

Association of Alpha B-Crystalline (CRYAB) Genotypes with Breast Cancer

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Abstract: Breast cancer is the most frequent cancer affecting women all over the world and its susceptibility is conferred by multigenic variations of the genome. Mammalian alpha B-crystallin (CRYAB) is a member of the small heat-shock protein family and a molecular chaperone continuously expressed in various tissues. CRYAB gene, encodes a major structure protein of the lens that can function as a molecular chaperon, has been identified as a tumor suppressor gene in several types of cancer, including breast cancer. However, the association of their genotypes and cancer risk is seldom studied. In this study, 40 samples from breast cancer patients and 20 samples of healthy people were genotyped via polymerase chain reaction and sequencing. Then, the association of C802G (rs14133) and intron2 (rs2070894) polymorphisms with breast cancer risk was investigated. The results found that, those individuals with CRYAB C802G CG and GG genotypes have a significant increase risk for breast cancer than those with the CC genotype. As for intron2 polymorphisms, there was no significant association of the genotype with breast cancer risk. In allelic frequency analysis, the G allele CRYAB C-802G conferred a significantly ($p=21 \times 10^{-7}$) increased risk of breast cancer. The results provided that the G allele of CRYAB C802G is correlated with breast cancer risk and Single Nucleotide Polymorphism (SNPs) of genes associated with breast cancer can be used as a potential tool for improving cancer diagnosis and treatment planning.

Key words: Alpha b-crystallin • Breast cancer • Polymorphism

INTRODUCTION

Breast cancer is a complex disease that is caused by abnormal growth and uncontrolled division of cells within the terminal duct and lobular of the breast [1]. It is included about 10 percent of all cancers and 23 percent of women cancers in developed countries [2]. Over 15% of healthy women have at least one first-degree relative with breast cancer and experimental data showed that the risk of breast cancer in women has been doubled in recent years [3]. It is believed that breast cancer is largely multicausal and its susceptibility is conferred by multigenic variations of the genome, each contributing to the overall breast cancer risk. Mammalian alpha B-crystallin (CRYAB) is a member of the small heat-shock protein (sHSP) family and a molecular chaperone continuously expressed in various tissues [4, 5]. Among the various sHSPs, Hsp22, Hsp27 and CRYAB (HspB5) are true heat-shock proteins whose synthesis is increased

in response to stress. Up to date, the most studied sHSPs are Hsp27 and CRYAB. CRYAB gene, encodes a major structure protein of the lens that can function as a molecular chaperon [6], has been identified as a tumor suppressor gene in several types of cancer, including breast and ovarian cancer [6-8]. However, the association of their genotypes and cancer risk is seldom studied [8]. Grown body of researcher reported that CRYAB expression is strongly associated with lymph node metastasis in breast cancer [9]. This result was further supported by the high abundance of CRYAB in basal like breast carcinoma, which represents a subgroup of breast cancers with bad prognosis and high Metastatic potential [10]. Furthermore, this research group demonstrated activation of the MEK/ERK pathway through CRYAB over-expression in immortalized human mammary epithelial cells, which may lead to increased cell migration and invasion. Together with six other genes, CRYAB was down regulated in breast tumors and metastases [11].

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Table 1: Primers used for CRYABC-802G (rs14133) and CRYAB intron2 (rs2070894)

CRYABC-802G (rs14133) primer		CRYAB intron2 (rs2070894) primer	
Forward primer	Reverse primer	Forward primer	Reverse primer
5'-TTGACCATCACTGCTCTCTT-3'	5'-TTGGCAATGTGACACATACC-3'	5'-GTCTAGAAGACTAAGTTAGG-3'	5'-AGAGAAGTCACAACCTCAAGT-3'

In 2009, a comparison between tumor interstitial fluid and normal interstitial fluid demonstrated that expression of CRYAB was clearly lower in the tumor interstitial fluid of breast cancer patients [12]. Yet, the genomic status of CRYAB and the linkage between its genotype and clinical outcome are largely unknown. In order to understand the genomic role of CRYAB in breast cancer, we have chosen two polymorphic loci of CRYAB, one promoter loci C802G (rs14133) and one in the intron region, intron2 (rs2070894) and we investigated their genotypic distribution in a Jordanian breast cancer population.

MATERIALS AND METHODS

Study Population and Sample Collection: The present study was carried on the Alpha b-crystalline (CRYAB) genotypes. The standard guidelines of the King Abdullah Hospital and Jordan University (Faculty of Medicine, Jordan University) were implemented in paraffin embedded tissues.

Samples Collection: The present work consists of 40 breast cancer and 20 cancer-free samples which collected from King Abdullah University. All paraffin embedded block samples of patients and control were collected between 2008 and 2014 and were defined by expert Dr. Mu'ath M.A. Al-Rajoub, Department of Pathology and Medicine Laboratory, King Abdullah University Hospital, Jordan.

Methods

DNA Extraction: Genomic DNA was extracted according to the method of Yun [13] by using ZR FFPE DNA MiniPrep™ according to manufacturer's procedures.

Genotyping Assays: Genotyping and further processes were done according to the previous studies [14, 15]. Briefly, the following primers (Table 1) were used for CRYAB C802G (rs14133) polymorphisms with the following reagents: DNTP MIX 1000µL PROMEGA USA GoTaq®, DNA Polymerase 100µL PROMEGA USA, Nuclease-Free-Water 100mL PROMEGA USA and Magnesium Chloride Solution 25mM, 25ml PROMEGA USA just use for intron2. The following cycling

conditions were performed using conventional polymerase chain reaction: 1 cycle at 94°C for 5 min, 35 cycles of 94°C for 1min, 55°C for 1min and 72°C for 1 min and a final extension at 72°C for 10 min. To check the length of the obtained sequences, PCR products were separated on 2% agarose gel stained with ethidium bromide and GeneRuler™ 100bp DNA ladder ready-to-use fermentas life sciences USA ladder was used and check by the ChemiDoc MP system, which is a full-feature instrument for gel.

Sequence Reading and Data Analysis: The obtained sequenced fragments of the 60 samples were read by the use of ABI software and then statistical analyses were done. In our study, only those matches with all single nucleotide polymorphism (SNP) data (Case/control=40/20) were selected for final analysis. Pearson's two-sided chi-square test or Fisher's exact test (when the expected number in any cell was less than five) was used to compare the distribution of the CRYAB genotypes between cases and controls. Data was recognized as significant when the statistical p-value was less than 0.05.

RESULTS

DNA Amplification by PCR: As shown in Fig. 1, the separations of PCR product by agrose gel electrophoresis revealed that the first fragment (C802G) at 363 bp and the second one (intron2) at 413 bp.

The Results of DNA Sequencing: Table 2 shows the distributions of the genotypic frequencies for CRYAB (C802G SNP), between controls and breast cancer patients. Statistical analysis revealed that there were significant different between breast cancer patients and control groups in (GG) genotypic polymorphism at level of (P=0.00543). Although, there is no statistical different (P>0.05) between breast cancer patients and control group in the distributions of the genotypic frequencies for CRYAB (intron2 SNPs) (Table 3), but the distributions of the CRYAB alleles were scored significant differences (P=0.00001) between breast cancers and control one (Table 4).

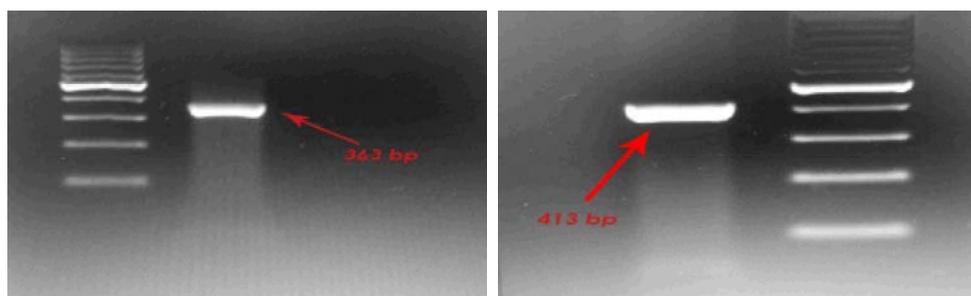


Fig. 1: PCR products of C802G (left) at 363 bp and inton 2 (right) at 413 bp which separated on 2% agarose gel and stained with ethidium bromide using 100 bp DNA Ladder.

Table 2: Distribution of CRYAB genotypes among breast cancer patients and control (C802G SNP)

Genotype	Patients		Controls		OR (95% CI)	P-value
	Frequency	Percentage	Frequency	Percentage		
CC	20	50%	13	65%	1 (reference)	--
CG	15	38%	4	20%	1.02(0.288-0.538)	--
GG	5	12%	3	15%	1.08(0.322-0.577)	0.00543

Which, OR stands for: Odds ratio, CI: confidence interval. P-value based on two-sided Chi-square test with Yate's correction or Fisher's exact test.

Table 3: Distribution of CRYAB genotypes among breast cancer patients and control (intron2 SNPS)

Genotype	Patients		Controls		OR (95% CI)	P-value
	Frequency	Percentage	Frequency	Percentage		
CC	16	40%	10	50%	1 (reference)	--
CT	6	15%	6	30%	1.1(0.118-0.818)	0.139
TT	18	45%	4	20%	1.1(0.121-0.801)	--

Which, OR stands for: Odds ratio, CI: confidence interval. P-value based on two-sided Chi-square test with Yate's correction or Fisher's exact test.

Table 4: Distribution of CRYAB alleles among breast cancer patients and controls (C802G)

C802G	Patients		Controls		OR (95% CL)	P-value
	Frequency	Percentage	Frequency	Percentage		
ALLELE C	55	69%	30	75%	1 (reference)	--
ALLELE G	25	31%	10	25%	1.67(1.54-2.1)	0.000

Which, OR stands for: Odds ratio, CI: confidence interval. P-value based on two-sided Chi-square test with Yate's correction or Fisher's exact test.

DISCUSSION

Overall, breast carcinogenesis is very complicated and varies with individual cases and should be investigated from multiple angles. The present study evaluated the association between two polymorphic genotypes of alpha B-CRYAB with the risk of developing breast cancer the polymorphic genotypes. Throughout the analysis, the study results provide evidence that G allele of CRYAB C802G is correlated with breast cancer risk. SNPs of genes associated with breast cancer can be used as a potential tool for improving cancer diagnosis and treatment planning. From the proteomic point view, CRYAB was found down regulated in breast tumors [11] and about 17.74 fold in the tumor interstitial fluid of breast

cancer patients. Indeed, the genomic contribution of CRYAB breast cancer needs more active studies. Over 60 SNPs have been identified which can slightly increase a woman's risk of breast cancer and the list continues to grow [16].

Actually, a large number of environmental and genetic factors are playing an important role in breast cancer development and considered as prognosis for its appearance. Currently, several breast cancer susceptibility genes have been identified with BRCA1 and BRCA2 are major genes related to 15% of hereditary breast cancer cases [17,18] and that strongly indicates we need further studies to identify other genes that have effective influence on breast cancer risk or giving prognosis for its appearance and playing active roles in risk prediction. In

this study, we selected SNPs of the *CRYAB* gene, *CRYAB* C802 G (rs14133) and *CRYAB* intron2 (rs2070894) and investigated their association with the susceptibility to the breast cancer in 60 individual of the sample, 40 patients and 20 as controls. Among the two selected polymorphisms, one was in the promoter region and the other was in the intron2 region. This is in agreement with the findings of Chen-hsien [19] in Taiwan, 2011, whereas, variant genotypes of *CRYAB* C802G were investigated through population who were significantly with breast cancer, the statistical results indicated that variant genotypes of *CRYAB* C802G were significantly associated with higher susceptibility for breast cancer. The C802G SNP is located in the *CRYAB* promoter region, where as two conserved heat-shock elements and several cis-acting regulatory elements have been identified [20, 21]. However, the detailed correlation between the different genotypes of *CRYAB* C802G and gene expression needs further verification, such as by promoter activity assay. In addition to play a role as a heat-shock protein, *CRYAB* is also considered to be an anti-apoptosis protein, interacting with multiple target proteins. The result of DNA sequencing showed that 50% of sample patients CC wild genotype have no changes in DNA sequence of sample patient with the reference genome and were looked at chemo photograph one peak. Although, it was found that CG heterozygotes genotype forms 38% of sample patients which have two peaks above each other one of them blue refer to allele C and another peak refer to allele G. Meanwhile, the GG homozygotes genotypes which were 12% of patient individuals and 15% of control sample, the DNA of patient samples showed different sequence allele compared with referenced sequence C to be G. The Alleles of *CRYAB* C802G were significantly associated with a higher susceptibility to breast cancer. This could be used as a biomarker to help in prevention and treatment of breast cancer. The clinical and proteomic evidences mentioned above indicate to the significance of *CRYAB* in breast carcinogenesis and raised our interest in investigating the genomic contribution of *CRYAB* to breast cancer.

The other version of *CRYAB* intron2 (rs2070894) showed that this genotype and the distributions of C and T alleles have no significance between patient and control samples. The frequency of CC in 40% of the patient has no significant different with its reference sequence, while heterozygous genotyping CT (15%) of patient and 30% of control samples were C mention to T in sequence as compared with sequence references. However, the homozygotes in intron2 SNP that consider when nucleoid

T be in samples DNA sequencing different with reference sequencing and one red peak which refers to nucleoid T. Our results showed that *CRYAB* C802G was associated with breast cancer susceptibility and since this SNP is located on the promoter of the *CRYAB* gene, its change may cause differential expression of the protein product. Phenotype assays, such as immunohistochemistry and western blotting are needed in fresh breast cancer tissues to provide more detail and realistic correlations with clinical outcomes. In the future, knowledge of *CRYAB* status, available from routine immunohistochemical examination of a tumor biopsy, may therefore be an invaluable marker for those at risk of breast cancer and for clinical outcomes.

CONCLUSION

It can be concluded that, this was the first study, which focuses on the SNPs of *CRYAB* and breast cancer in the Arab nation and the presence of the G allele of C802G was associated with a higher risk of breast cancer. The G allele of C802G could be used as a useful marker in breast oncology for cancer detection. From the researcher point view, further studies were recommended to identify biomarkers to date, particularly those attempting to correlate or predict patient outcome in order, to help patients from early discover into more speedily recovery.

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