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Polymorphic Analysis of MHC-Linked Heat Shock Protein 70 (HSP70-2 AND HSP70-HOM) Genes: Their Susceptibility and Prognostic Implications in Breast Carcinoma Cases of Kashmiri Population

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Abstract

Background: Heat Shock Proteins (HSP's) are expressed at high levels in wide range of tumors. They are determining factors for tumor cell survival as they promote autonomous cell proliferation, inhibit cell death pathways, delay senescence as well as influence the immune response to tumor cells.

Purpose of the study: We designed a large scale case-control study to characterize the frequency of two polymorphisms within the MHC classIII-linked HSP70 genes, Hsp70-2 and Hsp70-hom, in order to find any association of these genotypic variants for predisposition to and clinical outcome of breast carcinoma patients from Kashmir valley in North India. Polymerase Chain Reaction and restriction enzymes were utilized to characterize the frequency of two polymorphisms with in Hsp70-2 and Hsp70-hom genes in 114 breast carcinoma cases and 90 healthy controls from the same population of Kashmir. Association of high frequency allelic/genotypic variants of Hsp70genes with various clinicopathological features of prognostic significance was assessed by Chi-square test using SPSS software.

Results: In the present study, allelic frequency of Hsp70-2 A/G heterozygote (0.88) ($p=0.008$) was found to be significantly high in breast carcinoma cases compared to control (0.744) with a Relative Risk =2.67 fold. Conversely, the allelic frequency of Hsp70-2A/A allele in homozygous condition was significantly low in breast carcinoma cases and worked out to be 0.078 (Vs 0.244 in control) with $p=0.001$, implicating it as a protective allele against breast cancer in subjects with this genotype. Similarly, significantly high frequency of 0.50 (Vs 0.30 in control) of Hsp70-homC/C allele was found in homozygous condition in breast cancer cases suggestive of a positive relative risk associated with this genotype (RR=2.42) ($p=0.003$). The overall genotype frequency data analysis of Hsp70-2 and Hsp70-hom genes was significant ($\chi^2=11.46$, $p=0.003$; and $\chi^2=10.56$, $p=0.005$). The study also reveals considerable association of high frequency alleles of HSP70 genes, especially of Hsp70-2A/G or G/G in breast tumors with clinico-pathological features of poor prognosis.

Conclusion: These results indicate that the relative risk of breast cancer associated with Hsp70-2 and Hsp70-hom gene polymorphisms is confined to Hsp70-2 A/G or G/G and Hsp70homC/C haplotype in our population. The study, therefore, suggests Hsp70-2A/G orG/G and Hsp70homC/C genotypes as potential susceptibility markers and independent prognostic indicators in breast carcinoma patients in Kashmiri population.

Keywords: Breast cancer, Hsp-70-2, Hsp70-hom, Kashmir, India

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1. INTRODUCTION

Heat Shock Proteins (HSP's) are the products of several distinct gene families that primarily have molecular chaperone function i.e protein holding and protein folding function [1], [2]. However, HSP's also have key role in controlling cellular metabolism [3]. They are required for cell survival under stress situations and are, therefore, expressed at high levels after exposure to a variety of environmental and patho-physiological stressful conditions [4-6]. The induction of HSP's, for example, occur in wide range of tumors and the mechanism of increased transcription is by either reversal of repression of HSP gene promoter by mutant TP53 (wild type TP53 act as repressor of HSP70 gene) [7-10]; by increased transcription and stabilization of Heat Shock Factor1 {HSF1} [11-13]; and by positive regulation of HSP genes by protooncogenes like c-myc [14, 15]. The protein products of HSP's promote tumor growth by stabilizing its client proteins, most of which are mutant proteins (like TP53), protooncogenes, telomerase's; facilitating autonomous cell proliferation, inhibiting programmed death (PCD) and senescence, favoring angiogenesis, invasion and metastasis [16].

The 70Kdal HSP70 family includes three intronless genes, Hsp70-1, Hsp 70-2, Hsp 70-hom, that have been mapped within the Class III region of MHC complex on 6p21.3 [17, 18]. Hsp70-1 and Hsp70-2 genes are 12kb apart and lie 92kb telomeric to C2 gene whileas Hsp70-hom is located 4kb telomeric to Hsp70-1 [17, 18]. Hsp70-1 and Hsp70-2 encode identical 641 aminoacid proteins [19] whileas the Hsp70-hom encode a 641 aminoacid protein that shares a 90% sequence identity with other Hsp70 proteins [20]. Generally, HSP70 proteins are involved in protecting cell proteins from damage caused by various stressful stimuli by binding to denatured or improperly folded proteins. However, in tumors, the 70 Kdal HSP members, in particular, are involved in protecting tumor cell from Programmed Cell Death (PCD) and delaying senescence, improving thereby their survival. This is evident from inactivation or knockdown of Hsp70 genes in tumor cell that results in spontaneous activation of PCD [21].

Several reports also indicate an important role of HSP70 proteins in immunological mechanism affecting tumor cell survival and cancer pathogenesis. HSP's serve as target for specific immune recognition by both antibodies and T cells [22]. HSP70 proteins also have been shown to act as antigen presenting cell (APC) for tumor derived peptides leading to an antitumor immune recognition by cytotoxic T lymphocytes [23].

Genetic polymorphism in HSP70 genes may influence its anti-apoptotic and immune modulator function and, therefore, may have consequences on predisposition to and prognosis of the disease. The present work is a

case-control study to investigate a potential association of genetic variation of Hsp70-2 and Hsp70-hom genes with the risk to and prognosis of disease (disease outcome) in a cohort of breast cancer cases from Kashmir, North India. Our data indicate that genetic polymorphism in Hsp70-2 and Hsp70-hom genes may represent susceptibility and prognostic indicators.

2. MATERIAL AND METHODS

Patients and Controls:

The gene and allele frequencies of the Hsp70-2 and Hsp70-hom genes were determined in a group of 90 control subjects and 114 patients with breast carcinoma. The patients (111 females and 3males) were the ones recruited to Medical Oncology department of Sher-I-Kashmir Institute of Medical Sciences (SKIMS) from 2002-2003. The mean age of patients was 40.7 years. The majority of female were menopausal (60.52%), married (86.8%). Clinically majority of the cases were diagnosed as Infiltrating Ductal Carcinoma (IDC) (89.47%), 7.89% as Inflammatory Breast Carcinoma (IBC) and 2.63% as pagets disease with underlying ductal carcinoma. The axillary lymph node was involved in 76.3% and 10.5% of the cases develop secondaries to bone and liver. The majority of the cases belonged to Clinical Tumor Stage II (a,b) and III(a,b) (55.26% and 39.47%).The clinical diagnosis were confirmed by histopathological examination, majority of which were of grade I and II (89.46%). Clinico-pathological data of 114 breast cancer patients were collected from the department of Medical Oncology and Pathology of SKIMS, Kashmir, India. Control subjects (all females) were unrelated healthy blood donors having no evidence of any personal or family history of cancer (or any other serious illness) selected from the same population of Kashmir. The acceptance for the present work was taken from our Institutional (SKIMS) ethical committee as well as informed consent was taken from cases for the study.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes by standard procedure. 5 ml of heparinised blood was mixed with 15 ml of DNA lysis solution (155mM NH₄Cl , 10ml KHCO₃,0.1mM EDTA; PH 8.0).Leucocytes were spun down ; suspended in 10 ml of saline EDTA solution (75mM NaCl ,20mM EDTA PH 8.0),1 ml of 10% SDS, and incubated with proteinase K at 37°C in a water bath overnight. DNA was subsequently separated from proteins by phenol chloroform iso-amyl alcohol procedure. DNA in the supernatant fluid was precipitated with ethanol and the pellet was dissolved in 400 µl of DNA storage buffer and stored at 4°C

Polymorphism analysis of the Hsp70-2 and Hsp70-hom genes

Polymorphism within Hsp70-2 and Hsp70-hom genes has been characterized by Milner and Campbell (1992) who identified a polymorphic Pst1 site at position 1267A>G [=1249A>G, (GI=5123454)] of the Hsp70-2 gene and a polymorphic Nco1 site at position 2437(C>G) [=1630 C>G, (GI=27436929)] of the Hsp70-hom gene. The position 1267 of the Hsp70-2 gene lies in the coding region, but corresponds to a silent mutation. The polymorphic nucleotide 2437 of the Hsp70-hom gene corresponds to a Met>Thr aminoacid substitution. The coding sequence of this Hsp70-2 and Hsp70-hom genes were amplified from g-DNA using sequence specific oligo-nucleotide primers. For Hsp70-2, the 5' primer: 5' ACCCTG GAG CCC GTG GAG AA was used in combination with the 3' primer: 5' CAC CCG CCC GCC CCG TAG G. For Hsp70-hom, the 5' primer: GGA CAA GTC TGA GAA GGT ACA G-3' was used in combination with the 3' primer: 5' GTA ACT TAG ATT CAG GTC TGG 3'. The PCR mixture contained to a final concentration of 500 nano-grams of g-DNA, 200 μ M of dNTP's, 1.5mM MgCl₂, 1X Taq polymerase buffer, 10 picomoles/ul of each primer and 1U of Taq polymerase. Amplification was accomplished by initial incubation at 94 $^{\circ}$ C for 5min followed by 35 cycles of incubation at 94 $^{\circ}$ C for 30 sec.; annealing at 62 $^{\circ}$ C for 35sec and 58 $^{\circ}$ C for 45sec respectively for Hsp70-2 and Hsp-hom; and extension at 72 $^{\circ}$ C for 55sec. followed by final incubation at 72 $^{\circ}$ C for 7 min.. To assess the polymorphism of the Hsp70-2 at position 1267 and that of Hsp-hom at position 2437, the corresponding PCR products were digested with Pst1 (CTGCA \downarrow G; G \uparrow ACGCT) and Nco1 (C \downarrow CATGG; GGTAC \uparrow C) (Restriction enzymes, FERMENTAS, INC, USA) respectively. The presence of Pst1 in Hsp70-2 gene was indicated by the cleavage of the 189bp amplified PCR product to yield fragments of 116bp and 73bp products. The two allelic forms of Hsp70-2 Corresponding to the presence and absence of Pst1 site are referred to as Hsp70-2 A/A and Hsp70-2 G/G allele respectively. Similarly, the presence of Nco1 site in Hsp70-hom gene was indicated by the cleavage of 878bp amplified PCR product to yield fragments of 551bp and 327bp. The two allelic forms of Hsp70-hom corresponding to presence and absence of Nco1 site are referred to as Hsp70-hom C/C and Hsp70-homG/G allele respectively.

Statistical Analysis

All statistical analysis was performed using S-PLUS soft-ware. Chi-square test was used to test for a significant association between breast cancer and Hsp70-2 and Hsp70-hom genotypes (allelic frequencies). Relative risk associated with a particular genotype was estimated by the odds ratio formula [24]. Chi-square test was used to find any significant association of high frequency alleles in breast cancer

cases with various clinicopathological parameters of prognostic significance. The parameters included were Age; <50years Vs \geq 50 years; Clinical tumor Stage [II (a,b)] Vs [III(a,b)-IV] (AJCC,2002)[25]; lymph node status (positive Vs negative) and histopathological Grade [I Vs (II-III)] [26]. The level of significance was set at $p \leq 0.05$.

3. RESULTS

Polymorphism in HSP70.2 and HSP-hom genes as risk factors for Breast carcinoma.

HSP70-2 Polymorphism

The genotype frequencies of Hsp70-2 in patients with breast carcinoma and the control group from the population of Kashmir is shown in Table 1 (Figure 1 and Figure 2).

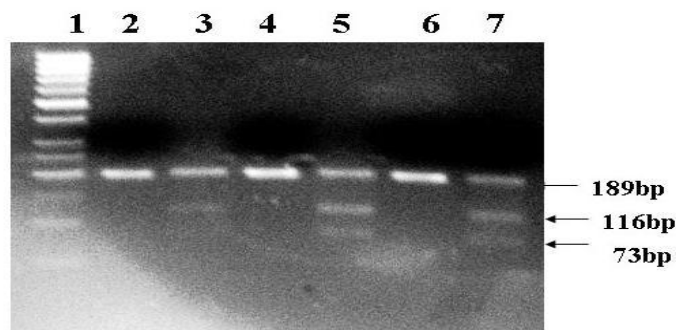


Figure 1

Figure 1: Restriction fragment length polymorphism analysis of Hsp70-2 gene: The Hsp70-2 PCR product (189bp) were digested with Pst 1. The Hsp70-2A/A allele corresponds to 116 and 73bp cleavage products (presence of Pst 1 site) and hsp70-2G/G allele corresponds to 189bp uncleaved product (absence of Pst 1 site). The reaction products electrophorised on 3% agarose gel are shown. Lane# 1: 50bp Molecular Weight Marker; Lane# 2,4,6: undigested HSP 70.2 189bp PCR product; Lane # 3, 5, 7 = Restriction enzyme Pst 1 digested HSP 70.2 PCR product (all showing heterozygous change, hsp 70-2A/G).

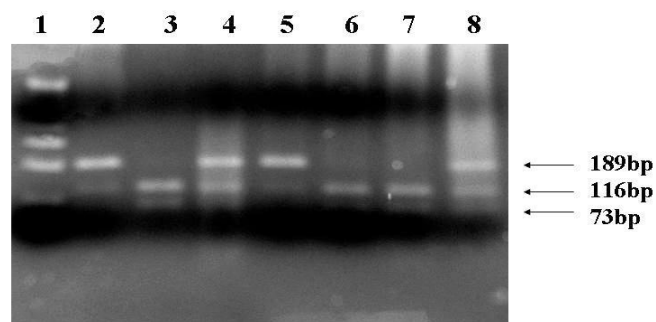


Figure 2

Figure 2: Restriction fragment length polymorphism analysis of Hsp70-2 gene: The Hsp70-2 PCR product (189bp) were digested with Pst 1. The Hsp70-2A/A allele corresponds to 116 and 73bp cleavage products (presence of Pst 1 site) and Hsp70-2G/G allele corresponds to 189bp uncleaved product (absence of Pst 1 site). The reaction products electrophorised on 3% agarose gel are shown: Lane# 1= 100bp Molecular Weight Marker; Lane# 2, 4, 8 = Restriction enzyme Pst 1 digested Hsp70-2 PCR product (all showing heterozygous change, Hsp70-2 A/G); Lane# 3, 6, 7 = Restriction enzyme Pst1 digested Hsp70-2 PCR product (homozygous change, Hsp70-2A/A; Lane# 5 = Restriction enzyme Pst1 undigested Hsp70-2 PCR product, Hsp70-2 G/G).

The frequency of Hsp70-2A/A allele in patients with breast carcinoma is 0.078 (Vs 0.244 in controls) resulting in a significantly negative relative risk (RR=0.29) associated with this genotype ($p=0.001$). An increase in frequency of Hsp70-2A/G heterozygote was observed in breast carcinoma cases (0.88) compared to control group(0.744).The allelic frequency of Hsp70-2A/G heterozygote was significantly more with a relative risk 2.67 fold in breast carcinoma cases ($p=0.008$) compared to control. Conversely the allelic frequency of Hsp70-2G/G allele was 0.035 in breast cancer cases (Vs 0.011in control) with R.R=3.24.The overall frequency of Hsp70-2G allele in homozygous or heterozygous condition was 0.456 in breast cancer cases (Vs 0.41 in control). These results indicate that Relative Risk of breast carcinoma associated with Hsp70-2 polymorphism is confined to Hsp70-2G allele in homozygous or heterozygous state while as Hsp70-2A allele in homozygous condition is rather a protective allele for breast carcinoma in our population.

Hsp70-hom Polymorphism

The allelic frequency of Hsp70-hom genotypes in breast carcinoma patients and control group given in **Table 2** (Figure 3 and Figure 4) reveal high frequency of Hsp70-homCC allele in homozygous condition in breast cancer cases 0.50 (Vs 0.30 in control) with a significantly positive relative risk associated with this genotype (RR=2.42)($p=0.003$).

Conversely, the significantly low frequency of Hsp-homG genotype in homozygous (0.08) or heterozygous condition (0.48) in breast cancer cases compared to control (0.04 and 0.65 respectively) suggest it rather a protective allele for breast cancer with negative relative risk (RR= 0.19, $p=0.102$; RR=0.49, $p=0.013$ respectively).These results indicate the relative risk of breast cancer associated with Hsp70-hom polymorphism is confined to Hsp70homCC genotype.

The overall Chi-square test used for comparative evaluation of Hsp70-2 and Hsp70-hom gene polymorphic analysis reveals that the data is quite significant ($\chi^2=11.46$, $p=0.003$; and $\chi^2=10.56$, $p=0.005$).

The presence of high frequency allelic variants of HSP70's (Hsp70-2A/G or G/G and Hsp70-homCC in our population) when compared with various clinico-pathological attributes of breast cancer patients showed statistically significant association of Hsp70-2A/G or G/G genotype with advanced Clinical Tumor Stage [III(a,b)-IV] ($p=0.005$) and histopathological Grade II-III ($p=0.001$), features that reflect poor prognosis (**Table 3**). However, Hsp70-homCC genotype, though found at high frequency among breast cancer cases, was significantly associated with early Clinical tumor stage II (a,b) ($p=0.005$) and histopathological grade I ($p=0.000$) (**Table 3**), which

suggest it rather a very low risk imposing genotypic variant of Hsp70-hom for breast cancer.

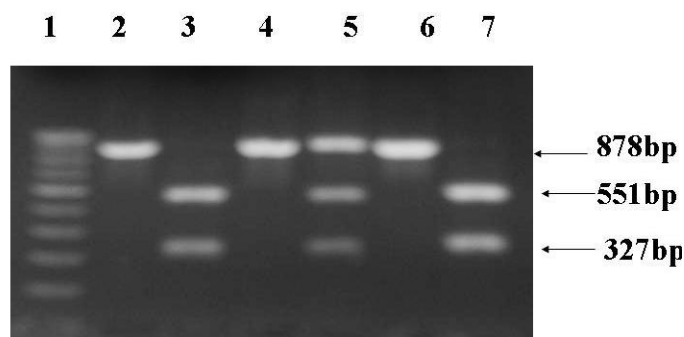


Figure 3

Figure 3: Restriction fragment length polymorphism analysis of hsp70-hom gene: The hsp70-hom PCR products (878bp) were digested with Nco1. The hsp70-homC/C allele corresponds to 551bp and 327bp cleavage products (presence of Nco1 site) and hsp70-hom G/G allele corresponds to 878bp uncleaved product (absence of Nco1 site). The reaction products electrophorised on 1.5% agarose gel are shown. Lane# 1: 100bp Molecular Weight Marker; Lane# 2, 4, 6: undigested HSP 70-hom 878bp PCR product; Lane # 3 & 7: Nco1 digested HSP70-hom PCR product (homozygous hsp 70-homC/C/allele); Lane#5: Nco1 digested HSP70-hom PCR product (heterozygous, hsp 70-hom C/G allele)

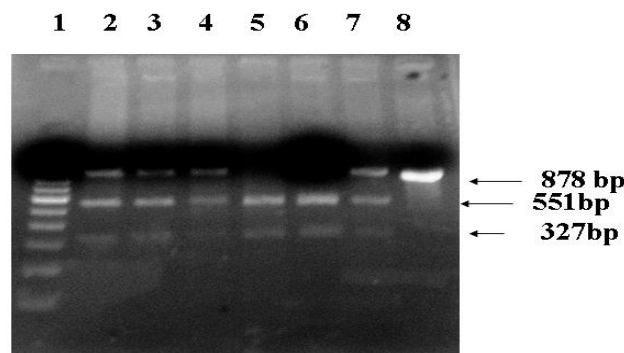


Figure 4

Figure 4: Restriction fragment length polymorphism analysis of hsp70-hom gene: The hsp70-hom PCR products (878bp) were digested with Nco 1. The hsp70-homC/C allele corresponds to 551bp and 327bp cleavage products (presence of Nco1 site) and hsp70-hom G/G allele corresponds to 878bp uncleaved product (absence of Nco 1 site). The reaction products electrophorised on 1.5% agarose gel are shown. Lane# 1: 100bp Molecular Weight Marker; Lane# 2,3,4, 7: Nco1 digested HSP70-hom PCR product(all heterozygous, hsp 70-hom C/G allele) Lane#5, 6 : Nco1 digested HSP70-hom PCR product (both homozygous hsp 70-homC/C/allele); Lane# 8: Nco1 undigested HSP70-hom product (hsp 70-hom G/G allele).

4. DISCUSSION

Several studies have shown statistical evidence of association between specific human leukocyte antigen (HLA) alleles and risk for or protection against various cancers [27]. This have further highlighted the presence of candidate genes for various cancers within or nearby the HLA. Given the chromosomal location of Hsp70 genes within HLA; their essential role in multiple steps involved in cancer pathogenesis and

their determining role in the immune response to tumor cells, they are rightly associated with cancer susceptibility [16, 17]. In the present study we report a strong association between specific Hsp70-2 and Hsp70-hom allelic variants and risk for or protection against breast cancers in Kashmiri population.

Comparison of Hsp70-2 allele and genotype frequencies in patients with breast cancer and control subjects from same population of Kashmir indicated a significant decrease of Hsp70-2A/A genotype in breast cancer cases, suggesting it rather a protective allele in homozygous state ($p=0.001$). Conversely, a high relative risk was found in breast cancer cases that carry Hsp70-2 G allele in heterozygous or homozygous state (**Table 1**).

Our finding is in agreement with the two separate reports from Tunisia, where also statistically significant breast cancer cases carry Hsp70-2 G allele in homozygous state (0.25) (Vs 0.02 in control) (RR=16.3, $P=0.0001$ [28] and 0.280 Vs 0.05 in control (RR=7.12; $p=0.0001$) [29]. The Hsp70-2 G allele in homozygous form has been also reported to impart risk in cancers other than Breast like Non-Hodgkins lymphoma (RR=18.2; $p=0.0001$) [28], nasopharyngeal carcinoma (RR=2.309; $p=0.006$) [27] and in diseases other than cancer [30-31].

Similarly, a striking difference in the frequency of Hsp70-hom genotypes was found in breast cancer patients of Kashmir when compared with controls (**Table 2**). Hsp70-homC/C genotype in breast cancer cases (0.50) (Vs 0.30 in controls) was found to impart significantly high relative risk to breast cancer (RR=2.42, $p=0.003$) and the risk decreases significantly as the allelic frequency of Hsp70-homG allele increases in homozygous or heterozygous state (RR=0.43, $P=0.005$). This is contrary to what has been reported from Tunisia where allelic frequency of Hsp70-homG allele in homozygous or heterozygous is more in breast cancer cases (0.13) compared to controls (0.05) (RR=3.4, $p=0.01$) [32]. More interestingly, Hsp70-2A/A and Hsp70-homG/G genotypes were found in negligible frequencies in breast cancer cases from our Kashmiri population hence are protective alleles to our population.

Comparison between the Hsp70-2 and Hsp70-hom haplotype frequencies in patients and in control subjects indicate almost double frequency of breast cancer cases (48/114) (0.42) harbour together Hsp70-2A/G or GG and Hsp70-homC/C haplotype when compared with controls (18/90) (0.20). These results suggest that Hsp70-2A/G or G/G and Hsp70-homC/C haplotype may represent a specific risk factor for breast cancer to our population of Kashmir.

Genotype	Controls (n=90) (f)	Breast carcinoma (n=114) (f)	Result		
			OR	χ^2	p-value
Hsp70-2	0.244	0.078	0.27	2.67	0.001
A/A	0.744	0.88	2.67	6.93	0.008
Hsp70-2	0.011	0.035	3.24	1.20	0.271
A/G					(NS)
Hsp70-2					
G/G					

$\chi^2=11.468, p=0.003$

Hsp= Heat Shock Protein; f= genotype frequencies; χ^2 =Chi -Square; OR=odds ratio;

NS=Not Significant. ¹The Chi-square test was used whether significant differences (p-value) in genotype frequencies were observed when patient group was compared with control subjects.

Table 1: Genotype frequencies of Hsp70-2 in control subjects and in Patients with breast carcinoma¹.

Genotype	Controls (n=90) (f)	Breast carcinoma* (n=114) (f)	Result		
			OR*	χ^2	p-value
Hsp70-hom	0.30	0.50	2.42	9.02	0.003
C/C	0.50	0.48	0.49	6.11	0.013
Hsp70-hom	0.65	0.08	0.19	2.68	0.102
C/G					(NS)
Hsp70-hom					
G/G					

$\chi^2=10.56, P=0.005$

Hsp= Heat Shock Protein; f= genotype frequencies; χ^2 =Chi -Square; OR=odds ratio; NS=Not Significant. ²The Chi-square test was used whether significant differences (p-value) in genotype frequencies were observed when patient group was compared with control subjects

Table 2: Genotype frequencies of Hsp70-hom in control subjects and in patients with breast carcinoma.²

Although the possible functional implications of HSP70 gene polymorphisms have not been studied, several features suggest that they may be among several factors dictating the function of HSP70. The polymorphic Pst I site in Hsp70-2, although a synonymous variation, has functional significance as per recent reports which show the influence of synonymous gene variation on the expression levels and enzyme activity, possibly, by affecting the secondary structure of mRNA, its stability and the timing of co translational folding that alters the substrate or inhibitor binding sites [32,33]. NcoI Hsp70-hom polymorphism corresponds to Met>Thr substitution at amino acid 493, which from a part of peptide binding domain of Hsp70-hom protein [20]. Based on the Hsp-70 structural model, a Met to Thr substitution at amino acid 493 could be associated with variation in peptide binding specificity of Hsp70-hom between haplotypes. The variation may influence antigen presentation of Hsp70-hom of tumor derived antigens to cytotoxic T lymphocytes.

Features	Hsp70-2A/G or G/G			Hsp70-homC/C				
	Genotype(%)	χ^2	p=value	odds ratio	Genotype(%)	χ^2	p=value	odds ratio
Age (yrs)								
<50	75/81(92.59)	0.091	0.762	1.25	45/81(55.55)	3.45	0.063	0.334
≥50	30/33 (90.90)				12/33(36.36)			
^aClinical Tumor Stage								
II (a,b)	54/63(85.71)	7.910	0.005	0.00	42/63(66.66)	25.86	0.000	4.8
III(a,b)- IV	51/51(100)				15/51(29.4)			
^bHistopathological Grade								
I	42/51(82.35)	12.07	0.001	0.00	36/51(70.58)	15.64	0.000	4.8
II-III	63/63 (100)				21/63(33.33)			
^cLymph node status								
(+ve)	81/87(93.10)	0.503	0.478	0.25	33/87(33.93)	21.40	0.000	0.076
(-ve)	24/27(88.88)				24/27(88.88)			

^aClinical Tumor stage (AJCC, 2002) : II(a,b)= when tumor size ranges from >2 and >5cm and metastasis to ipsilateral axillary nodes but no metastasis,

III(a,b)= tumor of any size and any no of nodes but no metastasis and IV: tumor extends to chest wall, any no of nodes involved and metastasis.

^bHistopathological Tumor Grade (WHO, IHC of Tumors,1988) : Determined based on pathological examination .

^cLymph node status: +ve= involved, -ve= not involved

Table 3: Association of high frequency Hsp70-2A/G or G/G and Hsp70-homC/C genotypes with various Clinico pathological features of breast cancer patients of Kashmir.

Molecular studies have shown that the 70Kdal HSP members mediate tumorigenesis through inhibition of PCD and delaying senescence [16]. Several PCD pathways, like the network mediated by p53 and bcl₂ families, induced by oncogenes like c-myc and Ras, must be inhibited to counter death signals and permit tumor progression [6], [34, 35]. This demands induction of HSP's, which have been reported in several malignant cell types [6]. The high frequency allelic variants of Hsp70s' in breast cancer, like Hsp70-homC/C and Hsp70-2A/G or G/G haplotypes in our population, might express well, possibly by increased stability of variant mRNA or by altering the timing of co-translational folding that produce protein with altered enzymatic activity, so as to facilitate anti-apoptosis and delay senescence, and hence support tumor development. Apart from the presumed influence of gene variations on their expression levels and enzymatic activity, HSP70s, under stress situations (like tumor), are induced to facilitate tumor progression by stabilizing (i) the emergence of mutant proteins (like TP53) (ii) by alternating the inflammatory nature of the tumor environment that influences antitumor cytotoxic T-cell response [36]. Since, wild type TP53 acts a repressor of HSP70 genes; mutation in it is reported to relieve the HSP70 genes from repression and hence increases its transcription [7,10]. In our previous study, we reported TP53 mutations in 44% of the sporadic breast cancer cases

[37], therefore, an increase in expression of HSP genes is very likely, which further facilitates the various steps involved in tumor development. Furthermore, HSP70s released from necrotic cells, at low levels, enhance tumor progression through activation of the nuclear factor, NF-kB, [38] and at high rate of tumor cell necrosis, such as those that occur after exposure to cytotoxic drugs, elicits specific CD4⁺T-cell mediated anti-tumor immune response that mediate tumor regression [39].

The Hsp70-homCC and Hsp70-2A/G or G/G haplotype in breast cancer cases of Kashmiri population might express to the extent to be a good inhibitor of PCD and a poor antitumor immune modulator and therefore posing risk to breast cancer as evident from their high frequency in breast cancer cases compared to controls. The presence of high frequency genotypes of HSP70 genes when compared with various clinical parameters of prognostic significance substantiates the role of Hsp70-2A/G or G/G genotype as risk imposing as evident from its presence in breast cancer cases bearing poor prognostic features. However, according to Manley's (2005) BADGE (Better Association for Disease and Gene) classification, the Hsp70-2 gene-disease association is of 3rd-4th class (based on p-values) that suggests low assurance of reproducibility [40]. Further, the presence of Hsp70-homCC genotype in breast cancer cases belonging to Clinical Tumor Stage II and pathological grade I, though highly

reproducible association (Manley, 2005)[40], is incongruous that suggests it rather a very low risk imposing genotype, comparatively of better prognosis, for breast cancer in our population.

5. CONCLUSION

The high frequency of Hsp70-2A/G or G/G and Hsp70-homCC haplotype in breast cancer cases compared to controls suggest them as susceptibility allelic/genotypic variants for breast cancer to our population. Further, the significant association of high frequency allelic variants of HSP70 genes in breast tumors with various clinicopathological parameters suggests them as prognostic indicators as well. The high frequency, especially, of Hsp70-2A/G or G/G genotype in breast tumors of patients with such clinic pathological features as advanced Clinical tumor stage [III(a,b) – IV] and histopathological grade II-III, is a finding which assumes significance in view of the fact that these features reflect poor prognosis. The study suggests Hsp70-2A/G or G/G and Hsp70-homCC genotypes as attractive susceptibility markers and independent prognostic indicators in breast cancer patients of Kashmiri population. Nevertheless, these observations need further investigations in a bigger cross section of the breast cancer patients and relevant controls.

Abbreviations: Heat Shock Proteins=HSP; Restriction Fragment Length Polymorphism =RFLP; Infiltrating Ductal Carcinoma=IDC; Inflammatory Breast Carcinoma =IBC;

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