Original Article

Tamoxifen side effects: pharmacogenetic and clinical approach in Mexican mestizos

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Background: Tamoxifen metabolism is translated into four genetic phenotypes (GP): genetic poor metabolizer (gPM); genetic intermediate metabolizer (gIM); genetic normal metabolizer (gNM); and genetic ultra-rapid metabolizer (gUM). Although *CYP2D6* is involved in tamoxifen biotransformation, its association with tamoxifen side effects (TSE) is limited. Therefore, we evaluated *CYP2D6* GP and clinical variables as potential predictors of TSE in Mexican Mestizo patients.

Methods: This cross-sectional study evaluated *CYP2D6* GP, clinical data, and self-reported TSE in 71 women. Potential predictors were tested in uni- and multivariable models.

Results: Hot flashes (57.75%), arthralgia (45.07%), headache (43.66%), and cramps (39.44%) were the most frequent TSE. Three GP were identified: gPM (2.8%); gNM (93.0%); and gUM (4.2%). In the univariate analysis, none of the GP was predictive of TSE. However, the uni- and multivariable models showed contraceptive use and chemotherapy treatment prior to tamoxifen therapy to be predictive. Two alleles were identified for the first time at unusually high frequencies: *CYP2D6*34* (13.2%); and **39* (14.7%).

Conclusions: Our findings indicate that CYP2D6 GP were not significantly predictive of TSE, though two clinical descriptors were. The present results are a valuable contribution to pharmacogenetic characterization of Mexican Mestizo populations who, like other Latin-American groups, are poorly represented in the literature.

Keywords: *CYP2D6*; mestizo population; tamoxifen side effects (TSE)

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Introduction

Tamoxifen has been widely used as an adjuvant treatment in patients diagnosed with estrogen receptor-positive breast cancer (ER+ breast cancer) (1). A selective estrogen receptor modulator (SERM), it is a prodrug extensively metabolized by enzymes belonging to the cytochrome P450 superfamily, particularly CYP2D6. Metabolism generates two main active metabolites: 4-hydroxytamoxifen and endoxifen (2).

The *CYP2D6* gene is highly polymorphic, attaining at least 130 single-nucleotide polymorphisms (SNP) that define >100 allelic variants (3). These are translated into four genetic phenotypes (GP): genetic poor metabolizer (gPM); genetic intermediate metabolizer (gIM); genetic normal metabolizer (gNM); and genetic ultra-rapid metabolizer (gUM) (4,5).

Although *CYP2D6* is clearly involved in tamoxifen biotransformation, its association with therapeutic efficacy is still debated, with various opinions for (6-8) and against (9,10).

Approved for clinical applications in 1970, tamoxifen has been shown to reduce recurrence indices and improve five-year disease-free survival rates (11). Results from the Adjuvant Tamoxifen Longer Against Shorter trial highlight the need for a possible extension to a ten-year treatment period (12). Several tamoxifen side effects (TSE) are generated by its pro- and anti-estrogenic activity (13). These TSE include hot flashes (HF), headache, arthralgia, cramps, retinopathy, vomiting, dizziness, night sweats, myelosuppression, thromboembolism, and endometrial cancer (14). The most frequent symptom in a diverse patient population has been HF, and it has consequently been proposed as an indicator of tamoxifen therapeutic efficacy, although conclusions have varied (8,9,15).

Some clinical studies have evaluated the predictive potential of *CYP2D6* for TSE occurrence, but results have been contradictory. For instance, gPM patients are reported to have a lower HF incidence (8), which is plausible since CYP2D6 is responsible for tamoxifen activation to endoxifen. In another study, gUM patients developed a higher number of TSE than the other phenotypes (16). Other authors, however, have not identified any association between *CYP2D6* and TSE (9,15). In addition, variable results have been observed in evaluations of potential associations between HF and *CYP2D6* (8,9,17). Much of this interstudy inconsistency can be attributed to lack of methodological rigor; for example, some studies have not considered CYP2D6 inhibitor use and therapeutic compliance (18).

Decision-making for patients under adjuvant treatment would be better informed with more pharmacogenetic data on widely used therapies. However, substantial pharmacogenetic data for drug metabolizing enzymes are only available for certain populations, and is lacking or poor for many others. In Mexico, some studies have been done to redress this shortcoming though none has properly tested their clinical impact. Instead, they were designed to describe the most prevalent alleles in some CYP genes, including nonfunctional variants related with the gPM status such as: *CYP2C19*2* (9.20%) and **3* (0.10%) (19); *CYP2C9*2*, **3*, and **6* with frequencies of 7.00%, 1.50%, and 0.50%, respectively (20); and *CYP2D6*3* (0.90–1.50%), *CYP2D6*4* (11.00–13.00%), and **5* (1.00–2.00%) (21,22).

This diversity in nonfunctional variants in various drug metabolizing enzymes could be attributed to the ethnic heterogeneity of Mexico's population which is the result of intermixing of various Amerindian populations with predominantly European emigrants, creating what are known as Mestizo populations (23). Genetic variation in Mexico responds to these Amerindian and European ancestries, and exhibits some degree of grouping based on geography; differences have been reported between regions in the north (e.g., Sonora, Nuevo Leon, and Durango) and south (e.g., Chiapas, Quintana Roo, and Yucatan) (23). The state of Yucatan is notable in this respect because its Mestizo group has been heavily influenced by the Maya, a major Amerindian population in the region (23). This substantial contribution from a single group may explain the genetic diversity observed in Mestizos from Yucatan, and may have produced inter-individual variability in genes such as *CYP2D6* (23,24).

Genetic heterogeneity may be associated with variability in and presentation of TSE. To our knowledge, no research that complies with international recommendations for non-prospective studies, in such a poorly represented group in literature as the Mestizos from Yucatan, Mexico, has been reported on the predictive value of *CYP2D6* (or other clinical variables) for TSE (18). Although the influence of absent/decreased activity in *CYP2D6* genotypes has been extensively explored to test the efficacy of tamoxifen treatment (mostly in Caucasian and Asian groups), very limited attention has been given the relationship between *CYP2D6* polymorphisms and TSE (25). The present study aim was to analyze *CYP2D6* genotypes and phenotypes, and clinical characteristics, as potential predictors of TSE in highly adherent ER+ breast cancer Mestizo patients from Yucatan, Mexico, without CYP2D6 inhibitor intake.

Methods

Study design and patients

A cross-sectional, analytical study was done of ER+ breast cancer women from Merida, Yucatan, Mexico, under adjuvant tamoxifen (20 mg/day) treatment. Considering a 10% gPM prevalence in Mexican Mestizos (22,26),

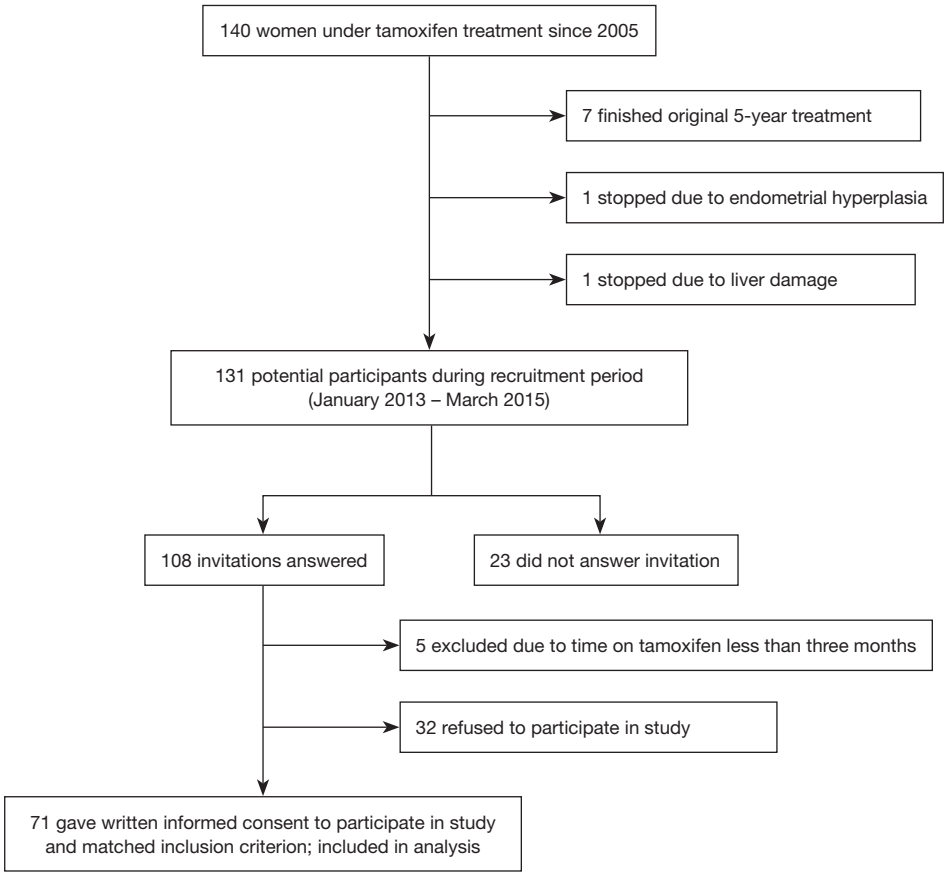

Figure 1 Flow chart of overall study population recruitment process.

and assuming a type 1 error (α=0.05) and 5% accuracy; the minimum estimated sample size was 71 patients (27). Study design power to detect significant pharmacogenetic associations was estimated in 60% using the QUANTO software (28) with the following conditions: sample size =71, type I error of 0.007 (after Bonferroni correction, taking into account the four studied polymorphic loci); and a dominant genetic model.

Inclusion criteria included high patient adherence to tamoxifen treatment. Exclusion criteria included CYP2D6 inhibitor/inductor therapy (*Table S1*), and/or a tamoxifen treatment period of less than three months (18). Participant recruitment resulted in a total of 71 women consenting to participate (*Figure 1*). None was metastatic at the time of recruitment (January 2013 to March 2015). All participants were seen at the Oncology Department of the Specialty Medical Unit (Unidad Médica de Alta Especialidad - UMAE), and family practice clinics, of the Mexican Social Security Institute (Instituto Mexicano del Seguro Social - IMSS) in Merida, Yucatan, Mexico.

All procedures complied with the ethical standards of the IMSS National Clinical Research Ethics Committee (R-2013-785-057), the Dr. Hideyo Noguchi Regional Research Center Review Board for Ethical Research with Human Subjects, and the 1964 Helsinki Declaration and its amendments. After explanation of the study objectives, its contribution to improved understanding of tamoxifen metabolism, data confidentiality, and the possible complications of venipuncture, written informed consent was obtained individually from all study participants.

Data collection: demographic, socioeconomic, side effects, and adherence

A questionnaire was applied to participants to collect personal data; height (m); weight (kg); body mass index (BMI, kg/m^2); medical history; previous breast cancer treatment; tamoxifen use duration; TSE; and treatment adherence. Adherence was defined as a ≥80% dosage compliance during the previous month as quantified by

pill count (29-32). Questionnaire items included yes/no questions on the occurrence of six TSE: HF; headache; cramps; arthralgia; dizziness; and vomiting. Items also addressed TSE severity, which was rated on a Likert-type scale based on the Common Terminology Criteria for Adverse Events of the U.S. National Cancer Institute: 1 (mild), 2 (moderate), and 3 (severe) (33). Patients were instructed to report the TSE only if they had begun to appear after tamoxifen treatment initiation.

Socioeconomic status was assessed with the Graffar's classification system (34), which evaluates education level, occupation, family income source, and household characteristics.

Menopausal status was assigned as follows: (I) pre-menopausal, having had at least one menstrual period during the prior three months with no changes in regularity during previous year; and (II) post-menopausal, amenorrhea during at least six continuous months (15).

Laboratory procedures—genomic DNA extraction and genotyping

A single 4 mL sample of peripheral blood was collected from each participant for DNA isolation. Samples were kept at 4–8 ℃ until processed. Genomic DNA was extracted from EDTA-anticoagulated total blood with the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) following manufacturer instructions. Genotyping of *CYP2D6* was done with the TaqMan® Allelic Discrimination Assay (Applied Biosystems, Foster City, CA, USA) using a StepOne device (Applied Biosystems) and following supplier specifications. Based on the most frequent alleles described in Mexican Mestizo populations, a set of seven SNP were used for identification of *CYP2D6*1*,* *2*, **3*, **4*, **5*, **10*, **17*, **29*, and other alleles (21,22,26,35,36). TaqMan® probes (Applied Biosystems) were used for this purpose (*Table S2*). *CYP2D6* duplications/multiplications and gene deletion (*CYP2D6*5*) were evaluated with the copy number variation assay (Applied Biosystems) (*Table S2*).

Statistical analysis

Population characteristics were described employing category variable frequencies; comparisons were made by using a Chi-squared or Fisher exact test (when needed), at a significance level of P≤0.05. For continuous variables, the mean and median were calculated; comparison between variables was assessed by a Kruskal-Wallis test, with an *a posteriori* Dunn's test (when necessary) at a significance level of P≤0.05.

Analyses of SNP allelic and genotypic frequencies, Hardy-Weinberg equilibrium (HWE; Chi-squared test at a significance level of P≤0.05), and linkage disequilibrium (LD) were done with the Arlequin ver. 3.0 software (37). Haplotype frequencies were estimated with the PHASE ver. 2.1 software and the EM algorithm (38,39), and by visual inspection according to the reference haplotype from the Pharmacogenomics Knowledge Base (PharmGKB) (40); diplotypes were conformed as previously suggested (41), and GP inferred with the metabolic activity score (MAS) following the Gaedigk *et al.* method (4). This method scores each allele in the *CYP2D6* diplotype, assigning a value of 0 to null activity alleles, 0.5 to those with decreased activity, and 1.0 to those with normal activity. The score sums of each diplotype are categorized as 0 (gPM); 0.5 (gIM); 1–2 (gNM); and >2 (gUM).

The association between the evaluated TSE and the genetic and non-genetic variables of interest was measured with the two-sided Chi-squared test in univariate models at a P≤0.05 significance level. Comparative parametric and non-parametric tests were applied when needed. Binary logistic regression using dichotomous categories was applied to predict TSE occurrence. Odds ratios (OR), P values, and 95% confidence intervals (95% CI) were calculated. These analyses were run with the SPSS ver. 20 statistical software (SPSS, Inc., Chicago, IL, USA).

Results

Study population

Mean patient age was 50.7 years (range, 31–82 years), with similar proportions of pre- and post-menopausal women (*Table 1*). Based on Graffar's socioeconomic scale, 97.2% of participants belonged to classes II–IV, and only 2.8% to class I. Most (77.5%) participants had received chemotherapy prior to initiating tamoxifen treatment, while only 21.1% had exhibited at least one chronic disease. Median length of tamoxifen use was 21 months (range: 3–108 months).

TSEs

Most participants (90.14%) reported at least one TSE. The four most frequent TSE were HF (57.75%), arthralgia (45.07%), headache (43.66%), and cramps (39.44%). All the remaining TSE occurred at a <20% frequency. Moreover, no

Table 1 Study population information

Characteristics	Value
Age (years)	50.7±11.4
BMI (kg/m^2)	29.6±5.2
Age at menarche (years)	12.3±1.4
Socioeconomic status	
Class I	2 (2.8)
Class II	25 (35.2)
Class III	23 (32.4)
Class IV	21 (29.6)
Menopausal status	
Pre-menopausal	36 (50.7)
Post-menopausal	35 (49.3)
Chemotherapy before tamoxifen	
Yes	55 (77.5)
No	16 (22.5)
At least one other chronic disease	
Yes	15 (21.1)
No	56 (78.9)
Contraceptive therapy use	
Yes	26 (36.6)
No	45 (63.4)
Time using contraceptive	
Therapy (months)	60 [1–180]
Time using TAM (months)	21 [3–108]

Data are shown as mean ± SD, number (percentage) or median [range]. BMI, body mass index; SD, standard deviation.

significant difference in TSE occurrence was identified based on the two tamoxifen treatment length categories (P>0.19) (*Table 2*). Notably, three patients had been following a 10-year treatment protocol and all had experienced TSE. A relatively small proportion of participants reported severe TSE: 4.23% for HF; 1.41% for arthralgia; 1.41% for headaches; and 1.41% for vomiting (*Table S3*). No significant difference was found in TSE severity according to length of tamoxifen use (P>0.10; *Table S3*).

CYP2D6 genotypes/phenotypes

Four SNP corresponded to the HWE (P>0.2), and three were monomorphic (rs59421388, rs35742686, and rs28371706) (*Figure S1*). Linkage disequilibrium (LD) analysis identified disequilibrium between rs16947 and rs3892097 and rs1065852 (D'=0.99; P<0.05). In addition, disequilibrium was present between rs1065852 and rs3892097 (D'=1.00; P=0.0001). The *CYP2D6* haplotype was therefore inferred using only the polymorphic variants (*Table 3*).

Fourteen diplotypes were identified in the population, the most prevalent (32.4%) being *CYP2D6*1/*2* (*Table 3*). Using the MAS method (4) phenotypes were inferred for the four metabolizer categories: gIM (0%); gPM (2.8%); gUM (4.2%); and gNM (93.0%).

TSEs: predictive value of CYP2D6 and other clinical variables

After grouping the cases by their inferred GP no differences were observed in age (P=0.23) or the median number of TSE exhibited per each category (P=0.41) (*Table S4*); although six patients (9.09%) in the gNM group (n=66) exhibited up to five different TSE. All six studied TSE were reported by participants in the gNM group; arthralgia, vomiting, and cramps were not present in the gUM group; and arthralgia, vomiting, and dizziness were not present in the gPM group. No differences in TSE presence/absence were identified between these three GP (P>0.16) (*Table S4*).

Uni- and multivariable analyses showed the *CYP2D6* GP to be inadequate predictors of the occurrence of at least one TSE (*Table S5*), and inadequate predictors specifically of HF (*Table 4*), as well as of the remaining symptoms (data not shown). However, these models did identify chemotherapy prior to tamoxifen initiation as a strong predictor of at least one TSE (*Table S5*). Chemotherapy and contraceptive use during reproductive age were strong predictors of HF occurrence (*Table 4*). In the multivariable analysis, chemotherapy was predictive only of headache (*Table S6*), and pre-menopausal status only of cramps (*Table S6*).

Discussion

One of the main challenges to compliance in adjuvant hormone therapy (e.g., tamoxifen treatment) is the occurrence of TSE during initial treatment stages (14,42). However, this was not observed among the participants, since TSE occurred regardless of the point they were at in the treatment. Indeed, TSE prevalence were higher (P=0.005) in the participants studied here (89%) than in a

Table 2 Tamoxifen side effects exhibited in the studied population and by length of tamoxifen treatment

TSE	Tamoxifen length of treatment (months)			P value†
	Overall (n=71)	3–21 (n=36)	>21 (n=35)	
One TSE, n (%)				0.43
Yes	64 (90.14)	31 (86.11)	33 (94.29)	
No	7 (9.86)	5 (13.89)	2 (5.71)	
Hot flashes, n (%)				0.39
Yes	41 (57.75)	19 (52.78)	22 (62.86)	
No	30 (42.25)	17 (47.22)	13 (37.14)	
Arthralgia, n (%)				0.91
Yes	32 (45.07)	16 (44.44)	16 (45.71)	
No	39 (54.93)	20 (55.56)	19 (54.29)	
Headache, n (%)				0.19
Yes	31 (43.66)	13 (36.11)	18 (51.43)	
No	40 (56.34)	23 (63.89)	17 (48.57)	
Vomiting, n (%)				0.43
Yes	7 (9.86)	5 (13.89)	2 (5.71)	
No	64 (90.14)	31 (86.11)	33 (94.29)	
Nausea, n (%)				0.96
Yes	12 (16.90)	6 (16.67)	6 (17.14)	
No	59 (83.10)	30 (83.33)	29 (82.86)	
Dizziness, n (%)				0.61
Yes	16 (22.54)	9 (25.00)	7 (20.00)	
No	55 (77.46)	27 (75.00)	28 (80.00)	
Cramps, n (%)				0.29
Yes	28 (39.44)	12 (33.33)	16 (45.71)	
No	43 (60.56)	24 (66.67)	19 (54.29)	

†, Chi-squared test calculated between 3 and 21 and >21 groups. TSE, tamoxifen side effects.

study of U.S. patients (73%) (15).

Hot flashes (HF) are the most prevalent and widely studied vasomotor symptom (15,43-45), and have been proposed as a suitable marker of therapeutic efficacy (8,43). This agrees with the present findings in which HF were the most prevalent TSE (57.75%). Occurrence did not differ between pre- and post-menopausal women, which do not agree with a study largely attributing HF to pre-menopausal women (15). Only 4.23% of the participants in the present results reported HF as severe, whereas up to 21% were reported as severe in U.S. patients (15).

Some authors have suggested that HF occurrence depends on the adequate endoxifen levels, since this is the most active metabolite produced from tamoxifen biotransformation (8,16), and is associated to *CYP2D6* integrity. However, this association has not been found in other studies (9). The present results were more in accordance with in that the *CYP2D6* GP were not predictive of any of the studied TSE. By contrast, Goetz *et al.* (8) reported that gPM patients had lower HF incidence than gNM and the randomized Breast International Group (BIG) 1-98 Trial found that gPM and gIM patients exhibited

Table 3 *CYP2D6* haplotype/diplotype frequencies and inferred genetic phenotypes (n=71)

Inferred alleles	Frequencies (%)	Functional status	Diplotypes	Number (%)	MAS	GP (n, %)
**4*	5.9	No function	**4J/*5*	2 (2.8)	0	gPM (2, 2.8)
**4J*	5.2		**34/*4J*	3 (4.2)	1	gNM (66, 93.0)
**5*	2.2		**1/*4*	6 (8.5)		
**10*	0.8	Decreased function	**2/*4*	3 (4.2)		
**1*	37.5	Normal function	**39/*5*	1 (1.4)		
			**1/*10*	1 (1.4)	1.5	
			**1/*1*	10 (14.1)	2	
**2*	18.4		**1/*2*	23 (32.4)		
			**2/*2*	4 (5.6)		
**34*	13.2		**34/*34*	7 (9.9)		
			**39/*39*	8 (11.3)		
**39*	14.7		**1×N/*2*	1 (1.4)	3	gUM (3, 4.2)
			**2×N/*34*	1 (1.4)		
**1×N,*34×N,*39×N*	2.2	Increased function	**39×N/*39*	1 (1.4)		

GP, genetic phenotype; MAS, metabolic activity score; gPM, genetic poor metabolizer; gIM, genetic intermediate metabolizer; gNM, genetic normal metabolizer; gUM, genetic ultra-rapid metabolizer.

a higher risk of HF than gNM patients (46). Of note is that two of the patients assigned to the gPM group in the present study lacked TSE predictive value. In another study, SNP suggestive of *CYP2D6*41* (related to the gIM phenotype) were associated with fatty liver disease in breast cancer patients under tamoxifen treatment (47).

Since gNM and gUM efficiently generate endoxifen, they would, in theory, be the groups at highest risk of TSE occurrence (48). One study of Italian gUM patients found them to have a higher number of TSE when compared with gPM, gIM and gNM groups (16). Again, the present results exhibited no statistical difference between median TSE numbers among the three identified phenotypes. One study that coincides with the present results is derived from the Adjuvant Tamoxifen and Exemestane in Early Breast Cancer trial and found no association was found between CYP2D6 genotypes/phenotypes and TSE (9). As mentioned above, most previous research in this area has mainly involved populations of Caucasian and Asian origin, leaving geographic areas, such as Latin America acutely underrepresented. How *CYP2D6* influences TSE remains unclear and will require extensive further research to determine if it has predictive relevance in clinical practice.

It was noteworthy that tamoxifen use with previous chemotherapy was found to be a strong predictor of HF, an association that remained significant even after adjusting for other variables such as CYP2D6 phenotype. This does not coincide with results from the BIG 1-98 group in which an association was found between HF and *CYP2D6* in the group which had received chemotherapy prior to tamoxifen use (46). Indeed, even when taking into account patients who had received chemotherapy (n=55), phenotype was not associated with HF in the present study; although contraceptive use during reproductive age was still a predictive factor (*Table S6*). Though not a direct contrast, the fact that presence of contraceptive use during reproductive age in the present results was identified as a protective factor against HF development in both the uni- and multivariable models is not supported by a study in the US in which previous use of post-menopausal hormone replacement therapy was reported as a risk factor for development of TSE (15).

Of the alleles responsible for the absence of an association between TSE and *CYP2D6* GP found here, two

Table 4 Uni- and multivariable models for prediction of tamoxifen-induced hot flashes

Explanatory variables	Hot flashes (yes/no)			
	Unadjusted		Adjusted[a]	
	OR (95% CI)	P	OR (95% CI)	P
Age (years)				
>40	Ref.		Ref.	
≤40	0.80 (0.25–2.51)	0.70	0.46 (0.10–2.04)	0.31
BMI (Kg/m^2)				
>30	Ref.		Ref.	
≤30	0.91 (0.34–2.39)	0.84	0.71 (0.22–2.32)	0.57
Months using TAM				
>21	Ref.		Ref.	
≤21	0.66 (0.26–1.70)	0.39	0.75 (0.24–2.38)	0.34
Pre–menopausal				
No	Ref.		Ref	
Yes	1.16 (0.44–3.04)	0.77	1.70 (0.44–6.49)	0.44
Contraceptive therapy				
No	Ref.		Ref.	
Yes	0.22 (0.08–0.61)	0.003	0.21 (0.07–0.67)	0.008
Genetic phenotype				
gNM/gUM	Ref.		Ref.	
gPM	–	–	–	–
Chemotherapy				
No	Ref.		Ref.	
Yes	6.17 (1.74–21.83)	0.003	6.98 (1.68–29.09)	0.008

[a], Odds ratio adjusted for all variables listed in table. Ref, reference category; OR, odds ratio; CI 95%, 95% confidence interval; BMI, body mass index; gNM, genetic normal metabolizer; gPM, genetic poor metabolizer; gUM, genetic ultra-rapid metabolizer.

apparent functional variants are worth noting: *CYP2D6*34* (13.2%) and **39* (14.7%). The **34* variant was originally reported in 1997 in a European population at a <2.7% frequency (49), and **39* was named a "functional allele" in 2008 after *in vitro* evaluation (50). Both alleles were also reported in 2010 in a Brazilian population at <1% (51). Samples inferred as *CYP2D6*39* could be analyzed with an extended SNP panel including rs1058164, exclusive to *CYP2D6*39* (52). Additional analyses could target other variants recently reported in Mexico: rs769258 for *CYP2D6*35* (53); rs28735595 for *CYP2D6*41* (53); or rs267608 for *CYP2D6*53* (21).

The 2.8% frequency for gPM in the present population does not differ (P=0.47) from that reported in a study of women in the US (~5%) (15). In Asians, gPM barely reaches 0.2%, while in Caucasians it is higher (~10%) (54). In another study, *CYP2D6*4* was the main allele (70–90% of cases) representing the gPM group (55), but in the present study it was the genotype **4J/*5*. To our knowledge this is the first report of a sub-variant form of the **4* allele in Mexican Mestizo patients. The original report for **4J* was at a <2% frequency (49), which is lower than the 5.1% observed here. Allele **5* was detected in 2.2% of the present cases. Its frequency varies widely between different populations (2.6–12.5%) (46,56-59), but the present results coincide with those reported for a general population in

Mexico (1.3–2.67%) (60).

A possible inherent limitation to the present study is the small sample size of the cross-sectional design, which still had 60% of the power needed to detect significant pharmacogenetic associations (see Methods). This enabled it to identify the less common variants in *CYP2D6* (of which only two were classified as gPM). As described in previous paragraphs, the association has been inconsistent between studies. The limited sample size is due to the fact that only current tamoxifen users could be included in the study; this is a result of institutional limitations within the Mexican public health system and the cross-sectional approach. This means no data was collected about patients who could not tolerate this drug, had finished or stopped the treatment with it, or had switched to an aromatase inhibitor. One way to begin addressing this shortfall would be to implement retrospective analyses within the studied population to build a more integrated perspective of tamoxifen efficacy.

Phenotype inference is a complex process that depends heavily on the criteria used. In the present results, for instance, even though no gIM-related alleles (*CYP2D6*17*, **29*, **41* or **59*) were identified, the MAS system would have classified them as gNM in the presence of normal function alleles (4). Application of other criteria might have classified them as gIM rather than gNM (41), which could agree with the allele-dosage effect of reduced-function *CYP2D6* alleles on reduced plasma endoxifen concentrations (61). The gIM group is therefore operationally defined by separating the gNM/gPM genotype from the gNM (41). Under this scenario the gNM group (93.0%) in the present results would account for 76.1% of the total while the gIM group would be 18.3%. The gIM GP is also very rare in Mexican Mestizos; it has not been reported at all in the country's southeast (53), and attains only 2.0% frequency in the north (62). Moreover, when the analysis included all four GP (i.e., gPM, gIM, gNM and gUM) they still had no effect on HF prediction (data not shown). Variation in phenotype inference criteria can clearly generate incongruous results in different data sets. Further pharmacokinetic research will help to clarify the causes behind this variation and to better define phenotype groups.

The present findings on side effects in women under tamoxifen treatment are in conspicuous disagreement with some previous research. Generating a broader data foundation that would allow drawing firm conclusions will require a larger sample size that captures the genetic complexity of Mexican Mestizos, and evaluation of the "real" metabolic phenotype.

Conclusions

Hot flashes were the most frequent TSE in the present sample, but *CYP2D6* genetic phenotypes were not effective predictors of side effect occurrence or frequency. Chemotherapy prior to tamoxifen treatment and contraceptive use during reproductive age were the only two predictive biomarkers of hot flashes. Allele distribution results showed *CYP2D6*34* and **39* to have unusually high frequencies (even after adjusting for the possible presence of other variants reported in other groups from Mexico) in the studied women, which have not been reported in other populations. In conjunction with other reports, the present study contributes to pharmacogenetic characterization of Mexico's Mestizo populations, which are poorly represented in the literature. Further research is sorely needed to better elucidate the controversial association between *CYP2D6* and tamoxifen metabolism.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2018.12.27). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures complied with the ethical standards of the IMSS National Clinical Research Ethics Committee (R-2013-785-057), the Dr. Hideyo Noguchi Regional Research Center Review Board for Ethical Research with Human Subjects, and the 1964 Helsinki Declaration and its amendments (as revised in 2013). A

written informed consent was obtained individually from all study participants.

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Supplementary

Table S1 CYP2D6 inhibitors and inductors considered for the study

Class	Drugs
Inhibitors	
Anti-arrhythmic	Amiodarone, propafenone
Antibiotics	Quinidine, terbinafine, chloroquine, quinacrine
Anticancer drugs	Doxorubicin, lomustine, vinblastine, vincristine, vinorelbine
Antihistamines	Chlorphenamine, diphenhydramine, cimetidine, ranitidine
Anti-hypertensives	Labetalol, mibefradil
Antipsychotics	Chlorpromazine, haloperidol, thioridazine, levomepromazine, fluphenazine
Antiretrovirals	Ritonavir, delavirdine
Calcium-channel blockers	Diltiazem
Inhibitor of monoamine-oxidase	Moclobemide
Nonsteroidal anti-inflammatory drugs	Celecoxib
Other	Bupropion, metoclopramide, lobeline, yohimbine, encapone
Selective serotonine reuptake inhibitors	Citalopram, fluoxetine, paroxetine, fluvoxamine
Steroidal anti-inflammatory drugs	Methadone, codeine, dextropropoxyphene
Tricyclic antidepressants	Clomipramine, imipramine, desipramine
Inductors	
Antibiotics	Glucocorticoids, griseofulvin, rifabutin, rifampicin, nafcillin, sulfadimidine
Anticonvulsive	Carbamazepine, ethosuximide, phenytoin, primidone, oxcarbazepine, Phenobarbital
Antidiabetics	Troglitazone
Antigout	Sulfinpyrazone
Antiretrovirals	Nelfinavir, nevirapine
Hormone replace drugs	Progesterone
Nonsteroidal anti-inflammatory drugs	Phenylbutazone, rofecoxib
Steroids	Dexamethasone

Table S2 TaqMan® probes and their *CYP2D6* allele targets

SNP	SNP rs	TaqMan probes	*CYP2D6* allele targets
100 C/T	rs1065852	C__11484460_40	*CYP2D6*4, *10*
1023 C/T	rs28371706	C__2222771_40	*CYP2D6*17*
4180 G/C	rs1135840	C__27102414_10	*CYP2D6*2,*4, *10, *17, *29, *39*
2850 C/T	rs16947	C__27102425_10	*CYP2D6*2, *17, *29, *34*
1846 G/A	rs3892097	C__27102431_D0	*CYP2D6*4*
2549 Del	rs35742686	C__32407232_50	*CYP2D6*3*
3183 C/T	rs59421388	C__34816113_20	*CYP2D6*29*
XN/Del		Hs00010001_cn	*CYP2D6*XN/CYP2D6*5*

SNP, single-nucleotide polymorphism; XN, multiplications; Del, deletion.

Table S3 Tamoxifen side effects severity as reported by study participants and by length of tamoxifen treatment

TSE	Tamoxifen length of treatment (months)			P value[†]
	Overall (n=71)	3–21 (n=36)	>21 (n=35)	
Hot flashes, n (%)				0.77
Mild	28 (39.44)	16 (44.44)	12 (34.29)	
Moderate	9 (12.68)	4 (11.11)	5 (14.29)	
Severe	3 (4.23)	2 (5.56)	1 (2.86)	
No	31 (43.66)	14 (38.89)	17 (48.57)	
Arthralgia, n (%)				0.90
Mild	19 (26.76)	9 (25.00)	10 (28.57)	
Moderate	12 (16.90)	7 (19.44)	5 (14.29)	
Severe	1 (1.41)	0 (0.00)	1 (2.86)	
No	39 (54.90)	20 (55.56)	19 (54.29)	
Headache, n (%)				0.11
Mild	18 (25.41)	13 (36.11)	5 (14.29)	
Moderate	12 (16.93)	6 (16.67)	6 (17.14)	
Severe	1 (1.41)	0 (0.00)	1 (2.86)	
No	40 (56.34)	17 (47.22)	23 (65.71)	
Vomiting, n (%)				0.30
Mild	4 (5.63)	3 (8.33)	1 (2.86)	
Moderate	2 (2.82)	2 (5.56)	0 (0.00)	
Severe	1 (1.41)	0 (0.00)	1 (2.86)	
No	64 (90.14)	31 (86.11)	33 (94.29)	
Nausea, n (%)				0.58
Mild	6 (8.45)	2 (5.56)	4 (11.43)	
Moderate	6 (8.45)	4 (11.11)	2 (5.71)	
Severe	0 (0.00)	0 (0.00)	0 (0.00)	
No	59 (83.10)	30 (83.33)	29 (82.86)	
Dizziness, n (%)				1.00
Mild	13 (18.31)	7 (19.44)	6 (17.14)	
Moderate	3 (4.23)	2 (5.56)	1 (2.86)	
Severe	0 (0.00)	0 (0.00)	0 (0.00)	
No	55 (77.46)	27 (75.00)	28 (80.00)	
Cramps, n (%)				0.29
Mild	14 (19.72)	8 (22.22)	6 (17.14)	
Moderate	14 (19.72)	6 (16.67)	7 (20.00)	
Severe	0 (0.00)	0 (0.00)	0 (0.00)	
No	43 (60.56)	22 (61.11)	22 (62.86)	

[†], Fisher's exact test. TSE, tamoxifen side effects.

Table S4 Reported tamoxifen side effects by inferred phenotype

TSE	gNM (n=66)	gUM (n=3)	gPM (n=2)	P value
Age (years)	48.50 [31.00–82.00]	57.00 [48.00–57.00]	60.00 [55.00–65.00]	0.23[b]
No. of TSE	2 [0–5]	2 [0–3]	1 [1–1]	0.41[b]
Hot flashes				0.63[c]
Yes	37 (56.06)	2 (66.67)	2 (100.00)	
No	29 (43.94)	1 (33.33)	0 (0.00)	
Arthralgia				0.16[c]
Yes	32 (48.48)	0 (0.00)	0 (0.00)	
No	34 (51.52)	3 (100.00)	2 (100.00)	
Headache				0.30[c]
Yes	28 (42.42)	1 (33.33)	2 (100.00)	
No	38 (57.58)	2 (66.67)	0 (0.00)	
Vomiting				1.00[c]
Yes	7 (10.61)	0 (0.00)	0 (0.00)	
No	59 (89.39)	3 (100.00)	2 (100.00)	
Nausea				0.75[c]
Yes	10 (15.15)	1 (33.33)	1 (50.00)	
No	56 (84.85)	2 (66.67)	1 (50.00)	
Dizziness				0.73[c]
Yes	15 (22.73)	1 (33.33)	0 (0.00)	
No	51 (77.27)	2 (66.67)	2 (100.00)	
Cramps				0.38[c]
Yes	27 (40.91)	0 (0.00)	1 (50.00)	
No	39 (59.09)	3 (100.00)	1 (50.00)	

Data presented as median [range] or number (percentage). [b], Kruskal-Wallis test; [c], Fisher's exact test. gNM, genetic normal metabolizer; gPM, genetic poor metabolizer; gUM, genetic ultra-rapid metabolizer; TSE, tamoxifen side effects.

Table S5 Uni- and multivariable models for clinical predictors of tamoxifen side effects

Explanatory variables	One TSE (yes/no)			
	Unadjusted		Adjusted[a]	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (years)				
>40	Ref.		Ref.	
≤40	0.64 (0.11–3.66)	0.63	0.03 (0.001–1.03)	0.052
BMI (kg/m^2)				
>30	Ref.		Ref.	
≤30	0.24 (0.03–2.14)	0.24	0.08 (0.005–1.25)	0.07
Months using TAM				
>21	Ref.		Ref.	
≤21	0.38 (0.07–2.08)	0.43	0.29 (0.04–2.38)	0.11
Pre-menopausal				
No	Ref.		Ref	
Yes	2.38 (0.49–11.56)	0.42	12.78 (0.67–244.60)	0.09
Contraceptive therapy				
No	Ref.		Ref.	
Yes	0.75 (0.15–3.64)	1.00	0.50 (0.07–3.87)	0.51
Genetic phenotype				
gNM/gUM	Ref.		Ref.	
gPM	–	–	–	
Chemotherapy				
No	Ref.		Ref.	
Yes	5.78 (1.14–29.30)	0.04	24.28 (1.80–330.26)	0.02

[a], Odds ratio adjusted for all variables listed in table. Ref, reference category; OR, odds ratio; 95% CI, 95% confidence interval; BMI, body mass index; gNM, genetic normal metabolizer; gPM, genetic poor metabolizer; gUM, genetic ultra-rapid metabolizer; TSE, tamoxifen side effects.

Table S6 Uni- and multivariable models for prediction of tamoxifen-induced headache, cramps, and hot flashes in chemotherapy-treated patients

Explanatory variables	Headache (yes/no) (n=71)				Cramps (yes/no) (n=71)				Hot flashes (yes/no) (n=55)[†]			
	Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]		Unadjusted		Adjusted[a]	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Age (years)												
>40	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
≤40	0.83 (0.26–2.64)	0.75	0.30 (0.07–1.27)	0.10	0.18 (0.04–0.86)	0.02	0.16 (0.03–0.94)	0.04	0.37 (0.11–1.29)	0.11	0.30 (0.06–1.54)	0.15
BMI (Kg/m^2)												
>30	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
≤30	0.59 (0.22–1.54)	0.28	0.33 (0.10–1.08)	0.07	0.71 (0.27–1.90)	0.50	0.67 (0.22–2.07)	0.48	0.63 (0.19–2.15)	0.46	0.71 (0.17–2.99)	0.64
Months using TAM												
>21	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
≤21	0.53 (0.21–1.38)	0.19	0.45 (0.14–1.42)	0.17	0.59 (0.23–1.55)	0.29	0.47 (0.15–1.44)	0.19	0.54 (0.17–1.70)	0.29	0.66 (0.17–2.59)	0.55
Pre–menopausal												
No	Ref.		Ref		Ref.		Ref		Ref.		Ref.	
Yes	2.00 (0.74–5.41)	0.17	2.95 (0.80–10.89)	0.11	0.34 (0.12–0.91)	0.03	0.39 (0.12–1.29)	0.12	0.71 (0.21–2.43)	0.59	1.42 (0.28–7.13)	0.67
Contraceptive therapy												
No	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
Yes	1.17 (0.44–3.10)	0.75	1.90 (0.58–6.24)	0.29	1.20 (0.45–3.23)	0.71	2.37 (0.73–7.78)	0.15	0.12 (0.03–0.42)	0.0001	0.13 (0.04–0.50)	0.003
Genetic phenotype												
gNM/gUM	Ref.		Ref.		Ref.		Ref.		Ref.		Ref.	
gPM	–	–	–	–	1.56 (0.10–25.93)	1.00	0.89 (0.04–18.61)	0.94	–	–	–	–
Chemotherapy												
No	Ref.		Ref.		Ref.		Ref.		NR		NR	
Yes	7.80 (1.62–37.65)	0.004	12.33 (2.06–73.65)	0.006	1.58 (0.48–5.17)	0.45	3.13 (0.83–11.83)	0.09				

[a], Odds ratio adjusted for all variables listed in table; [†], Calculated in chemotherapy-treated patients. Ref, reference category; OR, odds ratio; CI 95%, 95% confidence interval; BMI, body mass index; gNM, genetic normal metabolizer; gPM, genetic poor metabolizer; gUM, genetic ultra-rapid metabolizer; NR, no required.

Reference (SNP)	Genotypic frequencies (n)			Allelic frequencies (n)	
rs1135840 (4180 G/C)	CC	CG	GG	C	G
	0.31 (22)	0.45 (32)	0.24 (17)	0.54 (76)	0.46 (66)
HWE: P=0.48					
rs16947 (2850 C/T)	AA	GA	GG	G	A
	0.17 (12)	0.42 (30)	0.41 (29)	0.62 (88)	0.38 (54)
HWE: P=0.45					
rs3892097 (1846 G/A)	CC	CT	TT	C	T
	0.80 (57)	0.17 (12)	0.03 (2)	0.89 (126)	0.11 (16)
HWE: P=0.20					
rs1065852 (100 C/T)	AA	GA	GG	G	A
	0.03 (2)	0.18 (13)	0.79 (56)	0.88 (125)	0.12 (17)
HWE: P=0.25					
rs59421388 (3183 C/T)	CC	Monomorphic			
rs35742686 (2549 Del/A)	TT	Monomorphic			
rs28371706(1023 C/T)	GG	Monomorphic			

Figure S1 Genotype and allele frequencies with Hardy-Weinberg equilibrium from the evaluated SNP. HWE, Hardy-Weinberg Equilibrium.