DECODING THE GENETIC ALTERATIONS IN PRAME GENE FAMILY AND ITS ASSOCIATION WITH HEAD AND NECK SQUAMOUS CELL CARCINOMA

ABSTRACT

To identify the genetic alterations, the pattern of gene expression and the consequence of mutations documented in the PRAME family of genes in HNSCC patients and to compare the mutation frequency with that of the normal population. Database used was cBioportal, Oncoprint data analysis was done which identifies variations in genes, ExAc Analysis which provides the population screening for observed mutations, pathogenicity of variants was identified using Mutpred and IMutant analysis and Gene expression profiling was done in which mRNA expression levels of queried gene with highest frequency of mutations could be observed. The survival analysis was done using the UACLAN database. The results were statistically analyzed. PRAME gene expression levels significantly correlated with the tumor grade, size, nodal involvement and the clinical states of HNSCC patients. Hence, there exists a strong association between the mutations identified with that of the disease phenotype. The present study provides preliminary data on the involvement of the PRAME family of genes with HNSCC, which has been validated using experimental evidence in the South Indian population. The p value was found to be 0.0095 which is less than 0.01 and hence there is a significant difference in survival rate of patients with a differential expression pattern of PRAME protein.

Key words: head and neck cancer, association, PRAME gene expression, genetic alterations.

INTRODUCTION

Squamous cell carcinomas of the head and neck (HNSCCs) are an invasive genetically complex phenotype with an incidence rate reaching a steep increase in the developing nations. Despite several treatments options, the primary treatment choices for most patients are surgery and radiotherapy. But these therapies are associated with significant morbidity and a decline in quality of life. Radiotherapy resistance is most commonly observed in HNSCC patients [1]. Major malignant growths in the HNSCC are precipitated after the upper aerodigestive epithelium is exposed to carcinogenic agents such as tobacco and liquor. The human papillomavirus (HPV16,18) was unambiguously implicated in a subset of these malignant growths as a causative factor. Treatment options for an individual are decided based on some parameters such as the capability to tolerate treatment, concurrent sickness and the awaited practical results [2]. Squamous cell carcinomas (HNSCC) are around 90% of all head and neck malignant growths. HNSCC is the world's 6th driving occurrence disease. Cancer of nasal cavity and paranasal sinus, nasopharyngeal, laryngeal cancer, hypo-pharyngeal cancer, oropharyngeal, cancer of salivary gland are the major types of head and neck cancers. Other types of HNSCC include brain tumour, esophageal eye cancer, parathyroid cancer, sarcoma, and thyroid cancer [3]. The proportion of male and female ranges somewhere in the range of 2:1 and 4:1. Such malignancies are unequivocally connected with certain hazard factors, *viz.,* smokeless tobacco, pan, gutka, alcoholism, and certain other environmental factors [4]. The identification of genetic alterations

in crucial genes known to be associated with HNSCC would open new avenues towards identification of potential targets to develop therapeutic leads.

In line with the above fact, PRAME (preferentially expressed melanoma antigen) is a member of the cancer-testis antigen family has been selected for the present study. It is predicted that the *PRAME* gene has been alterably progressed and emerged due to tough conflict between quickly splitting cells during oogenesis and spermatogenesis [5]. This gene is concealed within the human immunoglobulin lambda gene locus on the reverse strand of chromosomes 22 wraps an area of about 12 kilo-bases [6]. Protein binding and receptor binding of retinoic acid is said to be the PRAME gene's molecular functions. This gene negatively regulates the signaling pathways of the retinoic acid receptor, apoptotic process and cell differentiation. It also up-regulates the growth and proliferation of cells [7]. The signaling pathway stimulates PRAME gene that are highly operated in response to inflammation or infection and the resultant may have double role as a histone binding protein [8]. It is perceived to be an immunocyte tumour-associated antigen. This prompts the cytotoxic T cells response which accelerates the growth of tumors [9].

The escalated PRAME expression is correlated with raised denying to chemotherapeutic procedures in Hodgkin's disease and large B cell lymphomas. PRAME gene is also aimed for the immunotherapy of acute leukemia [10]. The PRAME expression is less in normal tissues yet has been recognized in a diverse types of solid organs cancers including breast, skin, lung, neck and head cancer [11]. The PRAME gene manifestation and modulation is poorly interpreted, therefore the expression of PRAME gene in virulence is still undisclosed.

The objectives of this study was to identify genetic alterations in the PRAME family of genes in HNSCC patients, to compare the mutation frequency with that of the normal population, to identify the pattern of gene expression, to identify the consequence of mutations documented and

to derive an association between the genotype and phenotype. Therefore, we hypothesized that there could be a strong association between the mutations identified with that of the disease phenotype.

MATERIALS AND METHODS

Data source:

The present study follows a retrospective design of the observational study. The source of patient data was obtained from the database cBioportal [12,13]. This site contains an exhaustive array of descriptions of patients from different cohorts. The TCGA, Firehose legacy data set consisted of 528 cases of the head and neck squamous cell carcinoma, of which 512 tumor samples had sequencing and copy number alteration data. For each of the cases in the data set there was a full profile of mutated, amplified, deleted genes. In table 1 the demographic information of the cases in the dataset is presented. The database "HUGO Gene Nomenclature Committee at the European Bioinformatics Institute" (www.genenames.org/data/) contained a list of essential genes belonging to the PRAME gene family. In the cBioportal database, user-defined queries based on these genes were submitted, and the resulting Oncoprint data was used for further analysis.

Oncoprint data analysis:

Oncoprint evidence provides information on the frequency distribution of differences in each of the identified genes, degree of variability, improvements in amino acid protein coding, gene amplification, deletions, insertions, frameshifts, splice site mutations etc.Such information can be used to (a) determine a putative relation between the phenotype of the disorder and the genotype,

(b) classify the differences in mechanisms or genes otherwise known, and (c) recognize any new variants that may be correlated with the phenotype.

Protein stability analysis:

I-Mutant v3.0 is a support vector machine (SVM)based method for automated detection of improvements in protein stability through single point mutations. The predictions of the algorithm are focused upon the sequence of proteins. The predictions were categorized into three classes: favorable mutation (−0.5 kcal / mol), significant decrease (< −0.5 kcal / mol) and large increase (> 0.5 kcal / mol) respectively. The free energy shift (DDG) expected by I-Mutant 3.0 is based on the discrepancy between mutant and native protein (kcal / mol) free energy increase in Gibbs unfolding [14].

MutPred analysis:

MutPred v2 is a standalone and online framework developed to identify substitutes of amino acids as pathogenic or healthy in person. The FASTA-format wild-type protein sequence is used for the function and the specified substitution locations. The possibility of mutation is stated to be deleterious [15].

gnomAD analysis:

Dataset gnomAD v2.1.1 consists of an array of 125,748 exomes and 15,708 individual sequencing genomes. Such research was used to search for the occurrence of the missense variants found in the HNSCC data in other persons for whom the sequencing data is available. Variations across 141,456 human exomes and genomes show the continuum of resistance and loss of function across human protein coding genes [16].

UALCAN analysis:

the expression of the gene in HNSCC was analysed using the UALCAN (http://ualcan.path.uab.edu/cgi-bin/TCGA-survival1.pl?genenam=*SERPINB5*&ctype=HNSC) database. Survival curve analysis based on the tumor grade and expression profile was performed to demonstrate the putative role of *SERPINB5* gene with HNSC [16]. Gene expression data is expressed as transcripts per million (TPM) which is a normalization method for RNA- seq data. The TPM values used for the generation of box-whisker plots were also used to determine the significant difference between the groups. The t test was performed using PERL script with the comprehensive perl archive network (CPAN) module. Combined survival effect analysis of gene expression and other clinical parameters such as race, gender, tumor grade, cancer subtypes were assessed using multivariate Kaplan- Meier survival analysis [17].

RESULTS AND DISCUSSION

Oncoprint data analysis:

In oncoprint analysis data (figure 1), the grey box indicates no alterations, the red box indicates amplification, the blue box indicates deep deletion, the green box indicates missense mutation (It is a point mutation in which single nucleotide change results in different amino acid

production) and this box indicates truncating mutation (nonsense mutation that results in premature stop codon). The *PRAME* and *PRAMENP* genes were found to have numerous sites amplifications. The genes *PRAME, PRAMEF1, PRAMEF2, PRAMEF6, PRAMEF7, PRAMEF10, PRAMEF11, PRAMEF12, PRAMEF17* and *PRAMEF18* exhibited missense mutation/variation. *PRAMEF11* was found to have the highest alteration level of all genes examined (4%). The majority of *PRAMEF11* alterations were missense mutation and deep deletion (Figure 1). Since, the protein sequence of PRAME11 could not be retrieved from the public domain, further analysis was carried in the gene with the next highest frequency which was *PRAME.* The *PRAME* and *PRAMENP* genes were found to have numerous deep deletions among the analyzed set of genes. The *PRAMEF18* gene was found to exhibit most of the truncating mutations comparatively. In addition, the variants found in this analysis were contrasted with the non-synonymous variants in the gnomAD database in order to decide if the variant is novel or documented in the general population. Numerous truncating and missense variants have been reported of unknown significance. The missense mutation observed in oncoprint analysis was taken into consideration and then analyzed for the stability and pathogenicity using mutpred and imutant analysis (Table 3).

Protein stability largely impacts the protein's biological function. For all of the non-synonymous variants found in the analysis, therefore, protein stability was assessed. In PRAME gene, the alteration L119R that is Leucine transformation to Arginine- the protein stability was observed to be decreased hence gained a negative score. For the same pathogenesis tends to be deleterious. So if an alteration shows the protein stability decreased and if its pathogenicity is also deleterious then it may be a disease causing variation. And in the same way, if the protein stability is

decreased or increased and if its pathogenicity is neutral - it tends to have no effect or the alteration itself won't be enough to cause a disease. Surprisingly, most of the deleterious effect was shown by the genes *PRAME, PRAMEF2, PRAMEF4, PRAMEF6, PRAMEF10, PRAMEF12 and PRAMEF18.*

The gnomAD analysis is a platform for identifying the novel mutation and the mutation that has already been discovered. gnomAD analysis for the family of the *PRAME* genes reveals a number of novel mutations. This analysis helps identify the type of alteration, the loci, the percentage of alteration and the frequency of the variant allele (Table 2). Majority of the novel variations were observed in the *PRAMEF2* gene. *PRAME, PRAMEF1, PRAMEF6, PRAMEF10, PRAMEF12, PRAMEF17, PRAMEF18* and *PRAMEF26* were also found to exhibit novel variations among the analyzed set of genes.

In figure 2, the expressions of the PRAME gene across different tumors are compared. The comparison of head and neck cancer with other types of cancers caused by the PRAME gene was done by PAN cancer analysis. Here the TCGA (The Cancer Genome Atlas) is taken into consideration, with that the similarities and differences among the genomic alterations found across the diverse tumor types were analyzed. The gene expression analysis (figure 3) is based on the grade and the level of frequency of mRNA transcription in each grade of cancer. The *PRAME* expression was shown to increase with grades of cancer in comparison to the normal group. Figure 4 denotes the survival growth curve analysis chart, here the red line indicates high expression and blue line indicates low/medium expression. And their p value was found to be 0.0095 which is less than 0.01 and hence confirming the fact that there is a significant difference

in survival rate of patients with a differential expression pattern of PRAME protein. The high level expression also related to poor survival probability in HNSC patients.

Various evidence have indicated an in depth relationship between PRAME and tumorigenesis. PRAME acts as a ligand-dependent co-repressor within the important retinoic acids receptor (RAR) pathway. When PRAME is absent, the activation of the RAR pathway by retinoids will cause proliferation arrest, cell differentiation and apoptosis. Conversely, the RAR pathway is inhibited when PRAME is abnormally present, leading to incessant cell proliferation and tumorigenesis [18]. Oral carcinogenesis may be a multifactorial process involving numerous genetic processes which will alter the function of oncogenes, tumor suppressor genes, and other related molecules. The resulting anomalies can increase the assembly of growth factors and therefore the number of cell surface receptors, and/or increase transcription or intracellular messenger factor levels. These changes can, in turn, cause a loss of tumor suppressor activity and provide rise to a phenotype capable of accelerating cellular proliferation, weakening cell cohesion, and causing local infiltration and metastasis [19]. In the other paper, the authors also studied recent duplications within the human genome and located that Cancer / testis antigen genes were represented during this gene set, including the family of PRAME (preferentially expressed antigen of melanoma) genes located on chromosome 1 and expressed within the testis and during a sizable amount of tumors. Duplicated *PRAME* genes are hominid-specific genes that have arisen in the genome of humans since the chimp divergence. The family of *PRAME* genes also spread in other Eutheria. Chimp and mouse have orthological clusters of *PRAME* genes on their respective chromosomes 1 and 4 [20].

PRAME may be a germinal tissue-specific gene that's also expressed at high levels in hematological malignancies and solid tumors. The physiological functions of PRAME in normal and tumour cells are unknown, although a task within the regulation of retinoic acid signaling has been proposed. Sequence homology and structural predictions suggest that PRAME is said to be the leucine-rich repeat (LRR) family of proteins, which have diverse functions. PRAME continues to function as both a useful prognostic marker in acute leukemias and solid tumors, and a beautiful target for potential immunotherapy [21]. Down-regulation of PRAME promotes tumorigenicity of leukemic cells in nude mice [22]. a completely unique gene, *PRAME* encodes the antigen, HLA-A24 it's expressed during a large proportion of tumors and also in some normal tissues at a lower level [23]. PRAME expression in neuroblastoma is awfully common and was universally seen in patients with advanced-stage disease [24]. The epitopes PRAME and HLA- A* 0201 (human histocompatibility leukocyte antigen) are expressed on cancer cells of diverse histologic origin, making them attractive targets for immunotherapy of cancer [25].

Neuroblastoma cells which are modulated by interaction with active NK cells, that the presence of endogenous levels of PRAME is sufficient for productive peptide presentation to T-cells. Altogether, we envision these T-cells to eliminate neuroblastoma tumor cells that were made immunogenic by preceding NK cell therapy [26]. 93% of primary lesions and 100% of advanced cases over expressed PRAME [27]. PRAME wasn't expressed on chondrosarcoma tumors [28]. PRAME is an independent prognostic biomarker which identifies increased metastatic risk in patients with class 1 or disomy 3 tumors [29]. PRAME is usually observed in human cancers confers growth or survival advantages by antagonising RAR signalling [30]. Computational approach has been widely preferred in recent years to locate genetic alterations and identify

candidate genes associated with the disease phenotype [31]. Although the study revealed a lot of information about the putative association of PRAME gene family with HNSC, there exist certain limitations viz., the dataset included a diverse population which included patients from different ethnic groups or races. Since the allele frequencies tend to deviate in different populations, screening of one population or group is essential to draw the association pattern. Furthermore, cancer is a complex phenotype with a complex interplay between the genetic and epigenetic factors. A deep probing into the epigenetic marks related to this gene family could provide additional information on the role of these genes in the process of tumorigenesis. With all the limitations addressed, the present study opens new avenues towards identification of target genes which can serve as both therapeutic as well as diagnostic markers.

REFERENCES

[1] Alsahafi E, Begg K, Amelio I, Raulf N, Lucarelli P, Sauter T, et al. Clinical update on head and neck cancer: molecular biology and ongoing challenges. Cell Death Dis 2019;10:540.

[2] Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. Lancet 2008;371:1695–709.

[3] Tobias JS. Cancer of the head and neck. BMJ 1994;308:961–6.

[4] Biswas R, Halder A, Ghosh A, Ghosh SK. A comparative study of treatment outcome in younger and older patients with locally advanced oral cavity and oropharyngeal cancers treated by chemoradiation. South Asian J Cancer 2019;8:47–51.

[5] Birtle Z, Goodstadt L, Ponting C. Duplication and positive selection among hominin-specific PRAME genes. BMC Genomics 2005;6:120.

[6] Roszik J, Wang W-L, Livingston JA, Roland CL, Ravi V, Yee C, et al. Overexpressed

PRAME is a potential immunotherapy target in sarcoma subtypes. Clin Sarcoma Res 2017;7:11.

[7] Fulton J, Heery DM. PRAME (Preferentially Expressed Antigen In Melanoma). Atlas Genet Cytogenet Oncol Haematol 2014. https://doi.org/10.4267/2042/54374.

[8] Wadelin FR, Fulton J, Collins HM, Tertipis N, Bottley A, Spriggs KA, et al. PRAME is a golgi-targeted protein that associates with the Elongin BC complex and is upregulated by interferon-gamma and bacterial PAMPs. PLoS One 2013;8:e58052.

[9] Van Baren, Chambost, Ferrant, Michaux, Ikeda, Millard, et al. PRAME , a gene encoding an antigen recognized on a human melanoma by cytolytic T cells, is expressed in acute leukaemia cells : Rapid Paper. Br J Haematol 1998;102:1376–9.

[10] Ding K, Wang X-M, Fu R, Ruan E-B, Liu H, Shao Z-H. PRAME Gene Expression in Acute Leukemia and Its Clinical Significance. Cancer Biol Med 2012;9:73–6.

[11] Matsushita M, Ikeda H, Kizaki M, Okamoto S, Ogasawara M, Ikeda Y, et al. Quantitative monitoring of the PRAME gene for the detection of minimal residual disease in leukaemia. Br J Haematol 2001;112:916–26.

[12] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.

[13] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:l1.

[14] Capriotti E, Fariselli P, Casadio R. I-Mutant2.0: predicting stability changes upon mutation from the protein sequence or structure. Nucleic Acids Res 2005;33:W306–10.

[15] Pejaver V, Urresti J, Lugo-Martinez J, Pagel KA, Lin GN, Nam H-J, et al. MutPred2:

inferring the molecular and phenotypic impact of amino acid variants. bioRxiv 2017:134981. https://doi.org/10.1101/134981.

[16] Karczewski KJ, Francioli LC, Tiao G, Cummings BB, Alföldi J, Wang Q, et al. Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv 2019:531210. https://doi.org/10.1101/531210.

[17] Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi BVSK, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia 2017;19:649–58.

[18] Chang T-C, Yang Y, Yasue H, Bharti AK, Retzel EF, Liu W-S. The expansion of the PRAME gene family in Eutheria. PLoS One 2011;6:e16867.

[19] Pérez-Sayáns M, Somoza-Martín JM, Barros-Angueira F, Reboiras-López MD, Gándara Rey JM, García-García A. Genetic and molecular alterations associated with oral squamous cell cancer (Review). Oncol Rep 2009;22:1277–82.

[20] Dobrynin P, Matyunina E, Malov SV, Kozlov AP. The novelty of human cancer/testis antigen encoding genes in evolution. Int J Genomics Proteomics 2013;2013:105108.

[21] Wadelin F, Fulton J, McEwan PA, Spriggs KA, Emsley J, Heery DM. Leucine-rich repeat protein PRAME: expression, potential functions and clinical implications for leukaemia. Mol Cancer 2010;9:226.

[22] Tajeddine N, Gala J-L, Louis M, Van Schoor M, Tombal B, Gailly P. Tumor-associated antigen preferentially expressed antigen of melanoma (PRAME) induces caspase-independent cell death in vitro and reduces tumorigenicity in vivo. Cancer Res 2005;65:7348–55.

[23] Ikeda H, Lethé B, Lehmann F, van Baren N, Baurain JF, de Smet C, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity 1997;6:199–208.

[24] Oberthuer A, Hero B, Spitz R, Berthold F, Fischer M. The tumor-associated antigen PRAME is universally expressed in high-stage neuroblastoma and associated with poor outcome. Clin Cancer Res 2004;10:4307–13.

[25] Kessler JH, Beekman NJ, Bres-Vloemans SA, Verdijk P, van Veelen PA, Kloosterman-Joosten AM, et al. Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. J Exp Med 2001;193:73–88.

[26] Spel L, Boelens J-J, van der Steen DM, Blokland NJG, van Noesel MM, Molenaar JJ, et al. Natural killer cells facilitate PRAME-specific T-cell reactivity against neuroblastoma. Oncotarget 2015;6:35770–81.

[27] Pollack SM, Li Y, Blaisdell MJ, Farrar EA, Chou J, Hoch BL, et al. NYESO-1/LAGE-1s and PRAME are targets for antigen specific T cells in chondrosarcoma following treatment with 5-Aza-2-deoxycitabine. PLoS One 2012;7:e32165.

[28] Field MG, Decatur CL, Kurtenbach S, Gezgin G, van der Velden PA, Jager MJ, et al. PRAME as an Independent Biomarker for Metastasis in Uveal Melanoma. Clin Cancer Res 2016;22:1234–42.

[29] Epping MT, Wang L, Edel MJ, Carlée L, Hernandez M, Bernards R. The human tumor antigen PRAME is a dominant repressor of retinoic acid receptor signaling. Cell 2005;122:835–47.

[30] Khateeb EE, Morgan D. Preferentially Expressed Antigen of Melanoma (PRAME) and

Wilms' Tumor 1 (WT 1) Genes Expression in Childhood Acute Lymphoblastic Leukemia, Prognostic Role and Correlation with Survival. Open Access Maced J Med Sci 2015;3:57–62.

[31] Vijayashree P, Paramasivam A. Virtual screening of mutations in antioxidant genes and its putative association with HNSCC: An *in silico* approach. Mutat Res Fund Res Fund Mol *M.* 2020: 111710 [Epub ahead of print]

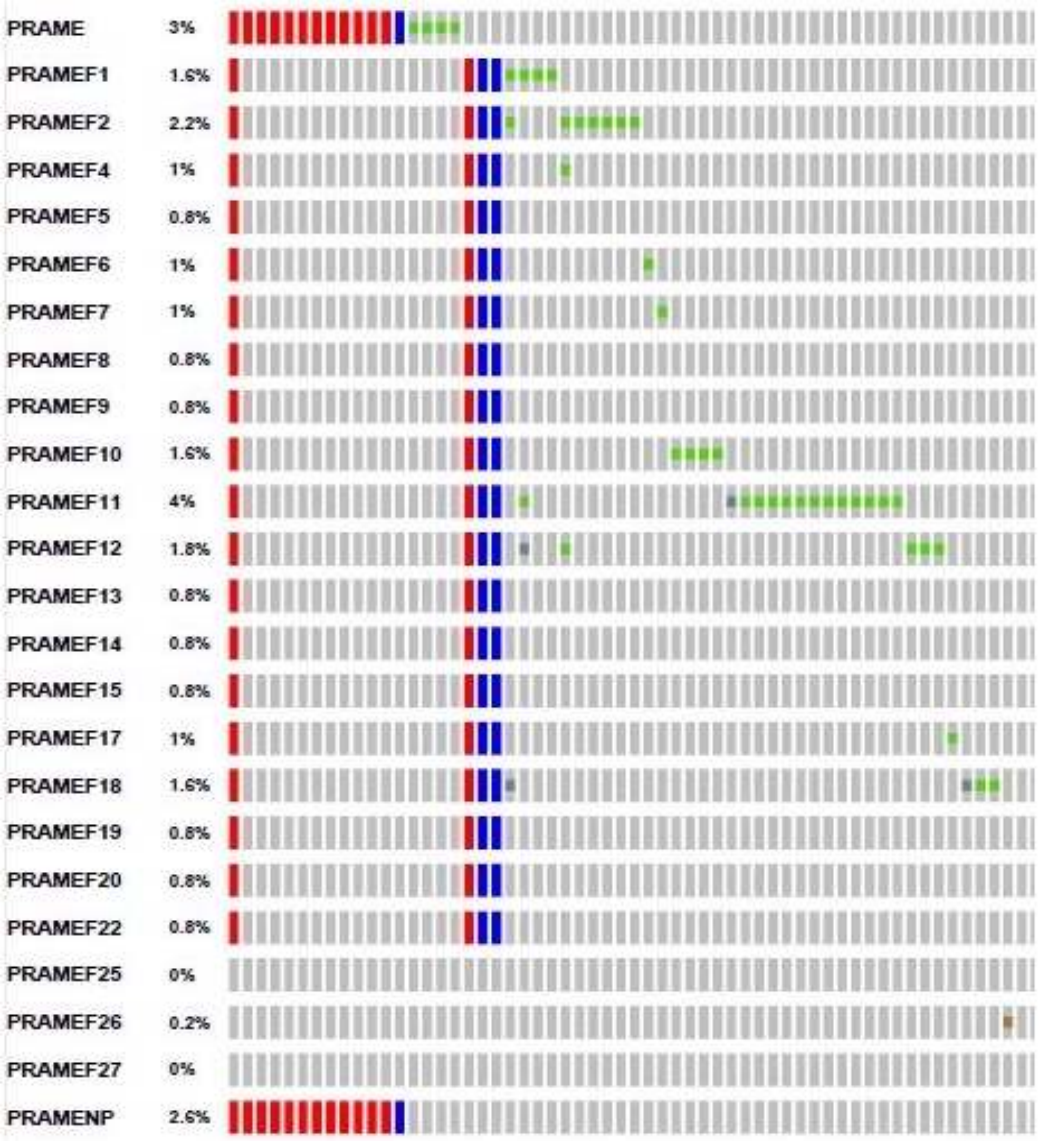

Figure 1 The oncoprint data depicting different types of gene alterations in the *PRAME* family of genes. *PRAMEF11* was found to have the highest alteration level of all genes examined (4%). The *PRAME* and *PRAMENP* genes were found to have numerous sites of amplifications. The genes *PRAME, PRAMEF1, PRAMEF2, PRAMEF6, PRAMEF7, PRAMEF10, PRAMEF11, PRAMEF12, PRAMEF17* and *PRAMEF18* exhibited missense mutation which was then analyzed for the stability and pathogenicity using mutpred and imutant analysis.

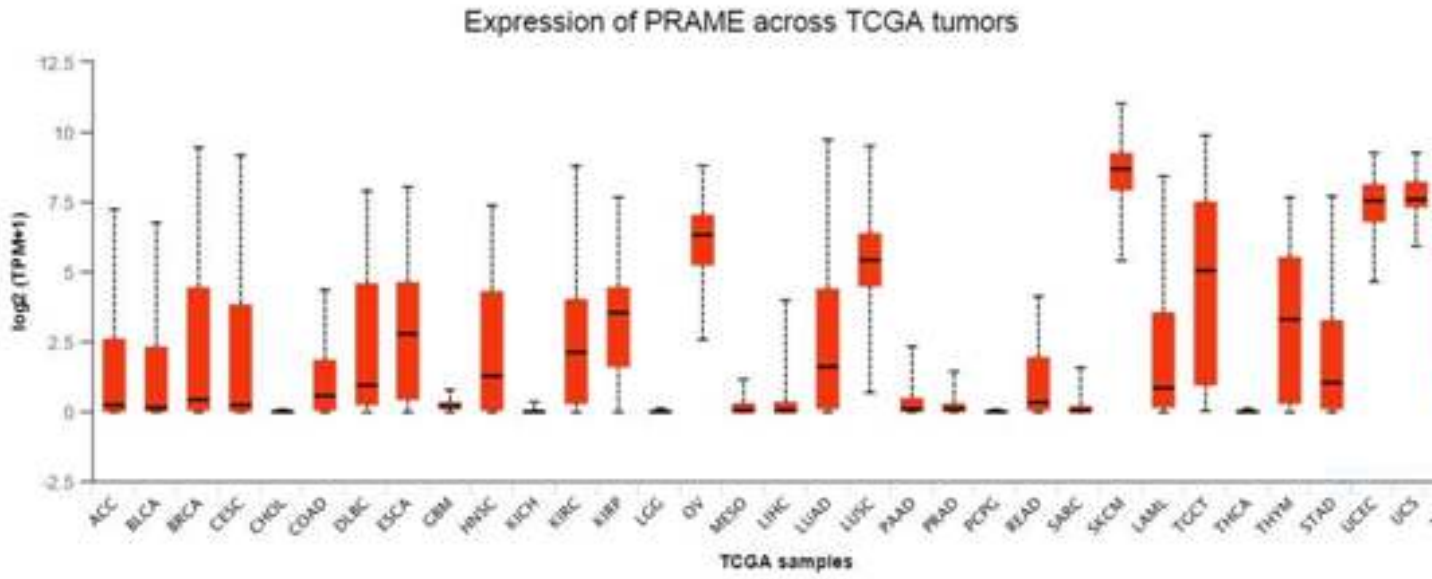

Figure 2 represents the comparison of expression of PRAME across different tumors types. X axis represents TCGA samples and Y axis represents the PRAME gene expression among the various types of tumors.

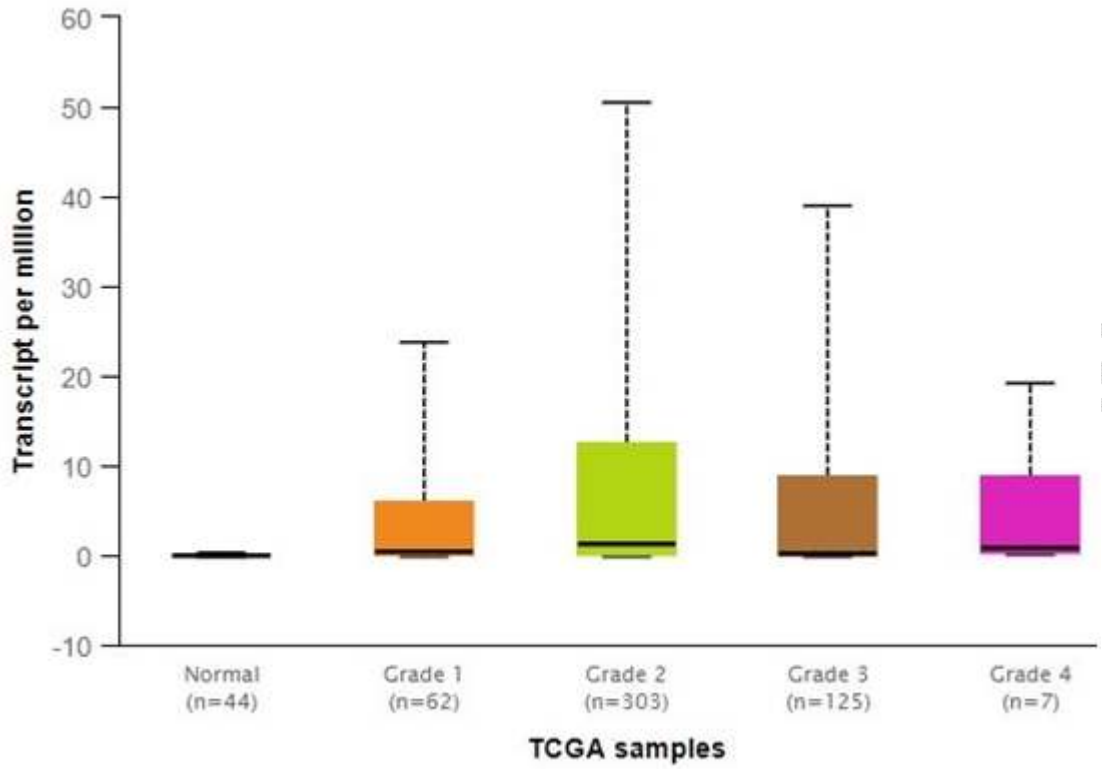

Figure 3 Box-whisker plot representing the differential gene expression pattern of the *PRAME* gene across different tumor grades. X axis represents the different grades of HNSCC samples from the TCGA data set and Y axis represents the *PRAME* gene expression in HNSC in transcript per million (TPM). A significant difference in the gene expression profile was observed between normal vs grade 1 ($p = 8.6 \times 10^{-4}$), normal vs grade 2 ($p = < 10^{-12}$) and normal vs grade 3 (3.125×10^{-8}). A p value less than 0.05 is considered to be significant.

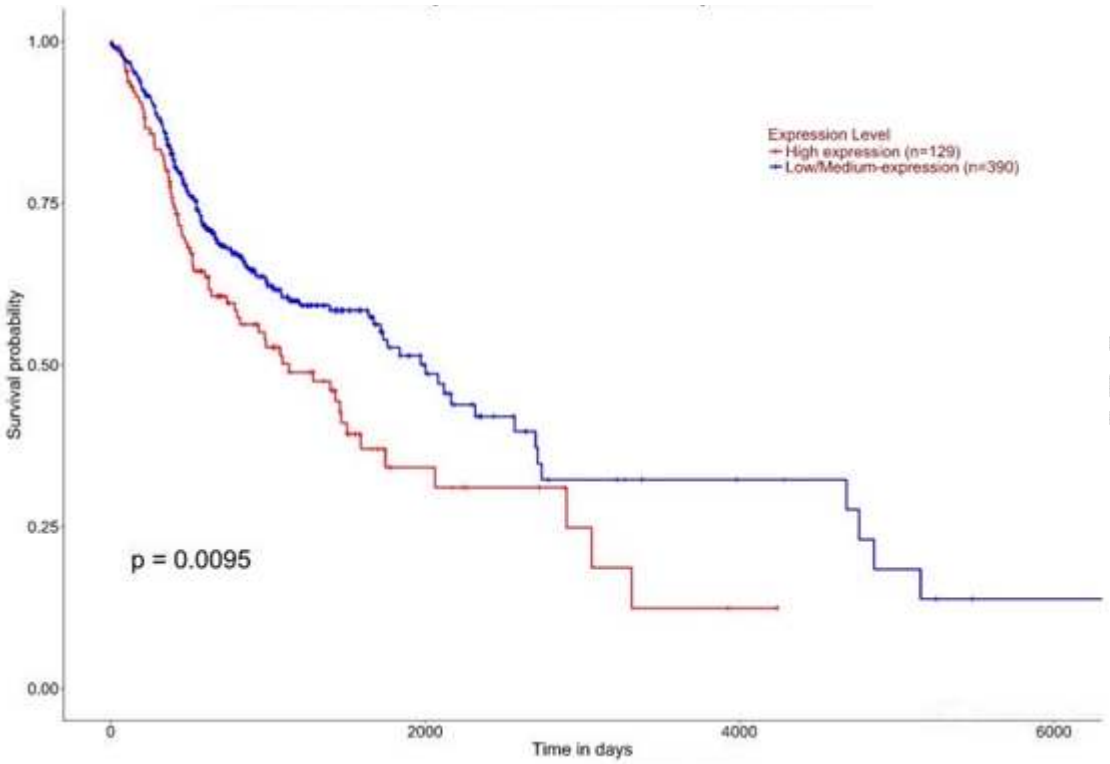

Figure 4 Kalplan-Meier plot showing the association of altered PRAME expression with HNSC patient's survival. The x - axis represents time in days and y - axis denotes the survival probability in HNSC patients. The red line corresponds to high level expression and the blue line represents low/medium level expression. A significant association was observed between high and low/medium level expression of PRAME (p = 0.0095). A p value less than 0.05 is considered to be significant.

Table 1: Demographic details of patients analysed in the present study (as obtained from the cBioportal site)

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

* Neoplasm histologic grade available for 452 patients in the TCGA provisional dataset

Table 2: Frequency and type of genetic alteration in the *PRAME* family of genes

Gene	Protein	Alteration	Cytogenetic location	Percentage of alteration	Variant allele frequency in tumor sample	gnomAD frequency data
PRAME	Preferentially expressed Antigen in Melanoma	Amplification	22q11.22		-	-
		Deep deletion			-	-
		L119R		3	0.12	
		Q287P			0.30	Novel
		L313R			0.50	Novel
		R125Q			0.06	Novel
PRAMEF1	PRAME family member 1	Amplification	1p36.21	1.6	-	-
		Deep deletion			-	-
		V171I			0.23	Novel
		E338K			0.23	Novel
		T72K			0.38	rs149382773
		N297K			0.03	Novel

PRAMEF2	PRAME family member 2	Amplification Deep deletion Y194H H268R A114P E11Q L313I R174M T381A	1p36.21	2.2	- - 0.02 0.19 0.39 0.19 0.19 0.04 0.06	 Novel Novel Novel Novel Novel Novel Novel
PRAMEF4	PRAME family member 4	Amplification Deep deletion P418L	1p36.21	1	- - 0.02	 rs753793229
PRAMEF5	PRAME family member 5	Amplification Deep deletion	1p36.21	0.8	-	-
PRAMEF6	PRAME family member 6	Amplification Deep deletion V135E	1p36.21	1	- - 0.10	- - Novel
PRAMEF7	PRAME family member 7	Amplification Deep deletion S317N	1p36.21	1	- - 0.13	- - rs779669158
PRAMEF8	PRAME family member 8	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF9	PRAME family member 9	Amplification Deep deletion	1p36.21	0.8	- -	- -

PRAMEF10	PRAME family member 10	Amplification Deep deletion Q270L M46R L266P R96S	1p36.21	1.6	- - 0.20 0.61 0.23 0.10	- - Novel rs1167071023 Novel Novel
PRAMEF12	PRAME family member 12	Amplification Deep deletion R94C Q4* S307W P238L S128I	1p36.21	1.8	- - 0.27 0.25 0.26 0.21 0.29	- - rs752095583 rs757917825 Novel Novel Novel
PRAMEF13	PRAME family member 13	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF14	PRAME family member 14	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF15	PRAME family member 15	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF17	PRAME family member 17	Amplification Deep deletion S117A	1p36.21	1	- - 0.15	- - Novel

PRAMEF18	PRAME family member 18	Amplification Deep deletion N448Tfs*? N448Qfs*19 L354V L373M	1p36.21	1.6	- - 0.33 0.36 0.28 0.03	- - Novel Novel rs1384433084 Novel
PRAMEF19	PRAME family member 19	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF20	PRAME family member 20	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF22	PRAME family member 22	Amplification Deep deletion	1p36.21	0.8	- -	- -
PRAMEF25	PRAME family member 22	Amplification Deep deletion	1p36.21	0	- -	- -
PRAMEF26	PRAME family member 26	K159del	1p36.21	0.2	0.14	Novel
PRAMEF27	PRAME family member 22	Amplification Deep deletion	1p36.21	0	- -	- -
PRAMENP	PRAME N-Terminal like, Pseudogene	Amplification Deep deletion	22q11.22	2.6	- -	- -

Table 3: Protein stability and pathogenesis of variants identified in *PRAME* family of genes

Gene	Alteration	Protein stability	Score	Pathogenicity	Score
PRAME	L119R	Decrease	-0.95	Deleterious	-5.964
	Q287P	Decrease	-1.13	Deleterious	-5.407
	L313R	Decrease	-1.30	Deleterious	-5.843
	R125Q	Decrease	-1.07	Deleterious	-2.571
PRAMEF1	V171I	Decrease	-0.06	Neutral	-0.844
	E338K	Increase	0.15	Neutral	-2.075
	T72K	Decrease	-0.32	Neutral	-1.305
	N297K	Decrease	-0.60	Neutral	-1.822
PRAMEF2	Y194H	Decrease	- 0.76	Neutral	1.041
	H268R	Increase	0.36	Deleterious	-4.565
	A114P	Decrease	-1.40	Neutral	-1.356
	E11Q	Increase	0.45	Neutral	-1.601
	L313I	Increase	0.51	Neutral	-1.854
	R174M	Decrease	-0.57	Deleterious	-5.405
	T381A	Decrease	-0.17	Deleterious	-3.315
PRAMEF4	P418L	Decrease	-0.91	Deleterious	-9.315
PRAMEF6	V135E	Decrease	-1.35	Deleterious	-2.89
PRAMEF7	S317N	Decrease	-2.18	Neutral	0.691

PRAMEF10	Q270L	Decrease	-0.37	Deleterious	-5.046
	M46R	Increase	0.02	Neutral	2.204
	L266P	Decrease	-0.88	Deleterious	-5.697
	R96S	Decrease	-1.55	Deleterious	-5.633
PRAMEF12	R94C	Decrease	-0.99	Deleterious	-4.786
	S307W	Increase	0.25	Deleterious	-5.728
	S128I	Increase	0.80	Neutral	-2.302
PRAMEF17	A117S	Increase	0.09	Neutral	-2.11
PRAMEF18	L354V	Increase	0.97	Deleterious	-2.818
	L373M	Decrease	-0.07	Neutral	-1.943