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CLINICAL TRIAL

Association between *CYP2D6* genotype and tamoxifen-induced hot flashes in a prospective cohort

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Abstract Women with reduced CYP2D6 activity have low endoxifen concentrations and likely worse long term benefits from tamoxifen. We investigated the association between *CYP2D6* genotype and tamoxifen-induced hot flashes in a prospective cohort. We collected hot flash frequency and severity data over 12 months from 297 women initiating tamoxifen. We performed *CYP2D6* genotyping using the AmpliChip CYP450 test and correlated inherited genetic polymorphisms in *CYP2D6* and tamoxifen-induced hot flashes. Intermediate metabolizers had greater mean hot flash scores after 4 months of tamoxifen therapy (44.3) compared to poor metabolizers (20.6, $P = 0.038$) or extensive metabolizers (26.9, $P = 0.011$). At 4 months, we observed a trend toward fewer severe hot flashes in poor metabolizers compared to intermediate plus extensive metabolizers ($P = 0.062$). CYP2D6 activity may be a modest predictive factor for tamoxifen-induced hot flashes. The presence or absence of hot flashes should not be used to determine tamoxifen's efficacy.

Keywords Breast cancer · Tamoxifen · CYP2D6 · Hot flash · Genotype

Introduction

The selective estrogen receptor modulator tamoxifen is one of the mainstays for treatment and prevention of hormone receptor positive breast cancer [1]. Tamoxifen is a pro-drug

The description of the study design can be found on http://www.clinicaltrials.gov (NCT00228930).

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that is converted to active metabolites by cytochrome P450 (CYP) enzymes, primarily CYP2D6 [2]. Endoxifen (4-hydroxy N-desmethyl tamoxifen) is believed to be a key active metabolite of tamoxifen. Endoxifen has equal affinity for ER and equal in vitro anti-cancer activity as the other primary metabolite, 4-hydroxy tamoxifen, but endoxifen is present in five to tenfold higher concentrations than 4-hydroxy tamoxifen [2]. Most but not all studies reported to date suggested that subjects with reduced or absent CYP2D6 activity have reduced serum concentrations of endoxifen, and may have worse long term tamoxifen-associated benefits than those with normal enzyme activity [2–10].

One of the most bothersome tamoxifen-associated toxicities is hot flashes, reported by over 50% of women [1]. A retrospective analysis has suggested that moderate or severe hot flashes are significantly less common in women homozygous for the *4 null variant of *CYP2D6* [4]. Because patients with reduced CYP2D6 activity have worse breast cancer outcomes and are less likely to report hot flashes, it has been suggested that presence of hot flashes during tamoxifen therapy may predict for superior breast cancer outcomes [11, 12]. Based on these data, we hypothesized that inherited germline variants in the *CYP2D6* gene that cause reduced or absent enzymatic activity would be associated with lower hot flash scores in tamoxifen-treated women.

Methods

We enrolled 297 women initiating tamoxifen in a prospective multicenter observational study designed to identify associations between *CYP2D6* germline variants and tamoxifen-related phenotypes. Complete details regarding the design and conduct of this study and *CYP2D6* genotyping methods have been previously reported [13, 14]. The protocol was approved by the institutional review boards of all three participating study sites. All enrolled patients provided written informed consent.

In brief, patients initiating therapy with tamoxifen for breast cancer treatment or prevention were enrolled and treated with tamoxifen 20 mg orally per day for 12 months. Participants recorded the number of hot flashes that were mild, moderate, severe, or very severe [15] over 7 days prior to initiation of tamoxifen therapy and after 1, 4, 8, and 12 months of tamoxifen therapy. Hot flash score, which is a summary of hot flash frequency and severity, was calculated as previously described [16].

Whole blood samples were obtained from each patient at baseline. The women underwent comprehensive genotyping for 33 *CYP2D6* alleles using the AmpliChip CYP450 test (Roche Diagnostics, Basel, Switzerland) and the xTAG CYP2D6 assay (Luminex Corp, Austin, Texas; 16 alleles) [14]. Each *CYP2D6* allele was assigned a value from 0 (for nonfunctional alleles) to 1 (for fully functional alleles) based on its relative activity for dextromethorphan *O*-demethylation [17]. For each subject, the two allele scores were summed [18]. Patients were classified as poor metabolizers (PMs) if their total score was <1, intermediate metabolizers (IMs) if the score was 1 to <2, and extensive metabolizers (EMs) if the score was ≥ 2.

The primary endpoint for this study was to assess the relationship between *CYP2D6* germline variants and change in hot flash score during the initial 4 months of tamoxifen therapy. This time period was selected and used in all our analyses because tamoxifen serum concentrations reach steady state by 4 months, and to limit the confounding effect of concomitant medication usage and premature trial discontinuation [13]. A general linear model was used to test the differences in hot flash frequency and score among the three genotype groups. Fisher's exact test was used to compare the hot flash severity distribution of the three groups (no hot flashes, mild/moderate, and severe/very severe) between cohorts divided by CYP2D6 metabolizer status (PM vs. EM/IM). The effect of CYP2D6 metabolizer status on hot flash-free survival during the first year of tamoxifen treatment was tested using Kaplan–Meier plots and log-rank tests. Time to hot flash was treated as a time-to-event outcome. Subjects with hot flashes at baseline or who were taking medications known to affect hot flashes were excluded from the survival analyses.

Results

Change in hot flash score by *CYP2D6* genotype

For the entire cohort, mean weekly hot flash score increased within 1 month of initiating tamoxifen therapy and remained elevated throughout treatment [13]. In an intent to treat analysis, we did not observe differences in mean absolute hot flash score at baseline among women according to CYP2D6 metabolizer status (Fig. 1). At the 4 month time-point, IMs reported significantly higher mean weekly hot flash scores (44.3 ± 10.2) compared to either EMs (26.9 ± 8.8, $P = 0.011$) or PMs (20.6 ± 16.9, $P = 0.038$). When patients on SSRIs known to inhibit CYP2D6 were omitted from the analysis, a higher hot flash score was still noted in IMs compared to EMs ($P = 0.0395$, data not shown).

In an intent to treat analysis, we observed a significant increase in mean hot flash score in IMs (41.8 ± 6.2) compared to EMs (25.3 ± 4.7) after 4 months of tamoxifen therapy relative to baseline ($P = 0.040$). In addition, in the

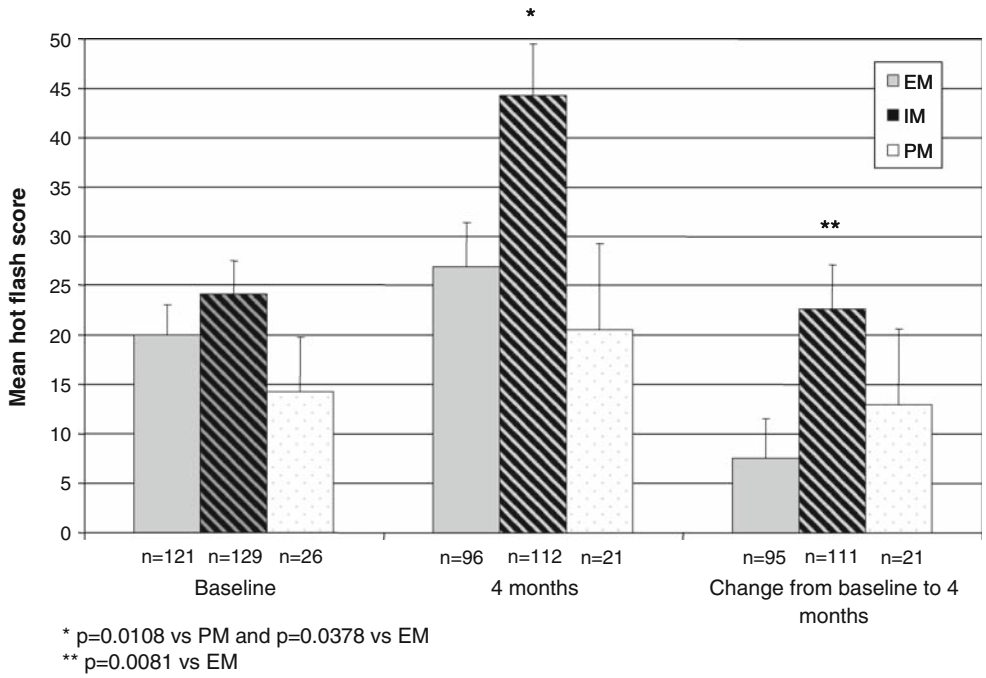

Fig. 1 Mean weekly hot flash score at baseline, 4 months after initiating tamoxifen, and change in hot flash score from baseline to 4 months by CYP2D6 metabolizer group derived from *CYP2D6* genotype for all study participants. Number of subjects evaluated at each time point listed below the *bars*. *Error bars* signify standard error. *EM* extensive metabolizer (*solid bars*), *IM* intermediate metabolizer (*diagonal bars*), *PM* poor metabolizer (*dotted bars*)

subset of subjects not on concomitant medications known to treat hot flashes or to affect CYP2D6 activity at any time during study participation ($n = 109$), we observed a trend suggesting that EMs and PMs were more likely to remain free of hot flashes during tamoxifen therapy compared to IMs ($P = 0.100$ and $P = 0.089$, respectively; Fig. 2).

Change in hot flash severity by *CYP2D6* genotype

Since hot flash score is the product of hot flash severity and frequency, it is possible that *CYP2D6* genotype may preferentially influence hot flash severity, as has previously been reported in patients homozygous for the most prevalent *CYP2D6* null variant in Caucasians, *CYP2D6*4* [4]. At 4 months, we observed that PMs were less likely to develop severe or very severe hot flashes compared to EMs and IMs combined (9.5 vs. 29.8%, $P = 0.062$; Table 1). When potential associations between *CYP2D6* genotype and hot flash frequency were analyzed, the findings were similar to that seen for hot flash score (data not shown).

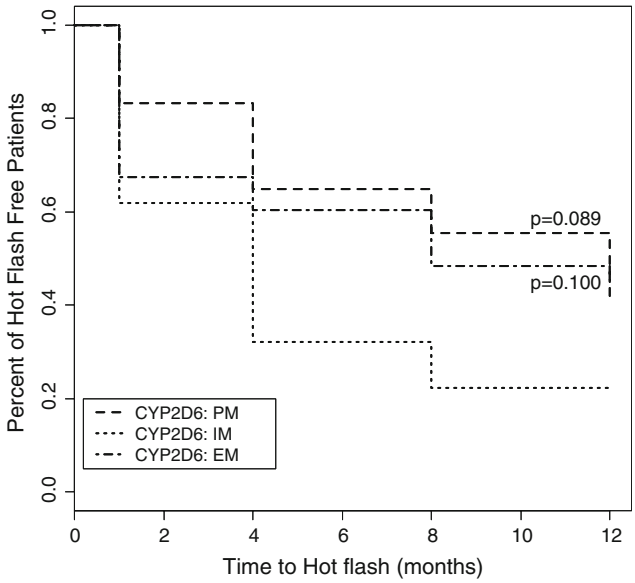

Fig. 2 Kaplan–Meier curves for the effect of *CYP2D6* genotype on hot flash-free survival during the first year of tamoxifen treatment ($n = 109$). Subjects with hot flashes at baseline or who were taking medications known to affect hot flashes or tamoxifen metabolism were excluded from the analysis. *EM* extensive metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer. *P* values represent comparison of EM or PM to IM

Conclusions

In this prospective observational study, we detected an association between *CYP2D6* intermediate metabolizer phenotype and tamoxifen-associated hot flashes (Fig. 1). Though this association of hot flash score with IMs, but not EMs, is unexpected, it may reflect reduced adherence to therapy in subjects with the EM phenotype. One potential

Table 1 Mean hot flash severity at baseline and after 4 months of tamoxifen therapy by CYP2D6 metabolizer group derived from *CYP2D6* genotype for all study participants

Severity	Month 0		Month 4	
	EM/IM	PM	EM/IM	PM
No hot flashes	42.4% ($n = 106$)	53.9% ($n = 14$)	24.0% ($n = 50$)	42.9% ($n = 9$)
Mild/moderate	37.6% ($n = 94$)	38.5% ($n = 10$)	46.2% ($n = 96$)	47.6% ($n = 10$)
Severe/very severe	20.0% ($n = 50$)	7.7% ($n = 2$)	29.8% ($n = 62$)	9.5% ($n = 2$)
P value	0.285		0.062	

EM extensive metabolizer, *IM* intermediate metabolizer, *PM* poor metabolizer

explanation is that subjects with the EM phenotype who experienced more severe hot flashes were more likely to discontinue therapy prior to the 4 month assessment, and therefore could not be included in the analysis. This explanation does not appear to be valid, however, since of the subjects for whom baseline hot flash data were available, similar numbers of EM and IM subjects were missing hot flash data at the 4 month time point (25 of 121 EMs and 30 of 129 IMs).

The strengths of this study include a prospective assessment of hot flashes using a validated hot flash diary [16] both prior to and during tamoxifen therapy. Limitations include heterogeneity of the patients with respect to menopausal status, prior chemotherapy, and concomitant medications, all of which are known to influence hot flashes.

These results differ from a recent preliminary report that EMs are more likely to experience hot flashes than PMs [19]. In that report from the Women's Healthy Eating and Living (WHEL) study, women with early stage breast cancer who had been taking tamoxifen for at least 4 months self-reported hot flash severity over the preceding 4 weeks at the time of enrollment. Our study differs from the WHEL report in multiple ways, including acquisition of hot flash data using a validated 7 days hot flash diary to capture both hot flash frequency and severity data, as well as assessment of hot flashes both prior to and during tamoxifen therapy. In addition, the use of concomitant medications that could affect CYP2D6 activity by the WHEL participants is unknown. These factors could potentially account for the different results noted in the two studies.

Our observation that PMs are less likely to report severe hot flashes than EMs and IMs are similar to those previously published in a retrospective report [4]. Two main differences between our study and the prior publication are that we performed more comprehensive *CYP2D6* genotyping and we prospectively collected patient-reported hot flash frequency and severity. In summary, these results suggest that CYP2D6 activity may influence severity of tamoxifen-associated hot flashes, although it is unclear whether this CYP2D6 effect is through a differential tamoxifen metabolism or through known effects of this enzyme in the brain [20].

Our results suggest that CYP2D6 activity is likely not the sole determinant of tamoxifen-associated hot flashes. Instead, the development of tamoxifen-associated hot flashes is likely multifactorial, including factors involved in tamoxifen metabolism as well as estrogen metabolism and signaling. Indeed, we have previously demonstrated an association between polymorphisms in the estrogen receptor beta gene *ESR2* and likelihood of developing hot flashes [13]. In addition, women with hot flashes at the time of menopause have been shown to be more likely to experience tamoxifen-induced hot flashes [21]. Additional studies are required to determine the factors involved in tamoxifen-associated hot flashes and to further elucidate the relationship between hot flashes and breast cancer outcomes. Until additional data are available, clinicians should not use the presence or absence of hot flashes in tamoxifen-treated women to predict possible long term benefits related to the drug.

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Conflicts of interest statement N.L.H. is on the Scientific Advisory Board of Otsuka Pharmaceuticals and has received research funding from AstraZeneca and Lilly Pharmaceuticals. J.M.R. has received research funding from Pfizer and speaking honoraria for Roche Diagnostics. T.S. has received speaking honoraria for Roche Diagnostics. AMS is a member of the speaker's bureau and receives research funding from Glaxo-Smith Kline and is a consultant to Eli

Lilly and Company. D.A.F. is on the Scientific Advisory Boards of Labcorp, Inc. and Otsuka Pharmaceuticals, is a consultant to Roche Molecular Diagnostics, and has received research funding from Pfizer and Novartis. D.F.H. has received research funding from AstraZeneca, Glaxo-Smith Kline, Pfizer, and Novartis. V.S. is on the Scientific Advisory Board of Otsuka Pharmaceuticals, has served as a consultant to Wyeth Pharmaceuticals, Concert Pharmaceuticals and JDS Pharmaceuticals, and has received research funding from Glaxo-Smith Kline, Pfizer, and Novartis.

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