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ORIGINAL ARTICLE

Clinical relevance of telomerase polymorphism for breast cancer: A systematic review

Katherine de Souza Rodrigues[1], Joao Nunes de Matos Neto[2], Rodrigo Haddad1, Diego Madureira de Oliveira[1]

[1]Faculty of Ceilandia, University of Brasilia - UnB, Brasilia, Federal District, Brazil; [2]Faculty of Medicine, University of Brasilia - UnB, Brasilia, Federal District, Brazil

Summary

Purpose: *To perform a systematic review to explore the clinical relevance of hTERT polymorphisms for breast cancer (BC).*

Methods: *Twenty-nine polymorphic regions were evaluated after comprehensive searching of 1236 articles, and selection of 9 publications (total of 12986 cases and 16758 controls).*

Results: *About the influence of hTERT variants in BC risk, 3 studies showed that the variant rs2736098 was associated with increasing risk. The variants rs10069690 and rs2853676 were also described as risk factors for BC. Only one variant rs2736100 presented as risk factor for BC. MNS16A genotype influenced the risk of BC in an Iranian, but not in the Greek and American populations. The associations of 5 hTERT variants with expression of hormone receptors were also evaluated in some studies. One study showed that the variant rs10069690 was associated to the estrogen receptor (ER)-negative and triple negative subtype, but other authors did not find the same results. In addition, the association of rs273618 with ER-/progesterone receptor (PR)+ cases, and rs10069690, rs2735940, rs4246742 and rs2736100 with both negative receptors were described. After data reanalyses, we found that the variant rs2735940 and rs2736100 were associated with ER-/PR- cases among patients with BC. Also, the variant rs2736100 was associated with ER+/PR+ cases and the variant rs2736118 was associated to ER+/PR+ and ER-/PR+ cases.*

Conclusions: *The associations between hTERT variants and BC risk and outcomes could be useful since a polymorphism can be identified before the diagnosis, but the heterogeneity of data and analyses found in different studies lead to many controversies.*

Key words: *breast cancer, polymorphisms, telomerase, TERT*

Introduction

Every day, many women are diagnosed globally with BC, the most common cancer in females and the leading cause of cancer-related deaths in many countries [1]. Early diagnosis of BC is mandatory for a better prognosis due to its complex and multifactorial pathogenesis [1,2]. Thus, efforts to improve BC diagnosis has led to search for new markers in an attempt to minimize the aggressiveness of the treatment administered and to reduce mortality rates [2].

Telomeres are special nucleoprotein structures essential for protecting chromosomal terminals against degradation, fusion and rearrangement from end to end [3,4]. Several studies have reported that the length of telomeres in solid tumor tissues may be a potential marker of prognosis [5,6]. In addition, telomere shortening is associated with several prognostic factors in BC [7].

The enzyme telomerase recognizes the 3' hydroxyl (3'OH) at the end of the telomere G-chain

Correspondence to: Diego Madureira de Oliveira, PhD. Universidade de Brasilia - UnB, Faculdade de Ceilandia – FCE, Centro metropolitano, conjunto A, lote 1, Brasilia, DF– Brasil. CEP: 72220-275.
Tel: +55 61 9 8575 6904, E-mail: dmadureira@unb.br
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overhang and adds repeat sequences at the chromosomal ends. Functional telomerase is composed of the TERT protein (telomerase reverse transcriptase) and the telomerase RNA component (TERC) that acts as a template for DNA synthesis [3]. The expression of human TERT (hTERT - the catalytic subunit of human telomerase) is able to restore the telomere's length, avoiding the senescence process and making the cell immortal. This process is vital for the maintenance of replicating cells in the tumor, since it maintains telomere integrity and enables such cells to divide continuously [3,8]. Therefore, telomerase is characterized as a key enzyme in the process of carcinogenesis, and a potential molecular marker for the prognosis of various types of cancer.

Given the fundamental role of TERT in oncogenesis, polymorphisms of genes related to telomerase may influence the expression levels of this enzyme, influencing the host's susceptibility to tumor progression and metastasis [9,10]. Some studies have linked these polymorphisms with susceptibility and/or survival of BC [9,11-18]. Thus, we performed a systematic review to explore a more precise estimation of the clinical relevance of hTERT polymorphisms for BC.

Methods

Study design

A systematic review evaluating the clinical relevance of polymorphisms of hTERT gene as risk factor and/or prognostic indicator for BC was undertaken in accordance with scientific standards, using the following protocol:

Eligibility criteria

Studies that reported some telomerase polymorphism in patients diagnosed with BC were selected. No language or time search restrictions were set. Acceptable study designs included cohort, case-control, and cross-sectional studies.

Studies were excluded for the following reasons: (1) cancers other than BC; (2) non-telomerase related gene polymorphism; (3) reviews, letters, personal opinions, book chapters, conference abstracts, posters and patents.

Information sources and search strategy

Studies to be considered for inclusion were identified using the search strategy for each of the following electronic databases: CAPES, PubMed and Wiley Online Library. All databases were searched up to June 30, 2017. The strategies of keyword were: ("Polymorphism") AND ("Telomerase") AND Breast Cancer, for the PubMed and Wiley Online Library; for CAPES the terms were: ("Polymorphism telomerase") AND Breast Cancer.

Studies selection and data collection process

Eligibility of the selected articles was determined in two phases. In phase 1, titles and abstracts identified in all electronic databases were chosen independently. Articles that appeared to meet the inclusion criteria based on their abstracts were selected. In phase 2, the full text of all selected articles were read and studies that did not meet the inclusion criteria were excluded. One author (K.S.R.) collected the required information from the selected articles; a second author (D.M.O.) cross-checked the information to confirm the quality of the data extraction. Any disagreement was resolved by discussion with a third author (J.N.M.N.). A fourth author (R.H.) was involved when assistance was required in the final decision. Subsequently, important information of each included article, such as main author, title, year of publication, study population, samples (cases and controls), genetic variants studied and single nucleotide polymorphisms (SNPs) related to TERT were collected.

Additional analyses / Statistics

Studies with data available to quantitative approach were selected for meta-analysis. The odds ratio (OR) and hazard ratio (HR) values were extracted or calculated. The Hardy-Weinberg equilibrium (HWE) in the controls was tested by the Chi-square test for goodness of fit when allele and genotypic frequencies were given ($p<0.05$ was considered as statistically significant). hTERT variants cited in 3 or more studies were grouped for forest plot only if at least one of them presented statistically significant association. Cases were segregated according to hormonal receptor expression status when applicable and the OR for each case was calculated separately. Association of polymorphisms with other clinical features was also searched.

Results

Studies' selection and characteristics

After comprehensive searching of 1236 articles, we identified 9 relevant publications including 12986 cases and 16758 controls to evaluate the association between telomerase polymorphisms and BC. The selection procedure is represented in Figure 1. These studies were conducted in US Americans (8801 subjects, 29.59%), Iranian (491 subjects, 1.65%), Turkish (217 subjects, 0.73%), Greek (237 subjects, 0.8%), African ancestry (2895 subjects, 9.73%) and Polish (4291 subjects, 14.42%), or involved more than one population (12812 subjects, 43.07%). All characteristics of the selected studies are summarized in Table 1. Twenty-nine among 120 polymorphisms studied were related to telomerase (28 hTERT and 1 hTERC). The list of genetic variants covered in the studies is shown in Table 2.

hTERT polymorphisms and breast cancer risk

The most frequently cited hTERT variant rs2735940 showed no association with BC risk [12,14,16]. On the other hand, the genotypes AG and GG for the variant rs2736098 were associated with increasing risk by 3 out of 4 studies (Figure 2).

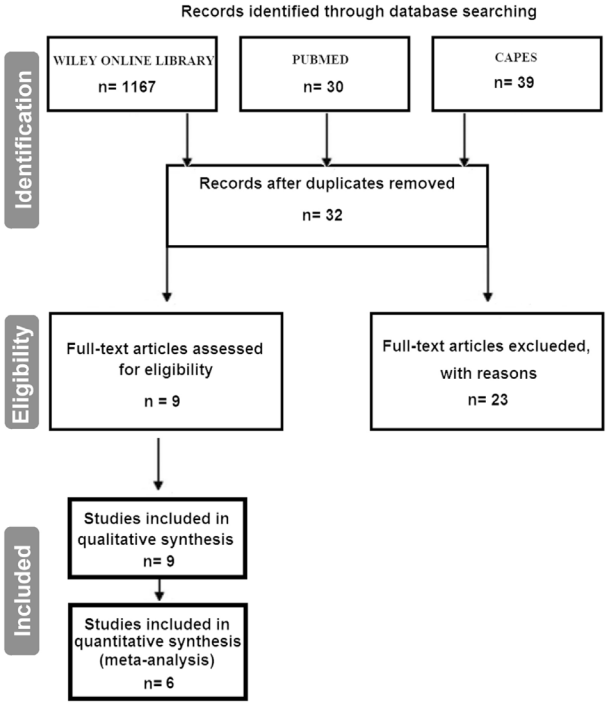

Figure 1. Flow chart of study identification, inclusion, and exclusion.

In fact, in the Turkish, Polish and Iranian populations, the GG genotype significantly increased the risk of BC (OR=1.88, 95%CI 1.04-3.40 and p=0.034; OR=0.76, 95%CI 0.58-1.00 and p= 0.05; and OR=1.87, 95%CI 1.19-2.94 and p=0.006, respectively) compared to the AA [12,13,16]. In women of African descent no association was detected [18].

The variant rs10069690 was also described as risk factor for BC (OR=1.18 per allele). Additionally, it was found that the frequency of risk alleles in African-American population (0.57) was higher than in women of European descent (0.26) [11]. Pellatt et al. [19] confirmed this association in the US non-Hispanic white and U.S. Hispanic and Mexican population (OR=1.13, 95%CI 1.03-1.24). In this same population the variant rs2853676 was modestly associated with overall BC risk (OR=1.23, 95%CI 1.00-1.5), but it was not confirmed in African ancestry (OR=1.03, 95%CI 0.91-1.16) [18].

Three studies testing the variant rs2736100 as risk factor for BC were found, but only one had statistically significant results (Figure 3). The association was absent in the Turkish and ancestral African [13,18], but it is a possible risk factor to postmenopausal women (OR=1.20, 95%CI 1.01-1.42 in US non-Hispanic white and US Hispanic and Mexican) [19]; two other hTERT variants with significant association in these popu-

Table 1. Summary of descriptive characteristics of included studies

Author	*Year*	*Ethnicity*	*Cases*	*Controls*	*Polymorphisms*	*Methodology*
Haiman, C.	2011	European and African	2722	6415	1	Illumina Infinium 1M Duo, Illumina 660W array, Illumina CNV370 SNP array or Illumina 550-Duo SNP array
Hashiemi, M.	2014	Southeast Iranian	266	225	3	RFLP and digested using Bsp120I restriction enzyme and ARMS-PCR method
Oztas, E.	2016	Turkish	107	110	3	Roche LightCycler 480 real-time PCR platform or by PCR-RFLP methods, and digested with SfcI restriction enzyme or ApaI restriction enzyme
Pellatt, A	2013	US non-Hispanic white and U.S. Hispanic and Mexican	3592	4183	22	Multiplexed bead array assay format based on GoldenGate chemistry (Illumina)
Savage, A.	2007	Polish	1995	2296	24	TaqMan or MGB Eclipse platforms
Shen, J.	2012	American US	1026	0	52	BioTrove OpenArray™ system or by TaqMan assays
Varadi, V.	2009	Polish and Swedish	1656	2019	3	Allele-specific PCR-based KASPar SNP genotyping system (KBiosciences, Hoddesdon, UK)
Zagouri, F.	2012	Greek	113	124	1	Determined by classifying the DNA amplicons
Zheng, Y.	2012	African ancestry	1509	1386	11	Illumina GoldenGate Genotyping platform (Illumina Inc., San Diego, CA, USA)
Total			12986	16758	29*	

*Types of TERT polymorphisms evaluated

lations were rs426742 and rs2242652 (OR=0.85, 95%CI 0.77-0.93 and OR=1.51, 95%CI 1.11-2.04, respectively) [19].

Hashiemi et al. [12] found that the MNS16A genotype influences the risk of BC in an Iranian population in southeast Iran; the MNS16A L/S and S/S+S/S genotypes decreased the risk of BC (OR=0.51, 95%CI 0.35-0.75, p<0.001 and OR=0.55, 95%CI 0.38-0.81, p=0.002, respectively) compared to the L/L genotype. However, in the Greek and American populations, the hTERT MNS16A polymorphism was not associated with risk for BC [15,17].

Two hTERT variants, rs2853669 [13,14,16] and rs2736109 [14,16,18], were included in 3 studies and showed no association with BC risk. Other hTERT variants tested as risk factors with no statistically significant results were: rs2853690, rs7712562, rs2853677, rs13167280, rs2075786, rs2736099 [16]; rs3816659, rs4975616, rs402710, rs401681, rs31489, rs11133719, rs7726159 [18].

hTERT polymorphisms as prognostic factors in breast cancer

hTERT variant rs2853677 was significantly associated with reduced trend for breast cancer-specific mortality (T-allele; HR=0.57, 95%CI 0.39-0.84) [15]. The variant rs2735940 had no significant association with clinical-pathological parameters [12].

The association of 5 hTERT variants (rs 273618, rs10069690, rs2735940, rs4246742 and rs2736100) with expression of hormone receptors were evaluated in some studies. Haiman et al. [11]

Table 2. hTERT variants analyzed in the included studies

Polymorphism	*Gene*	*Number of reviews*
rs2735940	TERT	5*
rs2736098	TERT	4⁺
rs2853669	TERT	4‡
MNS16A	TERT	3⁺
rs2736100	TERT	3*⁺
rs2736109	TERT	3
rs10069690	TERT	2*⁺
rs2853676	TERT	2⁺
rs2853690	TERT	2
rs7712562	TERT	2
rs2853677	TERT	2‡
rs13167280	TERT	2
rs2075786	TERT	2
rs3816659	TERT	2
rs2736118	TERT	1*
rs4246742	TERT	1*⁺
rs2242652	TERT	1⁺
rs2736099	TERT	1
rs4975616	TERT	1
rs402710	TERT	1
rs401681	TERT	1
rs31489	TERT	1
rs2736109	TERT	1
rs11133719	TERT	1
rs7726159	TERT	1
rs2293607	TERC	1
rs33954691	TERT	1
rs1801075	TERT	1
rs2735845	TERT	1

‡ Association with survival;
* Association with hormone receptors (ER and PR);
⁺ Association with risk of breast cancer

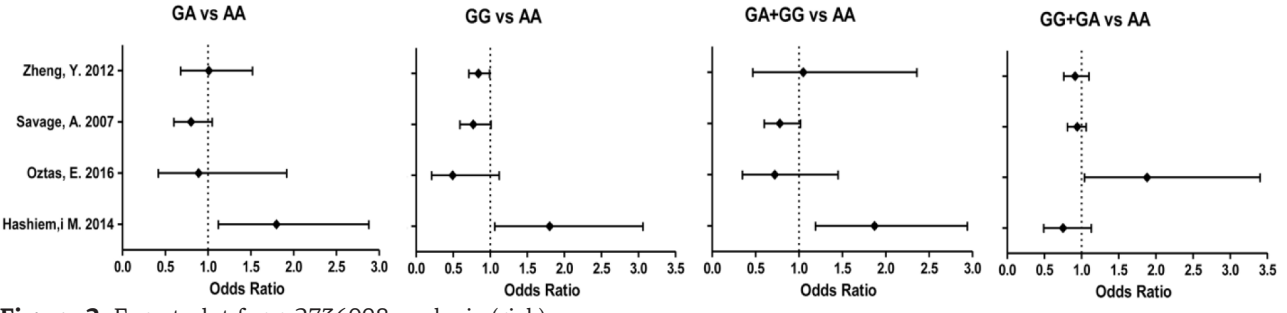

Figure 2. Forest plot for rs2736098 analysis (risk).

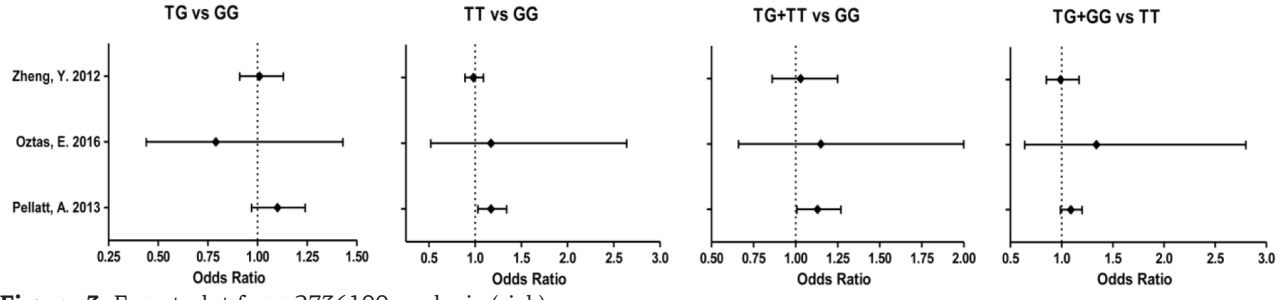

Figure 3. Forest plot for rs2736100 analysis (risk).

found that the variant rs10069690 was associated to the ER-negative and with triple negative (ER-negative, PR-negative and human epidermal growth factor-2 (HER2)-negative) subtypes of BC. In addition, the risk allele (T allele) of rs10069690 locus may be responsible for an incidence rate of 15% (95%CI 10-20) higher than ER-negative or triple-negative BC in African women compared to women of European ancestry. Hashiemi et al. [12] and Oztas et al. [13] also tested the association between hormone receptors expression and these polymorphisms, but found no significant results.

Since receptors expression status is very important pathological information, Pellat et al. [19] evaluated the implications of many hTERT polymorphisms on this status and described association of rs273618 with ER-/PR+ cases, and rs10069690, rs2735940, rs4246742 and rs2736100 with both negative receptors. However, the OR values calculated were relative to control group. Then, we extracted the data and analyzed the impact of hTERT polymorphisms in each subtype of tumor (ER+/PR+, ER+/PR-, ER-/PR+, ER-/PR-) relative to all cases. We found that CC genotype for the variant rs2735940 and TT genotype for the variant rs2736100 were associated with ER-/PR- cases among patients with BC (OR=1.52, 95%CI 1.21-1.91, p=0.0003 and OR=1.5, 95%CI 1.2-1.87, p=0.0007, respectively). TT genotype for the variant rs2736100 was associated with ER+/PR+ cases (OR=0.78, 95%CI 0.64-0.95, p=0.018). Using this same approach, we also found association between AA genotype for the variant rs2736118 and ER+/PR+ and ER-/PR+ cases (OR=1.23, 95%CI 1.02-1.48, p=0.034 and OR = 0.19, 95%CI 0.09-0.4, p<0.0001, respectively).

Discussion

Cancer is a multifactorial disease, and genetics can play a significant role in the appearance, development and outcomes of oncogenic disorders. Studies involving genetic factors, such as polymorphisms, are essential in order to prevent serious cases, anticipate diagnosis and establish clinical practices and health policies. In fact, several genes have been implicated in hereditary breast cancer syndromes [20], and studies on polymorphisms as risk factors for BC are increasingly common. The hTERT gene is strategic for tumor maintenance and is involved in the pathogenesis of almost all kinds of cancer, which makes it a great target for studies. Some of the polymorphisms approached in this article have direct impact on gene functionality and, consequently, on cell phenotype [6]. The associations between hTERT variants and clinical features such as hormonal receptors expression or mortality could be useful since a polymorphism can be identified before the disease diagnosis. If confirmed the susceptibility to triple negative subtype of patients with specific genotypes, the therapeutic strategy can be adopted early, improving the chance of successful outcome. However, this study showed that, despite some significant associations, there are still a lot of controversies about this issue, with conflicting results, variability among different populations and very discrete associations. The bulk of studies remains a limitation, but the *omics* works will certainly generate more precious data soon.

Conflict of interests

The authors declare no conflict of interests.

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