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Genetic Alterations in HSPA Family of Genes and their Putative Association with HNSCC

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Head and neck squamous cell carcinoma (HNSCC) is an aggressive life-threatening disease associated with high mortality rates. Several genes related to stress response have been studied extensively to reveal their possible association with HNSCC. Members of the *HSPA* (heat shock protein family A) family are exclusively expressed under stress conditions suggesting that these are specialised to function in the stress response.
Aim: The aim of the present study is to demonstrate the genetic alterations in the *HSPA* gene family and their putative association with HNSCC.
Materials and Methods: The source of the patient's data was procured from the cBioportal database. The TCGA Firehose Legacy comprised 528 head and neck squamous cell carcinoma cases. Oncoprint data analysis can be used to derive a putative association between the disease phenotype and genotype, to identify the variations and to identify any novel variations which can be associated with the disease phenotype. The gnomAD data analysis was used to compare between the observed variants documented in the present study with that of reported variants deposited in the gnomAD repository.

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Results and Discussion: The *HSPA1A&HSPA1B* genes harboured high frequency of amplification and deep deletions. The expression of two genes *HSPA8* and *HSPA13* was found to be up-regulated in the primary tumor sample in comparison to normal tissues. In Kaplan Meier analysis, *HSPA8* gene expression was compared with gender, here, high expression in females shows low survival rate. Similarly, *HSPA13* gene expression was compared with race, here low expression in africanamerican population shows low survival rate.
Conclusion: The present study provides preliminary data on the involvement of the *HSPA* family of genes with HNSCC, which has to be validated using experimental evidence in other populations.

Keywords: Carcinoma; gene expression; heat shock proteins; novel mutations; protein stability.

1. INTRODUCTION

The association between genetic alterations and human cancer was first observed decades ago [1]. Cytogenetic studies revealed that specific chromosomal abnormalities were linked to the development of certain cancers. In addition, substantial genetic disruption leads to chromosome aberrations, rearrangements, and aneuploidy and these are observed in tumour cells. However, it was not clear whether this widespread genetic instability was a cause or a consequence of the cancer phenotype [2]. An understanding of the role of genetic alterations in cancer development arose out of studies of oncogenic viruses and hereditary cancers.

Head and neck squamous cell carcinoma (HNSCC) is an aggressive life-threatening disease associated with high mortality rates. It is the sixth most common form of cancer as reported world-wide [3]. Most HNSCCs arise in the epithelial lining of the oral cavity, oropharynx, larynx and hypopharynx. About 600,000 new cases, including approximately 50,000 in the United States, are diagnosed each year. In males in their 50s or 60s, HNSCC occurs more frequently, but the occurrence in younger individuals is growing [4]. HNSCC follows a similar genetic pattern for progression in its development from premalignant lesions such as leukoplakia, dysplasia, erythroplakia and lichen planus [5]. These cancers are strongly associated with certain environmental and lifestyle risk factors like tobacco and alcohol consumption [6]. Several *in silico* studies have revealed novel variants which may act as putative drivers involved in transformation of normal cells to malignant ones [7]. In this respect, the therapeutic potential of targeting *HSPA* and modulating *HSPA* expression is attracting much interest [8].

All organisms, except some hyperthermophilic archaea, contain the family of *HSPA* chaperones [9]. The heat shock proteins function as molecular chaperones and are involved in the process of translation and transport of proteins across membranes. Members of the HSPA family are expressed under stress conditions indicating that they are specialized to function in the stress response [10]. Extracellular heat-shock proteins are sensed by the immune system as damage-associated molecular patterns (DAMPs) [11]. The surge of *HSPA* in the peripheral circulation and serum is observed during different kinds of exercise, and excessive use of mobile phones. Concordant increase in the levels of serum C-reactive protein was also observed and can be used as potential biomarkers in systemic inflammation. Although the *HSPA* family of genes have been implicated in HNSCC, the underlying molecular mechanism is not clearly understood. In the essential gene families, the present research recognises genetic alterations. In laboratory work, any differences found in the sample can be repeated to arrive at definitive proof of the interaction of the variations/mutations with the phenotype of the disorder. The present *in silico* research was therefore intended to examine the genetic variations in the proposed gene family in order to establish the putative relation between the disease phenotype and genotype. Our team has extensive knowledge and research experience that has translated into high quality publications [12–33]. Hence, the aim of the study is to demonstrate the genetic alterations in the *HSPA* gene family and their putative association with HNSCC.

2. MATERIALS AND METHODS

2.1 Data Source

Retrospective study design has been followed in the present study. The source of the patient's data is procured from the cBioportal database [34]. This database includes an extensive array of patient information from different cohorts. 528

HNSCC cases were found in 'The Cancer Genome Atlas' (TCGA) where sequencing and the copy number alteration data are available for tumour samples. The list of vital genes areobtained from literature search. The genes includes, *HSPA1A, HSPA1B, HSPA1L, HSPA2, HSPA4, HSPA4L, HSPA5, HSPA6, HSPA7, HSPA8, HSPA9, HSPA12A, HSPA12B, HSPA13, HSPA14, HSPH1 and HYOU1*. The user described queries based on these were submitted to the cBioportal database, and the resulting oncoprint data are used for further analysis.

2.2 Oncoprint Data Analysis

Oncoprint data analysis provides information on frequency distribution, variations in each of selected genes, type of variation, changes in protein coding amino acids, gene amplification, deletions, insertions, frameshifts, splice site mutations, etc. These information are used for possible correlation between genotype and phenotype, to identify the differences of less known mechanisms/genes and to know the novel variations that may be associated with the phenotype of the disease.

2.3 gnomAD Data Analysis

The data compares the observed variants documented in this study with the reported variants deposited in gnomAD repository [31].

2.4 Gene Expression and Survival Analysis

The expression of the gene presenting with highest frequency of gene alteration in HNSCC was analysed using the UALCAN (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival) database. Survival curve analysis based on the tumor grade and expression profile was performed to demonstrate the putative role of heat shock protein family of genes with HNSCC. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using log-rank test that generated a p value which was further used to indicate statistical significance of survival correlation between groups [35].

3. RESULTS

The primary database, cBioportal and several other datasets are selected for the present study. The TCGA dataset contained information of 528 HNSCC patients. The demographic details of the patients are given in Table 1. Oncoprint data analysis is performed to analyse the genetic alterations and the variations seen in the gene family. Here, *HSPA8* and *HSPA13* genes were observed with high levels of variation (Fig. 1). The gene amplification was seen in 2 genes, where, both *HSPA1A* and *HSPA1B*harboured equal frequency of gene amplification. Two genes *HSPA1A* and *HSPA1B* demonstrated deep deletions. *HSPA8* and *HSPA13* genes showed the highest number of variations/ mutations among all the genes identified with alterations (Table 2). Various truncating and missense variants with unknown significance are documented in the present study (Fig. 1). Genetic alterations of the genes associated with the *HSPA* family are documented. Several novel variants were identified, along with a few reported variants in *HSPA4 (rs144576995, rs377082440), HSPA4L (rs773358656), HSPA6 (rs1454026580),* and *HSPA12A (rs782770261, rs1161715269, rs781956168).*

The primary tumour in both the genes showed higher expression than the normal tumour. This implies the fact that these rare variants are associated with the risk of particular disease. The gene expression profile of *HSPA8* and *HSPA13* showed significant differences as compared to paired normal tissuesfrom patients with a value of 8.36×10^{-4} and 10^{-12} (Fig. 2) respectively. The Kaplan - Meier plot shows the effect of gene expression level classified based on gender and race on HNSCC patient survival. The patients were stratified based on gender, race, grade of tumor to correlate with the expression profile (high, low or medium). Fig. 3 shows the expression level of *HSPA8* based on gender. A significant difference observed between the four groups is 0.0042 (p-value), wherein females showing higher expression of HSPA8 were accompanied by low survival rate. Further, Fig. 4 showed that the expression level of *HSPA13* in African American 0.026 (p-value) is related to low survival rate.

4. DISCUSSION

It has been postulated that the minimum constellation of mutations required for oncogenic transformation in humans includes inactivation of TP53 and RB, activation of RAS (or other members of that pathway), and constitutive expression of hTERT. More than five million people are affected and 379,000 deaths have

occurred globally due to head and neck cancer [36]. Recently, our team has identified a prognostic marker associated with HNSCC (34). The HPV, i.e., the human papilloma virus has emerged as a major cause of HNSCC among non-smokers and light drinkers [37]. According to the GLOBOCAN survey, 2018, the incidence of head and neck squamous cell carcinoma was found to be clustered in specific regions worldwide with a high incidence rate recorded in the south asian countries [38,39]. A large number of tumor suppressor genes and oncogenes have now been identified and characterized through the analysis of tumor cell DNA [40]. *HSPA* is expressed in very low levels in non-stressed cells and is dramatically induced in hyperthermia [41]. *HSPA* is the most conserved hsp throughout the evolution of prokaryotes and eukaryotes [42]. Several studies have shown that the thermosensitivity of cells is altered if the expression of *HSPA* is enhanced or reduced [43].

Table 1. Table represents the demographic details of patients analysed in the present study (as obtained from the cBioportal site). The paired normal tissue found adjacent to the tumor was used for comparison

Gender	Male (n = 386) Female (n = 142)
Mutation count	6-3181
Diagnosis age	19-90 years
Smoking status	Smokers: 515 Data not available: 12 Unknown: 1
Alcohol history	Yes – 352 No – 165 Data not available: 11
Neoplasm Histologic grade	Grade 1: 63 Grade 2: 311 Grade 3: 125 Grade 4: 7 Grade GX: 18 Data not available: 4
Race category	White: 452 African: 48 Asian: 11 American Indian or Alaska native: 2 Data not available: 15

Table 2. Table represents the gene alterations in heat shock proteins family of genes

Gene	Protein coded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
HSPA1A	Heat shock protein family A (Hsp70) member 1A	6p21.33	1.2	Amplification Deep deletion	- -	-
HSPA1B	Heat shock protein family A (Hsp70) member 1B	6p21.33	1.2	Amplification Deep deletion	- -	-
HSPA1L	Heat shock protein family A (Hsp70) member 1 like	6p21.33	2.4	E320K A414D L126F V84D	0.13 0.09 0.15 0.05	Novel Novel Novel Novel

Gene	Protein coded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
				Q603H	0.21	Novel
				K389R	0.18	Novel
HSPA2	Heat shock protein family A (Hsp70) member 2	14q23.3	2.2	E306Q	0.34	Novel
HSPA4	Heat shock protein family A (Hsp70) member 4	5q31.1	1.8	D831Y	0.18	rs144576995
				Q514*	0.46	Novel
				S784*	0.21	Novel
				S828L	0.32	rs377082440
				S40C	0.17	Novel
HSPA4L	Heat shock protein family A (Hsp70) member 4 like	4q28.1	1.4	D566H	0.34	Novel
				D611Y	0.38	Novel
				E694Q	0.28	Novel
				H806Q	0.16	Novel
				A495V	0.24	Novel
				R261Q	0.26	rs773358656
HSPA5	Heat shock protein family A (Hsp70) member 5	9q33.3	2	D610N	0.11	Novel
				D355N	0.21	Novel
				E622K	0.41	Novel
				K122N	0.14	Novel
HSPA6	Heat shock protein family A (Hsp70) member 6	1q23.3	2.2	R313C	0.14	Novel
				F70L	0.22	rs1454026580
				H242Y	0.28	Novel
				R195H	0.21	Novel
				F94Sfs*4	0.33	Novel
HSPA7	Heat shock protein family A (Hsp70) member 7 (pseudogene)	1q23.3	0	-	-	-
HSPA8	Heat shock protein family A (Hsp70) member 8	11q24.1	3	R311C	0.30	Novel
				E110Q	0.09	Novel
				K597I	0.38	Novel
				A161D	0.15	Novel
				M621_G624del	0.15	Novel
					0.19	Novel
				N306D	0.07	Novel
				R299C	0.19	Novel
				E644Q	0.04	Novel
				E600Q	0.71	Novel
				P640S		
HSPA9	Heat shock protein family A (Hsp70) member 9	5q31.2	0.4	K288N	0.22	Novel
HSPA12A	Heat shock protein family A (Hsp70)	10q25.3	1.2	L480V	0.25	Novel
				D575H	0.29	Novel

Gene	Protein coded	Cytogenetic loci	% of genetic alterations	Gene alterations	Variant allele frequency	gnomAD frequency
	member 12A			R531C	0.33	rs782645684
				E191V	0.20	Novel
HSPA12B	Heat shock protein family A (Hsp70) member 12B	20p13	1	K165N	0.36	Novel
HSPA13	Heat shock protein family A (Hsp70) member 13	21q11.2	3	E401D	0.39	Novel
				E160K	0.14	Novel
				S143F	0.08	Novel
				P321R	0.47	Novel
				A208S	0.35	Novel
				A11V	0.14	Novel
				V12Gfs*21	0.41	Novel
HSPA14	Heat shock protein family A (Hsp70) member 14	10p13	1.8	R245*	0.33	Novel
				S395*	0.12	Novel
HSPH1	Heat shock protein family H (Hsp110) member 1	13q12.3	1.4	E826G	0.29	Novel
				V799F	0.29	Novel
				D294H	0.04	Novel
				T700S	0.56	Novel
				M762I	0.03	Novel
HYOU1	Hypoxia up-regulated 1	11q23.3	2.6	A416T	0.54	rs782770261
				P168L	0.16	rs1161715269
				E184K	0.12	Novel
				S888*	0.04	Novel
				E535K	0.07	rs781956168

The expression of *HSPA*s can be induced by insults other than thermal stress, including ischemia, heavy metals, nutrient deprivation, irradiation, infections, inflammation, and exposure to organics and oxidants [44]. Members of the *HSPA* family are known to control all aspects of cellular protestations such as nascent protein chain folding, protein import into organelles, recovering of proteins from aggregation, and assembly of multi-protein complexes [45]. Extracellular *HSPA*s are stimulators of innate immune responses [46]. Studies have linked the expression of *HSPA*s to several phenotypes of carcinoma, viz., therapeutic resistance, metastasis, and poor clinical outcome. In malignant cells, *HSPA* protects cells from the stress associated with rapid proliferation, suppress cellular senescence, and resistance to stress-induced apoptosis including protection against cytostatic drugs and radiation therapy [47].

HSPA1 is among the best characterized cancer-related chaperones, while the significance of *HSPA2* for cancer remains poorly understood. Previously researchers demonstrated that in primary NSCLC, *HSPA1* was associated with good prognosis while *HSPA2* correlated with bad prognosis, suggesting different roles of these proteins in cancer [48]. The changes in the induction of *HSPA* transcription are closely associated with the aging process. Various authors have studied the heat inductibility of different genes in HSPA family, such as, *HSPA1A/1B* and *HSPA6*, following the stress conditions [49]. Vidyashri et.al., has done a similar research based on genetic alterations of *SPARC* gene family and its association with HNSCC. Longxiang et.al., showed that downregulation of *HSPA2* inhibits proliferation via ERK ½ pathway and endoplasmic reticular stress in lung adenocarcinoma [50]. In present study, the expression of the primary tumour of 2 genes, i.e., *HSPA8* and *HSPA13* has shown upregulation and among them, the highly

significant one is the expression of *HSPA13* gene which shows the p value as 10^{-12}. Shan Lu et.al has studied the regulation of *HSPA* gene and have concluded that androgen receptor and its signaling regulates *HSPA* expression in prostate cancer cells and that *HSPA1B* could be an androgen receptor target gene [51]. Dorota et.al., has discussed the expression, function and regulation of *HSPA2* protein in spermatogenic, somatic and cancer cells [52]. JG-98, a novel class of allosteric inhibitors of *HSPA*s, causes effective disruption of *HSPAs-BAG3* interaction. Hence, *HSPA-BAG3* complexes can be considered as broad-acting regulators of cancer cell signaling and a promising anticancer target [53]. With an ever increasing trend of head and neck cancers in Asian population [54], it has become a crucial point to identify the molecular targets involved in the pathogenesis of the disease. Accumulating evidences have proved the effect of several markers [55] in different types of patients with [56] and without habits. An extensive probing into each of these genomic signatures would aid in limiting the number of markers directly associated with the disease phenotype.

The present study demonstrated the effect of highly expressed genes of the HSPA family i.e., *HSPA8* and *HSPA13* under gender and race. Under gender, high expression in females has shown low survival rate and under race, low

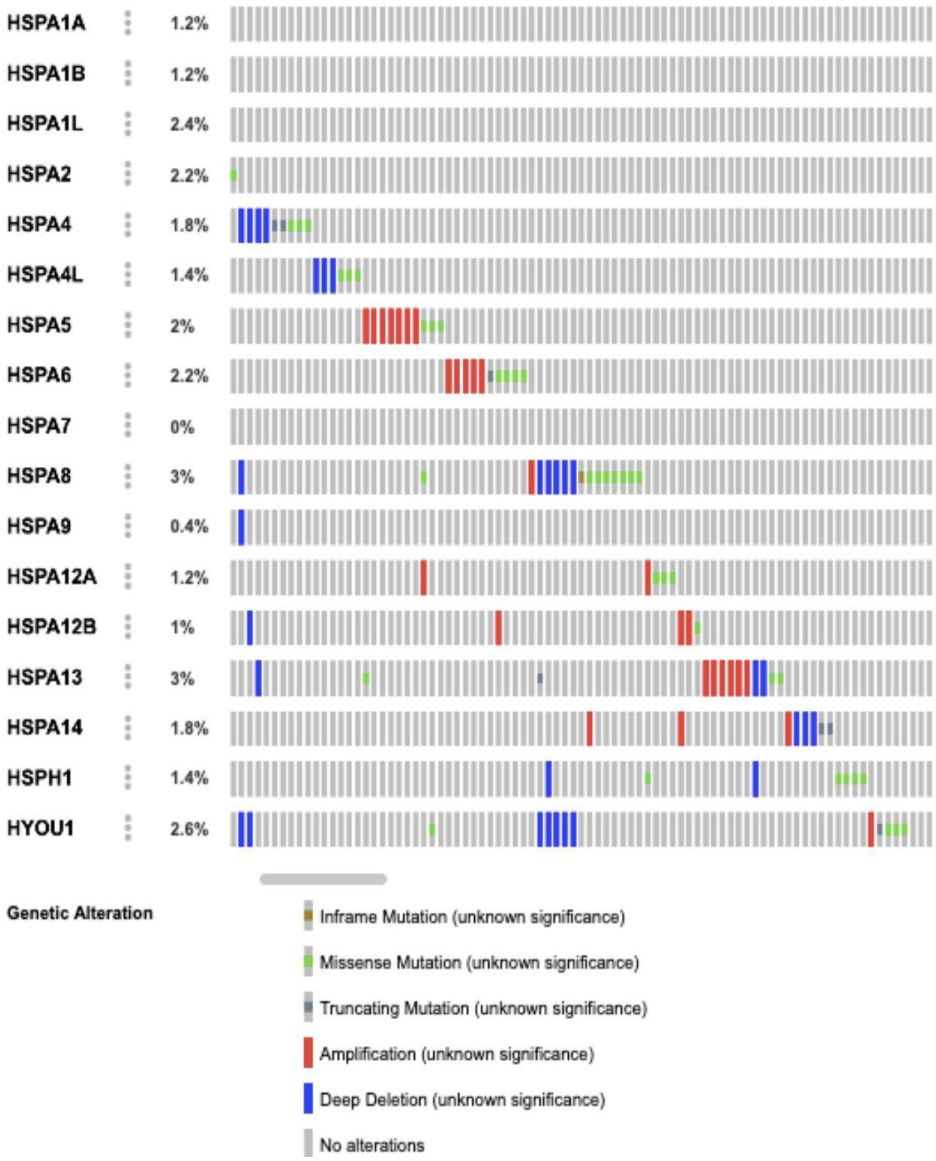

Fig. 1. The figure shows the oncoprint data depicting the genetic alterations in heat shock protein gene family A in HNSCC patients

expression in African American population has shown low survival rate. The above mentioned previous researches have helped in doing this present study. The study identifies the genetic alterations in the crucial gene family. Any variations observed in the study can be replicated in experimental work so as to arrive at conclusive evidence on the association of the variations/mutations with the habit of individuals are the factors that influence the study results.

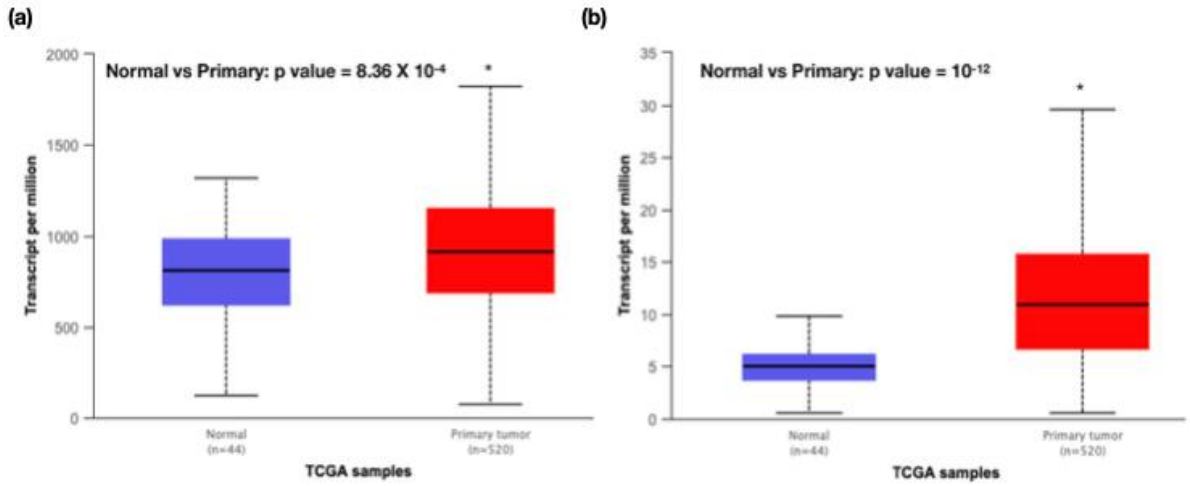

Fig. 2. Box-Whisker plot showing relative expression profile of *HSPA8 (a)* and *HSPA13 (b)* gene (Normal vs primary tumor). The X axis denotes the TCGA samples (blue bar indicates normal and red bar indicates primary tumor) and Y axis denotes the transcripts per million values. The comparison of gene expression patterns between normal vs primary tumor was found to be significant in both the genes assessed *HSPA8* ($p = 8.36 \times 10^{-4}$) and *HSPA13* ($<10^{-12}$). A p value less than 0.05 was considered to be significant

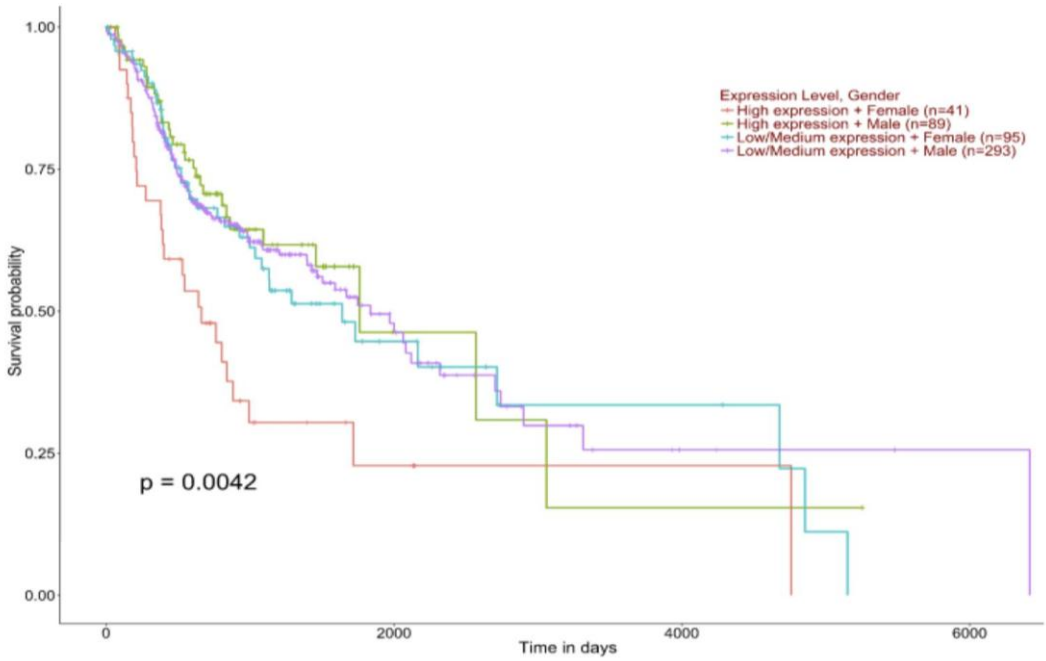

Fig. 3. Kaplan–Meier plots showing the association of *HSPA8* expression classified based on the gender of HNSCC patient with respect to survival probability. The x-axis represents time in days and the y-axis shows the survival probability. A significant difference in the survival probability was observed in female patients with high level expression of *HSPA8* demonstrating low survival when compared to the other groups (p value = 0.0042)

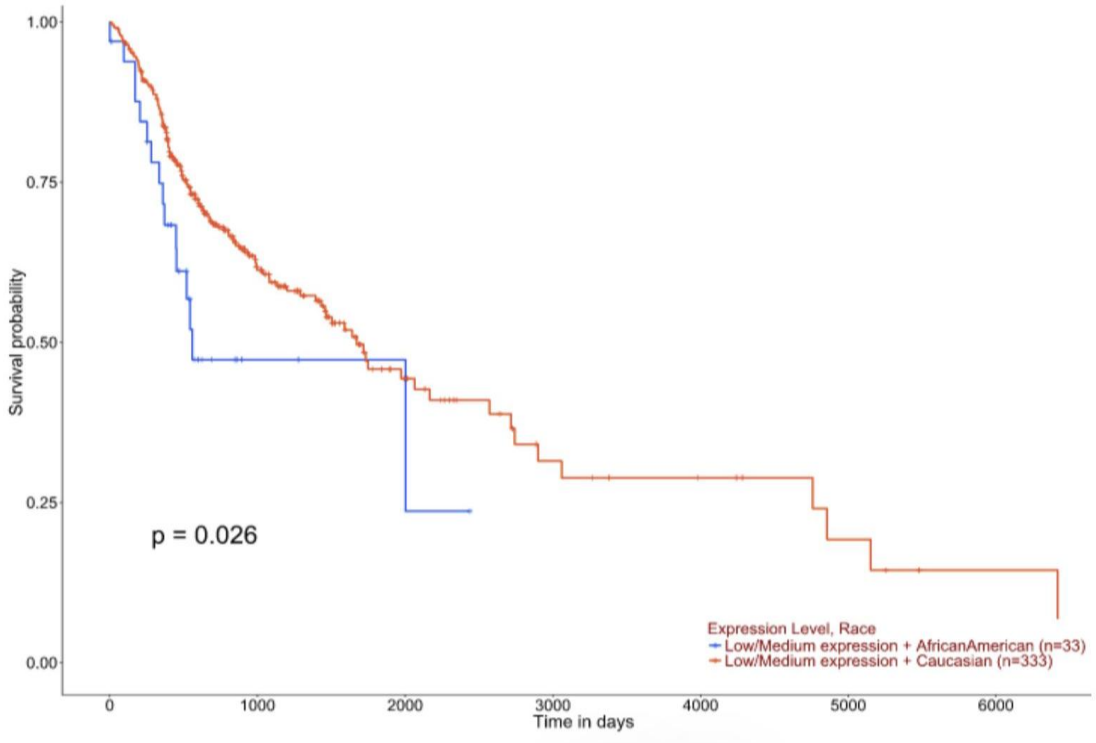

Fig. 4. Kaplan–Meier plots showing the association of *HSPA13* expression classified based on the ethnicity of HNSCC patients with respect to survival probability.The x-axis represents time in days and the y-axis shows the survival probability. A significant difference in the survival probability was observed in African-American patients exhibiting low/medium level expression of *HSPA13* with low survival rate when compared to the other groups (p value = 0.026)

5. CONCLUSION

The present study has discussed the relationship between the various *HSPA* genes about the expression, regulation, mutations and variations. It also provides preliminary data on the involvement of the HSPA family of genes with Head and neck squamous cell carcinoma (HNSCC), which has to be validated using experimental evidence.

FUNDING

With an ever increasing trend of head and neck cancers in Asian population [54], it has become a crucial point to identify the molecular targets involved in the pathogenesis of the disease. Accumulating evidences have proved the effect of several markers [55] in different types of patients with [56] and without habits. An extensive probing into each of these genomic signatures would aid in limiting the number of markers directly associated with the disease phenotype.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

We conducted our research after obtaining proper IEC approval.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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