ERCC1 intron 1 was associated with breast cancer risk

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Abstract

Introduction: There are numerous studies addressing associations of polymorphisms in DNA repair genes and cancer risks because accurate and efficient DNA repair is crucial to genomic integrity and fidelity. ERCC1 is important in DNA nucleotide excision repair.
Material and methods: We genotyped constitutive variants of ERCC1 in approximately 300 adults with breast adenocarcinoma and 126 controls of Iranian women. In total, 426 Iranian sporadic breast cancer affected women compared to the control group were studied by PCR-RFLP for ERCC1 variant.
Results: The genotype ERCC1 TT has the highest frequency in both groups (36.6 in patients and 8.5 in controls). The genotype ERCC1 was the most important risk factor in our population [GG/AA odds ratio: 0.692, 95% confidence interval (CI): 0.4-1.199, p = 0.188; GG/AG odds ratio: 3.333, 95% CI: 1.917-5.795, p = 0.001; AA/AG odds ratio: 0.208, 95% CI: 0.124-0.348, p = 0.342].
Conclusions: Our patients was associated with breast cancer risk.

Key words: ERCC1 gene, polymorphism, breast cancer, MboII, polymerase chain reaction-restriction fragment length polymorphism, susceptibility factor.

Introduction

Breast cancer is the second most common cancer in the world and the most common cancer in Iranian women in rate [1, 2]. There are many studies on polymorphisms in DNA repair genes and cancer risks [1].

The DNA repair system is complex many of which are polymorphic [3] once of DNA repair genes, ERCC1 (ASE-1), whose products are important in nucleotide excision repair lie.

ASE-1 is positioned in an anti-sense orientation to and overlaps with the gene for ERCC1 and is possibly involved with the RNA polymerase I transcription complex.

Variation in efficiency of these processes might influence cancer development. Defective or inefficient repair could lead to accumulation of deleterious mutations in the absence of apoptotic destruction of DNA-damaged cells or cancer progression, by any of the previously mentioned mechanisms or by more efficient repair reducing effectiveness of chemotherapy aimed at DNA damage and resultant reduction of cancer cell killing.

We report here the allele frequencies of ERCC1 in breast cancer patients in Iran.

Material and methods

Patient data

Analyses were conducted for 300 patients and 126 controls genotyped for ERCC1; ages were 35-55 years.

This study was ethically approved by the local Ethical Committee of Islamic Azad University from the point of view of patients' and also control group members' rights.

All patients in stage II (tumour 2-5 cm) participated in the Special Medical Centre, part of chemotherapy, Tehran, Iran. A questionnaire including questions on breast cancer risk factors was completed and each patient filled in a consent form. Blood samples were collected from patients and controls prior to the start of treatment.

Subjects were genotyped for ERCC1 using genomic DNA extracted from peripheral blood lymphocytes. DNA was isolated from peripheral blood using FelxiGene DNA extraction kit (Qiagen Germany).

Genotyping

The polymorphisms were detected using a modified polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method [4, 5]. The PCR primers were synthesized by TAG Copenhagen A/S. Primers were for each polymorphism as follows. ERCC1 intron 1 (*ASE-1*) (rs3212981) (255 bp) [6]. Forward: TAGTTCCTCAGTTTCCCG, Reverse: TGAGCCAATTCAGCCACT. The cycling conditions were 94°C, 30 s; 55.5°C, 30 s (35 cycles); 72°C, 60 s. The PCR products were digested with 1 unit of MboII, and separated on a 6% acrylamide gel.

This method is able to detect all three possible genotypes for the polymorphism: homozygous wild type, heterozygous variant type and homozygous variant type.

Table I. ERCC1 genotype frequencies [*n* (%)] for cases and control: analyses of 300 affected women and 126 controls for ERCC1 genotype frequency shows that ERCC1 AA genotype has the highest frequency in both groups (36.5 in patient and 8.5 in control group). The *AA* genotype is in the patient group 21.1 compared with controls 7.0. *AG* genotype is lower in the case group (12.7) compared to the control group (14.1)

Genotype	Patients *n* (%)	Control *n* (%)	Total *n* (%)
n	300	126	426
ERCC1			
GG	90 (21.1%)	30 (7.0%)	120 (28.2%)
AG	54 (12.7%)	60 (14.1%)	114 (26.8%)
AA	156 (36.6%)	36 (8.5%)	192 (45.1%)

Table II. Comparison between genotypes, odds ratio and p value showed that genotype ERCC1 *AA* was the most important risk factor in our population; *AA* odds ratio: 3.333, 95% CI: 1.917-5.795, $p = 0.001$; *GG* odds ratio: 0.692, 95% CI: 0.4-1.199, $p = 0.188$; *AG* odds ratio: 0.208, 95% CI: 0.124- 0.348, $p = 0.342$. After genotype ERCC1 AA, genotype GG was the most important risk factor in our population

Genotype ERCC1	Odds ratio	95% confidence interval	Value of *p*
AG	0.208	0.124-0.348	0.342
GG	0.692	0.4-1.199	0.188
AA	3.333	1.917-5.795	0.001

Statistical analysis

The genotypes and allelic frequencies of ERCC1 polymorphisms in patient and control groups were analysed by χ^2 and Fisher's exact tests.

Results

Analyses of affected and controls show that genotype ERCC1 AA has the highest frequency in both groups (36.6 in patients and 8.5 in controls).

The frequency of genotype ERCC1 AG in patients was 12.7 compared with 14.1 in controls (Tables I-II, Figures 1-2).

The genotype ERCC1 was the most important risk factor in our population. Comparison between genotypes, odds ratio and p value showed that the genotype ERCC1 GG/AG was the most important risk factor in our population: GG/AA odds ratio: 0.692, 95% confidence interval (CI): 0.4-1.199, $p = 0.188$; GG/AG odds ratio: 3.333, 95% CI: 1.917-5.795, $p = 0.001$; AA/AG odds ratio: 0.208, 95% CI: 0.124-0.348, $p = 0.342$].

We conclude that A/A and then G/G in our patients were associated with breast cancer risk but there is no relation between presence of A/G and increase of breast cancer risk.

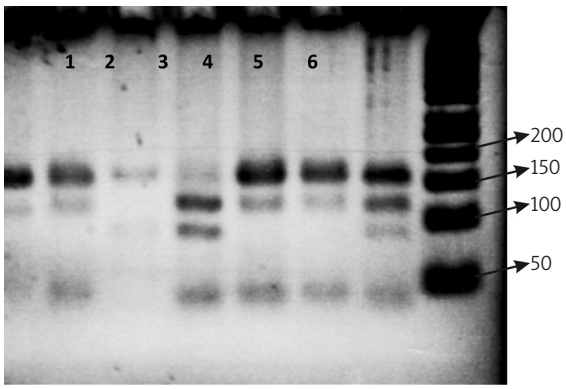

Figure 1. Polymorphism analysis of the *ERCC1 intron* 1. The PCR products were digested with restriction enzyme MboII in groups 1, 4, 5 and 6; *AG* genotype (167 bp, 114 bp, 83 bp, 49 bp, 6 bp), 3; *AA* genotype (114 bp, 83 bp, 49 bp, 6 bp) 2; *GG* genotype (167 bp, 83 bp, 6 bp); (ladder 50 bp)

Discussion

Numerous studies address associations of polymorphisms in DNA repair genes and cancer risk [1]. Accurate and efficient DNA repair is crucial for genomic integrity and fidelity.

The DNA repair system is complex, governed by more than 125 genes, many of which are polymorphic [7-9]. Two DNA repair genes, ERCC2 and ERCC1, whose products are important in nucleotide excision repair, lie on chromosome 19q13.3 [10].

ERCC1 (ASE-1) is positioned in an anti-sense orientation to and overlaps with the gene for ERCC1 and is possibly involved with the RNA polymerase I transcription complex. Recent studies show that base excision repair is required to repair DNA lesions induced at low N-nitrosodiethylamine concentrations [11].

To our knowledge, no studies have published the associations of ERCC1 rs3212981 polymorphisms and breast cancer risk.

There have been several studies of ERCC2 variants with other cancers, most notably with head and neck cancers, lung cancer, and skin cancer [1, 3, 12-16] but none have reported about ERCC1 intron 1. So we examined ERCC1 intron 1 and the association with breast cancer risk.

However, Hansen did not find associations between the ERCC1 polymorphisms, the haplotype and risk of colorectal cancer but ERCC1 Asn118Asn has been associated with risk of breast cancer and lung cancer [17].

In another study, Zhou found that excision repair cross-complementing group 1 (ERCC1) is the lead enzyme in the nucleotide excision repair process, and low expression of ERCC1 mRNA levels has been associated with higher risk of cancer. On the other hand, ERCC1 8092C > A polymorphism may modify the associations between cigarette smoking and lung cancer risk [18].

On the other hand, in another study Yu *et al.* suggested that alteration at codon 118 within the ERCC1 gene may exist in platinum-sensitive and platinum-resistant ovarian cancer tissues [19].

In another study it was seen that the expression of ERCC1 was particularly lower in TNBCs than other types of breast cancers [20]. But in one study by Schöffski, there was seen a low BRCA1 and high ERCC1 in a population of sarcomas [21].

We previously reported a significant association of the ERCC2 polymorphism K751Q and R156R with breast cancer risk [22], but now, in this paper we have reported about the association between ERCC1 C8092A and breast cancer risk.

In this report, we examine ERCC1 polymorphism with breast cancer and controls. In this study we conclude that there is a relation between presence of T/T of ERCC1 genotype and increase of breast cancer risk.

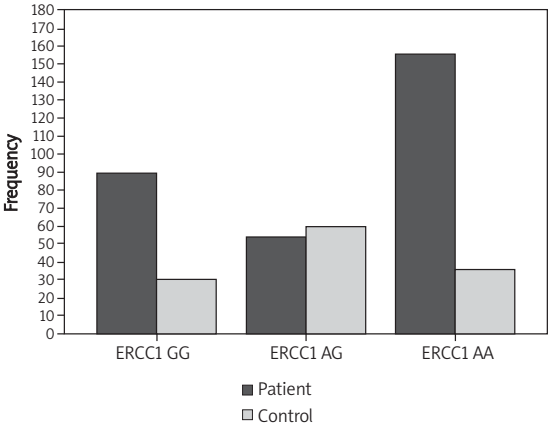

Figure 2. ERCC1 genotype frequencies [*n* (%)] for cases and controls: Analyses of 300 affected women and 126 controls for ERCC1 genotype frequency shows that *AA* genotype has the highest frequency in the patient group and *AG* genotype has a decrease in the patient group compared to the control group control and another group

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