Comparative study of polymorphism frequencies of the *CYP2D6*, *CYP3A5*, *CYP2C8* and *IL-10* genes in Mexican and Spanish women with breast cancer

Aim: Pharmacogenetic studies in breast cancer (BC) may predict the efficacy of tamoxifen and the toxicity of paclitaxel and capecitabine. We determined the frequency of polymorphisms in the *CYP2D6* gene associated with activation of tamoxifen, and those of the genes *CYP2C8*, *CYP3A5* and *DPYD* associated with toxicity of paclitaxel and capecitabine. We also included a *IL-10* gene polymorphism associated with advanced tumor stage at diagnosis. **Patients & methods:** Genomic DNAs from 241 BC patients from northeast Mexico were genotyped using DNA microarray technology. **Results:** For tamoxifen processing, *CYP2D6* genotyping predicted that 90.8% of patients were normal metabolizers, 4.2% ultrarapid, 2.1% intermediate and 2.9% poor metabolizers. For paclitaxel and the *CYP2C8* gene, 75.3% were normal, 23.4% intermediate and 1.3% poor metabolizers. Regarding the *DPYD* gene, only one patient was a poor metabolizer. For the *IL-10* gene, 47.1% were poor metabolizers. **Conclusion:** These results contribute valuable information towards personalizing BC chemotherapy in Mexican women.

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KEYWORDS: breast cancer ■ capecitabine ■ DNA ■ paclitaxel ■ pharmacogenetics ■ polymorphism ■ tamoxifen

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Breast cancer (BC) is the most common cancer in women worldwide, with more than 1,380,000 new cases each year and a mortality of approximately 500,000 in 2008 [101]. Clinically, BC is an extremely heterogeneous disease, where the determination of histologic type [1,2], tumor size and the status of lymph nodes, hormones and growth factor receptors contribute to the pathological diagnosis and prompt predictions, forecasts and, ultimately, therapy recommendations [2,3]. Approximately 50% of women diagnosed with primary BC will eventually relapse and develop metastatic or advanced disease. In addition, approximately 10% of patients will already present metastatic disease at the time of diagnosis [4]. In spite of a correct histologic diagnosis, many patients are over- or under-treated as a result of the different individual responses to drug therapy.

Many factors contribute to individual variability in relation to treatment, including age, BMI, diet, compliance with therapy and the characteristics of the drug target. However, genetic variations such as SNPs in genes related to drug-metabolizing enzymes greatly influence the individual response to drugs. Variations mainly in four genes, *CYP2D6*, *CYP2C8*, *CYP3A5* and *DPYD*, have been demonstrated to be very strong predictors of the effectiveness of tamoxifen and the toxicity of capecitabine and paclitaxel [5–9], which are among the most commonly used drugs in BC treatment. Therefore, it would be helpful to analyze the patient's genetic profile in order to personalize treatment based on their ability to efficiently metabolize these drugs.

Tamoxifen, a selective estrogen receptor modulator, requires metabolic activation by enzymes before its conversion into its active metabolites, 4-hydroxytamoxifen and endoxifen. Genetic polymorphisms in the *CYP2D6* gene affect the catalytic activity of its enzyme, resulting in different plasma concentrations of the active metabolites of tamoxifen [10].

Four CYP2D6 phenotypes are observed on the basis of their metabolic capabilities: poor metabolizer (PM) – absent or very low metabolic activity predicted from the presence of two inactive alleles; intermediate metabolizer (IM) – decreased metabolic activity predicted from the presence of an active allele combined with a decreased activity or a no activity allele, two decreased-activity alleles or the combination of a decreased activity and a no activity allele; normal or extensive metabolizer (EM) – functional metabolic activity predicted from the combination of two active alleles; and ultrarapid metabolizer (UM) – increased metabolic activity predicted from the presence of three or more functional alleles due to extra

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copies of the gene [9,11]. More than 80 different alleles have been described, but the most frequent with functional activity are **1*, **2* and **35*, while **3*, **4*, **5*, **6*, and less frequently **14*, **18*, **21* and **44* are responsible for the loss of metabolic activity. Furthermore, alleles **10*, **17* and **41* have reduced activity, whereas duplication of the gene (**1xN* and **2xN*) leads to multiple copies and therefore to corresponding increased enzyme activity.

The frequency of the alleles varies between different populations. The PM phenotype is present in 5–10% of individuals in Caucasian populations (alleles **3*, **4*, **5* and **6* of the *CYP2D6* gene) [12]. By contrast, less than 1% of individuals from Asian populations show this phenotype [6]. In fact, most Asians are classified as IM owing to the high frequency of allele *CYP2D6*10* carriers [13].

The frequencies of individuals who are UM, ranges from 10 to 15% in individuals from Caucasian populations [14]. Patients who exhibit this type of drug metabolism may be more susceptible to episodes of hot flashes during tamoxifen therapy [15].

Another common drug used in BC is paclitaxel. This is a mitotic inhibitor [16,17] metabolized in the liver by isoforms encoded by the genes *CYP2C8* and *CYP3A* and then transported by P-gp. CYP2C8 is the principal enzyme that catalyzes the formation of the major metabolite, 6α-hydroxypaclitaxel. CYP3A4 and CYP3A5 enzymes have also been shown to contribute to the metabolism of paclitaxel to a lesser extent. The dose-limiting toxicities are neuropathy and neutropenia; however, the interindividual variability in toxicity and survival is considerable [18]. The *CYP2C8*3*, *CYP2C8–hapC* and *CYP3A5*3* variants have been associated with paclitaxel-induced neurotoxicity and myelosuppression. Carriers of *CYP2C8*3* had 11% lower paclitaxel clearance than noncarriers ($p = 0.03$) [19,20].

Despite the results mentioned above, other authors have found that the haplotype C (rs1113129, G>C) of *CYP2C8* and the allele *CYP3A5*3* are associated with protection, while *CYP2C8*3* has an increased risk of paclitaxel neurotoxicity [20,21]. Therefore, more research is required to define the contribution of the different alleles to serious adverse effects.

Recently, the *CYP2C8*13* and *CYP2C8*14* alleles have been described in the Japanese population. The *CYP2C8*14* allele reduces the affinity of the CYP2C8 enzyme to paclitaxel and may influence the clinical response to drugs [22].

Another drug used in chemotherapy against BC is capecitabine. This is an oral fluoropyrimidine. The activity of DPYD, the key enzyme in the catabolism of this drug, is a determining factor for the occurrence of severe toxic reactions to 5-fluorouracil (5-FU) and its derivatives, including capecitabine. Therefore, the *DPYD* gene polymorphism contributes to the heterogeneity of DPYD enzyme activity. More than 30 SNPs have been identified, although most of these variations do not have functional consequences in enzyme activity.

The intronic variant IVS14+1G>A (*DPYD*2A*) has been associated with poor DPYD enzyme activity and has been found in up to 40–50% of patients who developed grade 4 neutropenia [23]. G/G genotype is associated with normal enzyme activity, while G/A and A/A genotypes are associated with reduced or null activity. In addition, other variants of the *DPYD* gene have been associated with 5-FU toxicity. These are c.775A>G (K259E) and four other missense mutations, c.85T>C (C29R), c.496A>G (M166V), c.1601G>A (S534N) and c.1627A>G (I543V), as well as one silent mutation, c.1896T>C, which affects the F632 codon. Interestingly, the K259E mutation does not cause a decrease in the DPYD enzyme function in heterozygous individuals [24].

Although not directly related to drug metabolism, variation in the *IL-10* gene has also been proposed as being of prognostic value in BC. The A/A genotype of this gene has been associated with larger malignant tumors at the time of diagnosis [25]. Therefore, it is interesting to include this polymorphism in studies of genetic risk for this tumor.

The objective of this study was to determine the frequencies of the different polymorphisms in *CYP2D6*, *CYP3A5*, *CYP2C8*, *DPYD* and *IL-10* genes in women with BC from northeastern Mexico. It is a prerequisite to compare them with the corresponding frequencies described for the Spanish population, since this is an important component of the ethnic mix of our northeastern population. To carry out this study, we chose as a genomic screening tool, a DNAchip developed on the basis of allele frequencies in Spanish population. By attempting to achieve this goal, we are contributing valuable information in support of the adoption of personalized medicine strategies for fighting BC, capitalizing on the genetic and biochemical individuality of each patient to optimize effectiveness and reduce toxicity of chemotherapy.

Patients & methods

Subjects & samples

The subjects were BC patients selected among women receiving chemotherapy at the Centro Universitario Contra el Cáncer, Hospital Universitario Dr José E González (HU), Universidad Autónoma de Nuevo León and the Hospital de Especialidades #25 of the Instituto Mexicano del Seguro Social (IMSS), both located in Monterrey, Nuevo León, Mexico. Both are reference centers for patients affected by this neoplasm from all over northeastern Mexico. The HU Institutional Research Committee approved the study (registration no. BI10–002), which was also subsequently approved by Hospital #25. Of the patients who were interested in participating, 241 women gave written informed consent and voluntarily donated 5 ml of venous blood for DNA extraction. A commitment was made with the subjects doctors to send back customized reports of the findings revealed in the present study and to provide technical assistance for interpreting them. All women who participated in this study were Mexican and their parents and grandparents were also from Mexico. The allelic frequencies of Spanish women were obtained from the Progenika Biopharma SA database.

DNA preparation

Genomic DNA was extracted from whole blood of all 241 patients enrolled in the study, either with the QIAmp DNA Blood Kit (Qiagen Inc., CA, USA) according to the manufacturer's instructions, or using TSNT lysis buffer (1% Triton, 1% SDS, 100 mM NaCl, 10 mM Tris-HCl [pH 8.0] and 1 mM EDTA) followed by phenol–chloroform extraction and ethanol precipitation. DNA concentrations were adjusted with the spectrophotometer to 20 ng/µSl in purified nuclease-free water.

Analysis of DNA polymorphisms

The analysis of *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* gene polymorphisms was performed using a DNA microarray, PHARMAchip®, according to the manufacturer's instructions (Progenika Biopharma SA, Derio, Spain). PHARMAchip® was selected for use as it was one of the first pharmacogenetics tracking devices on the market. This pharmacogenetic device and its accompanying software are used to identify the predicted phenotype deduced from SNPs in genes related to drug metabolism that are common in European individuals, including the encoding enzymes CYP2D6, CYP2C8, CYP3A5, DPYD, and the cytokine IL-10. For example, based on the activity of the enzyme, the alleles **1*, **2* and **35* of the *CYP2D6* gene are characterized as of extensive activity; **9*, **10*, **17*, **29*, **41*, **10XN*, **17XN* and **41XN* as of reduced activity; **3*, **4*, **5*, **6*, **7*, **8*, **11*, **14A*, **15*, **19*, **20*, **36*, **40* and **4xN* as of null activity; **1xN*, **2xN*, **35XN* as of increased activity, and finally, **14B*, **25*, **26*, **30* and **31* as of unknown activity. For the rest of the genes investigated, this chip analyzed all the polymorphisms shown in Table 1.

Statistical analysis

Once the frequencies of the polymorphisms were obtained, the χ^2 test was used to compare the distribution of frequencies in Mexican patients with those reported for Spanish patients. First, Hardy–Weinberg equilibrium tests were carried out for all the different genotypes described. Only SNPs that conformed to Hardy–Weinberg equilibrium in both study groups were included.

In addition, association tests between SNP allelic frequencies (allelic associations) in Mexican and Spanish patients were carried out using the standard contingency χ^2 test and p-values.

All genetic analyzes were performed using HelixTree® software (Golden Helix, Inc., MT, USA) and SPSS (SPSS Inc., IL, USA) version 14.0.

Results

The age range of participants was between 40 and 60 years and clinical stages IIB and IIIB were the most frequently found. The pharmacogenetic analysis of their DNA efficiently revealed their *CYP2D6* gene variations. We identified that 90.8% of the study population had a normal metabolic profile for the processing of tamoxifen. However, 2.9% were PMs, resulting in an inability to convert tamoxifen to endoxifen. Finally, 4.2% of the patients turned out to be UM and another 2.1% IM (Table 2); in

Table 1. Allelic variants studied with PHARMAchip®.

Gene	Allelic variants
CYP2D6	**1*, **2*, **3*, **4*, **5* (deletion), **6*, **7*, **8*, **9*, **10*, **11*, **14A*, **14B*, **15*, **17*, **18*, **19*, **20*, **25*, **29*, **31*, **35*, **41*, **1XN* (duplication), **2XN*, **4XN*, **10XN*, **17XN*, **35XN* and **41XN*
DPYD	IVS14+1 G>A
IL-10	G-1082A
CYP2C8	**1*, **2*, **3* and **4*
CYP3A5	**1*, **3*, **6*, **9* and **10*

Table 2. *CYP2D6* genotypes and predicted phenotypes from Mexican and Spanish women.

CYP2D6 genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
**1/*1*	EM	40.2	15.0	6.19×10^{-14}
**1/*2*	EM	20.5	12.0	0.001
**1/*4*	EM	11.2	11.7	0.948
**1/*5*	EM	2.0	1.4	0.475
**1/*6*	EM	0.4	0.3	0.751
**1/*9*	EM	0.4	1.1	0.379
**1/*10*	EM	1.6	1.1	0.543
**1XN/*1*	UM	1.6	1.1	0.524
**1XN/*2*	UM	2.2	1.9	0.597
**1XN/*15*	EM	0.4	0.0	NA
**1/*17*	EM	1.2	1.3	0.139
**2/*2*	EM	1.6	4.1	0.101
**2/*4*	EM	0.8	8.2	9.22×10^{-5}
**2/*5*	EM	1.2	0.8	0.581
**1/*35*	EM	1.6	4.1	0.101
**1/*41*	EM	2.4	6.0	0.049
**4/*4*	PM	1.6	4.9	0.406
**4/*5*	PM	0.8	0.3	0.326
**5/*9*	IM	0.4	0.3	0.751
**2/*41*	EM	0.4	2.5	0.057
**4/*10*	IM	0.4	0.0	NA
**4/*4XN*	PM	0.4	0.0	NA
**4/*41*	IM	1.2	2.7	0.231
**6/*35*	EM	0.8	0.0	NA
**17/*35*	EM	0.4	0.6	NA
**2/*10*	EM	0.4	0.5	0.837
**1/*3*	IM	0.0	0.8	NA
**1XN/*41*	EM	0.0	0.3	NA
**1XN/*9*	EM	0.0	0.3	NA
**2/*3*	IM	0.0	0.8	NA
**2/*35*	EM	0.0	3.5	NA
**2/*6*	IM	0.0	0.3	NA
**2/*9*	IM	0.0	1.6	NA
**2XN/*10*	EM	0.0	0.3	NA
**2XN/*2*	UM	0.0	1.6	NA
**2XN/*4*	EM	0.0	1.1	NA
**2XN/*5*	EM	0.0	0.3	NA
**3/*4*	PM	0.0	0.3	NA
**35/*35*	EM	0.0	0.3	NA

EM: Normal or extensive metabolizer; IM: Intermediate metabolizer; NA: Not applicable; PM: Poor metabolizer; p χ^2: Probability of χ^2; UM: Ultrarapid metabolizer.

Table 2. *CYP2D6* genotypes and predicted phenotypes from Mexican and Spanish women (cont.).

CYP2D6 genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
**35/*41*	IM	0.0	1.1	NA
**4/*35*	IM	0.0	2.2	NA
**4/*9*	IM	0.0	2.2	NA
**41/*41*	IM	0.0	0.5	NA
**4XN/*5*	PM	0.0	0.3	NA
**5/*35*	IM	0.0	0.3	NA
**5/*41*	IM	0.0	0.3	NA
**9/*35*	IM	0.0	0.5	NA
**9/*9*	IM	0.0	0.3	NA
**10/*41*	IM	0.0	0.3	NA
**17/*41*	IM	0.0	0.3	NA

EM: Normal or extensive metabolizer; IM: Intermediate metabolizer; NA: Not applicable; PM: Poor metabolizer; p χ^2: Probability of χ^2; UM: Ultrarapid metabolizer.

both cases, dose adjustment is recommended to maintain optimal levels.

As for the *CYP2C8* gene, **1/*1* was the most frequent genotype (Table 3) related to the metabolism of paclitaxel and it corresponds to an EM (75.3%); **1/*2*, **1/*3* and **1/*4* genotypes correspond to an IMs and accounted for 23.4% of the population sampled; whereas PMs (**3/*3* and **3/*4* genotypes) accounted for 1.3% of the cases.

With regards to the *CYP3A5* gene, 94.9% of the patients carry at least one *CYP3A5*3* allele (Table 4) and 61.3% are **3/*3* homozygous, corresponding to PMs. In addition, one patient was found to have the **1/*6* (0.4%) genotype, which also corresponds to a PM phenotype. An EM profile was observed in only 4.6% of cases.

In the case of the *DPYD* gene, which is related to capecitabine metabolism, only one patient showed the A/A genotype (corresponding to PMs), while all the others showed the G/G genotype (which suggests patients will be EMs) (Table 5).

As for the variation in the *IL-10* gene (Table 6), which codes for a cytokine and whose A/C genotype has been associated with increased tumor size and lymphatic invasion at the time of diagnosis, 47.1% of the patients presented this genotype (poor production of the cytokine), while the rest had lower risk genotypes, A/G and G/G.

Comparison of allele frequencies of the genes studied (*CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10*) in European and Mexican women showed mostly nonsignificant differences (Tables 2–6).

Discussion

Tamoxifen is the most widely used drug for the prevention of BC in patients with estrogen-receptor positive tumors [26,27]. Irvin and colleagues showed evidence of the usefulness of tamoxifen dosing guided by genotyping of the *CYP2D6* gene [28]. By doubling the dose of tamoxifen from 20 to 40 mg/day, IMs reached endoxifen levels comparable to those of EMs (from normal tamoxifen doses), something that did not occur with PMs. This finding was confirmed in a cohort of 1325 patients treated with tamoxifen as adjuvant monotherapy, in what represents the largest study published to date that includes the state of tumor progression [29]. According to the genotyping of the *CYP2D6* gene, 2.9% of the patients were found to have a predicted phenotype as PMs. The

Table 3. Comparison of *CYP2C8* genotypes and predicted phenotypes between Spanish and Mexican women.

CYP2C8 genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
**1/*1*	EM	75.3	55.1	6.09×10^{-7}
**1/*2*	IM	1.8	0.8	0.284
**1/*3*	IM	13.2	26.9	8.25×10^{-5}
**1/*4*	IM	8.4	10.4	0.419
**3/*3*	PM	0.4	3.2	0.024
**3/*4*	PM	0.9	1.9	0.335
**2/*4*	PM	0	0.3	NA
**4/*4*	PM	0	1.6	NA

EM: Normal or extensive metabolizer; IM: Intermediate metabolizer; NA: Not applicable; PM: Poor metabolizer; p χ^2: Probability of χ^2.

Table 4. *CYP3A5* genotypes and predicted phenotypes from Mexican and Spanish women.

CYP3A5 genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
**1/*1*	EM	4.6	0.3	1.45×10^{-4}
**1/*3*	PM	31.9	13	6.23×10^{-10}
**1/*6*	PM	0.4	0	NA
**3/*3*	PM	61.3	85.9	1.19×10^{-7}
**3/*6*	PM	1.7	0.8	0.472

EM: Normal or extensive metabolizer; NA: Not applicable; PM: Poor metabolizer; p χ^2: Probability of χ^2.

genotypes involved were **4/*4* (1.6%), **4/*5* (0.8%) and **4/*4xN* (0.4%). Because of this genetic condition, patients are not candidates for treatment with tamoxifen. On the other hand, the 2.1% of patients who are IMs can benefit from dose adjustment. The other genotypes observed in this study were **5/*9* (0.4%), **4/*41* (1.2%) and **4/*10* (0.4%). This indicates that pharmacogenetic knowledge can be successfully used to adjust the concentrations of drugs in women with IM and UM, and to detect those patients in whom the drug will not be useful (PM). The latter is of utmost importance since tamoxifen is administered from 2 to 5 years as postchemotherapy treatment. Providing this treatment to women with poor tamoxifen metabolism implies leaving them unprotected, with the risk of tumor recurrence and disease progression.

CYP2C8 is the major enzyme responsible for paclitaxel metabolism. There are at least 20 SNPs identified in this gene. The ones defining the *CYP2C8*2* and *CYP2C8*3* alleles contain mutations that affect the sequence of the encoded protein. The *CYP2C8*2* mutant allele has an allelic frequency of 18% in African–Americans, while the *CYP2C8*3* allele has a frequency of 13% in Caucasians and 2% in African–Americans. Both *CYP2C8*2* and *CYP2C8*3* have a lower catalytic constant and a higher intrinsic clearance of paclitaxel, compared with the wild-type *CYP2C8*1* allele [30]. Clinical studies in patients have demonstrated significant variability in the pharmacokinetics of paclitaxel between individuals. Part of this variability could be explained by *CYP2C8* mutant alleles that reduce the metabolism and elimination of paclitaxel in the body. These mutant alleles could also increase the toxicity of this treatment. Only 0.4% of the patients presented a **3/*3* genotype against 3.2% in Spaniards. These patients require careful monitoring.

CYP3A5 protein expression is reduced in the case of the presence of a *CYP3A5*3* allele as a result of improper splicing of the pre-mRNA and the reduced translation of mRNA deficiently processed into a functional protein. The frequency of the *CYP3A5*3* allele varies from approximately 50% in African–Americans, up to 90% in Caucasians [31]. Detection of different alleles in the *CYP3A5* gene and knowledge of allelic frequency in specific ethnic groups are important requirements for establishing the clinical relevance of screening tests for these polymorphisms and optimizing pharmacotherapy. For example, in a previous study, the frequency of a *CYP3A5*3* defective allele in a Dutch–Caucasian population was 91%, followed by *CYP3A5*2* (1%) and *CYP3A5*6* (01%), while *CYP3A5*4*, **5* and **7* were not detected [32]. In our sample, patients carrying *CYP3A5*3* accounted for 94.9%, distributed in the following genotypes: **3/*3* (61.3%), **1/*3* (31.9%) and **3/*6* (1.7%).

DPYD gene homozygosity (genotype G/G) is associated with normal metabolism of capecitabine; 99.6% of all patients had this genotype. Only one case (0.4%) had the A/A homozygous genotype. However, four patients out of nine showed evidence of toxicity with the use of this drug. Because the mutation included in the DNAchip corresponds to the most common in the Spanish population, it is possible that some of the patients, who were negative for this 'Spanish' mutation, carry a different, still unknown, mutation that explains this toxicity in a Mexican population.

Finally, 47.1% of patients had the -1082A/A genotype for the *IL-10* gene, this being a multifunctional cytokine with both immunosuppressive and antiangiogenic functions. Polymorphisms in the promoter of *IL-10* genetically determine interindividual differences in cytokine production. In Chinese women, this genotype was associated with a significantly increased risk of affecting the lymph nodes and increased tumor size at the time of diagnosis. In the haplotype analysis of *IL-10*, patients carrying the ATA haplotype showed greater impairment in lymph nodes and a higher tumor stage

Table 5. *DPYD* genotypes and predicted phenotypes from Mexican and Spanish women.

DPYD genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
A/A	PM	0.4	0	NA
A/G	IM	0	0.5	NA
G/G	EM	99.6	99.5	0.832

EM: Normal or extensive metabolizer; IM: Intermediate metabolizer; NA: Not applicable; PM: Poor metabolizer; p χ^2: Probability of χ^2.

at the time of diagnosis, in comparison with other patients with different haplotypes (ACC, GTA and GCC) [23]. Subsequently, a meta--analysis suggested a lack of association between two SNPs (rs1800896 and rs1800872) in the promoter of the *IL-10* gene and BC [33]. Therefore, this association is still under study. In addition, some authors have shown that methylation of the *IL-10* gene is lower in cancer tissue than in normal and benign breast tissues, and this hypomethylation promotes gene activation. Consequently, hypomethylation of the *IL-10* gene may be involved in the process of breast carcinogenesis [34]. Further studies are required to clarify the role of *IL-10* in BC, either with a larger number of samples or with samples whose DNA analysis include other SNPs/haplotypes in the *IL-10* gene and study its methylation status.

The distribution of the allele frequencies of the *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* genes in patients with BC from northeastern Mexico is very similar to that reported for Spanish patients with the same neoplasia, presenting only minimal differences. The study will now be extended to central and southern regions of the country. Because these regions have a smaller Spanish component, it can be assumed that the DNAchip will be less useful and that newer versions with oligonucleotide probe sets for different ethnic mixes should be developed.

It is important to consider that our population is Mestizo. Using molecular and nonmolecular nuclear DNA markers, Mexican Mestizo populations are genetically homogeneous and the admixture contributions are Spanish (50–60%), Amerindian (37–49%) and African (1–3%) depending on the region of the country studied [35,36]. Furthermore, comparing the genetic structure of Mexican women with breast cancer with previously reported data of Mexican populations using SNPs, similar allele frequencies were found [37]. Therefore, it is important to characterize the distribution of polymorphisms in Mexican women.

In summary, the distribution of the allele frequencies of the *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* genes in patients with BC from northeastern Mexico is very similar to that reported for Spanish patients with the same neoplasia, presenting only minimal differences.

Table 6. Comparison of *IL-10* genotypes and predicted phenotypes between Spanish and Mexican women.

IL-10 genotype	Predicted phenotype	Mexican women (%)	Spanish women (%)	p χ^2
A/A	PM	47.1	37.5	0.017
A/G	IM	42.6	48.9	0.123
G/G	EM	10.2	13.6	0.218

EM: Normal or extensive metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer; p χ^2: Probability of χ^2.

Conclusion

SNPs present in northeastern Mexican populations were successfully traced by DNAchip with only 0.4% being unidentified alleles ('no calls'). The findings reveal that this pharmacogenomic tool is suitable for studying Mexican populations. At the present time, prospective studies are being conducted in order to relate the different polymorphisms in the different genes, whose variability was first revealed for Mexican populations in the present study. These will be useful to support potential benefits of personalized medicine for Mexican women affected by this disease.

Future perspective

Mexico is a developing country where the use of pharmacogenetics is not routine. It is important to implement measures to ensure that physicians are trained and learn about new tools of personalized medicine. In this research we used a DNAchip to identify polymorphisms in the *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* genes in patients with breast cancer in northeastern Mexico. This information will be useful to support the potential benefits of personalized medicine for Mexican women.

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- Many breast cancer (BC) patients are over- or under-treated as a result of different individual responses to drug therapy. Four CYP450 genes (*CYP2D6*, *CYP2C8*, *CYP3A5* and *DPYD*), have been demonstrated to be strong predictors for the effectiveness of tamoxifen and the toxicity of capecitabine and paclitaxel.
- Genetic polymorphisms in the *CYP2D6* gene affect the catalytic activity of its encoded enzyme, resulting in different plasma concentrations of the active metabolites of tamoxifen. Paclitaxel is metabolized in the liver by isoforms encoded by the genes *CYP2C8* and *CYP3A*.
- DPYD is the key enzyme in the catabolism of capecitabine. The intronic variant IVS14+1G>A (*DPYD*2A*) has been associated with poor DPYD enzyme activity.
- IL-10 is not directly related to drug metabolism; however, variation in the *IL-10* gene has been proposed to be of prognostic value in BC.

Patients & methods

- The aim of this study was to determine the frequencies of the different polymorphisms in *CYP2D6*, *CYP3A5*, *CYP2C8*, *DPYD* and *IL-10* genes in women with BC from northeastern Mexico. Patients (n = 241) gave written informed consent and voluntarily donated 5 ml of venous blood for DNA extraction. *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* gene polymorphisms were analyzed using a DNA microarray.

Results

- We found that 91% of the study population had a normal metabolic profile for the processing of tamoxifen, 2.9% showed a poor metabolism, 4.2% of the patients turned out to be ultrarapid metabolizers and another 2.1% were intermediate metabolizers.
- As for the *CYP2C8* gene, in relation to the metabolism of paclitaxel, we identified that 75.3% had a normal phenotype, 23.4% correspond to an intermediate metabolism and 1.3% were poor metabolizers. For the *CYP3A5* gene, 95.3% of the patients correspond to poor metabolizers and 4.6% of cases had a extensive metabolizer profile.
- In the case of the *DPYD* gene, which is related to capecitabine metabolism, only one patient showed a genotype associated with poor metabolism, while the rest of the patients had an extensive or normal metabolism.
- For the *IL-10* gene, 47.1% of the patients presented the A/C genotype, which is associated with poor production of the cytokine, while the rest had lower risk genotypes.

Discussion

- The distribution of the allele frequencies of the *CYP2D6*, *CYP2C8*, *CYP3A5*, *DPYD* and *IL-10* genes in patients with BC from northeastern Mexico, is very similar to that reported for Spanish patients with breast cancer. Pharmacogenomics studies will be useful to support the potential benefits of personalized medicine for Mexican women affected by this terrible disease.

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