Association of Alpha B-Crystallin (CRYAB) Genotypes with Breast Cancer Susceptibility in Taiwan

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Abstract. *Aim: Alpha B-Crystallin (CRYAB) is purported to be a metastasis suppressor protein, and lack or lower CRYAB expression is a prognostic biomarker for several types of cancer, such as that of the prostate and head and neck. However, the association of genomic variation of CRYAB and breast cancer is not well studied. The aim of this study was to evaluate the association of polymorphic genotypes of CRYAB with breast cancer within a Taiwanese population. Patients and Methods: In this hospital-based study, 1232 patients with breast cancer and an equal number of healthy controls in central Taiwan were genotyped via polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) and the association of CRYAB A-1215G (rs2228387), C-802G (rs14133) and intron 2 (rs2070894) polymorphisms with breast cancer risk in a central Taiwanese population was investigated. Results: Those individuals with CRYAB C-802G CG and GG genotypes had 1.50- and 2.22-fold risk for breast cancer than those with the CC genotype. As for the A-1215G and intron 2 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 ($p=5.63\times10^{-10}$) increased risk of breast cancer. Our results provide evidence that the G allele of CRYAB C-802G is correlated with breast cancer risk and this polymorphism may be a useful marker for early detection of breast cancer in clinical practice.*

Breast cancer is the most frequent cancer affecting women all over the world. Accumulation of specific, and often largely unknown, genomic alterations is responsible for abnormal cell proliferation, genomic instability and acquisition of increasingly invasive and drug-resistant phenotypes. 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 (1, 2). Among the various sHSPs, Hsp22, Hsp27 and CRYAB (HspB5) are true heat-shock proteins whose synthesis is increased in response to stress. To date, the most studied sHSPs are Hsp27 and CRYAB. *CRYAB* gene, encoding a major structure protein of the lens that can function as a molecular chaperon (3), has been identified as a tumor suppressor gene in several types of cancer, including breast and ovarian cancer (3-5). However, the association of their genotypes and cancer risk is seldom studied. In 2006, it was reported that CRYAB, together with six other genes, was down-regulated in breast tumors and metastases (6). 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 by 17.74-fold (7). As 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 three polymorphic loci of *CRYAB*, two promoter loci, A-1215G (rs2228387) and C-802G (rs14133), and one in the intron region, intron 2 (rs2070894), and investigated their genotypic distribution in a relatively large Taiwanese breast cancer population.

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Key Words: Alpha B-crystallin, polymorphism, breast cancer, carcinogenesis.

Table I. *Characteristics among breast cancer patients and controls.*

Characteristic	Controls (n=1232)			Patients (n=1232)			*P*-value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age at onset (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at first birth of child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Site							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history							
First degree (mother, sister and daughter)				55	4.5%		
Second degree				6	0.5%		
No history				1171	95%		
Habit							
Cigarette smokers	86	7.0%		170	13.8%		<0.0001[a]
Alcohol drinkers	91	7.4%		162	13.1%		<0.0001[a]

Statistic results based on [a]Chi-square or [b]unpaired Student's *t*-test.

Patients and Methods

Study population and sample collection. The study population consisted of 1232 breast cancer patients and 1232 age-matched cancer-free control volunteers. The patients, diagnosed with breast cancer, were recruited at the Outpatient Clinics of General Surgery between 2004 and 2008 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. The clinical characteristics of the patients, including their histological details, were all graded and defined by expert surgeons (Dr. Wang, Liu and Su). All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. The same number of age-matched non-breast cancer healthy volunteers as controls were selected after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included habits.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (8-14). Briefly, the following primers were used: *CRYAB* A-1215G (rs2228387): 5'-ACCTG-TTGGAGTCTGATCTT-3' and 5'-ATGCACCTCAATCAC ATCTC-3'; *CRYAB*C-802G (rs14133): 5'-TTGACCATCACTGCTC TCTT-3' and 5'-TTGGCAATGTGACA CATACC-3'; *CRYAB* intron 2 (rs2070894): 5'-GTCTA GAAGACTAAGTTAGG-3' and 5'-AGAGAA GTCACAAC-TCA AGT-3'. The following cycling conditions were performed: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s, and a final extension at 72˚C for 10 min. The PCR products were studied after digestion with *Fau*I, *Fat*I, and *DPN*I, restriction enzymes for A-1215G (cut from 212 bp A type into 67+145 bp G type), C-802G (cut from 363 bp G type into 85+278 bp C type), and intron 2 (cut from 363 bp T type into 74+339 bp C type), respectively.

Statistical analyses. In our study, only those matches with all single nucleotide polymorphism (SNP) data (case/control=1232/1232) were selected for final analysis. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *CRYAB* SNPs in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. 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

The clinical characteristics and analysis of the 1232 recruited breast cancer patients and 1232 age- and gender-matched controls are shown in Table I. There were no significant differences between groups in their age, or sex; regarding habits, there were significantly more smokers and drinkers in the patient group (Table I). The distributions of the genotypic frequencies for *CRYAB* A-1215G, C-802G, and intron 2 polymorphisms between controls and breast cancer patients are shown in Table II. The genotypic distribution of the different genetic polymorphisms of *CRYAB* C-802G is significantly different between breast cancer and control groups ($p=3.4\times10^{-8}$), while those for A-1215G or intron 2

Table II. *Distribution of CRYAB genotypes among oral cancer patients and controls.*

Genotype	Controls	%	Patients	%	OR (95% CI)	*P*-value[a]
A-1215G (rs2228387)						
GG	1220	90.0%	1219	98.9%	1.00 (Reference)	
AG	12	1.0%	13	1.1%	1.08 (0.49-2.39)	
AA	0	0.0%	0	0.0%		0.8407
C-802G (rs14133)						
CC	877	71.2%	748	60.7%	1.00 (Reference)	
CG	305	24.8%	389	31.6%	1.50 (1.25-1.79)	
GG	50	4.0%	95	7.7%	2.22 (1.56-3.18)	3.4×10^{-8}
Intron 2 (rs2070894)						
CC	848	68.8%	812	65.9%	1.00 (Reference)	
CT	333	27.0%	365	29.6%	1.14 (0.96-1.37)	
TT	51	4.1%	55	4.5%	1.13 (0.76-1.67)	0.3014

OR: Odds ratio; CI: confidence interval. [a]*P*-value based on two-sided Chi-square test with Yate's correction or Fisher's exact test.

Table III. *Distribution of CRYAB alleles among breast cancer patients and controls.*

Allele	Controls	%	Patients	%	OR (95% CI)	*P*-value
A-1215G (rs2228387)						
Allele G	2452	99.5%	2451	99.5%	1.00 (Reference)	
Allele A	12	0.5%	13	0.5%	1.08 (0.49-2.38)	0.8411
C-802G (rs14133)						
Allele C	2059	83.6%	1885	76.5%	1.00 (Reference)	
Allele G	405	16.4%	579	23.5%	1.55 (1.35-1.79)	5.63×10^{-10}
Intron 2 (rs2070894)						
Allele C	2029	82.4%	1989	80.7%	1.00 (Reference)	
Allele T	435	17.6%	475	19.3%	1.11 (0.96-1.29)	0.142

OR: Odds ratio; CI: confidence interval. [a]P-value based on two-sided Chi-square test with Yate's correction.

were not significant (p=0.8407 and 0.3014, respectively). In detail, these with C-802G CG and GG genotypes were at 1.50- and 2.22-fold greater risk for breast cancer than those with the CC genotype (Table II).

In Table III, the distributions of the allelic frequencies for the *CRYAB* A-1215G, C-802G and intron 2 polymorphisms between controls and oral cancer patients are presented. Consistent with the findings in Table II, the G allele of the *CRYAB* C-802G is associated with 1.55-fold higher susceptibility for breast cancer compared with the C allele (Table III).

Discussion

Overall, breast carcinogenesis is very complicated and varies with individual cases, and should be investigated from multiple angles. From the proteomic viewpoint, *CRYAB* was found to be down-regulated in breast tumors (6), and in the tumor interstitial fluid of breast cancer patients by 17.74-fold (7). As yet, the genomic contribution of *CRYAB* to breast cancer has not been well studied. In this study, we selected three SNPs of the *CRYAB* gene, and investigated the associations with the susceptibility to breast cancer in a population of central Taiwan. Among the three polymorphisms selected, two were in the promoter region, and one was in the intron 2 region. The result indicated that variant genotypes of *CRYAB* C-802G were significantly associated with a higher susceptibility to breast cancer (Tables II and III). The C-802G SNP is located in the *CRYAB* promoter region, where two conserved heat-shock elements and several cis-acting regulatory elements have been identified (15-17). However, the detailed correlation between the different genotypes of *CRYAB* C-802G and gene expression needs further verification, such as by promoter activity assay. In addition to playing a role as a heat-shock protein, CRYAB is also considered to be an anti-apoptosis protein, interacting with multiple target proteins. In 2001, crystallins, including CRYAB, were shown to prevent apoptosis induced by various agents, including hydrogen peroxide, tumor necrosis factor, and staurosporine (18). In addition, CRYAB has been shown to inhibit the autoproteolytic cleavage of caspase3, therefore suppressing the activation of caspase3 (19). Furthermore, CRYAB can also interact directly with the pro-apoptotic members BAX and BCL-XS *in vitro* and *in vivo*, with

sequestration of these proteins preventing translocation to mitochondria and hence reduction of overall apoptosis progress (20). The clinical and proteomic evidence mentioned above indicates the significance of *CRYAB* in breast carcinogenesis, and arose our interest in investigating the genomic contribution of *CRYAB* to breast cancer.

In this study, our results showed that *CRYAB* C-802G 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.

In conclusion, this is the first study, which focuses on the SNPs of *CRYAB* and breast cancer in Taiwan, and the presence of the G allele of C-802G was associated with a higher risk of breast cancer. The G allele of C-802G may be a useful marker in breast oncology for cancer detection.

Acknowledgements

We thank Fang-Jing Li, Yeong-Ruey Chu, Tzu-Shin Weng and Tissuebank in China Medical University for their technical assistance. This study was supported by research grants from the Terry Fox Cancer Research Foundation, China Medical University Hospital (CMU-100-179) and the National Science Council (NSC 98-2320-B-039-010-MY3).

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Received July 6, 2011
Revised August 22, 2011
Accepted August 23, 2011