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Pharmacogenomic diversity of tamoxifen metabolites and estrogen receptor genes in Hispanics and non-Hispanic whites with breast cancer

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Abstract

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Ethical standards: Experiments comply with current U.S. laws. All patients provided informed written consent prior to participation, and the study was conducted in accordance with the ethical standards of the University of Texas Health Sciences Center at San Antonio Institutional Review Board and the San Antonio Cancer Institute Protocol Review Committee.

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Ethnic differences in patient genetics and breast cancer (BC) biology contribute to ethnic disparities in cancer presentation and patient outcome. We prospectively evaluated SNPs within phase I and phase II tamoxifen (TAM) metabolizing enzymes, and the estrogen receptor gene (ESR1), aiming to identify potential pharmacogenomic ethnicity patterns in an ER-positive BC cohort constituted of Hispanic and Non-Hispanic White (NHW) women in South Texas. Plasma concentrations of TAM/metabolites were measured using HPLC. CYP2C9, CYP2D6 and SULT1A1 genotypes were determined by DNA sequencing/Pyrosequencing technology. ESR1 PvuII and XbaI SNPs were genotyped using Applied Biosystems Taqman® Allelic Discrimination Assay. Hispanics had higher levels of TAM, 4-hydroxytamoxifen, and endoxifen than NHWs. There was a higher prevalence of CYP2D6 EM within Hispanics than NHWs, which corresponded to higher endoxifen levels, but no differences were verified with regard to CYP2C9 and SULT1A1. We found a higher incidence of the wild type forms of the ESR1 in Hispanics than NHWs. The performance status, the disease stage at diagnosis, and the use of aromatase inhibitors might have overcome the overall favorable pharmacogenomics profile of Hispanics when compared to NHWs in relation to TAM therapy responsiveness. Our data strongly point to ethnical peculiarities related to pharmacogenomics and demographic features of TAM treated Hispanics and NHWs. In the era of pharmacogenomics and its ultimate goal of individualized, efficacious and safe therapy, cancer studies focused on the Hispanic population are warranted because this is the fastest growing major demographic group, and an understudied segment in the U.S.

Keywords

Tamoxifen; Metabolites; Estrogen receptor gene; Hispanics; Pharmacogenomics

Introduction

Breast cancer (BC) is the most frequently diagnosed malignancy among women worldwide, with an annual estimation of 1.4 million new cases and half a million disease-related deaths [1]. Seventy-five percent of all breast tumors are of the estrogen receptor (ER α)-positive luminal subtype. ER α regulates the transformed phenotype of luminal BC, so that inhibitors of ER α -driven signaling, such as the selective ER modulator tamoxifen (TAM) and aromatase inhibitors (AIs) are a mainstay of BC treatment. Nonetheless, 30–40 % of ER α -positive tumors exhibit resistance to TAM after prolonged exposure in the metastatic setting [2, 3].

Mechanisms governing TAM resistance and tumor progression remain elusive. Activating ESR1 ligand-binding domain mutations were identified in hormone-resistant metastatic BC [4, 5]. Moreover, single nucleotide polymorphisms (SNPs) in the ESR1 gene, such as the intronic SNPs rs2234693 (PvuII; T > C) and rs9340799 (XbaI; A > G) [6], could influence the tumor response to the drug by a yet unexplored mechanism. Additionally, studies have addressed a genetic link in the metabolism of TAM and the risk of death from BC [7-23].

TAM is extensively metabolized by hepatic phase I and phase II enzymes [7, 8]. The affinity of TAM to ER α is only 1.8 % of that of 17 β -estradiol [8]. However, TAM is converted to 4-hydroxytamoxifen, mainly by CYP2D6 and CYP2C9, which has 100-fold higher affinity to

ER α and 30- to 100-fold greater potency in suppressing estrogen-dependent BC proliferation than TAM [7, 9–11]. Endoxifen (4-hydroxy-N-desmethyltamoxifen), formed from N-desmethyltamoxifen (N-DMT) by CYP2D6, is equipotent to 4-hydroxytamoxifen [9, 10, 12–15] but is likely the principal active metabolite of TAM due to its plasma concentration that exceeds that of 4-hydroxytamoxifen by several folds [9, 11, 16]. TAM and its metabolites are further metabolized and inactivated by phase II enzymes, as SULT1A1 [17, 18]. Genetic variations within these drug-metabolizing enzymes affect TAM metabolism and effectiveness against ER-positive BC, and further pharmacogenomic studies are needed to clarify the available controversial data [19–23].

TAM pharmacology might also be affected by ethnicity. Survival disparities have been reported amongst BC patients with different ethnicity, and Hispanics remain at increased risk of BC death than non-Hispanic Whites (NHWs) [24]. The Hispanic population is the fastest growing major demographic group in the U.S., and by 2050, should triple from 46.7 million to 132.8 million, thus representing 30 % of the U.S. Population [25], and from which one-fifth are Texas Latinos [26]. Hence, there is an urgent need for clinical data that relate genotype to clinical outcome, as Hispanics are currently under-represented in most cancer genomic and pharmacogenomic studies [27]. We prospectively evaluated polymorphisms within both phase I and phase II TAM metabolizing enzymes, and within ESR1, aiming to identify potential pharmacogenomic ethnicity patterns in an ER-positive BC cohort constituted of Hispanic and NHW women in South Texas.

Materials and methods

Study cohort

ER-positive BC patients, diagnosed between January 2004 and January 2005, and receiving TAM (20 mg daily) for at least 8 weeks were prospectively enrolled. Of these, 29 % were Hispanics and 72 % NHWs. Two blood samples were collected for genotyping and HPLC analysis. Demographic and clinical data were collected. All patients provided informed written consent prior to participation, and the study was conducted in accordance with institutional ethical standards.

Sample preparation

DNA was extracted from whole blood using the Qiagen DNA Midi Kit (Qiagen, Valencia, CA) according to the manufacture. Plasma was isolated from whole blood by centrifugation for HPLC analysis. Samples were stored at -80°C until processed.

Genotyping TAM metabolizing enzymes (CYP2C9, CYP2D6, and SULT1A1) and cellular target (ESR1)

CYP2C9 and CYP2D6 genotypes were determined by duplex and multiplex real-time DNA sequencing based on Pyrosequencing technology assay [28]. Alleles *1, *2, *3, *4, *6 were evaluated for CYP2D6, whereas alleles *1, *2 and *3 were investigated for CYP2C9, using Pyrosequencing 96HS system (Biotage) and Pyrosequencing PSQTM 96MA system (Biotage), respectively. Patients were genotyped for the SULT1A1*1 and SULT1A1*2 alleles, following the assay developed by our group [29]. The intronic ESR1 SNPs

rs2234693 (PvuII; C > T) and rs9340799 (XbaI; A > G) were genotyped using Applied Biosystems Taqman® Allelic Discrimination Assay (Applied Biosystems, Foster City, CA) in MJ Research DYAD thermocycler (MJ Research) and Applied Biosystems 7900HT Sequence Detection System (Applied Biosystems).

HPLC assay

Plasma concentrations of TAM and its metabolites were measured using a previously reported HPLC assay [30]. Two standard curves were evaluated, one for TAM and N-DMT with concentrations of 15–600 ng/ml, and a second curve for the 4-hydroxytamoxifen and endoxifen with concentrations ranging from 0.25 to 40 ng/ml.

Statistical analysis

Z-score/Chi square tests were used to assess significant differences between the Hispanics and NHWs with respect to categorical variables, whereas *t* tests were used to analyze differences regarding continuous variables (two-tailed significance level $p < 0.05$). Analyses were conducted using GraphPad Prism® Software Version 6.04.

Results

Patient demographics and clinical information

The cohort examined was comprised of 260 ER-positive and TAM-treated BC patients, 74 Hispanics and 186 NHWs from South Texas. Demographical and clinical information of the two ethnical subpopulations were compared, demonstrating their homogeneity with the following exceptions: women's menopausal status, performance status, and the disease stage at diagnosis (Table 1).

TAM and metabolites measurements

Hispanics had higher circulating levels (ng/mL) of TAM (130.7 ± 66.20 vs. 109.5 ± 54.10 ; $p = 0.016$; Fig. 1a), 4-hydroxytamoxifen (2.787 ± 1.917 vs. 2.018 ± 1.261 ; $p = 0.0019$; Fig. 1c), and endoxifen (7.19 ± 4.299 vs. 4.990 ± 3.934 ; $p = 0.0002$; Fig. 1d) than NHWs. There was no significant difference between their plasma concentration of N-DMT (214.3 ± 113.0 vs. 206.3 ± 100.9 ; $p = 0.5966$; Fig. 1b).

CYP2D6, CYP2C9 and SULT1A1

We questioned whether there were pharmacogenomic differences between Hispanics and NHWs that could affect TAM metabolism. Whereas there was a higher prevalence of CYP2D6 extensive (EM) than intermediate (IM) or poor (PM) metabolizers within Hispanics than NHWs (79 vs. 55 %, 15 vs. 37 %, 5 vs. 8 %, respectively; $p = 0.0020$; Fig. 2a), no significant differences were verified amongst the frequencies of CYP2C9 (EM: 91 vs. 83 %, IM: 8 vs. 16 %; $p = 0.0815$; Fig. 2b) and SULT1A1 (*1/*1: 36 vs. 49 %; *1/*2: 46 vs. 39 %, *2/*2: 18 vs. 12 %; $p = 0.1522$; Table 2). CYP2C9 PM was not considered for statistical analyses due to the low frequency in both ethnicities. We further evaluated CYP2D6 activity using a scoring system that incorporates CYP2D6 genetic variation and CYP2D6 mediated drug-drug interactions [31]. In brief, the scoring system provides a more

accurate method to evaluate CYP2D6 activity, which activity score (AS) is the product of the gene score (GS) based on the enzyme genotype and the inhibitor factor (IF) of concurrent medications taken by the patients in use of TAM. As proposed by the authors, CYP2D6 EM, IM and PM were given the GS 1, 0.5 and 0, respectively [31]. For the calculation of the IF, we followed the rules for CYP2D6 genotype input, as follows: (i) if the patient was not using any CYP2D6 inhibitor, IF = 1; (ii) if the patient was using a weak CYP2D6 inhibitor, IF = 0.75 for CYP2D6 GS = 0.5–1 or IF = 1 for CYP2D6 GS = 0; (iii) if the patient was using a strong CYP2D6 inhibitor, IF = 0 for CYP2D6 GS = 0.5–1 or IF = 1 for CYP2D6 GS = 0 [31]. For the classification of the CYP2D6 inhibitor as weak or strong, we followed the Food and Drug Administration Guidance for Industry Drug Interaction Studies—Study Design, Data Analysis, and Implications for Dosing and Labeling (2006) (<http://www.fda.gov/cder/guidance/6695dft.pdf>). We observed 4 and 8 cases of phenocopy (a shift from EM to PM phenotype following the CYP2D6 scoring system) within Hispanics and NHWs, respectively. However, there was still a higher rate of CYP2D6 EM amongst Hispanics than NHWs. As shown in the insert of Fig. 2A, we found an incidence of 65 versus 42 % of CYP2D6 AS 1, 8 versus 9 % of CYP2D6 AS 0.75, 12 versus 31 % of CYP2D6 AS 0.5, 2 versus 3 % of CYP2D6 AS 0.375, and 14 versus 16 % of CYP2D6 AS 0 in Hispanics versus NHWs, respectively ($p = 0.0082$). We then evaluated the endoxifen plasma concentrations measured in patients with CYP2D6 AS 1 (EM), 0.5 (IM) or 0 (PM). Hispanic patients with CYP2D6 AS 1 had higher plasma levels of Endoxifen than NHWs (8.603 ± 4.852 vs. 6.394 ± 4.031 , $p = 0.0146$), but no statistically significant differences were found in patients with CYP2D6 AS 0.5 or 0 in both ethnicities (6.509 ± 3.261 vs. 5.318 ± 4.090 , $p = 0.3719$ for AS 0.5, and 2.409 ± 2.041 vs. 1.967 ± 2.158 , $p = 0.5882$ for AS 0) (Table 3). Our results indicate that the higher plasma concentrations of 4-hydroxytamoxifen and endoxifen detected within Hispanics than NHWs derives from their higher activity of CYP2D6.

ESR1 PvuII and XbaI SNPs

The genotypes frequencies in Hispanics versus NHWs were PP 14 versus 21 %, Pp 40 % versus 52 %, pp 47 % versus 29 % for the PvuII SNP ($p = 0.0270$), and XX 10 % versus 11 %, Xx 31 % versus 47 %, xx 59 % versus 42 % for the XbaI SNP ($p = 0.0453$). Taken together, these data supports the higher incidence of the wild type forms of the ESR1 in Hispanics than NHWs.

Patients factors associated with BC recurrence

The apparent overall favorable profile of the Hispanics compared to NHWs in relation to TAM therapy responsiveness, that is a higher rate of CYP2D6 EM, higher active TAM metabolites plasma concentrations, and wild type ESR1, is puzzling because there was no statistically significant difference in disease free survival between the two populations. Although the numbers analyzed were not sufficient to reach statistical power, there is a trend within Hispanics to have a higher risk of BC recurrence than NHWs. We then sought to better understand the referred trend by comparing patients factors, including pharmacogenomic (ESR1 PvuII SNP, P allele; ESR1 XbaI SNP, X allele; CYP2D6 EM; HER2 overexpression) and clinical parameters (Advanced disease stage, DS 2 and 3; Poor patients' performance status, PS 1; Aromatase inhibitor, AI use) amongst the recurrent BC

cases. Our analysis revealed no differences in the frequency of ESR1 PvuII P allele (71 vs. 70 %), ESR1 XbaI X allele (57 % vs. 50 %), CYP2D6 EM (71 % vs. 60 %), and HER2 overexpression status (0 % vs. 10 %), Hispanics versus NHWs. On the other hand, there was statistical significance differences in the frequency of DS 2/3 (86 % vs. 50 %), PS 1 (29 % vs. 0 %), and AI use (57 % vs. 30 %) (Hispanics vs. NHWs; $p < 0.0001$) (Fig. 4). Our data suggest that the poor patients' performance status and the advanced disease stage at diagnosis of Hispanic patients seem to overcome their overall favorable profile in relation to TAM therapy responsiveness.

Discussion

The present study combines, for the first time, comprehensive analyses of the plasma concentrations of TAM and its metabolites and the polymorphisms within phase I and phase II TAM metabolizing enzymes, and within ESR1, in an ER-positive BC cohort constituted of Hispanic and NHW women in South Texas. The incidence of BC varies by race/ethnicity, with higher rates in NHWs (130.6 per 100,000) than in Hispanics (90.1 cases per 100,000) [25, 32]. The number of BC cases in Hispanics was 73 % higher in our cohort than that recorded nationwide, a fact that could be justified by the high concentration of Latinos in Texas [26], thus enabling a more inclusive evaluation of BC peculiarities within this minority population.

Despite the lower risk of BC among U.S. women from minority populations, including Hispanics, their risk of mortality is higher than that of NHWs, mostly due to diagnosis of BC in more advanced stages [25, 32-37]. The evaluation of the demographic and clinical information of the populations studied is in agreement with a poorer performance status and advanced disease stage at diagnosis of Hispanics than NHWs (Table 1). On the other hand, despite the fact that there was no statistically significant difference in the disease free survival between the two populations, we found a trend of higher incidence of BC recurrence among Hispanics than NHWs (Table 1; Fig. 4). We acknowledge that, although the majority of the women in our cohort had complied with the standard recommendation of 5 years of therapy with TAM, the mean number of years of follow-up after diagnosis and after initiation of TAM, both of ~6 years (Table 1), were below the disease free survival interval expected for TAM-treated BC patients. Indeed, this could have compromised our analysis as it has been well established that patients with primary BC treated with TAM have demonstrated significantly decreased recurrence and BC mortality for 15 years after primary diagnosis [2].

Differences in genetics and biology of BC are likely to be significant contributors to the disparities in BC presentation and outcome between Hispanic and NHW women [37, 38]. However, Hispanics remain under-represented in most cancer genomic and pharmacogenomic studies [25, 27, 39]. We, herein, present novel and comprehensive data concerning TAM metabolism in Hispanics and NHWs. Hispanics have higher mean plasma concentrations of TAM, 4-hydroxytamoxifen and endoxifen than NHWs (Fig. 1a, b, d), but there was no difference in the levels of N-DMT (Fig. 1c). At steady state, the average concentration, in ng/mL, of TAM, 4-hydroxytamoxifen and N-DMT has been reported to range from 93.95 to 202.32, 273.00 to 450.54, and 1.387 to 9.01, respectively. Plasma levels

of endoxifen, in turn, vary widely, from 2.33 to 36.9 ng/mL, and are dependent on the activity of CYP2D6 [9, 16, 40–42]. Therefore, our data are in concordance with previously published results. The higher plasma levels of TAM that we observed in Hispanics than NHWs are intriguing. This is because one might expect that Hispanics with a higher prevalence of CYP2D6 EM to have lower TAM levels accounting for the higher levels of 4-hydroxytamoxifen and endoxifen. Potential polymorphisms in other genes involved in TAM metabolism might be responsible for our findings. CYP3A4/5 is predominantly responsible for the conversion of TAM to N-DMT. A SNP in CYP3A5 (*3, 6986 G > A, rs776746) with decreased enzyme activity is prevalent (52 %) in Hispanics [43]. Additionally, TAM undergoes N-oxidation catalyzed by FMO to form tamoxifen-N-oxide, which is converted back to TAM by CYP450's, such as CYP1A6 and CYP1A1 [44]. FM03, the major adult hepatic FMO enzyme, exhibits significant ethnic variation. FM03 (11177 C > A, N61 K), devoid of enzyme activity, has an allelic frequency of 5.2 % in NHWs but is absent in Hispanics [45]. Two functional variants of CYP1A1 with increased enzyme activity (*2A and *2C), known as CYP1A1 *2B, has a prevalence of 8 % in Hispanics, whereas it is not observed within NHWs [46].

In previous CYP2D6 studies, approximately 50 % of women were EM, 43 % were IM, and 7 % were PM [7, 16]. These results are in concordance with ours, which revealed an incidence of 55 % CYP2D6 EM, 37 % CYP2D6 IM, and 8 % CYP2D6 PM phenotypes in NHWs (Fig. 2a). Considerable ethnic differences in the CYP2D6 allele frequencies have been reported with a prevalence of the PM phenotype in Hispanics ranging from 2.2 to 6.6 %, whereas it varied from 8.9 to 10.0 % among NHWs [Reviewed in 47]. These data are in agreement with our observations of an incidence of 5 and 8 % CYP2D6 PM in Hispanics and NHWs, respectively (Fig. 2a). We have further evaluated CYP2D6 activity using a scoring system that incorporates CYP2D6 genetic variation and CYP2D6 mediated drug-drug interactions [31], and, despite the occurrence of 4 and 8 cases of phenocopy within Hispanics and NHWs, respectively, there was still a higher rate of CYP2D6 EM amongst Hispanics than NHWs (Fig. 2a, insert). It is noteworthy to emphasize that our study is the first to demonstrate a higher prevalence of the CYP2D6 EM phenotype amongst Hispanic than NHW BC patients. We have further confirmed that the observed pharmacogenomic profile accounts for higher endoxifen levels detected in Hispanics than NHWs (Table 3). Despite the novelty of our data, we could not evaluate the impact of CYP2D6 pharmacogenomic profiling as a TAM therapy responsiveness marker that could ultimately be translated into clinic due to the low number of recurrent cases in our cohort, as aforesaid. Hence, the clinical value of genotyping CYP2D6 in BC patients remains controversial. On the other hand, our findings point to the inexistence of ethnic differences in CYP2C9 (Fig. 2b) and SULT1A1 genotypes (Table 2). Our group has previously reported that there is no correlation among SULT1A1 genotypes and the levels of TAM and its metabolites [29]. As well, it has been documented that CYP2C9 genotyping has no clinical value in predicting the clinical outcome of patients under TAM therapy [Reviewed in 22]. Whereas additional studies designed for following up TAM treated patients for up to 15–20 years after BC diagnosis is of fundamental importance to determine the actual clinical relevance of our findings, 20 % and 45 % (IM + PM), respectively of our Hispanic and NHW population were potentially at risk for subtherapeutic levels of TAM (endoxifen). CYP2D6 phenotype-

adjustment tamoxifen dosing for IM or PM patients and/or concomitant use of endoxifen might be reasonable alternatives treatment approaches for these patients [47].

Endoxifen targets ER α for degradation by the proteasome, blocks ER α -mediated transcriptional activation and inhibits estrogen-induced BC cell proliferation even in the presence of TAM, 4-hydroxytamoxifen and N-DMT at their steady state concentrations [48]. Of importance, concentrations of endoxifen detected in CYP2D6 EM phenotype carriers are much more potent than those of CYP2D6 PM phenotype [48]. Polymorphisms in ESR1 might result in alterations in its expression profile and in the sequence of the coded ER α , thus compromising the effectiveness of TAM therapy [48-50]. In this context, the presence of the dominant T allele of PvuII (Wild type pp), and the dominant A allele of XbaI (Wild type xx) SNPs can enhance ER α activity [49-51], as well as the antiestrogen activity of TAM and its metabolites [48]. In contrast, it has been suggested that the ESR1 PvuII wild type allele correlates with increased ESR1 gene dosage, negative status of progesterone receptor (PgR) status, and to TAM resistance [51]. Furthermore, it has been reported that the occurrence of the PvuII SNP produces a binding site for *myb* transcription factor, thus increasing the ER transcriptional activity and potentially correlating to a satisfactory response to the therapy with SERMS [52]. In the present work, we found no correlation amongst ESR1 PvuII and XbaI SNPs and the PgR status (Data not shown). Moreover, considering the low recurrence rate of BC in our population, genotyping ESR1 PvuII and XbaI SNPs in our cohort is unlikely to serve as a TAM responsiveness predictor marker. On the other hand, we found a higher prevalence of ESR1 wild type gene within Hispanics than NHWs (Fig. 3a, b). Onland-Moret and colleagues (2005) have observed the genotypes Pp, pp, Xx and xx in frequencies of 36.4, 19.8, 36 and 29.3 %, respectively, among Caucasians [53]. In turn, Slaterry and colleagues (2007) have shown different allele frequency between Hispanic and NHW women, in which the former have a greater frequency of the wild type allele $\times$ than the later [54]. To our knowledge, we are pioneer in demonstrating the phenomena related to the PvuII SNP higher frequency of the allele p within Hispanics than NHWs. The clinical relevance of the findings remains unknown.

The apparent overall favorable profile of the Hispanics in comparison with the NHWs in relation to TAM therapy responsiveness has intrigued us due to the lack of statistically significant difference in disease free survival between the two populations studied. As previously discussed, although the numbers analyzed were not sufficient to reach statistical power, there is a trend within the Hispanics to have a higher risk of BC recurrence than NHWs. In this matter, our results suggest that the poor patients' performance status and the advanced disease stage at diagnosis of Hispanic patients seem to overcome the overall favorable profile of the former when compared to the NHW population in relation to TAM therapy responsiveness (Fig. 4). With lower income and educational levels, Hispanics, specifically Texas Latinos, are less prone to identify early symptoms of BC, and to be submitted to early screening exams periodically [34]. Likely important, considering that AI pharmacology is not affected by CYP2D6 polymorphisms, and that there is a higher number of AI users among Hispanic than NHW recurrent cases, there might be other pharmacogenomic variables, such as CYP19A1/aromatase polymorphisms [Reviewed in 55 and 56], that could influence BC patients outcome.

In conclusion, our data strongly point to ethnical peculiarities with regard to pharmacogenomics and demographical features of TAM treated Hispanic and NHW BC patients. Thus, in the era of pharmacogenomics and its ultimate goal of individualized, efficacious and safe therapy, cancer studies focused on the Hispanic population are warranted because this is the fastest growing major demographic group, and understudied segment in the U.S. [25, 27]. Further studies should consider the heterogeneity of the Hispanic population, which is highly diverse with respect to genetic ancestry that presents both as a challenge and an opportunity for research to eventually minimize cancer health disparities.

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Fig. 1.

TAM and TAM metabolites plasma concentration in Hispanic and NHW breast cancer patients. TAM and metabolites plasma concentrations were assessed by HPLC in both Hispanics ($n = 74$), and NHWs ($n = 186$) Data are expressed as mean $\pm$ SD. Statistical significance was verified applying unpaired t test ($p < 0.05$)

Fig. 2. CYP2D6 and CYP2C9 phenotypes in Hispanic and NHW breast cancer patients. CYP2D6 and CYP2C9 phenotypes were assessed within Hispanics and NHWs. CYP2D6 activity was also assessed using a scoring system that incorporates CYP2D6 genetic variation and CYP2D6 mediated drug–drug interactions, in which the activity score (AS) is the product of the gene score (GS) based on the enzyme genotype and the inhibitor factor (IF) of concurrent medications taken by the patients in use of TAM [32] (Fig. 2a, *insert*). *EM* extensive metabolizers, *IM* intermediate metabolizers, *PM* poor metabolizers. For CYP2D6, $n = 65$ (Hispanics) and $n = 178$ (NHWs), and for CYP2C9, $n = 74$ (Hispanics) and $n = 186$ (NHWs). CYP2C9 PM was not included on the statistical analyses due to the low frequency of the phenotype in both ethnicities. Data are expressed as phenotype frequencies (%). Statistical significance was verified applying the z score/Chi square test ($p < 0.05$)

Fig. 3.

ESR1 PvuII and XbaI SNPs frequencies in Hispanic and NHW breast cancer patients.

Hispanics ($n = 73$) and NHWs ($n = 179$) were genotyped for ESR1 PvuII SNPs and ESR1 XbaI SNPs. The P and X alleles represent the occurrence of SNPs within the ESR1 gene, whereas the homozygous pp and xx genotypes refer to the wild type ESR1 gene. Data are expressed as genotype frequencies (%). Statistical significance was verified applying the z score/Chi square test ($p < 0.05$)

Fig. 4.

Patients factors associated with breast cancer recurrence. To better understand the trend within the Hispanics that likely have a higher risk of breast cancer recurrence than NHWs, patients factors, including pharmacogenomic (ESR1 PvuII SNP, P allele; ESR1 XbaI SNP, X allele; CYP2D6 EM; HER2 overexpression) and clinical parameters (Advanced disease stage, DS 2 and 3; Poor patients' performance status, PS 1; Aromatase inhibitor, AI use) were compared. Data are expressed % of breast cancer recurrent cases ($n = 7$ for Hispanics, and $n = 10$ for NHWs). Statistical significance was verified applying the z score/Chi square test ($p < 0.05$)

Table 1
Patient demographics and clinical history of the study population

Clinical parameter	Hispanics	NHWs	<i>p</i> value
Mean age at diagnosis	58.72 ± 12.13	58.89 ± 12.11	0.9159
Menopause status (MS)			0.0044 ^a
Pre menopause	23 %	30 %	
Post menopause	77 %	65 %	
Unknown	0 %	9 %	
Mean number of years of follow-up after diagnosis	6.581 ± 1.490	6.672 ± 1.766	0.6746
Mean number of years of follow-up after D1 on TAM	6.014 ± 1.503	6.237 ± 1.534	0.2851
Duration of TAM treatment (20 mg/day)			0.5506
<5 years	42 %	46 %	
5 years	43 %	44 %	
>5 years	15 %	10 %	
Aromatase inhibitor therapy			0.6710
Yes	51 %	54 %	
No	49 %	46 %	
Performance status (PS)			0.0177 ^a
PS 0	92 %	98 %	
PS 1	8 %	1.5 %	
PS 2	0 %	<1 %	
Disease stage (DS)			0.0363 ^a
DS 0	0 %	2 %	
DS 1	54 %	57 %	
DS 2	35 %	39 %	
DS 3	11 %	2 %	
Histological grade (HG)			0.8286
HG 1	20 %	21 %	
HG 2	37 %	32 %	
HG 3	29 %	29 %	
HG 4	14 %	18 %	
Disease recurrence			0.2828
Recurrence	10 %	5 %	
Disease-free	90 %	95 %	

Data are expressed as mean ± SD or percentage of cases, and were analyzed using unpaired *t* test or z score/Chi square test, respectively

^a Statistically significant, Hispanics vs. NHWs

Table 2
Allelic distribution and genotypic frequencies of CYP2D6, CYP2C9, and SUL1A1 in Hispanic and NHW Breast Cancer Patients

CYP2D6	*1/*1	*1/*2	*1/*3	*1/*4	*1/*6	*2/*2	*2/*3	*2/*4	*2/*6	*3/*4	*4/*4	*4/*6
Hispanics (n = 65)	24 (36.9)	22 (33.9)	0 (0)	4 (6.2)	0 (0)	5 (7.7)	0 (0)	6 (9.2)	1 (1.5)	1 (1.5)	2 (3.1)	0 (0)
NHW's (n = 178)	39 (21.9)	34 (19.1)	4 (2.2)	34 (19.1)	2 (1.1)	25 (14.0)	2 (1.1)	22 (12.4)	1 (0.6)	0 (0)	13 (7.3)	2 (1.1)
CYP2C9	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3						
Hispanics (n = 74)	58 (78.4)	9 (12.2)	6 (8.1)	0 (0)	1 (1.3)	0 (0)						
NHW's (n = 186)	121 (65.1)	33 (17.7)	27 (14.5)	2 (1.1)	1 (0.5)	2 (1.1)						
SUL1A1 (p = 0.1522)	*1/*1	*1/*2	*2/*2									
Hispanics (n = 74)	27 (36.5)	34 (45.9)	13 (17.6)									
NHW's (n = 186)	91 (48.9)	73 (39.3)	22 (11.8)									

Number (Percentage)

CYP2C9 *2/*3 and *3/*3 genotypes were not considered for further phenotype statistical analyses due to the low frequency in both ethnicities

For CYP2D6, the genotypes *1/*1, *1/*2 and *2/*2 accounted for the EM phenotype; *1/*3, *1/*4, *1/*6, *2/*3, *2/*4 and *2/*6 were considered for the IM phenotype; *3/*4, *4/*4 and *4/*6 were classified as the PM phenotype

For CYP2C9, the genotypes *1/*1 and *1/*2 accounted for the EM phenotype; *1/*3, *2/*2 were considered for the IM phenotype; *2/*3 and *3/*3 were classified as the PM phenotype

Table 3
Endoxifen concentrations, in ng/mL, in Hispanic and NHW breast cancer patients with different CYP2D6 activity scores

CYP2D6 activity score	Hispanics	NHWs	<i>p</i> value
AS 1	8.603 ± 4.852	6.394 ± 4.031	0.0146 ^a
AS 0.5	6.509 ± 3.261	5.318 ± 4.090	0.3719
AS 0	2.409 ± 2.041	1.967 ± 2.158	0.5882

CYP2D6 activity score is the product of the gene score based on the enzyme genotype and the inhibitor factor of concurrent medications taken by the patients, as described by Borges et al. (2010) [31]

AS 1 = EM; AS 0.5 = IM; AS 0 = PM. Data are expressed as mean endoxifen concentrations ± SD (ng/mL), and were analyzed using unpaired *t* test

^a Statistically significant, Hispanics versus NHWs