifen users

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Abstract Retrospective studies suggest that single nucleotide polymorphisms in the cytochrome P450 2D6 (CYP2D6) gene predict reduced tamoxifen metabolism, better tolerance and worse treatment outcome. We hypothesized that women with this genotype lack tamoxifen-induced endometrial and biochemical changes in follicle-stimulating hormone (FSH) and sex hormone-binding globulin (SHBG). We identified 56 breast cancer patients attending the follow-up clinic with a homozygous mutant (HM) status for the CYP2D6*4 null variant. Here, we report a detailed assessment of tamoxifen activity in 19 CYP2D6 HM women, while they were using tamoxifen either for metastatic ($n = 5$) or for early disease ($n = 14$). We assessed response to tamoxifen in metastatic disease. Endometrial appearances and serum levels of FSH and SHBG were assessed from retrospective and prospective testing. Our findings do suggest that the presence of two CYP2D6*4 alleles does not exclude a durable response of tamoxifen in metastatic breast cancer. The transvaginal ultrasonographic appearance of the endometrium in CYP2D6*4/*4 patients on tamoxifen is similar as seen in the normal population of tamoxifen users. The endometrium is increased in thickness with subepithelial cysts and endometrial polyps. Serum levels of FSH and SHBG in CYP2D6*4 HM tamoxifen users were in the range of what would be expected during tamoxifen treatment in the general population. Our findings do show CYP2D6*4/*4 carriers to have activity of tamoxifen on breast cancer, endometrium and serum levels of FSH and SHBG. They support clinical trials prospectively testing the effect of CYP2D6 genetic variability in response to tamoxifen before denying this drug to breast cancer patients only based on their CYP2D6*4 status.

Keywords Breast cancer · Tamoxifen · CYP2D6*4 · Biomarkers · Endometrium

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Introduction

Breast cancer is the most common cancer among females and an important cause for cancer death, also in our country [1]. Most tumors are estrogen receptor (ER) positive and their growth is stimulated by estrogens. Tamoxifen, a selective estrogen receptor modulator, is widely used at all ages and in all stages to treat ER-positive breast cancer

saving so far over half a million of lives worldwide, when given following early breast cancer diagnosis. It interferes with estrogen binding at the ER and consequently has an estrogen antagonistic effect in breast tissue [2]. In other organs like bone, uterus, pituitary and liver, its interaction with the ER may induce estrogen agonistic effects. After menopause, it increases bone mineral density and endometrial thickness with an increased risk to develop endometrial hyperplasia, polyps and even cancer [3]. Its estrogenic effect on the postmenopausal pituitary and liver, respectively, lowers circulating follicle-stimulating hormone (FSH) and increases sex hormone-binding globulin (SHBG) [4]. One of its most important short- and long-term side effects is an increase in hot flashes, but the exact mechanism remains unknown and is probably multifactorial [5].

Tamoxifen is a prodrug that is metabolized to active metabolites, of which 4-hydroxy-*N*-desmethyltamoxifen (endoxifen) is the most potent and abundant. The biotransformation of tamoxifen to endoxifen is catalyzed through cytochrome P450 (CYP450) enzymes. CYP3A4 and CYP3A5 are the most important for the demethylation, and CYP2D6 is the most important for the hydroxylation reactions [6, 7]. Sulfotransferases and UDP-glucuronosyltransferases are important for increasing the solubility and facilitate the excretion of tamoxifen and its metabolites [8, 9].

A lot of variation regarding clinical response and side effects is seen among women receiving tamoxifen. One of many possible explanations is variation in the metabolism of tamoxifen due to mutations in CYP450 enzymes important for converting tamoxifen to its active metabolites. One of the single nucleotide polymorphisms (SNPs) believed to play a role is CYP2D6*4, although there remain some contradictory results in the current literature. Several studies found significantly lower endoxifen levels in patients with two null alleles [10–12]. Some retrospective studies also found a significant association between the CYP2D6*4/*4 genotype and a worse clinical outcome [13, 14]. On the other hand, other studies found no such association [15, 16]. Wegman et al. [17] even found a significant better clinical outcome in CYP2D6*4/*4 carriers compared to wild-type carriers.

This observational retrospective study describes 56 breast cancer patients with the CYP2D6*4/*4 genotype and assesses the link between this genotype and clinical response, endometrial appearances, FSH and SHBG in the subset population of women who received tamoxifen.

Patients and methods

DNA from pre- and postmenopausal Caucasian women, previously treated for early or metastatic breast cancer in the Leuven University Hospital and consulting for follow-up visits between August 2007 and February 2009, was consecutively collected. Peripheral blood was sampled in K2EDTA plastic vacutainer tubes, and after centrifugation, germ line DNA was extracted from the precipitated leucocyte cell fraction according to standard procedures. Genotyping for the CYP2D6*4 polymorphism (rs1800716, 1846G>A) was carried out at the Vesalius Research Institute using a Sequenom iPLEX platform, as described by the manufacturer.

The following demographics, reported histopathological breast cancer characteristics at breast cancer diagnosis and given therapies were retrospectively assessed in the subset of women homozygous mutant (HM) for CYP2D6*4: patient's age, the largest tumor diameter, tumor grade, axillary lymph node status, ER, PR, HER-2/neu status and the Nottingham Prognostic Index (NPI) score. Tumor grading was performed according to the Ellis and Elston grading system. Lymph node status was from axillary clearance or the sentinel lymph node procedure, where an axillary clearance was performed in case of a positive sentinel lymph node. NPI score was determined as follows: NPI = 0.2 × tumor size (cm) + grade (Ellis and Elston) + lymph node status. Immunohistochemistry (IHC) staining for ER, PR and HER-2/neu was carried out according to the Envision method as a standard procedure for clinical purposes using the NCL-ER-6F11/2, NCL-PgR-312 (Novocastra Laboratories Ltd, Newcastle upon Tyne, United Kingdom) and CB11 (Ventana Medical Systems, Tucson, AZ, USA) primary monoclonal antibodies, respectively. ER, PR and HER-2/neu IHC staining were evaluated semi-quantitatively. Using the Modified Quick score for ER and PR, a positive result was defined as a score of 3 or more. For Her2/neu, an IHC score of 2 or 3 was defined as positive, where after a fluorescent in situ hybridization (FISH) test was performed. The Her2/neu status was considered positive after a positive FISH test (ratio Her2/neu signals/chromosome 17 signals >2.0).

Data from HM patients were either retrospectively or prospectively collected, following our knowledge of the CYP2D6 status. Clinical response to tamoxifen was assessed using the Response Evaluation Criteria In Solid Tumors (RECIST) together with changes in CA 15-3, a breast cancer tumor marker. Endometrial data were obtained from transvaginal ultrasonography. Using transvaginal ultrasound, the uterus was completely assessed in sagittal and coronal planes with uterine size recorded in three diameters ($D_{1,2,3}$). Double endometrial thickness (DET) was measured in the sagittal plane, from one endometrial–myometrial interface to another excluding intracavitary fluid. Endometrial abnormalities, such as internal cysts and polyps were recorded. The presence of internal cysts was defined by visualization of more than

one anechogenic area greater than 2 mm. Transvaginal ultrasonographies were performed using a GE Voluson E8® (GE Healthcare, Zipf, Austria). FSH and SHBG from postmenopausal HM individuals on tamoxifen were measured with the Roche Modular E170® (F. Hoffmann-La Roche Ltd, Basel, Switzerland) and Orion Spectria IRMA® (Orion Diagnostica Oy, Espoo, Finland), respectively. They were compared with FSH and SHBG from postmenopausal tamoxifen users with a normal CYP2D6 genotype. The protocol was approved by the Committee for Medical Ethics and all patients provided written informed consent.

Results

Allele and genotype frequencies

A total of 1,361 breast cancer patients were genotyped for CYP2D6*4. Genotype success rate was 99.4% ($n = 1{,}353$) and 52 samples were analyzed in duplicate with a concordance of 100%. No significant deviations from Hardy–Weinberg were observed ($P = 0.75$). Fifty six were HM for CYP2D6*4 and therefore have, according to the current classification, a poor metabolizer status. The frequency of the CYP2D6*4 allele was 21.1%. The frequencies of the homozygous wild type, heterozygous and homozygous mutant carriers were 61.9% ($n = 837$), 34.0% ($n = 460$) and 4.1% ($n = 56$), respectively. These allele and genotype frequencies are similar to those found in other studies [12, 13, 18, 19].

To increase the number of poor metabolizers, five other null alleles in CYP2D6 were also genotyped in the whole patient population, i.e., rs4986774, rs1135824, rs5030655, rs5030866 and rs5030867. Genotype success rates were 98.9% ($n = 1{,}342$), 91.2% ($n = 1{,}247$), 99.3% ($n = 1{,}358$), 99.4% ($n = 1{,}359$) and 99.0% ($n = 1{,}344$), respectively. Allele frequencies were 1.5, 0.0, 1.2, 1.2 and 0.1%, respectively. The frequencies of homozygous wild-type carriers were 97.0, 99.9, 97.6, 97.6 and 99.9%, and the frequencies of heterozygous carriers were 3.0, 0.1, 2.4, 2.4 and 0.1%, respectively, but no patients were found homozygous for any of these variants. This paper thus focus on the 56 CYP2D6*4/*4 patients.

Population characteristics

Demographic and tumor characteristics of the 56 HM patients are shown in Table 1. Data about breast cancer therapy at first diagnosis are listed in Table 2. The median follow-up between first diagnosis of breast cancer and time of DNA analysis for these 56 patients was 16 months (range 2–276 months). Forty of 56 HM patients were in follow-up for early breast cancer, 1 had ipsilateral recurrence, 11 were followed for metastatic disease and 4 patients did have non-invasive breast cancer. Of 56, 29 received tamoxifen as part of their treatment; 11 in the past and 18 were on tamoxifen when DNA was analyzed (Table 3). Of 29 CYP2D6*4 HM tamoxifen users, 5 received it in the metastatic and 24 in the adjuvant setting. All 5 metastatic cases were included for tamoxifen response assessment and 14 of 24 in the adjuvant setting and 2 of 5 in the metastatic setting were included for endometrial data and/or serum levels of FSH and SHBG.

Response to tamoxifen according to RECIST and tumor marker

The five patients who received tamoxifen for metastatic disease received it as second ($n = 1$) or first line ($n = 4$). Each single case is shortly discussed. Response to tamoxifen was assessed using RECIST criteria and the tumor marker CA 15-3.

Patient 1: She was 34 years old, premenopausal and previously treated for 5 years with 20 mg of tamoxifen (Nolvadex D®) in the adjuvant setting for an invasive ER-positive lobular adenocarcinoma. Seven years after the initial diagnosis, she was diagnosed with bone metastases. She then received a bilateral salpingo-oophorectomy plus anastrozole (Arimidex®). After 9 months on therapy, she progressed and was again started on tamoxifen 20 mg daily. This therapy clearly led to a clinical benefit during 16 months. She appeared to have a very endocrine responsive breast cancer, as her CA 15-3 levels further regressed on third-line treatment with a daily intake of 160 mg of megestrol acetate (Megace®). After 18 months on megestrol acetate, she received exemestane (Aromasin®) during 9 months and fulvestrant (Faslodex®) during 4 months, after which she was started on chemotherapy.

Patient 2: This 53-year-old premenopausal patient received radiocastration plus tamoxifen (Nolvadex D®), as first line endocrine therapy for bone metastatic disease. The tumor marker CA 15-3 steadily increased from 488 to 647 kU/L over a period of 7 months following start of therapy. However, she did have an endocrine responsive tumor, as second-line anastrozole (Arimidex®) led to a significant decrease in CA 15-3 to 49 kU/L and a clinical benefit of 22 months. She progressed on further endocrine agents and was thereafter treated with several lines of palliative chemotherapy. The only drug which led to short-term tumor regression was doxorubicin (Adriamycin®). She died 3.5 years after diagnosis.

Patient 3: This 47-year-old premenopausal patient also had radiocastration plus tamoxifen (Nolvadex D®), following six courses of chemotherapy for a liver metastasized invasive ductal adenocarcinoma (IDA) to consolidate

Table 1 Demographic and tumor characteristics

Demographic and tumor characteristics	*N*	%
Number of patients	56	100
Age median	51 (range 31–78)	
Sex		
Female	54	96.4
Male	2	3.6
Tumor type		
Invasive ductal	45	80.4
Invasive lobular	3	5.4
Other	4	7.1
NA	4	7.1
Estrogen receptor expression		
Positive	43	76.7
Negative	10	17.9
NA	3	5.4
Progesterone receptor expression		
Positive	39	69.6
Negative	13	23.3
NA	4	7.1
HER2/neu status		
Positive	8	14.3
Negative	39	69.6
NA	9	16.1
Tumor stage		
1	13	23.2
2	20	35.7
3	11	19.6
4	2	3.6
NA	10	17.9
Tumor size invasive tumors		
>20 mm	26	50.0
≤20 mm	15	28.8
NA	11	21.2
Tumor size non-invasive tumors		
≥20 mm	3	75.0
<20 mm	1	25.0
Positive lymph nodes		
Yes	21	37.5
No	23	41.1
NA	12	21.4
NPI median ($n = 42$)	4.50 (range 2.16–7.60)	
Menopausal state at diagnosis		
Premenopausal	23	42.6
Postmenopausal	26	48.1
NA	5	9.3
Vital status		
Alive	53	94.6
Death	3	5.4

NA not available, *NPI* Nottingham prognostic index

Table 2 Therapy at first diagnosis

	N	%
Local therapy		
Operation procedure		
Mastectomy	19	33.9
Tumorectomy	28	50.0
None	9	16.1
Radiotherapy	37	66.1
Chemotherapy		
Neo-adjuvant	3	5.4
Adjuvant	20	35.7
Metastatic disease	2	3.6
Hormonal therapy		
Tamoxifen		
Metastatic	4	7.1
Adjuvant	20	35.7
Tamoxifen with switch to an aromatase inhibitor		
Adjuvant	4	7.1
Aromatase inhibitor		
Adjuvant	10	17.9
Herceptin		
Adjuvant	3	5.4
Metastatic disease	1	1.8

Table 3 Tamoxifen

	N	%
Tamoxifen adjuvant		
Previously	7	12.6
Currently	17	30.4
Tamoxifen for metastatic disease		
At diagnosis		
Previously	3	5.4
Currently	1	1.8
Recurrence		
Previously	1	1.8
No tamoxifen		
Aromatase inhibitor	10	17.9
ER negative	9	16.1
Not started with hormonal therapy yet	3	5.4
Study medication	1	1.8
No invasive tumor	4	7.2

ER Estrogen receptor

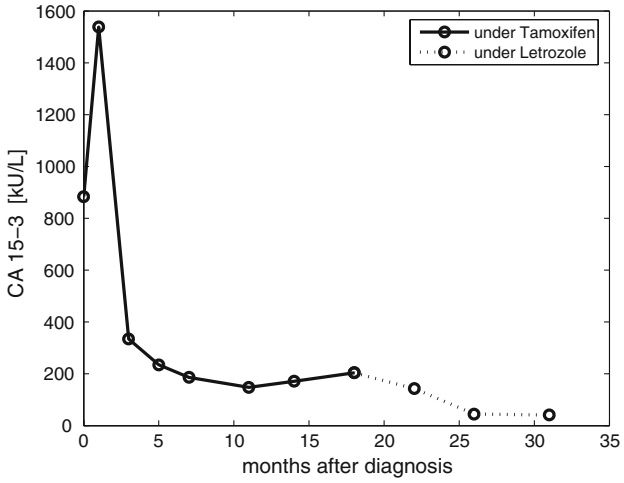

Fig. 1 Evolution of the tumor marker CA 15-3 since diagnosis for patient 5

the response obtained under chemotherapy. The tumor marker CA 15-3 continued to drop for 5 months, but slightly increased thereafter during 10 months. The patient was then switched to letrozole (Femara®).

Patient 4: She was also premenopausal and 46 years old when diagnosed with a primary liver metastatic ER-positive ductal adenocarcinoma of the breast. She started tamoxifen (Nolvadex D®) and goserelin (Zoladex®) in October of 2008. Her tumor marker initially increased from 171 to 458 kU/L during the first month of therapy and then decreased to 55 kU/L after 4 months. She clearly had a partial response, according to the RECIST criteria, to the combined anti-estrogen therapy, which she is still continuing.

Patient 5: She was a postmenopausal 73-year-old woman diagnosed with a bone and lung metastasized invasive ductal adenocarcinoma (IDA) in May 2006. She received tamoxifen (Nolvadex D®) for 14 months with a partial response according to RECIST criteria, while CA 15-3 significantly dropped from 1,538 to 148 kU/L without any other anti-cancer agent (Fig. 1). Previously, she did not receive chemo nor radiotherapy. At progression, she was put on letrozole (Femara®), which she is still taking. A repeat blood sample for DNA assessment confirmed her CYP2D6*4/*4 HM status.

Data on transvaginal appearance of the endometrium, FSH and SHBG

Data on FSH, SHBG and/or endometrial changes were available for 16 of the 29 patients ever or currently on tamoxifen (Table 4). Average FSH (33.9 IU/L, range 17.9–46.4 IU/L) and SHBG levels (89.1 nmol/L, range 50.7–156.2 nmol/L) did not differ from non-CYP2D6*4 HM patients on tamoxifen (data not shown).

Transvaginal ultrasonography showed a DET >5 mm in 7 patients, 6 women developed tamoxifen-like cysts and 2 women developed endometrial polyps. Two women had episodes of postmenopausal bleeding under tamoxifen and one woman 2 months after the cessation of tamoxifen treatment.

Patient 14 is a 57-year-old postmenopausal woman who was treated with radiotherapy and tamoxifen 20 mg daily in the adjuvant setting after breast conservative surgery for an IDA. After 25 months of tamoxifen, she developed an endometrial polyp and had a dilatation and curettage. Pathological investigation showed a large benign endometrial polyp compatible with tamoxifen use. Six months later, a new transvaginal ultrasound showed a DET of 7.4 mm with the typical tamoxifen appearance of the endometrium. Another curettage was performed confirming proliferative endometrial changes without atypia.

Discussion

This manuscript focused on efficacy and biomarkers of tamoxifen activity in 19 users with the CYP2D6*4/*4 genotype. Of the five patients with metastatic disease and eligible for response assessment, two patients received tamoxifen in combination with ovarian suppression. Response to this modality in one poor metabolizer can be entirely related to ovarian suppression alone. The lack of benefit from this combination but clear response from ovarian suppression plus anastrozole may be related to a poor metabolism of tamoxifen through her CYP2D6*4/*4 status but other factors may be involved as de novo resistance or poor compliance related to side effects of the combination. Two other HM patients had a clinical benefit from tamoxifen. Patient 3 had a continuing decrease followed by a small increase but still normal CA 15-3 during, respectively, 5 and 10 months following cessation of palliative chemotherapy. It is unlikely that the initial decrease in CA 15-3 during tamoxifen therapy is entirely due to a 5 months lasting effect of the previous chemotherapy. Furthermore, her biomarkers of tamoxifen activity like the transvaginal ultrasonographic appearance of the endometrium, low FSH and high SHBG levels were in the range of what could be expected in a postmenopausal woman on tamoxifen. Patient 1, with a clinical benefit and stable disease on tamoxifen for 16 months as second-line endocrine therapy following first-line progression on anastrozole, reflects tamoxifen efficacy in a so called poor metabolizer. However, critics may argue that response following cessation of an oral aromatase inhibitor in a poor tamoxifen metabolizer may come from raising estrogen levels. Although this argument has recently been backed by preclinical findings [20], the long lasting benefit of

Table 4 FSH, SHBG and endometrial changes

Patient	Number of months after start tamoxifen	Menopausal status and ovarian suppression	FSH (IU/L)	SHBG (nmol/L)	Endometrium	
					Before tamoxifen	Under tamoxifen
3	15 months	Premenopausal + radiocastration	34.5	156.2	NA	DET 4 mm
4	4 months	Premenopausal + goserelin	NA	NA	NA	DET 6.8 mm, tamoxifen-like cysts
6	8 months	Postmenopausal	40.5	NA	DET 9 mm	DET 15 mm, one episode of postmenopausal bleeding
	12 months		NA	NA		DET 12 mm, cysts and 2 polyps
7	7 months	Postmenopausal	26.3	50.7	NA	NA
8	4 months	Postmenopausal	46.4	103.4	NA	DET 4 mm, tamoxifen-like cysts
9	24 months	Postmenopausal	17.9	78.4	NA	NA
10	11 months	Postmenopausal + radiocastration	39.1	NA	DET 3 mm	DET 9 mm, tamoxifen-like cysts
11	28 months	Premenopausal	NA	NA	NA	DET 5.7 mm, tamoxifen-like
12	NA	Premenopausal	NA	NA	NA	Hypertrophic → HRT
13	NA	Postmenopausal	NA	NA	NA	Postmenopausal bleeding 2 months after stop tamoxifen
14	25 months	Postmenopausal	NA	NA	NA	Tamoxifen-like polyp of 25 mm → curettage
	31 months		NA	NA	NA	DET 7.4 mm and hyperplasia → curettage
15	17 months	Premenopausal + triptorelin	NA	NA	NA	DET 9.2 mm, tamoxifen-like cysts
16	10 months	Premenopausal + goserelin	NA	NA	NA	DET 8 mm, tamoxifen-like cysts
17	17 months	Postmenopausal	32.8	56.9	NA	DET 2 mm, tamoxifen-like cysts
18	10 months	Postmenopausal	NA	NA	NA	DET 2 mm, endometrial polyp, postmenopausal bleeding
	17 months		NA	NA	NA	DET 2.2 mm
	28 months		NA	NA	NA	DET 5 mm
19	3 months	Premenopausal	NA	NA	NA	6.3 mm, tamoxifen-like cysts

FSH follicle-stimulating hormone, *SHBG* sex hormone-binding globulin, *NA* not available, *DET* double endometrial thickness

tamoxifen is most likely related to activity of the drug. The most important patient from our study, i.e., patient 5, shows that some HM patients may respond to tamoxifen given as first-line endocrine treatment. A considerable clinical durable benefit and remarkable decrease of the tumor marker CA 15-3 from 1,538 to 148 kU/L, is in clear contrast to her CYP2D6 status as a poor tamoxifen metabolizer. It is obvious that one case does not reflect efficacy to tamoxifen of the entire CYP2D6*4 HM population, but it does confirm previous findings that CYP2D6*4 HM can in fact respond to tamoxifen treatment [17].

Indeed, the response to tamoxifen is also driven by other factors such as drugs interfering with tamoxifen metabolism, other variants in CYP2D6 and different metabolic pathways, which theoretically could take over once CYP2D6 lacks functionality [6, 12, 21]. According to the medical records, these patients did not take selective serotonin receptor inhibitors known to inhibit CYP2D6, but due to the retrospective nature of this study, data about concomitant medication may not always be reliable. Interesting is that three out of these five patients, including the one with a durable benefit from tamoxifen were heterozygous for CYP2C19*17, which is known to lead to an increased enzyme activity and increased metabolism of tamoxifen [22]. This highlights the importance of considering more than just the CYP2D6*4 variant when discussing tamoxifen as an efficacious drug for any patient with an ER-positive breast cancer.

Other issues when considering response to therapy are treatment related side effects and compliance. An inverse association between the occurrence of tamoxifen-related side effects and breast cancer recurrence with a potential link to SNP for CYP2D6 has previously been reported [14, 23–25]. Our findings of a benefit to tamoxifen in women with an HM status for CYP2D6*4 are compatible with this. An excellent compliance together with an excellent response might well be explained by active anti-cancer metabolites of tamoxifen not leading to severe side effects as seen in patients with an extensive tamoxifen metabolizer status [26]. Another aspect to keep in mind is that the

minimal level of endoxifen needed for a good response is not known. It has been reported that 5 mg instead of 20 mg of tamoxifen may well be as efficacious as the 20 mg dose we currently use compared with the 40 mg dose previously used [27]. Such aspects are of course difficult to evaluate in our retrospective study, but should be taken into account in future prospective studies and when interpreting results of published studies.

Our approach to assess response to tamoxifen by the CYP2D6*4 status is original and differs from others. The strength is that our approach reflects real life situations rather than statistical comparisons for disease free survival between groups with a different metabolizer status. We were not able to include in this small group of 29 patients a disease free survival calculation by the CYP2D6 status as events in the non-metastatic group were too small within the short follow-up from initial diagnosis. Also, this cohort is heterogeneous regarding predictive and prognostic markers for relapse as there are large variations in Nottingham Prognostic Index, age, menopausal status and other adjuvant therapies given prior to tamoxifen. To increase the number of patients with a poor metabolizer status, we also considered subjects homozygous for five other known CYP2D6 null alleles, but frequencies were low and no patient was found homozygous for any of these variants. The most common CYP2D6 null alleles were genotyped except the deletion (CYP2D6*5). This could have increased the group of poor metabolizers, but it has no influence on the results discussed here.

Apart from response assessment we looked at three possible biomarkers of tamoxifen activity with a potential to predict response. Absence of such an effect in a poor metabolizer may be another proof that the drug is not metabolized with the potential for this marker to serve as an early surrogate for poor treatment outcome. It is well known that tamoxifen intake induces uterine abnormalities like endometrial polyps, endometrial thickening and subepithelial cysts in a large proportion of postmenopausal tamoxifen users early in treatment [3, 28]. Also, tamoxifen leads to a decrease in serum levels of FSH and increase in serum levels of SHBG in postmenopausal women [4]. Until now, there is no evidence that such biomarkers can be regarded as predictors for clinical response to tamoxifen, but an absent effect might explain whether pathways for efficacy are the same as pathways of other tamoxifen-related effects.

Serum levels of FSH and SHBG and/or endometrial appearances in 16 HM patients on tamoxifen were very much tamoxifen-like. This is not what would be expected in patients with an inactive CYP2D6 enzyme if endoxifen would be responsible for those changes. Endometrial findings in CYP2D6*4 HM tamoxifen users have, to our knowledge, previously not been reported. However, a clear stimulatory effect of tamoxifen on the endometrium in postmenopausal Japanese women with the CYP2D6*10/*10 genotype, which is related to a decreased rather than absent CYP2D6 activity, has been reported. They found a significant increase in endometrial thickness between baseline and 1 year of tamoxifen treatment ($P < 0.05$) and no significant difference between the group of CYP2D6*1/*1 and CYP2D6*10/*10 patients [29]. Our findings for postmenopausal serum levels of FSH and SHBG in CYP2D6*4 HM carriers on tamoxifen are in agreement with previously reported levels of FSH and SHBG in tamoxifen users [4]. There were no differences in serum levels of FSH and SHBG between patients homozygous for CYP2D6*4 and those who were not. Again, also for these biomarkers it is likely that other metabolites of tamoxifen than endoxifen affect their concentrations.

Many retrospective studies on the CYP2D6 status and response to tamoxifen, resulted in inconclusive opinions, also during the latest Saint Gallen breast cancer conference, questioning its current importance as a predictor for response. We here gave further evidence that the CYP 2D6*4 status alone is probably not sufficient to predict lack of response to tamoxifen. We also showed that potential biomarkers for tamoxifen activity like endometrial changes, FSH and SHBG concentrations do not differ between wild type and CYP2D6*4/*4 subjects. Our findings clearly show that the only answer in this matter can come from a prospective study assessing the link between response to tamoxifen, the CYP2D6 status and endoxifen levels among several other variables as potential confounders.

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