

Genomics Testing in Head and Neck Cancers: Is there a Benefit?

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ABSTRACT

Introduction: Elucidation of the genomic basis of head and neck cancers (HNCs) may help in reducing cancer-related mortality and morbidity. This is because prognostication by predicting disease course and treatment response will help to individualize treatment protocols.

Materials and methods: This prospective pilot study used a 48-gene mutation panel on tumor tissue samples obtained from 18 patients suffering from HNCs. The clinical significance of these mutations was analyzed in terms of treatment resistance, presence of distant metastasis, family history, and disease recurrence.

Results: Two patients carried germline mutations, nine carried somatic mutations and seven samples had no mutation detected on the 48-gene panel. The genomic studies detected germline mutations in *BRCA* and *AIP*, and somatic mutations in *TP53*, *phosphatase and tensin homolog (PTEN)*, *RB1*, *STK11*, *GNA11*, and *HRAS*.

Conclusion: The study appears to validate early genomic testing of HNC cases to modify treatment protocols and offers more specific and personalized treatment options to patients.

Clinical significance: The study demonstrates the potential benefit of integrating genomic data with clinical details to map out a tailored treatment plan to benefit individual patients.

Keywords: Actionable mutations, Cancer genomics, Head and neck cancers, Head and neck squamous cell carcinoma (HNSCC).

International Journal of Head and Neck Surgery (2021): 10.5005/jp-journals-10001-1420

INTRODUCTION

Head and neck cancers (HNCs) contribute to a significant portion of cancer-related mortality and morbidity worldwide.¹ Despite advances in multimodality treatments, the overall survival has not improved significantly over time.² Genomic profiling of HNCs elucidates the biological basis of the individual tumor pathogenesis, identifies potential carcinogens, aids in the identification of diagnostic and prognostic markers and choice of targeted therapy³ hence, may impact on the clinical outcome.

We conducted a prospective pilot study on samples obtained from 18 HNC patients, to assess the nature of genetic alteration to clarify potential benefit.

MATERIALS AND METHODS

The patients of HNC, presenting to the Head and Neck clinic of Health Care Global Hospital, a tertiary referral oncology hospital, in Bengaluru, South India, from May 24, 2011, to November 11, 2016, were chosen for this study, which was funded internally as a proof-of-concept study.

The approval of the scientific research committee and Institutional Ethics Review Board (IERB) was obtained (No. 2017/02/06). Informed consent about the limited applicability to current treatment protocols, though with a possibility of future benefits but was obtained from patients. The clinical data of the patients including details of family history of cancers were obtained from the referring oncologist and, whenever possible through pretest genetic counseling by a genetic counselor.

The tissue samples (paraffin-embedded tissue/fresh frozen tissue) were obtained from 18 patients diagnosed with tumors of the head and neck. To assess tumor tissue quality, a small section of each biopsy (3–5 mm³) was cut, fixed in formalin, and stained with

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How to cite this article: Nayar RC, Pandit RV, Rao VUS, *et al.* Genomics Testing in Head and Neck Cancers: Is there a Benefit? *Int J Head Neck Surg* 2021;12(2):58–64.

Source of support: Nil

Conflict of interest: None

hematoxylin and eosin (H&E) for scoring by an oncopathologist (HCG). These slides were scored for the percentage volume of the tumor. The methodology for sequencing has been outlined in Appendix 1.

The genes included in the panel are listed in Table 1.

A brief explanation of the terms used.

Somatic and Germline Mutations

Germline Mutations

These are the mutations that occur within the germ cells. They are seen to be present in all the cells of an organism and are passed on to the next generation. Testing for germline mutations can be

Table 1: Distribution of mutations in various chromosomes

Chromosome number	Genes affected
1	<i>MPL, NRAS</i>
2	<i>ALK, ERBB4, IDH1</i>
3	<i>PIK3CA, MLH1, PDGFRA, VHL</i>
4	<i>FGFR3, KDR, KIT</i>
5	<i>CDH1, APC, CSF1R, NPM1</i>
7	<i>EGFR, BRAF, MET, SMO</i>
8	<i>FGFR1</i>
9	<i>NOTCH1, CDKN2A/B, ABL1, GNAQ, JAK2</i>
10	<i>FGFR2, PTEN, RET</i>
11	<i>HRAS, ATM</i>
12	<i>HNF1A, KRAS, PTPN11</i>
13	<i>FLT3, RB1</i>
14	<i>AKT1</i>
18	<i>SMAD4</i>
19	<i>GNA11, JAK3, STK11</i>
20	<i>SRC, GNAS</i>
22	<i>SMARCB1</i>

done on a blood sample or a buccal mucosal swab.⁴ Some germline mutations that are associated with HNCs are *FANC*,⁵ *p16/p14ARF*,⁶ *NSD1*,⁷ *CDKN2A*, and *ATR*.⁸

Somatic Mutations

These are acquired by individual cells. A population of cells derived by the asexual reproduction of this cell, forming a clone, generally comprise the cells of the tumor.⁹

Actionable and Non-actionable Mutations

A subset of driver mutations having diagnostic, prognostic, or therapeutic significance is known as actionable mutations, while those that are not as clinically significant are known as nonactionable mutations. These actionable mutations include those for which FDA approved drugs are available, even if recommended for use in tumors of other sites.¹⁰

RESULTS

The age distribution of the patients ranged from 9 to 74 years, with a median age of 45.94 years. There were 14 males and 4 females in the group. Family history was obtained from 17 patients of whom 4 had a family history of other cancers (2 patients had siblings with a history of other cancers and 2 had parents with a history of other cancers). Their origins were diverse (15 patients were Indians, 2 patients were of Middle Eastern origin, and 1 patient belonged to the island of Maldives).

There were nine patients with a history of exposure to tobacco, two with alcohol consumption history while the others did not report any risk factors.

The genomic study revealed germline mutations in *BRCA* (in a patient with carcinoma nasopharynx) and *AIP* (in a patient with adenoid cystic carcinoma of the right submandibular gland), somatic mutations in *TP53* in three patients with squamous cell carcinoma (one case of SCC of the tongue, one with SCC of the gingivobuccal sulcus, and the third involving the oropharynx and base of the tongue) and *HRAS* in three patients (in a case of metastatic papillary thyroid cancer, a case of malignancy of unknown origin, and a case of SCC of the tongue), while the other

mutations detected were *STK11* (in a patient with mucoepidermoid carcinoma of the hard palate), *GNA11* (in metastatic alveolar sarcoma of the tongue), *phosphatase and tensin homolog (PTEN)*, and *RB1* (in a case of SCC involving the gingivobuccal sulcus and the base of the tongue).

The remaining samples four cases of SCC (one involving the pyriform sinus, one involving both the oropharynx and pyriform sinus, one of the tongue, and the fourth, SCC of the buccal mucosa) and two cases of mucoepidermoid carcinoma (of the right parotid and one patient with carcinoma of the base of tongue) showed no mutation as seen by the 48-gene panel, while one sample belonging to a patient of papillary carcinoma thyroid failed quality control testing.

These mutations are described in Table 2 and their potential significance explained in the discussion.

DISCUSSION AND CLINICAL SIGNIFICANCE

TP53 is the most commonly mutated gene in HNC patients in studies conducted by Agrawal et al. and Stransky et al. on 32 and 74 samples, respectively. HPV-positive cancers were 100% positive for E6 and E7 oncogenes. The other mutations that were picked up in significant numbers were *SYNE1*, *NOTCH1*, and *HRAS*.^{11,12}

Pickering et al. conducted two studies on oral squamous cell cancer and on carcinoma tongue specimens in the years 2013 and 2014, respectively. Among the samples in both the studies, *CDKN2A* and *TP53* were the common mutations picked up.^{13,14} In a study conducted by Lin et al. on 128 samples of nasopharyngeal carcinoma, a mutation in *TP53* was seen in 17%, *CDKN2A* in 13%, and *ARID1A* in 11% samples. Less than 10% of tissue samples showed the presence of mutations in *SYNE1*, *ATG13*, *MLL2*, *PIK3CA*, *CCND1*, *NOTCH3*, and *FGFR2*.¹⁵

In 2015, Seiwert et al. and The Cancer Genome Atlas (TCGA) conducted genomic studies on both HPV-positive and -negative tumor tissue samples separately and found that in the HPV-negative group, the most commonly mutated gene was *TP53* followed by *CDKN2A*, while in the HPV-positive group, E6/E7 were positive in 100% samples and the second most commonly mutated gene was *PIK3CA*.^{7,16}

In 2018, a targeted sequencing conducted by Perdomo et al. on 180-paired diagnosed samples of head and neck squamous cell carcinoma (HNSCC) revealed the most frequently mutated genes to be *TP53*, *PIK3CA*, *NOTCH1*, *TP63* and *CDKN2A*.¹⁷

The mutations detected commonly in the above studies conducted from 2011 to 2015 were *TP53*, *EGFR*, *HRAS*, *NOTCH1/2/3*, *CDKN3A*, *CCND1*, *PIK3CA*, and *ATM*. E6 and E7 oncogenes were seen to be expressed in HPV-positive cancers (Table 3).

In our study, germline mutations were detected in two patients—*AIP* and *BRCA*, while somatic mutations were detected in nine patients. These were *TP53*, *PTEN*, *RB*, *STK11*, *GNA11*, and *HRAS*. *TP53* and *HRAS* were seen in 16.67% of patients each while the other mutations were detected in 5.5% of patients each.

SIGNIFICANCE OF GENE MUTATIONS DETECTED IN OUR STUDY

Germline Mutations

AIP

This is a tumor suppressor gene (TSG), a mutation in which was detected in 5.5% of patients in this study.

Table 2: Results—mutations detected and their potential significance

Age	Sex	Diagnosis	Histological type	TNM stage	Risk factors	Distant metastasis	Recurrence	Genes detected
27	M	Adenoid cystic carcinoma—right submandibular gland	Adenoid cystic carcinoma—grade I, submandibular gland	T1 N0 M0	None	Absent	No	<i>AIP</i>
22	F	Ca nasopharynx		N2	None	Absent	No	<i>BRCA</i>
72	M	Papillary carcinoma thyroid	Follicular variant		None	Cervical vertebrae	Yes	<i>HRAS</i> —poor response to cetuximab
42	M	Squamous cell carcinoma right gingivobuccal sulcus	Squamous cell carcinoma	T2 N0 M0	Alcohol, tobacco	Absent	No	<i>TP53</i>
41	M	Squamous cell carcinoma tongue	Squamous cell carcinoma of the tongue	pT2 N2b M0	Tobacco	Absent	No	<i>TP53</i> —poor response to cisplatin
9	M	Metastatic alveolar sarcoma of the tongue	Not done	M1	None	Lungs	No	<i>GNA11</i>
66	M	Squamous cell carcinoma neck with metastasis	Metastatic poorly differentiated carcinoma—left neck node biopsy	N1	None	Right kidney and adrenal, diaphragmatic crura, left lobe of liver, L2 vertebra, Rt renal hilar, retroperitoneal, periportal, portacaval, pancreaticoduodenal, retrocrural, and lower cervical lymph nodes	No	<i>HRAS</i>
63	M	Carcinoma oropharynx + base of the tongue	Metastatic squamous cell carcinoma differentiation	T1 N2b M0	Alcohol, tobacco	Absent	Yes	<i>TP53</i> —poor response to the combination of cisplatin and 5-fluorouracil
55	M	Squamous cell carcinoma tongue	Moderately differentiated squamous cell carcinoma	pT2 N1 M0	Tobacco	Absent	Yes	<i>HRAS</i> —poor response to cetuximab
53	M	Surgically operated case of left hard palate mucoepidermoid carcinoma (recurrent high-grade tumor)	S/o recurrence of mucoepidermoid carcinoma	T4a N0 M0	Tobacco	Absent	Yes	<i>STK11</i> —recurrence
27	M	Squamous cell carcinoma—left mandible	Moderately differentiated SCC of the left lower alveolus, gingivobuccal sulcus invading to a depth of 6 mm	T4 N0 M0	Tobacco	Absent	No	<i>PTEN, RB1</i>
74	M	Squamous cell carcinoma of the left pyriform fossa	Moderately differentiated keratinizing squamous cell carcinoma	T1N0M0	Tobacco	Lungs, bones—S1, S2	No	No mutation

Contd...



Contd...

Age	Sex	Diagnosis	Histological type	TNM stage	Risk factors	Distant metastasis	Recurrence	Genes detected
17	F	Mucoepidermoid ca base of tongue	Mucoepidermoid carcinoma-with mucinous component mostly	T1 N0 M0	None	Absent	No	No mutation
73	M	Ca left oropharynx + pyriform fossa	Squamous cell carcinoma	T3 N0 M0	None	Absent	No	No mutation
50	M	Mucoepidermoid tumor-right parotid gland	Mucoepidermoid tumor		Tobacco	Absent	No	No mutation
40	F	Squamous cell carcinoma-left buccal mucosa	Squamous cell carcinoma grade II		None	Absent	No	No mutation
49	M	Recurrent carcinoma tongue	Squamous cell carcinoma	T3 N0 M0	Tobacco	Absent	Yes	No mutation
47	F	Papillary carcinoma thyroid	Papillary carcinoma thyroid with metastasis to lymph nodes with extracapsular extension	T4 N1 M0	None	Absent	No	QC failed

The *AIP* gene product is involved in aryl hydrocarbon receptor-mediated signaling, the mutation of which is familial.¹⁸

AIP mutation in tumors is associated with an earlier onset, aggressive behavior, and poor response to treatment.¹⁹

In our study, a germline mutation in *AIP* was detected in a patient with adenoid cystic carcinoma-right submandibular gland who is only 27 years old. It was, however, not a recurrent tumor and responded well to therapy. He did not have any metachronous tumors or a family history of cancer. The patient is now being followed up closely.

BRCA

This is a TSG, a mutation in which was seen in 5.5% of patients in this study.

This gene product is involved in the repair of chromosomal damage with an important role in the error-free repair of DNA double-stranded breaks.²⁰ Germline mutations resulting in upregulation of TSG function, when left unchecked by repair genes, cause cancer.

The tumors harboring this mutation show resistance to cisplatin and poly (ADP-ribose) polymerase (PARP) inhibitors.²¹

A germline mutation of *BRCA* was detected in a case of carcinoma nasopharynx in a patient with a synchronous BIRADS 2 lesion in the left breast and a family history of two sisters having had breast cancer. No changes were recommended to the standard treatment protocol.

SOMATIC MUTATIONS

HRAS

This is an oncogene, a mutation in which was seen in 16.67% of patients in this study. A mutation in this gene was seen in 11% of patients as seen in a genomic study on HNCs conducted by Agrawal et al.¹¹ The activated allele of *HRAS* triggers the expression of transcription factors associated with proliferation.²² The expression

of *HRAS* is associated with treatment resistance. This mutation predisposes to cetuximab resistance in tumors which express EGFR mutations.²³ A mutation in *HRAS* is more common in Indian as compared to western patients, with a strong association with betel quid and smoking.²⁴

In our study, *HRAS* was detected in a case of SCC with recurrence and resistance to cetuximab and a case of MUO.

PTEN

This is a TSG, a mutation in which was seen in 5.5% of patients in this study.

Phosphatase and tensin homolog is a protein encoded by the *PTEN* gene. A reduced number of copies in a pseudogene of *PTEN*, *PPTEN1* has been detected in cases of HNSCCs.²⁵ The reduced expression of *PTEN* is associated with tobacco exposure, higher relapse rates, poor response, and survival rates.^{26,27}

In our study, *PTEN* was detected in a 27-year-old male patient suffering from moderately differentiated SCC of GBS invading the lower alveolus, with a history of tobacco use, family history of cancer, who responded well to chemotherapy. A closer follow-up to detect recurrences and counseling for tobacco avoidance was advised.

STK11

This is a TSG, a mutation in which was seen in 5.5% of patients in this study. The *STK11* gene is also known as the liver kinase B1 (LKB1) gene. It regulates cell polarity and also functions as a TSG.²⁸

It was detected in a recurrent case of mucoepidermoid cancer in this study.

RB

This is a TSG. A mutation in *RB* was seen in 5.5% of patients in this study. The *Rb* gene negatively regulates the cell cycle by inhibiting cyclin-dependent kinases. It blocks the entry of cells from the G1 phase to the S phase. It is also involved in epigenetic changes as it

Table 3: Gene mutations in head and neck cancers detected in various studies

Agrawal et al. ¹¹	2011	<i>TP53, NOTCH1, RELN, SYNE1, EPHA7, FLG, HRAS, PIK3AP1, RIMBP2, SI</i> <i>HPV+ : E6/E7, EPHB3, UNC5D, NLRP12, PIK3CA, TM7SF3, ENPP1, NRXN3, MICAL2</i>
Stransky et al. ¹²	2011	<i>TP53, CDKN2A, SYNE1, CCND1, MUC16, USH2A, FAT1, LRP1B, ZFX4, NOTCH1</i> <i>HPV+ : E6/E7, PIK3CA, RUFY1, EZH2, CDH10, THSD7A, FAT4, KMT2D, ZNF676, MUC16</i>
Cadoni et al. ³¹	2012	<i>CYP2E1, CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, EPHX1, ALDH2, ADH, XRCC1, XPD, Cyclin D1, P53, P73</i>
Pickering et al. ^{13,14}	2013–14	<i>TP53, CDKN2A, FAT1, TP63, CSMD1, PIK3CA, FADD/CCND1, MAML1, EGFR, NOTCH1, TNK2, AKT1, HLA-A, CASP8, SRC</i>
Lin et al. ¹⁵	2014	<i>TP53, CDKN2A/B, ARID1A, SYNE1, ATG13, MLL2, PIK3CA, CCND1, NOTCH3, FGFR2</i>
Seiwert et al. ¹⁶	2015	<i>TP53, CDKN2A, MDM2, MLL2, NOTCH1, CCND1, PIK3CA, PIK3CB, UBR5, EGFR, FGFR2</i> <i>HPV+ : E6/E7, PIK3CA, TP63, PIK3CB, FGFR3, NF1/2, SOX2, ATM, FLG, MLL3</i>
The Cancer Genome Atlas ⁷	2015	<i>TP53, CDKN2A, let-7c, PIK3CA, FADD, FAT1, CCND1, NOTCH1/2/3, TP63, EGFR, SMAD4, FGFR1</i> <i>HPV+ : E6/E7, PIK3CA, TP63, TRAF3, E2F1, let-7c, NOTCH1/3, FGFR3, HLA-A/B, EGFR</i>

HPV+: HPV-positive cancers

induces the enzyme histone deacetylase.²⁹ Mutations in RB have therapeutic implications. Pharmacologically targeting the RB-E2F pathway and the use of EGFR antagonists in the absence of E2F regulation are being currently studied.³⁰

P53

This is a TSG, a mutation of which was seen in 16.67% of patients in this study. A mutation in p53 was seen in 17% of cases in a study conducted by Lin et al., whereas various other studies show the incidence of this mutation in HNCs to be 66 to 94%.^{7,11–16,31} The inheritance of only one functional copy of the *TP53* gene results in the development of tumors in early adulthood, a disorder known as Li–Fraumeni syndrome.³² The presence of the germline mutation predisposes individuals to second cancer, around 30 years after the first. As it undergoes a germline mutation, it has also been identified in the normal epithelial cells in patients suffering from SCC.^{24,33} It is the most commonly mutated gene in human cancers.³⁴

This gene can also be modified by chemicals, radiation, viruses, or other mutagens.

Such modifications increase the likelihood of uncontrolled cell division. It is known to be associated with tobacco exposure, increased relapse rates, short survival, poor differentiation, and resistance to 5-FU and cisplatin.^{24,35,36} A mutation in p53 was detected in a recurrent case of SCC showing poor response to a combination of cisplatin and 5-FU. There is intratumor heterogeneity with respect to p53 mutations in tobacco-associated cancers. Therefore, targeted therapy in these cases might not be very successful.³⁷

GNA11

A mutation in this gene was seen in 5.5% of patients in this study.

The *GNA11* gene, along with its paralogues *GNAQ* and *GNAS*, code for proteins of the transmembrane G-protein-coupled receptors and their products. A mutated *GNA11* gene is seen to be associated with metastatic disease and poor prognosis. These are more common than *GNAS* and *GNAQ* mutations.³⁸ A mutation in *GNA11* was seen in a patient with alveolar sarcoma of the tongue which had metastasized to the lungs. The patient received chemoradiation. He was also treated with transarterial chemoembolization (TACE). He died of cardiac arrest during an embolization procedure for the tumor site bleed.

CONCLUSION

The current study identified somatic mutations in 9 out of 18 cases using a 48-gene panel of targetable mutations and 2 germline mutations. Though preliminary, the study appears to validate early genomic testing of HNC cases to modify treatment protocols (personalized medicine).

The discrepancy between mutations identified in the literature and our study, argues for the development of a larger customized panel of genes to be validated for personalized medicine in HNC cases in our setting. The rationale for a limited panel as against whole-genome sequencing would be the economic viability of the latter option.

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APPENDIX 1: METHODOLOGY FOR SEQUENCING

A total of 8 to 10 µg of intact double-stranded high-quality gDNA, quantified fluorometrically (Qubit) for each sample with an absorbance ratio (A 260/280) of ~1.8 to 2.0, and a minimum concentration of 100 to 500 ng/µL was utilized.

Work Flow for Sequencing Using Illumina Sequencing Platform

A custom panel (consisting of 48 genes) was designed by Triesta labs for this study based on target enrichment methods. A probe set containing pairs of oligonucleotides specific to the targeted regions were hybridized to each genomic DNA sample. The Amplicons were generated by connection of bound oligonucleotides by extension and ligation using a DNA polymerase and ligase, followed by PCR amplification. The PCR primers were flanked by index sequences for sample multiplexing as well as common adapters for sequencing cluster generation.

After PCR cleanup, the library quality was assessed on a Bioanalyzer Tape station (Agilent Technologies, Santa Clara,

California, USA). Each sample library was normalized according to the manufacturer's instructions, and equal volumes were pooled to generate the final sequencing library.

NGS Data Analysis

Each pooled library was sequenced on the Illumina sequencing instrument using paired-end sequencing design (2 × 100). The image processing and fastq file generation from raw read data were done with CASAVA version 1.8.2 and RTA version 1.17.28 (Illumina). The alignment of paired-end raw reads to the human hg19 genome assembly was performed with a BWA algorithm. The variant calling and coverage analysis was performed by an in-house bioinformatics pipeline (GATK/STRELKA) at Triesta laboratories. The variants were filtered and annotated by VARMINER and OncoMD software. The variants with a global minor allele frequency >1.0% were removed. The Integrative Genomics Viewer 16 (Broad Institute, Cambridge, Massachusetts, USA) was used to visualize variants against the reference genome.