RESEARCH COMMUNICATION

Single Nucleotide Polymorphisms in the p21 and bcl2 Cancer Susceptibility Genes and Breast Cancer Risk in Saudi Arabia

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Abstract

Certain single nucleotide polymorphisms (SNPs) in genes like p21 or bcl2 increase susceptibility to breast cancer but it has not, until now, been clear whether common polymorphic variants in the same genes also increase risk in Saudi Arabian population. The aim of this study was therefore to determine whether polymorphisms of p21 or Bcl2 might be associated with an increased risk of breast cancer in Saudi women. p21 (rs733590) C/T SNP was not found to be associated with breast cancer pathogenesis. However, we found that a reverse mutation T/C might be linked with breast cancer occurrence. Bcl2 genotypes were marginally associated overall with breast cancer risk. In addition, the alleles of this gene were significantly associated with risk of breast cancer. The allelic frequency of G was higher (0.68) in patients than in healthy women. AA vs. AG+GG genotype [OR=3.56 (1.24-10.68); P=0.008] was the dominant genotype. It is likely that these genes conferring measurably increased risks of breast cancer in our study population.

Keywords: Breast cancer - - p21 - bcl2 - SNPs - Saudia Arabia

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Introduction

The incidence of breast cancer shows geographical variation, even within areas of ethnic homogeneity. Comparison of mutation profile with other ethnic populations and regions reflected both differences and similarities indicating co-exposure to a unique set of risk factors. The differences could be due to exposure to particular environmental carcinogens including lifestyle, reproductive pattern; dietary or cultural practices. The incidence of breast cancer in Western countries, are about five times higher than rates in less developed countries (Bin Amer et al., 2008). In Saudi Arabia 21% of total female cancer diagnosis refers to breast cancer. It has been reported that young age in females is independent risk factor for breast cancer in the Saudi population (Elkum et al., 2007).

Recently there is great interest and attention to investigate the association of cancer with single nucleotide polymorphisms (SNPs)including p21, bcl2 or Mdm2. Several studies have already been attempted in some of these genes. One of the candidates, p21,the cyclin-dependent kinase (CDK), plays a direct role in mediating p53-induced G1 arrest (Roh et al., 2001). At least four polymorphisms have been identified for p21, the most studied being p21 Ser/Arg at codon 31, located in a highly conserved region of the gene (Chedid et al., 1994). Some studies have noted that the Arg/ Arg genotype in p21 is associated with a decreased risk of esophageal (Wu et al., 2009), endometrial and cervical cancer (Roh et al., 2001), while the p21 Arg/Arg genotype increased the risk of prostate cancer in a Taiwanese population (Huang et al., 2004).

The Bcl2 gene, located on chromosome 18q21.3, consists of three exons and two promoters, both of which have different functional properties. The protein Bcl-2 plays a major role in regulating apoptosis and cell cycle delay. Direct sequencing of DNA subjects from a Caucasian population has identified a novel single nucleotide polymorphism (SNP; –938C>A) in the inhibitory P2 promoter of the BCL2 gene (Park et al., 2004). Recently, Nuckel et al. showed that the –938C allele is significantly associated with increased P2 promoter activity and binding of nuclear proteins compared with the A-allele. Consistent with this suppressive effect of the P2 promoter, they found significantly increased Bcl-2 protein expression in B-cells from CLL patients carrying the –938AA genotype. In line with the finding that Bcl-2 overexpression is an unfavorable prognostic factor in B-CLL, this genotype was significantly associated with decreased survival (Nukel et al., 2006).

Despite the strong hypothesis for an involvement of these genes, to the best of our knowledge the association between these polymorphisms and the development

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of breast cancer in Saudi population has not yet been investigated. The aim of this study was firstly to determine whether previously identified breast cancer susceptibility alleles are associated with sporadic breast cancer in the Saudi Arabia and secondly to ascertain whether there are susceptibility alleles that predispose to breast cancers

Materials and Methods

Study Subjects

In this study, hundred breast cancer patients who were clinically and histopathologically diagnosed at KKUH from January 2009-October 2009 were included. All patients underwent complete staging procedures in the respective pathology laboratories. The study was approved by the Hospital Ethical-Committee Board. Written informed consent was obtained from all patients and control subjects included in this study. Eighty patients with primary breast cancer were randomly enrolled in this study. Eighty healthy females of similar age (50±5 years) as that of patients were voluntarily enrolled as controls and they were not receiving any prolonged medication, were not suffering from any genetic disorder nor had any family history of cancer. Both the study groups were drawn from Riyadh region of Saudi Arabia. The demographic data was collected using a structured questionnaire, which contained the details of age, stage, disease history, family history and other relevant details as required.

Sample collection

About 5mL of venous blood was drawn using sterile heparinized syringe from healthy individuals as control samples after clinical checkup. The blood samples of malignant cases were obtained after diagnosis from the pathology laboratory for genotyping using standard protocol.

Extraction of DNA

Genomic DNA was extracted using QIAamp mini blood DNA extraction kit as per the instructions of the manufacturer.

TaqMan Genotyping

Genotyping was carried out using TaqMan (Applied Biosystems) according to the manufacturer's instructions. Primers and probes were supplied directly by Applied Biosystems as Assays-by-Design™. All assays were carried out in 96-well plates. Cases and controls were arranged in a chequer board pattern on each plate to ensure even treatment during the assay procedure and each plate included negative controls (with no DNA). Plates were read on the ABI Prism 7500 Fast using the Sequence Detection Software (Applied Biosystems) using the following conditions: 50°C for 2 minutes, 95°C for 10 minutes, and then 40 cycles of amplification (92°C denaturation for 15 seconds, 62°C annealing/extension for 60 seconds). Failed genotypes were repeated. Assays in which the genotypes of duplicate samples did not show >95% concordance were discarded and replaced with alternative assays with the same tagging properties.

Statistical methods

For each SNP, deviation of genotype frequencies in cases and controls were compared by $\chi 2$ test for heterogeneity (two degrees of freedom) and test for trend (one degree of freedom), In order to evaluate the ethnicity-specific effect. Genotype specific risks were estimated as odds ratio (ORs) using unconditional logistic regression. No statistically significant differences were found (data not shown) and so the results have been combined and the risk associated with each SNP was estimated by allelic, dominant and recessive OR and associated 95% confidence intervals (CI). ORs with 95% confidence intervals (CIs) were calculated to assess the strength of the association between polymorphism and breast cancer risk. We explored the association for codominant model, dominant model, recessive model and allele versus allele, respectively. All statistical tests were based on two-sided probabilities using SPSS. We used a threshold of $P \leq 0.05$.

Results

We analyzed the polymorphic status of P21 and Bcl2 by TaqMan basaed Real Time PCR analysis. To the best of our knowledge, this is the first study in the Saudi population, which examined the association of in the P21or Bcl2 gene polymorphism with breast cancer.

p21 Polymorphism

As shown in Table1, the genotypic distributions were determined as 25.71% for the CC, 45.71% for the heterozygous CT status, and 28.57% for the TT, respectively.

The homozygous TT genotype was seen in lower percentage of patients with breast cancer (28.57%) when compared to healthy women (32.6%). Results showed that

Table 1. Distribution of p21 (rs733590) Genotypes and Allelic Frequencies of the Study Population

		Genotypes				Allelic Frequency		
		CC	CT	TT	Total	C	T	Total
p21	Controls (%)	21 (26.25)	33 (41.25)	26 (32.50)	80	0.46	0.53	160
(rs733590)	Patients (%)	18 (25.71)	32 (45.71)	20 (28.57)	70	0.48	0.51	140

Table 3. Distribution of bcl2 (rs956572) Genotypes and Allelic Frequencies of the Study Population

		Genotypes				Allelic Frequency		
		AA	AG	GG	Total	A	G	Total
bcl2	Controls (%)	6(8.60)	32(45.70)	32(45.70)	70	44(0.314)	96(0.685)	140
(rs956572)	Patients (%)	20(25)	28(35)	32(40)	80	68(0.425)	92(0.575)	160

Table 2. Odds Ratio with 95% CI of p21 (rs733590) Gene in Breast Cancer Patients

Genotype	Odds Ratio (CI 95%)	P-Value
p21 (rs733590)		
CC vs. CT	1.13 (0.47-2.71)	0.761
CC vs. TT	0.90 (0.35-2.31)	0.804
CT vs. TT	1.11 (0.43-2.87)	0.804
Dominant Model		
CC vs. CT+TT	1.03(0.46-2.28)	0.940
Recessive Model		
TT vs. CT+TT	1.20 (0.57-2.57)	0.602
C/T	0.93 (0.58-1.51)	0.769

Table 4. Odds Ratio with 95% CI of bcl2 (rs956572) Gene in Breast Cancer Patients

Genotype	Odds Ratio (CI 95%)	P-Value
bcl2 (rs956572)		
AA vs. AG	3.81 (1.22-12.4)	0.009
AA vs. GG	3.33 (1.08-10.8)	0.019
AG vs. GG	0.88 (0.41-1.88)	0.710
Dominant Model		
AA vs. AG+GG	3.56 (1.24-10.7)	0.008
Recessive Model		
GG vs. AA+AG	0.79 (0.39-1.59)	0.480
A/G	1.61 (0.98-2.67)	0.047

the genotype percentage among the patients and controls was the almost same, hence these groups were combined before statistical analysis (Table2). Data showed that CC vs. CT and CC vs. CT+TT genotypes did not exhibited a significant difference between patients and controls (P=0.761, P=0.94 respectively) and the CC genotype was not associated with the disease (OR, 1.03(0.46-2.28); (Table2).

The odds ratios (OR) for the TT homozygote and CT heterozygote were [OR=0.90 (0.35-2.31); P=0.804] and [OR=1.11 (0.43-2.87); P= 0.804] respectively, and that for the CC homozygote [OR= 1.13 (0.47-2.71); P= 0.761] in each case. Frequency of T allele was 0.51 and 0.53 among patients and controls respectively whereas, for C allele it was 0.48 and 0.46 among patients and controls respectively. None of the alleles C or T were significantly associated with breast cancer. However a lower percentage of TT genotype in patients suggests that transition mutation of C allele to T favors non-pathological condition for breast cancer. In other words a reverse mutation from T to C allele favors occurrence of breast cancer.

bcl2 Polymorphism

As shown in Table3, the genotypic distributions were determined as 25% for the AA, 35% for the heterozygous AG status, and 30% for the GG, respectively.

The homozygous AA genotype was seen in lower percentage of patients with breast cancer (8.60%) when compared to healthy women (25%). Results showed that the genotype percentage for AG and GG genotypes among the controls were almost same, but there was a border line difference among patients. These groups were combined before statistical analysis (Table4). Data showed that AA vs. AG (P= 0.009), AA vs. GG (P=0.019) and AA vs. AG+GG (P=0.008) genotypes exhibited a significant difference between patients and controls, and the AA genotype was associated with the disease (OR, 3.56(1.24-10.68); Table). However, GG vs AA+AG (p=0.48) genotype did not exhibit significant difference between patients and controls, therefore was not associated with disease (OR, 0.79 (0.39-1.59)

The odds ratio (OR) for the AA homozygote was [OR=3.1 (1.22-12.43); P=0.009] and that for the AG heterozygote was 1[OR=0.88 (0.41-1.88); P=0.710], and that for the GG homozygote [OR=3.33(1.08-10.776); P=0.019] in each case. Frequency of G allele was 0.68 and 0.57 among controls and patients respectively and was borderline association with breast cancer (OR, 1.61(0.98-2.67); P =0.047).

Discussion

Recently there has been great interest in the association of cancer with SNPs. SNPs are the most common sources of human genetic variation, and they may contribute to an individual's susceptibility to cancer (Wu et al., 2009). One of the candidates, p21 gene, plays a direct role in mediating p53-induced G1 arrest. Alteration in this gene may adversely affect the regulation of proliferation and elevate the susceptibility for cancer (Roh et al., 2001). The present study analyzes for the first time the polymorphism in Saudi women with breast cancer. To the best of our knowledge, this is the first study in the Saudi population, which examined the association of polymorphism of these genes with breast cancer. Our results indicated p21 (rs733590) C/T single nucleotide polymorphism was not found to be associated with breast cancer pathogenesis that p21. In agreement with our result Staalesen et al (2006) found that the p21G251A polymorphism was shown more frequently among breast cancer patients compared with controls, this difference did not reach statistical significance (Staalesen et al., 2006). However, we found that a reverse mutation T/C may be associated with breast cancer occurrence. Suggesting Genotypes were marginally associated overall with breast cancer risk and the alleles of this gene were significantly associated with risk of breast cancer. The combined effect of these polymorphisms, using the most common genotype as the reference, did not show any relationship with risk, and a test for interaction between these genes were insignificant. This suggests that there is inverse association of p21 gene polymorphism and confers a risk for the development of breast cancer.

Although, the p21G251A polymorphism was observed more frequently among breast cancer patients compared with controls, this difference did not reach statistical significance. The polymorphism of p21 is intimately related to cancer risk through G1-restriction (Keshava et al., 1997). The possibility that the polymorphism of p21 may contribute to tumor development has been raised. In this regard, positive correlations have been identified between the codon 31 polymorphism of the p21 and breast cancer (Facher et al., 1997)

There is a significant ethnic heterogeneity in the codon 31 polymorphism of the p21 gene. Allele frequency in Caucasian was 9-19% (Chedid et al., 1994; Facher et

al., 1997; Koopman et al., 1995; Hachiya et al., 1999) and 29-55% in populations of African and Asian origin (Hockenbery et al., 1990; Roh et al., 2001). Allele frequency in the Korean and in Japanese patients was 0.59/0.41 for the codon 31 Ser/Arg polymorphism (Hockenbery et al., 1990; Roh et al., 2001).

Both cancer initiation and neoplastic events are affected by genetic background. Expression of the antiapoptotic and antiproliferative protein Bcl-2 has been repeatedly observed to be associated with better clinical outcome in breast cancer. Bcl2 was one of the first genes discovered that regulate apoptosis (Metzger et al., 2004;). The present study investigated the frequency distribution of Bcl2 polymorphism in breast cancer. Here, patients had a higher frequency of bcl2 genotypes as compared to controls. Our results indicated that Bcl2 Genotypes were marginally associated overall with breast cancer risk. In addition, the alleles of this gene were significantly associated with risk of breast cancer. Bcl2 genotype is believed to increase cell survival without conferring any proliferative advantage to cells, thereby allowing cells to acquire additional mutations and facilitate cancer progression. Therefore, individuals with the G allele in the Bcl2 gene may be at slightly higher risk of developing breast cancer.

In conclusion, the data indicates bcl2 genes polymorphism were likely associated with breast cancer data and this polymorphism might be a candidate for the genetic marker to screen the risks of breast cancer. p21 (rs733590) C/T SNP was not found to be associated with breast cancer pathogenesis. However, we found that a reverse mutation T/C may be associated with breast cancer occurrence. These two polymorphisms appeared to contribute jointly to genetic susceptibility to breast cancer. The number of subjects enrolled for the present investigations may limit the power of study. Because the numbers of patients in some strata of stratification analysis were relatively small, these findings would be best considered as preliminary. Therefore, additional studies involving larger sample size should be carried out to elucidate the risk of p21 and Bcl2 genotypes and to validate of our findings. Future studies should also focus on gene--environment and gene--gene interactions among these genes and other cell cycle genes. After the clarification of their role in breast cancer, these polymorphisms may become a useful marker to predict the future development of breast cancer and to permit early therapeutic intervention in patients at high risk for the disease.

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