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Original Article

Polymorphism in the 3'-UTR of the thymidylate synthase gene and breast cancer risk in northeastern Mexico

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Abstract: Thymidylate synthase (TYMS) is a key enzyme in the process of DNA synthesis. A 6-bp deletion/insertion polymorphism in the 3'-untraslated region (3'-UTR) of the *TYMS* gene has been investigated for its association with breast cancer in a few number of studies. The purpose of this study was to determine whether an association exists between the *TYMS* 3'-UTR polymorphism and the risk of breast cancer in a population from northeastern Mexico, which has a higher European genetic background than other populations of the country. Genotypes were determined for 243 women with histologically confirmed breast cancer and 118 control subjects. Gene polymorphism was analyzed using a DNA microarray. Compared with the *del/del* homozygous genotype, a significant increase in the risk of developing breast cancer was associated with the *ins/ins* homozygous genotype (OR=2.52, 95% CI=1.24-5.13), and was almost associated with the *del/ins* heterozygous genotype (OR=2.04, 95% CI=1.00-4.14). Furthermore, there was a significant trend for an increased *ins* allele frequency with a higher risk of developing breast cancer (P for trend =0.030). In conclusion, we found an association between the *TYMS* 3'-UTR polymorphism and the risk of breast cancer in subjects from northeastern Mexico. Identification of inter-individual variability in *TYMS* polymorphisms may be useful for individualizing breast cancer genetic screening and therapeutic intervention.

Keywords: Breast cancer, thymidylate synthase (TYMS), *TYMS* 3'-UTR polymorphism, ethnicity, northeastern Mexico

Introduction

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related death among women worldwide [1]. The disease has important genetic and environmental components, most of them still unknown. The highly penetrant mutations in *BRCA1* and *BRCA2* account for small proportion of breast cancer cases [2]. Studies suggest that the interaction between low penetrant genetic polymorphisms and lifestyle or environmental risk factors may provide a plausible explanation for a much higher proportion of breast cancer cases [3].

Thymidylate synthase (TYMS) is a key enzyme in the conversion of deoxyuridine monophosphate to deoxythymidine monophosphate in the process of DNA synthesis. The conversion is essential for the production of thymidine, a nucleotide required for DNA repair and synthesis [4, 5]. A 6-bp deletion/insertion polymorphism in the 3'-untraslated region (3'-UTR) of the *TYMS* gene has been investigated for its association with breast cancer in a few number of studies, mainly in Caucasians and Asians [6-14]. In Mexico, only one related study has been carried out, in which only subjects from the State of Jalisco (located in the central-western area of the country) were analyzed [15].

The purpose of this study was to determine whether an association exists between the *TYMS* 3'-UTR polymorphism and the risk of breast cancer in population from throughout the northeastern area of Mexico, which has a higher European genetic background than other populations of the country.

Material and methods

Subjects

The subjects and methods have been described in detail in two previous reports [16, 17]. The

Table 1. Genotype and allele frequencies of the 6-bp deletion/insertion polymorphism in the 3'-untraslated region of the thymidylate synthase (*TYMS*) gene in breast cancer patients and healthy controls from northeastern Mexico

Genotype	Controls, n[a] (%)	Cases, n[a] (%)	P	OR (95% CI)
del/del	20 (17.5)	17 (8.6)	0.045	1.00
del/ins	52 (45.6)	90 (45.7)		2.04 (1.00-4.14)
ins/ins	42 (36.8)	90 (45.7)		2.52 (1.24-5.13)
Total	114 (100)	197 (100)		
del allele	0.4035	0.3147		
ins allele	0.5964	0.6853		
		p trend	0.030	

[a]Numbers may not sum to totals due to missing data. OR, odds ratio; CI, confidence interval.

patients were 243 women with histologically confirmed breast cancer who received chemotherapy at the University Cancer Center of the University Hospital "Dr José E González" of the Autonomous University of Nuevo Leon and the Hospital of Specialities number 25 of the Mexican Institute of Social Security, both located in Monterrey, Nuevo León, Mexico. Both are reference centers for breast cancer patients from throughout the northeastern area of Mexico, which includes the states of Zacatecas, San Luis Potosí, Tamaulipas, Coahuila, and Nuevo León. One hundred eighteen controls with no previous history of any type of cancer or other vital disease were also studied. This study conforms to the Declaration of Helsinki, was approved by the local ethics committee (registration HU BI10-002), and all participants provided informed written consent.

Polymorphism genotyping

Genomic DNA was obtained from peripheral blood samples either using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer protocol or using TSNT lysis buffer (1% Triton, 1% sodium dodecyl sulfate, 100 mM NaCl, 10 mM Tris-HCl [pH 8.0] and 1 mM EDTA) followed by phenol-chloroform extraction and ethanol precipitation. Analysis of *TYMS* 3'-UTR polymorphism was performed using the PHARMAchip® DNA microarray following manufacturer protocols (Progenika Biopharma SA, Derio, Spain).

Statistical analysis

Differences in genotype frequencies between patients and control subjects were analyzed by a Crosstab and Pearson's chi-square (χ^2) test. The data were input to SPSS, version 22.0 (SPSS Inc., Chicago, IL, USA) for handling and further statistical analyses. Hardy-Weinberg equilibrium (HWE) for allele frequencies was examined by the χ^2 test using the MAXLIK program [18]. Odds ratios (ORs) with 95% confidence intervals (95% CIs) were calculated to estimate the strength of associations using the Epi Info program (version 7. 1. 3, CDC, Atlanta, GA, USA). This software was also used to perform a χ^2 test to verify the existence of a linear trend between the presence of a given allele and the risk of developing breast cancer. In all analysis, differences were considered significant when *P* values were < 0.05.

Results

The genotype frequencies for *TYMS* 3'-UTR polymorphism were in agreement with HWE in the controls (χ^2=0.31, df=1, P=0.575). The *TYMS* 3'-UTR genotype frequencies were 17.5% *del/del*, 45.6% *del/ins*, and 36.8% *ins/ins* in control subjects and 8.6% *del/del*, 45.7% *del/ins*, and 45.7% *ins/ins* in breast cancer patients; the overall difference was statistically significant (P=0.045).

Compared with the *del/del* homozygous genotype, a significant increase in the risk of developing breast cancer was associated with the *ins/ins* homozygous genotype (OR=2.52, 95% CI=1.24-5.13), and was almost associated with the *del/ins* heterozygous genotype (OR=2.04, 95% CI=1.00-4.14). Furthermore, there was a significant trend for an increased *ins* allele frequency with a higher risk of developing breast cancer (P for trend =0.030). Results are summarized in **Table 1**.

Discussion

TYMS is a key enzyme in the process of DNA synthesis. Polymorphisms in *TYMS* gene may influence individual susceptibility to cancer in some populations. The purpose of this study was to determine whether an association exists between the *TYMS* 3'-UTR polymorphism and the risk of breast cancer in subjects from northeastern Mexico.

Zhou et al. analyzed *TYMS* allele frequencies reported in 39 studies and found that the *del* allele frequency among the controls was 71.0% in Asians and 33.5% in Caucasians [19]. The frequency of the *del* allele among the controls found in this study (40.3%, **Table 1**) was more similar to that in Caucasians than to that in Asians.

A few studies have investigated the association between the *TYMS* 3'-UTR polymorphism and risk of breast cancer, obtaining variable results. Although such an association has been found in some studies [7, 8, 10, 14], it has been discarded in others [6, 9, 11-13, 15]. The discrepancies among studies may be due to several factors, including ethnic differences among analyzed populations, differential exposure to specific environmental factors, and variation in methodologies employed. We observed a significant increased risk of developing breast cancer associated with the *ins/ins* genotype. In contrast, in another study carried out in Mexico [15], no associations were found. The differences between this study and our results are most probably related to ethnicity.

Mexican population exhibits high genetic variability. The admixture among Amerindians, Europeans, and Africans resulted in Mestizos, who represents more than 90% of the Mexican population [20]. Rubi-Castellanos et al. analyzed the genetic data of 13 combined DNA index system-short tandem repeats in subjects representing population samples from different regions of Mexico. They found significant genetic differentiation among Mestizos from different Mexican regions and observed genetic heterogeneity or asymmetric admixture throughout the country, displaying an increasing North-to-South gradient of Amerindian ancestry, and vice versa regarding the European component [21]. Moreno-Estrada et al. examined local patterns of variation from nearly 1 million genome-wide autosomal single-nucleotide polymorphisms (SNPs) for individuals from several populations, covering most geographic regions across Mexico. They demonstrated a high degree of fine-scale genomic structure across Mexico, shaped by pre-Columbian population dynamics and affecting the present-day genomes of Mexican mestizos, which is of both anthropological and biomedical relevance [22]. Our study examined subjects from northeastern Mexico, whereas in the previous study [15], subjects from the State of Jalisco (located in the central-western area of the country) were analyzed. Thus, ethnic diversity may have contributed to the discrepancies observed between these studies.

On the other hand, the *TYMS* 3'-UTR *del* allele has been associated with decreased mRNA stability, an enhanced rate of mRNA decay, and lower tumor TYMS expression [23]. Therefore, this *TYMS* variant has been hypothesized to increase risk of cancer, and in fact it has been associated in some studies with increased risk of breast cancer [7, 10, 14].

In contrast, we found a significant increase in the risk of developing breast cancer associated with the *ins/ins* homozygous genotype, and a significant trend for increased *ins* allele frequency with risk of developing this neoplasia. Similar results have been found in other cancer types [24, 25], and Akisik and Dalay found that the *TYMS* 3'-UTR *del* allele is associated with a significantly lower risk of breast cancer [8]. Thus, more research is necessary to determine the effect of the *TYMS* 3'-UTR polymorphism in TYMS production and function.

In conclusion, we analyzed the association between the *TYMS* 3'-UTR polymorphism and the risk of breast cancer in subjects from northeastern Mexico. A significant increase in the risk of developing breast cancer was associated with the *ins/ins* homozygous genotype, and there was a significant trend for an increased *ins* allele frequency with a higher risk of developing this neoplasia. Identification of inter-individual variability in *TYMS* polymorphisms may be useful for individualizing breast cancer genetic screening and therapeutic intervention. Analysis of a larger sample, inclusion of matched controls, and the consideration of gene-environment interactions could augment our understanding of the association between the *TYMS* 3'-UTR polymorphism and breast cancer risk in our population.

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Disclosure of conflict of interest

None.

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