Artículo original

Genetic polymorphisms in a Colombian women population with breast cancer and tamoxifen consumption

Polimorfismos genéticos en una población colombiana femenina con cáncer de mama y consumo de tamoxifeno

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ABSTRACT

Introduction: Breast cancer is positioned as the malignancy with the highest prevalence worldwide. In 2022, 2.2 million cases were reported. Colombia is considered the second country with the most frequent numbers of cancer in women. The epidemiological importance of this condition generates the need to establish in the region processes of demographic and clinical characterization, pharmacological intervention and identification of genetic variants, which determine a better understanding of cancer as a multifactorial situation.

Objective: To identify single nucleotide polymorphisms of CYP2D6*3, CYP2D*4 and ABCB1 C3435T in women with breast cancer with a history of tamoxifen consumption in the province of Caldas.

Methods: A descriptive and retrospective cross-sectional study was conducted in which 49 women from the department of Caldas, diagnosed with breast cancer on treatment with tamoxifen or aromatase inhibitors, who had a blood sample taken under the principles of

bioethics and established biosafety criteria and protocols. Subsequently, DNA extraction was performed, followed by amplification of the CYP2D6 *3,*4 and ABCB1 (C3435T) alleles. The digestion of each of the alleles was completed with the restriction enzymes MspI, MvaI and MboI, respectively.

Results: The CYP2D6*3 allele showed no polymorphisms in the analyzed population. The allele of the CYP2D6*4 gene presented polymorphism in the analyzed population: 78% were characterized as wild alleles (*1/*1) and 22% heterozygous alleles (*1/*4). 55.1% heterozygous alleles (TC) and 44.9% mutant homozygous alleles (TT) of the ABCB1 gene were found.

Conclusions: The alleles of the CYP2D6*4 gene were heterozygous in the population, finding association with intermediate or deficient metabolism, while not giving the adequate response to tamoxifen consumption, which led to the risk of breast cancer recurrence. The differences in the allelic frequencies of the ABCB1 gene suggest that half of the population analyzed presents the mutation and alteration in the membrane protein.

Keywords: breast cancer; tamoxifen; CYP2D6*3; CYP2D6*4; ABCB1 (C3435T).

RESUMEN

Introducción: El cáncer de mama se posiciona como la neoplasia maligna con mayor prevalencia a nivel mundial. En el año 2022 se reportaron 2,2 millones de casos. Colombia se considera el segundo país con cifras más frecuentes de cáncer en mujeres. La importancia epidemiológica de esta condición genera la necesidad de establecer en la región procesos de caracterización demográfica, clínica, intervención farmacológica e identificación de variantes genéticas, que determinen una mejor comprensión del cáncer como situación multifactorial.

Objetivo: Identificar polimorfismos de un solo nucleótido de CYP2D6*3, CYP2D*4 y ABCB1 C3435T en mujeres con cáncer de mama de la provincia de Caldas, con antecedente de consumo de tamoxifeno.

Métodos: Se realizó un estudio de tipo transversal descriptivo y retrospectivo en el que participaron 49 mujeres del departamento de Caldas, con diagnóstico de cáncer de mama en tratamiento con tamoxifeno o inhibidores de aromatasa, a quienes se les tomó una muestra

de sangre bajo los principios de la bioética y criterios y protocolos de bioseguridad establecidos. Posteriormente, se realizó la extracción de ADN, seguido de la amplificación de los genes CYP2D6 alelos *3,*4 y ABCB1 (C3435T). Se finalizó con la digestión de cada uno de los alelos con las enzimas de restricción MspI, MvaI y MboI, respectivamente.

Resultados: El alelo CYP2D6*3 no mostró polimorfismos en la población analizada. El alelo del gen CYP2D6*4 presentó polimorfismo en la población analizada: el 78 % fueron caracterizados como alelos silvestres (*1/*1) y el 22 % alelos heterocigotos (*1/*4). Se encontró un 55,1 % de alelos heterocigotos (CT) y un 44,9 % de alelos homocigotos mutantes (TT) del gen ABCB1.

Conclusiones: Los alelos del gen CYP2D6*4 fueron heterocigotos en la población, encontrándose asociación con metabolismo intermedio o deficiente, a la vez que no dio la respuesta adecuada al consumo de tamoxifeno, lo que conllevó al riesgo de recurrencia del cáncer de mama. Las diferencias en las frecuencias alélicas del gen ABCB1, sugieren que la mitad de la población analizada presenta la mutación y alteración en la proteína de membrana.

Palabras clave: cáncer de mama; tamoxifeno; CYP2D6*3; CYP2D6*4; ABCB1 (C3435T).

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Introduction

In 2020, a worldwide incidence of breast cancer of 47.8 per 100 000 women, a prevalence of 7.8 million and mortality of 685,000 was reported. In Colombia, 15,509 new cases were estimated, with a prevalence of 52,025 cases, and a mortality of 4,411, making it the leading cause of disease and death from cancer in women.[1,2]

For the therapeutic management of breast cancer, it is necessary, firstly, the identification of the status of estrogen receptors (ER+) and progesterone (PR); those ER+ tumors have a

favorable prognosis for hormone therapy with tamoxifen.[3] Tamoxifen, a nonsteroidal triphenylene derivative is a selective estrogen receptor modulator; which it has been used as an adjuvant therapy in breast cancer since 1977, leading to an improved disease-free survival and a significant decrease in tumor recurrence.[3]

Despite the therapeutic effect of the medication, it is observed that a significant number of women can develop progression of disease or recurrence, this phenomenon can be caused by inter-individual variations in genes of the cytochrome P450 family (CYP3A4, CYP3A5, CYP2C9, CYP2C19 and CYP2D6) associated with the metabolism of tamoxifen and / or genes of transporter proteins dependent on ATP hydrolysis, as ABCB1 (ATP-Binding Cassette B1).[4,5,6,7]

The CYP2D6 gene, associated with the biotransformation of tamoxifen, is located on the long arm of chromosome 22 (22q13.1); more than 100 allelic variants have been reported in this gene, although only a few alleles have clinical relevance such as *1 and *2 (functional alleles); *10 and *17 (reduced function alleles); *3, *4 and *5 (non-functional alleles).[8,9]

The polymorphism in CYP2D6*3 is presented by a deletion of an adenine at position 2549, generating a change in the reading frame; the polymorphism in CYP2D6*4 is caused by a substitution of a guanine for an adenine at position 1846.[10] Both polymorphisms in CYP2D6*3 and CYP2D6*4 cause non-functional proteins and are associated with the occurrence of adverse reactions or decreased response to medications.[11]

The ABCB1 gene (MDR1) is located on the long arm of chromosome 7 (7q21.12), encoding for a multidrug resistance protein, glycoprotein P.[12]

P-glycoprotein is a membrane transporter specialized in eliminating toxic substances and medications to the extracellular framework, generating a barrier to intestinal absorption of medications.[12,13,14] Some polymorphisms of clinical relevance are known from this gene, such as the transition of a cytosine by a thymine at position 3435 (C3435T) in exon 26, which is associated with a lower expression of glycoprotein P.[15]

Taking into account the epidemiological importance of breast cancer, the effect triggered by inter-individual variations in the response to tamoxifen and the little information available at the regional level of this type of variation.

The objective of this work was to identify single nucleotide polymorphisms in CYP2D6*3, CYP2D*4 and ABCB1 C3435T in women with breast cancer from the province of Caldas with a history of tamoxifen consumption.

Methods

Study population: 49 women with breast cancer treated in the specialized mastology and oncology service of the city of Manizales during the first half of 2016 who met the inclusion criteria after signing the informed consent.

Each patient, participated in a survey where the following information was tabulated: age, socioeconomic stratum, exposure time to tamoxifen, menopausal status, cardiovascular history, breast cancer family history, cardiovascular family history, concomitant medications, consumption of CYP inhibitors, cigarette exposure, Body Mass Index (BMI).

Identification of the single nucleotide polymorphism CYP2D6*3 (A2549del), CYP2D6*4 (G1846A) and ABCB1 (C3435T) by PCR-RFLP.

The DNA was extracted from peripheral blood, with the UltraClean kit® Blood DNA Isolation (MO BIO laboratories, Inc), following the methodology described by the manufacturer. Two single nucleotide polymorphisms of CYP2D6 (*3 and *4) and a single nucleotide polymorphism of ABCB1 (C3435T) were genotyped by PCR, using previously reported conditions. [16,17]

The PCR fragments from CYP2D6*3, CYP2D6*4 and ABCB1 (C3535T) were digested with the restriction enzymes MspI, MvaI (Jena Bioscience) and MboI (New England Biolabs® Inc) respectively,[16,17] according to the manufacturer's instructions.

Statistical analysis: the data were analyzed by Fisher's exact test, where $p < 0.05$ was considered statistically significant. Chi-square tests were performed to assess the goodness of fit among the observed and expected frequencies (Hardy-Weinberg equilibrium test). The analysis was performed with IBM SPSS licensed software® (Statistical Package for Social Sciences) version 25.

Calculation of allelic frequencies: The results obtained in the identification of the wild and mutant genotypes of the CYP2D6*4 and ABCB1 genes (C3435T) were tabulated, while the genotypic frequencies were calculated from the allelic frequencies

Equilibrium Calculations of the alleles analyzed: The data related to the frequency of the polymorphisms were contrasted by the Hardy Weinberg equilibrium for the determination of the expected frequencies for the sample, $\alpha = 0.05$ was established for the determination of the goodness of fit test of the frequencies found versus those ones expected.

Results

Genotyping of CYP2D6*3, CYP2D6*4 and ABCB1 genes (C3435T): A fragment of 270 bp, 355 bp and 244 bp was obtained for CYP2D6*3, CYP2D6*4 and ABCB1 (C3435T) respectively. Once the digestion for CYP2D6*3 was carried out with the MspI enzyme, a fragment of 188 bp and 82 bp was obtained in all samples corresponding to the wild type phenotype (*1/*1). For the CYP2D6*4 allele, the amplification of 355 bp was digested with the MvaI enzyme, the presence of the wild type genotype (*1/*1) was observed when obtaining two fragments of 250 bp and 105 bp, in addition heterozygous genotype (*1/*4) was evidenced when generating fragments of 335 bp, 250 bp and 105 bp. Finally, when performing the digestion of the 244 bp fragment of the ABCB1 gene with the MboI enzyme, where a 244 bp fragment was obtained, corresponding to the mutant homozygous variant (TT) as well as fragments of 244 bp, 172 bp and 68 bp corresponding to the heterozygous genotype (CT).

The allelic frequencies of the CYP2D6 gene (Homozygote *1/*1) was 77.6% while the CYP2D6 (Heterozygote *1/*4) was 22.4%; the allelic frequencies of the ABCB1 gene (C-T heterozygote) were 55.1% while the ABCB1 (T-T mutant homozygote) was 44.9%.

The statistical analysis determined that the proportions of the polymorphisms found do not correspond, in the case of the ABCB1 gene, with the Hardy Weinberg equilibrium, while CYP2D6 does correspond (tab. 1).

Table 1 - Hardy Weinberg equilibrium for the analyzed alleles

Frequencies		ABCB1	CYP2D6
Observed	Homozygote	22	38
	Heterozygote	27	11
	Homozygous variant	0	0
Expected	Homozygote	25.7	38,6
	Heterozygote	20	10
	Homozygous variant	3,7	0,6
p value		0,007768	0,376126

The descriptive analyses of the population allowed to determine the characteristics of the participants and the relationship in the state of the metabolized of the CYP2D6 gene (tab. 2).

Table 2 - Characteristics of the participants according to the Metabolizing Status cyp2D6

	Normal		Intermediate		
Age	**n**	**%**	**n**	**%**	***p* value**
30 - 40 years	2	5,3	1	9.1	0.479
30 - 40 years	2	5,3	1	9.1	0.479
41 - 50 years	8	21.1	4	36.4	---
> 50 years	28	73.7	6	54.5	---
Socioeconomic stratum					
Low	23	60.5	5	45.5	0.526
Middle	14	36.8	6	54.5	---
High	1	2.6	0	0	---
Exposure Time to Tamoxifen					
Up to 6 months	2	5.3	0	0	0.086
6 months - 1 year	2	5.3	3	27.3	---
> 1 year	34	89.5	8	72.7	---
Menopausal status					
Premenopausal	9	23.7	4	36.4	0.402
Postmenopausal	29	76,3	7	63.6	---
Cardiovascular history					
With CV background	13	34.2	5	45.5	0.496
No CV background	25	65.8	6	54.5	---
Breast Cancer Family history					
With background	11	28.9	4	36.4	0.638
No precedent	27	71,1	7	63.6	---
Cardiovascular family history					
With background	19	50	9	81.8	0.06
No precedent	19	50	2	18.2	---
Concomitant medications					
Up to 2 medications	28	73.7	7	63.6	0.165

3 - 4 medications	9	23.7	2	18.2	---
> 5 medications	1	1	2	18.2	---
Consumption of CYP inhibitors					
With exposure to inhibitors	5	13.2	3	27.3	0.265
No exposure to inhibitors	33	86.8	8	72.7	---
Introducing ADR					
With ADR	5	13.2	1	9.1	0.717
No ADR	33	86.8	10	90.9	---
Cigarette exposure					
Cigarette smoking	1	2.6	1	9.1	0.34
No cigarette consumption	37	97.4	10	90.9	---
Body Mass Index (BMI)					
Normal	16	42.1	4	36.4	0.687
Overweight	16	42.1	4	36.4	---
Obesity	6	15.8	3	3	---

CV: Cardiovascular. ADR: Adverse Drug Reaction. Source: Own elaboration.

The age of the patients was mainly above 50 years (74.1%) for the T-T ABCB1 phenotype. No family history of breast cancer was found for both the C-T and T-T phenotypes. A statistically significant association was found between presenting cardiovascular history (hypertension, diabetes or dyslipidemia) and the ABCB1 phenotype ($p < 0.05$). This association for the patients included in the analysis is reflected in the risk of medication use versus their bioavailability, mechanism of action and effect. Complementary information associated with demographic, clinical and general pharmacotherapeutic characteristics related to the ABCB1 phenotype are shown in table 3.

Table 3 - Characteristics of the participants in relation to the ABCB1 phenotype

	Heterozygote C-T		**Homozygous T-T Mutant**		
Age	**n**	**%**	**n**	**%**	***p* value**
30 - 40 years	1	3.7	2	9.1	0.64
41 - 50 years	6	22.2	6	27.3	---
> 50 years	20	74.1	14	63.6	---
Socioeconomic stratum					
Low	15	55.6	13	59.1	0.657
Middle	11	40.7	9	40.9	---
High	1	3.7	0	0	---
Exposure Time to Tamoxifen					
Up to 6 months	0	0	2	9.1	0.197
6 months - 1 year	2	7.4	3	13.6	---
> 1 year	25	92.6	17	77.3	---

Menopausal status					
Premenopausal	5	18.5	8	36.4	0.159
Postmenopausal	22	81.5	14	63.6	---
Cardiovascular history					
With CV background	14	51.9	4	18.2	0.015*
No CV background	13	48.1	18	81.8	---
Breast Cancer Family history					
With background	7	25.9	8	36.4	0.43
No precedent	20	74.1	14	63.6	---
Cardiovascular family history					
With background	15	55.6	13	59.1	0.804
No precedent	12	44.4	9	40.9	---
Concomitant medications					
Up to 2 medications	17	63	18	81.8	0.114
3 - 4 medications	9	33.3	2	9.1	---
> 5 medications	1	3.7	2	9.1	---
Consumption of ABC substrates					
With consumption	7	25.9	5	22.7	0.796
No consumption	20	74.1	17	77.3	---
Introducing ADR					
With ADR	5	18.5	1	4.5	0.138
No ADR	22	81.5	21	95.5	---
Cigarette exposure					
Cigarette smoking	1	3.7	1	4.5	0.882
No cigarette consumption	26	96.3	21	95.5	---
Body Mass Index (BMI)					
Normal	8	29.6	12	54.5	0.162
Overweight	14	51.9	6	27.3	---
Obesity	5	18.5	4	18.2	---

CV: Cardiovascular. ADR: Adverse Drug Reaction. *: Statistically significant. Source: Own elaboration.

In various pharmacological schemes presented to the patients, the capacity to generate inhibition of the pharmacokinetic process mediated by ABCB1 and CYP2D6 was presented a priori, independently of the functional state induced by alleles. A complex relationship between tamoxifen exposure and the ability to inhibit ABCB1 or behave as a substrate (prodrug) for CYP2D6 is presented. The investigation evidences a high burden for the inhibition process related to tamoxifen (43 prescriptions), verapamil (5 prescriptions) and atorvastatin (3 prescriptions). Complementary data between medication consumption and ABCB1 system activity and CYP2D6-mediated metabolism is presented in table 4.

Table 4 - Association between prescribed drug and ABCB1 and CYP2D6 inhibitory or inducing capacity

Medication	n (%)	ABCB1	CYP2D6
Tamoxifen	43 (93.9)	Inhibitor	Substrate
Verapamil	5 (10)	Inhibitor	Inhibitor
Atorvastatin	3 (6)	Inhibitor	Inhibitor
Amitriptyline	1 (2)	Substrate	Substrate
Sertraline	1 (2)	Inhibitor	Substrate
Nifedipine	1 (2)	Substrate	Inhibitor
Metoprolol	1 (2)	---	Substrate
Enalapril	4 (8)	Inhibitor	---
Omeprazole	3 (6)	Substrate	Inhibitor

In the presence of non-functional polymorphisms for ABCB1 and CYP2D6 there is a risk of limitation in the bioavailability of substances. In the case of tamoxifen, there is a high probability of decreasing its metabolic activation and therefore of not presenting its effect as an estrogen receptor inhibitor, exposing the population to breast cancer recurrence.

Discussion

Breast cancer is considered a public health problem of concern and importance to the general population. Currently there are clinical trials that allow the early detection of this pathology, through mammograms and physical examinations; treatments associated with this type of cancer are related to surgeries and adjuvant systemic therapy with hormone-therapy and chemotherapy, [18] in addition to the diagnoses and treatments, current knowledge allows the adoption of new strategies for the diagnosis and prognosis of the disease.

Hormone-therapy mediated by Tamoxifen works as a selective estrogen receptor (ER) modulator; indications are mainly focused on the treatment of breast cancer in postmenopausal women. Treatment with tamoxifen is used to prevent recurrences in women suffering from breast cancer, however, the efficiency of the intervention is estimated to be between 70% - 80%.[19] The role of cytochromes in the metabolism of medicines is important, that is why every day, the idea of using their characteristics in the field of precision

therapeutics is increasingly consolidated, both in the international and regional contexts of Latin America.[20]

Cytochrome proteins P450 (CYP) play an important role in tamoxifen metabolism; specific genes such as CYP2D6, CYP3A5 and CYP2C19 have been considered candidates where genetic variations have been associated with the efficacy of the treatment. The CYP2D6 gene is responsible for the metabolism of tamoxifen for the generation of the active metabolite, endoxifen. The polymorphisms CYP2D6*3 and CYP2D6*4 play an important role in the etiology of breast cancer and could help plan hormone therapy where tamoxifen is used.[21]

In this present study the alleles *3 and *4 of CYP2D6 were analyzed, according to the analysis of the CYP2D6 gene *3, it could be observed that all the samples analyzed, showed the same genotype, which indicates that the population does not present the mutation in this allele (homozygous population for the wild type genotype *1/*1).

The results in this investigation coincide with the reports of Kevin Hicks,[22] where it is indicated that individuals homozygous for the wild type genotype will present the same point mutation.

The high association between metabolism and genotype is evident, the proper functioning of the genes is related to the adequate metabolism of medications, for this reason, mutations of the CYP gene have been related to the different types of metabolizers.[23]

The findings in this present work allow an approach to the type of metabolism, where it was found that 77.6% of the CYP2D6*4 gene is in a homozygous state (*1/*1), a genotype that explains the extensive/normal metabolism of the medication, which would be expected for an important part of the population.

Specifically, in the participating population, it was found 22.4% of CYP2D6 genotype associated with intermediate or deficient metabolism, a condition that imposes on 11 women under chronic consumption of tamoxifen, risk of breast cancer recurrence in the 5 years following diagnosis. The committee for the clinical implementation of pharmaco-genetics indicates in the therapeutic adaptation guidelines for the use of tamoxifen, the change of therapy towards aromatase inhibitors in case of presentation of genotype *1/*4.[24]

In addition to CYP2D6, other enzymes of cytochrome P450 (CYP3A5 and CYP2C19) also contribute to the overall metabolism of tamoxifen and its metabolites, albeit in varying

degrees.[25] The membrane proteins of the ABCB1 gene are associated with the transport of specialized cell membrane; mutations in this gene are associated with a lower expression of glycoprotein P,[16] in the research 44.9% of the ABCB1 homozygous T-T gene (mutant) was found, which suggests the alteration in this type of proteins.

It is concluded that the alleles of the CYP2D6*3 genotype did not present polymorphisms, indicating that the population has a wild homozygous behavior for this characteristic.

The alleles of the CYP2D6*4 genotype responded as a heterozygous population, 77.6% of the analyzed population presented a wild genotype (*1/*1) and 22.4% of the analyzed population has a heterozygous genotype (*1/*4) associated with intermediate or deficient metabolism, which will not give the adequate response to the consumption of tamoxifen, which will lead to the risk of recurrence of breast cancer.

The allelic frequencies of the ABCB1 gene (Heterozygote C-T) were 55.1% while the ABCB1 (Mutant Homozygote T-T) was 44.9%, which suggests that about half of the population presents the mutation and therefore alteration in the membrane protein.

This work highlights the importance of finding biomarkers that allow predicting the functioning of genes and their association with the effects of medications, a great challenge for future works in personalized medicine.

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