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Medical Oncology

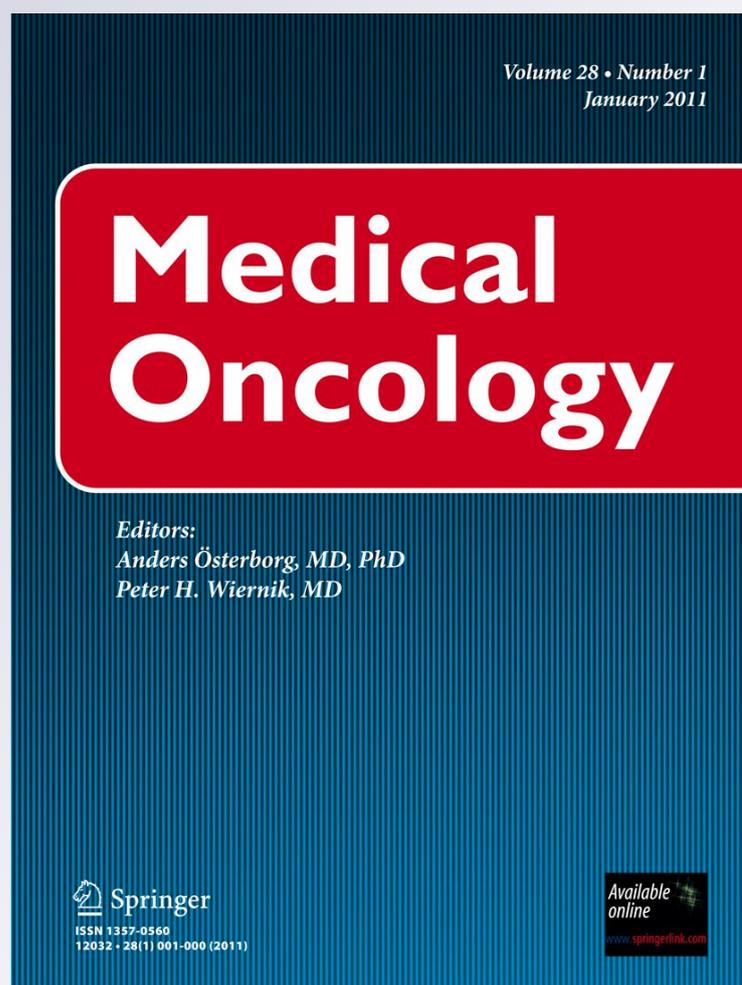
ISSN 1357-0560

Volume 29

Number 3

Med Oncol (2012) 29:1581-1585

DOI 10.1007/s12032-011-0111-x



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GGC_n polymorphism of *eRF3a/GSPT1* gene and breast cancer susceptibility

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Received: 9 October 2011 / Accepted: 2 November 2011 / Published online: 19 November 2011
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Abstract The significance of translation regulatory factors in elevating the risk of cancer has been recently recognized. Eukaryotic release factor 3a (eRF3a) is a translation termination protein that is encoded by G1 to S phase transition 1 gene (*GSPT1*). The *eRF3a/GSPT1* exon 1 contains a trinucleotide GGC repeat coding for a polyglycine expansion in the N-terminal of the protein. In the present study, we determined the allelic length of the GGC_n repeat in the *eRF3a* gene in 250 women with breast cancer and 250 age-matched controls. Our results show that the presence of the longer allele, 12-GGC, is correlated with threefold increased risk of breast cancer development. Our findings also suggest that women who are homozygous for 7-GGC allele are possibly at higher risk of developing breast cancer, especially before the age of 50. No significant effect of the allelic length of *eRF3a/GSPT1* polymorphism on inheritance or the grade of this disease was observed.

Keywords *eRF3a/GSPT1* · GGC repeat · Microsatellite · Breast cancer

Introduction

Worldwide, breast cancer is the most common neoplasm and also the primary cause of cancer death among women

[1]. It has been reported that globally over 1.15 million women are diagnosed with breast cancer and 502,000 die from the disease (World Health Organization 2008). Breast cancer incidence varies widely across geographic regions and racial groups, with the highest incidence in Caucasian women and lower incidence among African American and Korean women [2]. Women in the United States have the highest incidence rates of breast cancer in the world, with a 1 in 8 (12.5%) lifetime chance of developing invasive breast cancer and a 1 in 35 (3%) chance of breast cancer causing their death (American Cancer Society 2008). In Iran, breast cancer incidence is rising, and the age at onset is at least one decade lower than in the developed countries [3, 4].

Eukaryotic translation termination is governed by two release factors, eRF1 and eRF3, which associate in a complex which binds to the ribosomal A site. Eukaryotic RF1 promotes the release of the nascent polypeptide chain, and eRF3 as a small GTPase enhances eRF1 activity [5]. eRF3 also interacts with polyadenylation binding protein (PABP), which associates with the poly (A) tail of mRNAs for RNA stabilization [6]. More recent studies suggest that eRF3 may be involved in other cell processes such as cell cycle progression, apoptosis and cytoskeletal organization [7, 8]. Lee et al. demonstrated that eRF3 influences ASK1-mediated apoptosis by facilitating the dissociation of 14-3-3 from ASK1 [10]. Moreover, eRF3 is proteolytically processed into an isoform that contains a conserved N-terminal inhibitor of apoptosis (IAP) binding motif [11]. In humans, eRF3 has two distinct isoforms: eRF3a encoded by the *eRF3a/GSPT1* gene located in 16p13.1 and eRF3b encoded by the *eRF3b/GSPT2* gene located in Xp11.21–23 [12, 13]. eRF3a and eRF3b proteins share 87% identity, with most of the differences concentrated in their N-terminal domains [5]. It has been reported that eRF3a is

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present in all cell lines tested, whereas eRF3b is formed only in mouse brain. In addition, eRF3a depletion reduces the intracellular level of eRF1 protein by affecting its stability, suggesting that eRF3a is the major factor acting in translation termination [14]. It was previously reported that *GSPT1* mRNA levels are increased in intestinal-type gastric, breast and colorectal tumors [15, 16] and strongly decreased during human chondrocyte differentiation [18].

The N-terminal domain of human eRF3a contains a polyglycine expansion encoded by a trinucleotide GGC repeat in exon 1 of *eRF3a/GSPT1* gene. There are five known alleles of this gene, which encode for 7, 9, 10, 11 and 12 glycines. Previous studies have reported a strong correlation between the longest allele (12-GGC) and gastric, breast and colorectal cancer development [15, 16].

The purpose of this study was to evaluate the association between the GGC repeat polymorphism in exon 1 of *eRF3a/GSPT1* and the potential genetic susceptibility to the development of breast cancer.

Materials and methods

Sample collection and genotyping

Peripheral blood samples were collected from 250 women with breast cancer and 250 age-matched healthy blood donors. Breast cancer patients were all between 24 and 79 years old. Sample collection was done in Sayedoshohada Hospital in Isfahan city. Control subjects were randomly selected from women visiting hospitals for regular health checks and they had no family history of breast cancer.

DNA was extracted using a salting out method [19]. The (GGC)_n fragment was PCR-amplified via the previously reported primers [15], forward primer (5'-CATTCTCGCTCTCTGTCCAC-3') and reverse primer (5'-CTGGTCCAGCAGTCAGG-3'). A 25 µl of the PCR reaction mixture contained 100–250 ng of genomic DNA, 0.1 µM each of the forward and reverse primers, 200 µM dNTPs, 2.5 µl 10× PCR buffer, 2 mM MgCl₂, 2.5 µM 10% DMSO, 1M betaine and 2 U *smarTaq* DNA polymerase, all obtained from *CinnaGen Inc*, Iran. PCR conditions were as follows: 1 cycle of 94°C for 5 min, followed by 33 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 1 min and finally 1 cycle of 72°C for 10 min.

After analysis of the PCR products on polyacrylamide gel (10%), direct DNA sequencing of the selected alleles was performed to confirm their GGC repeat lengths. The sequenced alleles were used as allele-specific markers for exact determination of the number of GGC repeats in all the other samples.

Statistical analysis

SISA (<http://home.clera.net/sisa/>) and SPSS (ver.15) software were used for statistical analysis. Finally, the association between the genotype and different factors was examined by Pearson's χ^2 and odds ratio tests.

Results

GGC repeat polymorphism of *eRF3a/GSPT1*

Four different alleles of *eRF3a/GSPT1* for GGC repeat were detected, which encode 7, 10, 11 and 12 glycines (Fig. 1). The most frequent allele in both case and control groups was the 10-GGC allele. The (GGC)₈ allele was not detected in 500 individuals tested.

Allelic frequencies in patients were 5.8% 7-Gly, 64.2% 10-Gly, 27.2% 11-Gly and 2.8% 12-Gly. The distribution in controls was 5.4% 7-Gly, 68.8% 10-Gly, 24.8% 11-Gly and 1% 12-Gly.

The frequency distribution of the genotypes of GGC repeat length in patients and controls are shown in Fig. 2. The most common genotype in both cases and controls was homozygote 10 with frequencies of 44 and 48%, respectively, followed by heterozygous 10–11.

GGC repeat length and breast cancer risk

Compared with controls, patients had a higher frequency of the 12-Gly allele. Women carrying one allele of 12 (10/12, 11/12) are at significantly higher risk of developing breast cancer with odds ratio 2.85 (95% CI = 1.01–7.97; $P = 0.0371$) (Fig. 1). We did not observe any individuals homozygous for 12-Gly among 500 samples. In addition, there is a fivefold increase in the frequency distribution of homozygous 7 among patients (OR = 5.0816; 95% CI = 0.5894–43.8133, $P = 0.100$) (Fig. 2). Interestingly, 4 out of 5 of these patients were younger than 50 (OR = 3.6; 95% CI = 0.4028–33.1843; $P = 0.218$) (Table 1). However, due to the small sample size, this result does not achieve statistical significance. In a different set of experiment among 250 men controls, we have also detected one homozygous for 7-Gly, if we consider these controls as well the P value will be meaningful.

We also analyzed the correlation between the allelic length of *eRF3a/GSPT1* and age of onset and grade of tumor as well as inheritance of the disease. No association was found between the GGC repeat length and tumor staging or family history of breast cancer (data not shown).

Fig. 1 Allelic frequencies and odds ratio analysis of the *eRF3a/GSPT1* GGC repeat length alleles in the breast cancer patients and control individuals in Isfahanian population

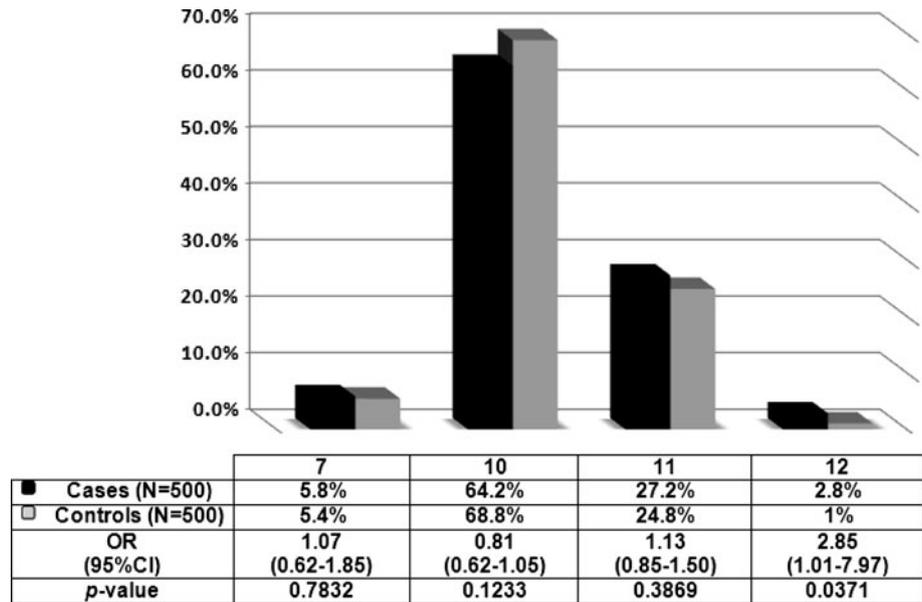


Fig. 2 Genotypic frequencies and odds ratio analysis of the *eRF3a/GSPT1* GGC repeat length in the Isfahanian breast cancer patients and controls

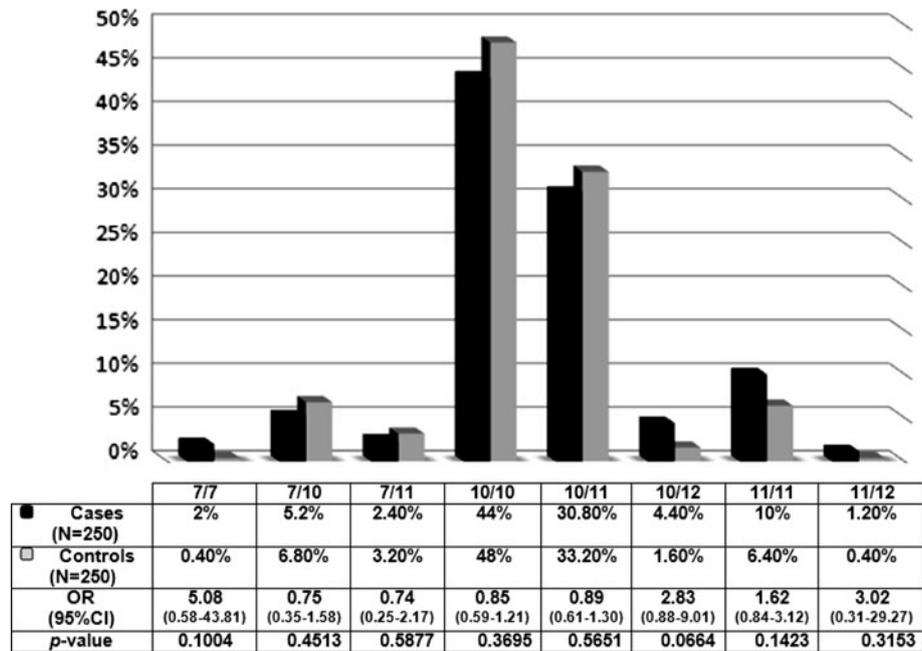


Table 1 Association between the allelic length of *eRF3a/GSPT1* and onset age of cancer

In patients	<50/≥50 years old	OR (95% CI)	P value
7/7 genotype	4/1	3.6562	0.2184
Other genotypes	128/117	(0.4028–33.1843)	

Discussion

In a population-based case–control study in Iran, we studied the GGC allelic length polymorphism in exon 1 of

the *eRF3a/GSPT1* gene and its association with the risk of breast cancer.

So far, there has been only one previous population-based study on the polymorphism of GGC and its association with cancers in Portugal [15–17]. There are some differences in allelic and genotypic distribution of GGC repeats between the two populations. The most common allele and genotype in both populations was 10-GGC repeats; however, the 9-GGC allele was not detected in our sample of 500 Iranian women. The frequency of this allele in the Portuguese population was 1%. Moreover, the frequency of the 12-GGC allele in the Iranian control group

was 1%, while this allele was not detected in the Portuguese control group.

The evidence from both studies suggests a positive association between a polymorphic GGC trinucleotide repeat in a translation factor (eRF3a) and breast cancer risk. Brito et al. [15] and Malta-Vacas et al. [16, 17] have detected the presence of the 12-GGC allele exclusively in patients with gastric, breast and colorectal cancers, corresponding to 12–20-fold increased risk of cancer development. In contrast, we observed the 12-GGC allele in 1% of control samples as well, but the frequency was higher in patients (2.8%). Because of the presence of the 12-GGC allele in controls, we found lower cancer risk of this allele (OR = 2.9). We also found no association between the 12-GGC repeat length and family history (data not shown). In addition, in contrast to the previous reports, our results suggest that women with two alleles of 7-Gly have much greater risk of developing breast cancer, especially before the age of 50.

eRF3a/GSPT1 mRNA levels have been reported to be upregulated in intestinal-type gastric, breast and colorectal tumors, relative to the normal adjacent tissues. Patients with the GGC₁₂ allele exhibited increased levels of mRNA expression relative to the shorter alleles [16, 17]. Also, it has been found that the mRNA levels of *eRF3a/GSPT1* with the GGC₁₂ allele were significantly upregulated both in primary lymphocyte cultures and in Jurkat cell line relative to the shorter alleles [17]. These elevated *eRF3a/GSPT1* mRNA levels are not the result of a coordinated upregulation of the translation termination machinery because the mRNA levels of the other translation termination factors were not changed [17].

In the *eRF3a/GSPT1* exon 1 GGC_n polymorphism, the number of CpG sites is directly proportional to the number of GGC repeats present. However, no correlation between the length of the alleles and methylation levels of the CpG sites inside the GGC expansion has been reported. *RF3a/GSPT1* gene copy number has also been determined in DNA samples from lymphocyte cultures of individuals with all possible genotypes, but no correlation has been found between the gene copy dosage and mRNA levels of expression [17]. So, other mechanisms must be responsible for the elevated levels of *eRF3a/GSPT1* mRNA.

RF3a/GSPT1 dysfunctions have been shown in cytoskeleton assembly and therefore in chromosome segregation defects in various organisms such as yeast and *Drosophila melanogaster* [9–20]. Micronuclei (MN) formation in dividing cells is the result of chromosome mal-segregation due to mitotic malfunction. So, micronucleus frequency is considered a predictive biomarker of cancer risk [21]. It has been found that cell lines with the longer alleles of *eRF3a/GSPT1* have higher frequencies of micronuclei in binucleated cells, which is probably a result of defects in mitotic spindle formation [17].

In summary, our findings suggest that Iranian women carrying the 12-GGC allele of *eRF3a/GSPT1* are at increased risk of breast cancer. Our results also suggest that homozygosity for the short 7-GGC repeat may be associated not only with the risk of breast cancer but also with the age of developing breast cancer in Iranian women. The relation between GGC repeat length polymorphism and risk of breast cancer is still unknown. Additional investigation is needed to understand the relationship between these genotypic variations, differential expression of *eRF3a/GSPT1* and risk of cancer development.

Acknowledgments The authors wish to thank all patients who contributed to this investigation and Professor Farzin Farzaneh (Kings College London) for valuable suggestions and critical reading of this manuscript. This work was supported by University of Isfahan.

Conflicts of interest The authors report no conflict of interest.

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