Frequency of 14 genetic variants associated with breast cancer risk and treatment in a Colombian population

Yeimy Viviana Ariza Márquez[1*], Fabio Ancízar Aristizábal Gutiérrez[2], Alberto Gómez-Gutiérrez[3], Piedad Elena Gómez[4], María Consuelo Casas Gómez[4], Ignacio Briceño Balcázar[5]

[1] Programa de doctorado en Biotecnología, Universidad Nacional de Colombia, sede Bogotá, Colombia, *E-mail: yvarizam@unal.edu.co.

[2] Departamento de Farmacia e Instituto de Biotecnología, Universidad Nacional de Colombia, sede Bogotá, Colombia.

[3] Instituto de Genética Humana, Facultad de Medicina, Pontificia Universidad Javeriana, Bogotá, Colombia.

[4] Instituto de Referencia Andino, Bogotá, Colombia.

[5] Facultad de Medicina, Universidad de la Sabana, Chía, Colombia.

Summary

Introduction: Genetic variations have been related to risk and treatment efficacy. Many polymorphisms in breast cancer are known to influence susceptibility, breast cancer risk and treatment outcome. Polymorphisms vary among populations; therefore, local studies are necessary.

Objective: To establish the frequency of polymorphisms associated to breast cancer risk and treatment pharmacogenomics in a group of Colombian individuals.

Methods: Data from microarray profiles including gene polymorphisms associated with breast cancer treatment were retrospectively collected (Pathway Genomics®). The frequency of marker CYP2D6 rs3892097 and a breast cancer panel (CAS8 rs1045485, CHEK21100delC, ESR1 rs2046210, FGFR2 rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662, AKAP9 rs6964587, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 delT, ESR1 rs3020314) were studied.

Results: Microarray data from **68** men and **92** women were analyzed. All polymorphisms were in Hardy-Weinberg equilibrium. Genotypic frequencies of CYP2D6 rs3892097 C/T, CAS8 rs1045485 G/C, and those of genes included in a breast cancer panel (CAS8 rs1045485, CHEK21100delC, FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 del T, ESR1rs3020314) did not significantly differ from previously published data. ESR1 rs2046210, with allele frequencies of C=0.04 and T=0.02, and AKAP9 rs6964587, with a frequency of A=0.005, were determined as rare.

Conclusions: The population studied was not significantly different in allele distribution from previously reported data at HapMap. Genotypes in Colombian population are similar to other previously studied groups of healthy subjects. Extended use of genotyping pharmacogenetic polymorphisms will prevent toxicity and adverse effects in tamoxifen treatment (for example in CYP2D6 rs3892097). Therefore, therapeutic alternatives should be evaluated based on individual pharmacogenetic studies.

Key words: Colombia; pharmacogenomics; breast Cancer.

Resumen

Frecuencia de 14 variantes genéticas asociadas con el riesgo y el tratamiento del cáncer de mama en una población colombiana

Introducción: las variaciones genéticas se han relacionado con el riesgo y la eficacia del tratamiento. Es sabido que muchos polimorfismos en cáncer de mama influyen en la susceptibilidad, el riesgo de cáncer y el resultado del tratamiento. Los polimorfismos varían entre las poblaciones, y por tanto, es necesario realizar estudios locales.

Objetivo: establecer la frecuencia de polimorfismos asociados al riesgo de cáncer de mama y la farmacogenómica del tratamiento en un grupo de individuos colombianos.

Métodos: los datos de los perfiles de microarreglos, incluidos los polimorfismos genéticos asociados con el tratamiento del cáncer de mama, se obtuvieron de forma retrospectiva (Pathway Genomics®). Se estudiaron la frecuencia del marcador CYP2D6 rs3892097 y un panel de cáncer de mama (CAS8 rs1045485, CHEK21100delC, ESR1rs2046210, FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662, AKAP9 rs6964587, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 delT, ESR1 rs3020314).

Resultados: se analizaron los datos de microarreglos de **68** hombres y **92** mujeres. Todos los polimorfismos siguieron el equilibrio Hardy-Weinberg. Las frecuencias fenotípicas de CYP2D6 rs3892097 C/T, CAS8 rs1045485 G/C, y aquellas de los genes incluidos en un panel de cáncer de mama (CAS8 rs1045485, CHEK21100delC, FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 del T, ESR1rs3020314) no difirieron significativamente de los datos publicados previamente. ESR1 rs2046210, con frecuencias alélicas de C = 0,04 y T = 0,02, y AKAP9 rs6964587, con una frecuencia de A = 0,005, se determinaron como raras.

Palabras clave: Colombia; farmacogenómica; cáncer de mama.

Introduction

Advances in technology have made multigene testing, or "panel testing", a practical option when looking for genetic variants that may be associated with a risk of breast cancer and treatment outcome. Use of pharmacogenomics will optimize treatment and lower health costs. In this context, it is necessary to study disease associations and pharmacogenetic polymorphisms in populations to optimize disease treatment. In the case of breast cancer, tamoxifen metabolism related polymorphisms are associated to treatment, but their frequency is poorly known in Colombian population.

Using different platforms to process and analyze samples can simultaneously identify genetic variants associated with susceptibility, clinical outcomes and drug response. For example, the spectrum of the frequency of the *MTHFR* gene polymorphism C677T in a group of healthy Colombian individuals was previously determined to evaluate the genetic background in relation to disease susceptibility and pharmacogenetic applications; the results for the C/C and C/T genotypes in a Colombian population were similar to other previously studied groups of healthy subjects. Subjects from Colombian populations might be at risk of developing diseases associated with MTHFR polymorphisms and might present toxicity and adverse effects if treated with MTX, which suggests the need to evaluate therapeutic alternatives based on individual pharmacogenetic studies [1].

In the case of breast cancer, the response to several hormone therapeutics, such as tamoxifen are mediated by the action of CYP2D6, which is highly polymorphic, and genotypes has been identified allowing classifying patients as ultra-rapid, normal and poor metabolizers.

Genetic variants in certain genes have been associated with early cancer risk [2], risk of developing cancer [3] and susceptibility to cancer [4]. In this way, CYP2D6*4 variant (rs3892097) is associated with response to tamoxifen, since patients carrying a T allele will present slow metabolism of this drug and would therefore need a dose adjustment or an alternative therapy [5]. In the case of CAS8 rs1045485, its C allele is associated with a lower risk of breast cancer [6,7]. On the other hand, ESR1 rs2046210 has been associated with risk of developing breast and endometrial cancer in Chinese women [8-10], and variant C has been associated with ER-negative but not ER-positive breast cancer ($P > 0.05$). These findings provide further evidence for distinct etiological pathways associated with invasive ER-positive and ER-negative breast cancer in younger women and women of African ancestry [11]. FGFR2rs1219648 showed a similar association with the risk of developing breast cancer in Chinese [12] and Indian populations [13]. Polymorphism rs13387042-2q35(G/A), has been associated with breast cancer risk only among postmenopausal women who never used hormone therapy [14]. Furthermore, the GG genotype of SNP rs13281615 plays a role in breast cancer through PVT1 expression during oncogenesis, and "protective" mutations could occur [15]. MSRP30 rs10941679 was significantly associated with BCIS (Breast cancer in situ) risk [16]. As for TNRC9 rs3803662, allele GG increased the risk of breast cancer in codominant inheritance (OR=2.19, 95% CI:1.19-4.02), and recessive genetic models (OR=2.06, 95% CI: 1.15-3.70) [17]. Moreover, the variant rs6964587 was associated with increased breast edema due to radiotherapy after the following 5 years (Beta, 0.22; 95% confidence interval, 0.09-0.34; P = 7×10^{-4}) [18] and increased to risk of breast cancer [19]. LSP1 rs3817198 has been associated with mammographic density in genome-wide studies [20]. Likewise, MAP3K1rs889312, has been associated with breast cancer risk in women of European ancestry [21] and ESR1rs3020314 showed stronger associations in ER-positive [22]. Finally, in AKAP9, the minor T allele of 7q21-rs6964587 was associated with breast cancer risk under a recessive model (OR 1.07, 95% CI 1.00 to 1.13, p = 0.04) [23].

Knowledge of Colombian disease associated polymorphism frequencies will allow to establish National Health policies to improve health and treatment outcome.

Methods

Population

A retrospective cross-sectional study in 160 healthy Colombian individuals over 18 years was conducted using molecular genetic profiling, age ranges between 18 and 74 years with a mean of 40.07 years. DNA samples were collected from saliva samples in Instituto de

Referencia Andino, as well as all corresponding applications from medical orders and/or individuals received between the years 2012 and 2014. All individuals signed an informed consent.

The Healthy Woman DNA Insight™ panel from Pathway Genomics® evaluates panel of breast cancer (CAS8 rs1045485, CHEK21100delC, ESR1 rs2046210, FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662, AKAP9 rs6964587, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 delT, ESR1rs3020314). The pharmacogenetic polymorphism panel from Pathway Genomics® in *Medication DNA Insight™genetic* test evaluates 15 metabolism associations for drug metabolism, including Tamoxifen (CYP2D6*4), in addition to Abacavir® hypersensitivity, aminoglycoside-induced ototoxicity, response to beta blockers, carbamazepine hypersensitivity, clopidogrel metabolism, estrogen supplementation, alpha interferon, metoprolol metabolism, metabolism and hypersensitivity to phenytoin, proton-pump inhibitor, simvastatin induced myopathy, metabolism of voriconazole and warfarin.

DNA microarrays were used to measure expression levels of probes, corresponding to alleles in CAS8 rs1045485, CHEK21100delC, ESR1 rs2046210,FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662,AKAP9 rs6964587, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 delT, ESR1rs3020314 genes. Each microarray position contains 10-12 picomoles of a specific DNA sequence under high stringency. Probe-target hybridization was detected and quantified by fluorophores attached to the probe.

The frequency of the polymorphism substitution in the genes markers was analyzed. Each participant was identified by a code used in a database, where the descriptive analysis for continuous variables such as age was performed with measures of central tendency and Shapiro-Wilk normality test. The description of categorical variables (origin, sex, genotype and allele) was performed by frequency analysis. Given the variable nature of the study association levels, they were evaluated by a Chi square test (X^2) with a confidence level of 95%. In addition, Hardy-Weinberg equilibrium was tested. Data base designed in Access includes the following variables that were Collected: Code anonymity of each individual, age, gender, date of birth, maternal ancestry, paternal ancestry, nationality, CYP2D6 rs3892097, panel for breast cancer (CAS8 rs1045485, CHEK21100delC, ESR1 rs2046210, FGFR2rs1219648, intergenic_2q35rs13387042, intergenic_8q24 rs13281615, MSRP30 rs10941679, TNRC9 rs3803662,AKAP9 rs6964587, LSP1 rs3817198, MAP3K1rs889312, PALBS1592 delT, ESR1rs3020314).

Statistical Methods

Available results were tabulated to perform the respective calculation of frequencies of each polymorphism and Hardy-Weinberg equilibrium was calculated using the online Curt Lab - HW calculator, and then a Genpop software was used in order to define genetic frequencies. Later on, contingency tables were established with a double entry and Chi squared distribution calculated. Results were transferred to the SPSS statistical program for Windows V20. They were not stratified by ethnic group or socioeconomic levels. Available frequencies were collected in HapMap Genome Browser release #28 (Phases 1, 2 and 3 - merged genotypes and frequencies) for the general population and also for the Colombian population for each of the variants tested in this review.

Ethical considerations

This study was approved by the Ethics Committee of Universidad Nacional de Colombia.

Results

No deviation from Hardy-Weinberg equilibrium is present; therefore, the population included in this study showed no significant migration, subgroup stratification, inbreeding or selection processes. Additionally, by contrasting data reported in Hap-Map for general population, Colombians of the present study have no significant differences with the sample of Colombian population reported in HapMap.

After evaluating each particular polymorphism, CYP2D6*4 rs3892097, CAS8 rs1045485, MSRP30 rs10941679, LSP1 rs3817198 and MAP3K1 rs889312, data found in the present study are similar to data report for general and Colombian population previously evaluated in HapMap. Allele Frequency Comparison between Present Study and Hap-Map is shown in Table 1.

Polymorphisms FGFR2 rs1219648, intergenic 2q35-rs13387042, intergenic 8q24-rs13281615, TNRC9 rs3803662 and ESR1 rs3020314, showed higher frequencies than those reported previously for the Colombian population in HapMap, and has greater similarity to those reported for the global population.

In addition, we identified in ESR1 rs2046210 variants C and G have frequencies of 0.04 and 0.02, respectively; similarly, AKAP9 rs6964587 allele A was found in a frequency of 0.005. The presence of these variants had not been previously reported in the Colombian population or the HapMap database.

Table 1. Allele frequency comparison between a Colombian population in the present study and HapMap database.

Gen - SNP	Ref SNP Alleles	MAF	Ancestral Allele	Population genetics ALL	1000 Genomes Browser Allele frequencies CLM	Alleles frecuencies IRA	HW
CYP2D6*4 rs3892097	C/T	T:0.09	C	C= 0.91 T= 0.09	C=0.8351 T=0.1649	C=0.903 T=0.087	X2 = 2,71 X2 test P value = 0,099
CAS8 rs1045485	C/G	C: 0.05	G	G= 0.95 C= 0.05	G=0.9149 C=0.0851	G=0.94 C=0.06	X2 =0,401 X2 test P value =0,526
CHEK21100delC	The protein-truncating						
ESR1 rs2046210	G/A	A: 0.41	A	G= 0.41 A= 0.59	G=0.7340 A=0.2660	G=0.74 A=0.20 C=0.04 T=0.02	X2 =3,082 X2 test P value = 0,079
FGFR2 rs1219648	A/G	G: 0.41	A	A= 0.59 G= 0.41	A=0.6436 G=0.3564	A= 0.64 G=0.36	X2 =0,149 X2 test P value = 0,699
intergenic_2q35 rs13387042	A/G	A: 0.47	G	A= 0.47 G= 0.53	A=0.4149 G=0.5851	A=0.42 G=0.58	X2 =0,169 X2 test P value = 0,68
intergenic_8q24 rs13281615	A/G	G: 0.49	A	A= 0.51 G= 0.49	A=0.4681 G=0.5319	A=0.46 G=0.54	X2 =0,865 X2 test P value = 0,352

(Continue)

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Table 1. Allele frequency comparison between a Colombian population in the present study and HapMap database (*continuation*).

Gen -SNP	RefSNP Alleles	MAF	Ancestral Allele	Population genetics ALL	1000 Genomes Browser Allele frequencies CLM	Alleles frecuencies IRA	HW
MSRP30 rs10941679	A/G (FWD)	G: 0.32	A	A= 0.68 G= 0.32	A=0.7021 G=0.2979	A=0.65 G=0.35	$X2 =0,048$ X2 test P value = 0,825
TNRC9 rs3803662	A/G	A: 0.44	G	A= 0.44 G= 0.56	A=0.4149 G=0.5851	A=0.39 G=0.61	$X2 =0,782$ X2 test P value = 0,376
AKAP9 rs6964587	G/T (FWD)	T:0.37	T	G= 0.63 T= 0.37	G=0.5585 T=0.4415	G=0.580 T=0.415 A=0.005	$X2 = 0,0059$ X2 test P value = 0,938
LSP1 rs3817198	C/T (FWD)	C: 0.22	T	T= 0.78 C= 0.22	T=0.7713 C=0.2287	T=0.75 C=0.25	$X2 = 1,865$ X2 test P value = 0,172
MAP3K1 rs889312	A/C (FWD)	C: 0.39	A	A= 0.61 C= 0.39	A=0.5638 C=0.4362	A=0.59 C=0.41	$X2 =0,085$ X2 test P value = 0,771
ESR1 rs3020314	C/T (FWD)	T: 0.38	C	C= 0.62 T= 0.38	C=0.5426 T=0.4574	C=0.45 T=0.55	$X2 = 0,552$ X2 test P value = 0,458

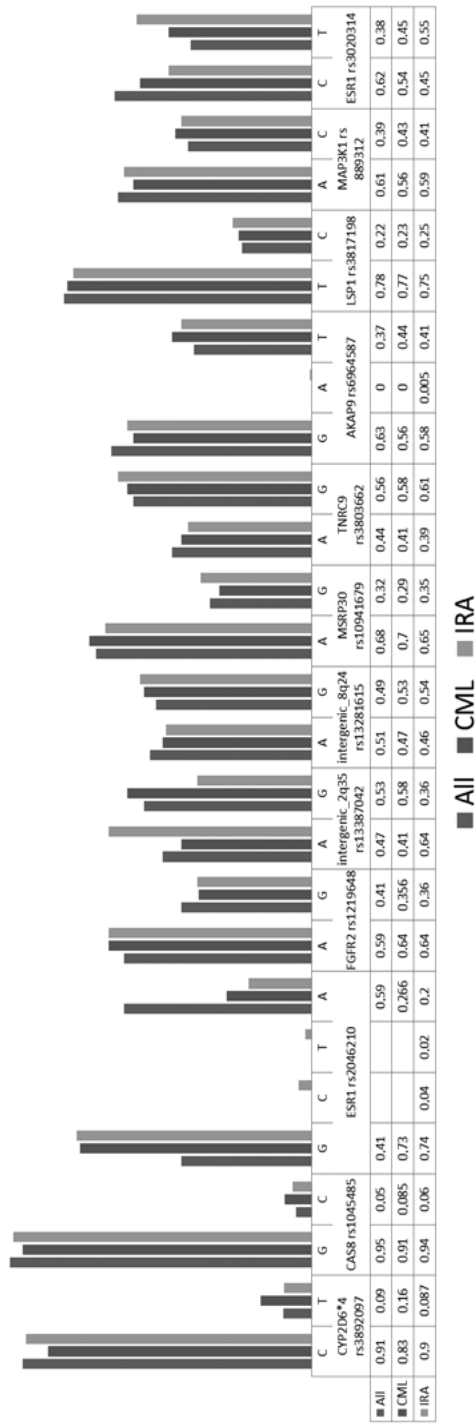|	CYP2D6*4 rs3892097		CAS8 rs1045485		ESR1 rs2046210				FGFR2 rs1219648		intergenic_2q35 rs13387042		intergenic_8q24 rs13281615		MSRP30 rs10941679		TNRC9 rs3803662		AKAP9 rs6964587			LSP1 rs3817198		MAP3K1 rs889312		ESR1 rs3020314	
	C	T	G	C	G	C	T	A	A	G	A	G	A	G	A	G	A	G	G	A	T	T	C	A	C	C	T
All	0.91	0.09	0.95	0.05	0.41			0.59	0.59	0.41	0.47	0.53	0.51	0.49	0.68	0.32	0.44	0.56	0.63	0	0.37	0.78	0.22	0.61	0.39	0.62	0.38
CML	0.83	0.16	0.91	0.085	0.73			0.266	0.64	0.356	0.41	0.58	0.47	0.53	0.7	0.29	0.41	0.58	0.56	0	0.44	0.77	0.23	0.56	0.43	0.54	0.45
IRA	0.9	0.087	0.94	0.06	0.74	0.04	0.02	0.2	0.64	0.36	0.64	0.36	0.46	0.54	0.65	0.35	0.39	0.61	0.58	0.005	0.41	0.75	0.25	0.59	0.41	0.45	0.55

■ All   ■ CML   ■ IRA

Figure 1. Bar chart for gene frequencies.

The graph shows the allele frequencies of the world population reported in HapMap in blue (All), the Colombian population in HapMap in red (CML) and the data from this study in green (IRA).

Discussion

As we move from gene disease discovery into the application of genetic knowledge, allele frequency studies in populations become more important for the design, implementation of studies and for determining treatment according to personal polymorphisms. Studies in Colombian population are scarce, and the present study determined the allele frequencies of 14 polymorphisms, previously associated with breast cancer status. Allele frequencies were compared with dbSNP and other data sources in HapMap.

Allele frequencies for minor alleles relevant for Colombian population are here reported, although the number of individuals within the categories is small. Local differences in allele frequencies should be considered when designing association studies in the future. One weakness of our analysis is the lack of treatment follow-up due to the diversity of places where patients are treated.

The number of useful biomarkers for predicting drug response will rapidly increase for improving treatment and settle the basis for personalized medicine. Pharmacogenetic profiles will be useful for prevention programs, and personalized therapies will reduce health costs and ensure a more efficient health care.

Conclusion

The population studied was not significantly different in allele distribution with previously reported data from HapMap. Genotypes in Colombian population are similar to other previously studied groups of healthy subjects. The use of genotyping pharmacogenetic polymorphisms will prevent toxicity and adverse effects in tamoxifen treatment (for example in CYP2D6 rs3892097), and therapeutic alternatives should be evaluated based on individual pharmacogenetic studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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How to cite this article: