

# Molecular Profile of Tongue Cancer in an 18-Year-Old Female Patient With No Recognizable Risk Factor

Melvin A. Ambele, PhD; Michael S. Pepper, PhD; Marlene B. van Heerden, NDipTech;  
Willie F. P. van Heerden, DSc 

**Background:** The occurrence of oral tongue squamous cell carcinoma (TSCC) in nonsmoking young adults, especially females, has increased. Yet, there is no clear evidence to support the existence of any single determinant. This case reports the presence of TSCC in an 18-year-old female with no recognizable risk factor for oral cancer development.

**Methods:** Histological examination and p16 immunohistochemistry were performed. Formalin-fixed paraffin-embedded sections were prepared from resected tissue and DNA was extracted for molecular OncoScan analysis.

**Results:** Histological and immunochemical analysis showed a p16-negative poorly differentiated keratinizing squamous cell carcinoma. OncoScan analysis of this tumor revealed a high confidence TP53:p.R213\*:c.637C>T somatic mutation as well as copy number alterations of chromosomal regions including gains of 1p, 3q, 5p, 7p, 8p, 8q, 11q, 15q, 17q, and 20p, and losses on 1p, 3p, 18q, and 22q.

**Conclusion:** The TP53:p.R213\*:c.637C>T mutation detected is indicative of a genetic predisposition to cancer and it is the first to be reported in TSCC in a nonsmoking young adult.

**Key Words:** Oral cancer, squamous cell carcinoma, OncoScan analysis, copy number variation, loss of heterozygosity.

**Level of Evidence:** Case report

## INTRODUCTION

There is an increase in the incidence of tongue squamous cell carcinoma (TSCC) among younger adults aged between 18 and 44 years. The typical associated risk factors such as alcohol and/or tobacco exposure in often are not present.<sup>1–5</sup> Females are more frequently affected.<sup>2,3,6</sup> The etiology of TSCC in these nonsmoking young individuals not related to human papillomavirus is unclear.<sup>7</sup> A recent review of the available molecular data on TSCC in both young and old patients showed no significance differences between the two groups despite the presence of several molecular markers and chromosomal abnormalities.<sup>8</sup> It has however been suggested that an increase of exposure to known risk factors like environmental smoke could be a driving factor of TSCC in young adults. A higher than expected risk of oral cancer development in females exposed to tobacco only has also been reported.<sup>9</sup>

The ability to metabolize harmful products resulting from environmental exposure to tobacco smoke varies among individuals, and this may play a role in the

development of TSCC in some nonsmokers.<sup>10</sup> The presence of TSCC in nonsmoking young female patients may suggest a genetic predisposition to the development of this cancer. A molecular assay (Affymetrix OncoScan) was performed on tumor tissue from a young female patient presenting with TSCC in the absence of typical associated risk factors to identify possible genetic aberrations (copy number variation and loss of heterozygosity [LOH]) characterizing the tumor in this patient.

## CASE REPORT

An 18-year-old female presented with an ulcerative lesion on the left lateral border of the tongue. An incision biopsy was performed and diagnosed as a poorly differentiated keratinizing squamous cell carcinoma (Figs. 1 and 2). The patient has no known risk factors and no history of previous malignancy or a family history of head and neck carcinoma. A hemiglossectomy with left modified radical neck dissection was performed. Lymph nodes from levels I, II, III, and V were received. The tumor was resected with clear margins although one positive lymph node was found at level V. The pathological staging was pT2N1M0. The patient received postoperative radiotherapy and is still tumor-free after 2 years.

The Affymetrix OncoScan assay was used to identify possible cancer-associated genetic changes in this tumor that could potentially contribute to the development of oral carcinogenesis and progression of TSCC in this patient. The whole genome view from OncoScan analysis showed detectable aberrations in certain chromosomes (Fig. 3). In summary, gains in some regions on chromosome 1p, 3q, 5p, 7p, 8p, 8q, 11q, 15q, 17q, and 20p along

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From the Department of Immunology, and SAMRC Extramural Unit for Stem Cell Research and Therapy, Faculty of Health Sciences (M.A.A., M.S.P.), Institute for Cellular and Molecular Medicine; and the Department of Oral Pathology and Oral Biology (M.A.A., M.S.P.), School of Dentistry, Faculty of Health Sciences, University of Pretoria, Pretoria, South Africa.

Conflict of interest: None.

Send correspondence to Willie F. P. van Heerden, Department of Oral Pathology and Oral Biology, School of Dentistry, University of Pretoria, PO Box 1266, Pretoria, South Africa. Email: willie.vanheerden@up.ac.za

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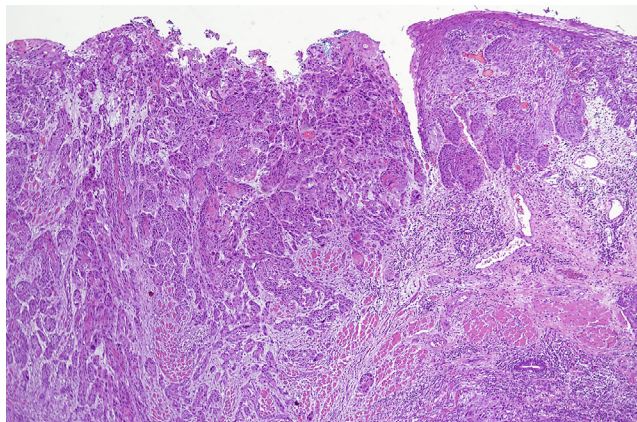


Fig. 1. Low power photomicrograph showing an ulcer containing squamous cell carcinoma islands in the base. A small fragment mucosa is present with associated carcinoma development (x40).

with losses on 1p, 3p, 18q, and 22q as well as LOH on 1p, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 10q, 11p, 13q, 14q, 15q, 16p, 17p, 17q, 21q, and 22q were detected in the genome (Fig. 2). No aberration was detected on chromosomes 6, 9, 12, 18, or 19. A high confidence somatic point mutation TP53:p.R213\*:c.637C>T was detected. This mutation is a clinical variant substitution mutation (nonsense) of the base cytosine by thymine (CGA>TGA) at position 637, resulting in a stop codon at the amino acid arginine. Some key genes associated with oral cancer development detected in this tumor are summarized in Table I.

## DISCUSSION

Smoking is known to be associated with TP53 mutation in many head and neck squamous cell carcinomas (HNSCC). The presence of TSCC in the nonsmoking young female patient we describe here likely points to either a genetic predisposition, an activating mutation on oncogenes, inactivating mutation on tumor suppressor genes (TSGs), or a combination of the above which leads to the development of this cancer. OncoScan analysis of

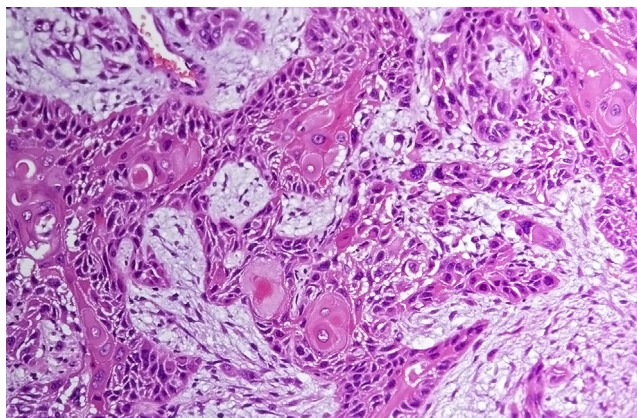


Fig. 2. Photomicrograph showing the features of a poorly differentiated keratinizing squamous cell carcinoma in the patient described herein (x400).

the tumor revealed a TP53:p.R213\*:c.637C>T mutation. This mutation has been reported in many cases to be a pathogenic germline mutation for Li-Fraumeni syndrome (autosomal dominant inheritance), which is a hereditary cancer-predisposing syndrome and is also present in many different types of Li-Fraumeni syndrome-associated malignancies.<sup>16-23</sup> The presence of the TP53 mutation in this nonsmoking teenager suggests the existence of a nonsmoking TP53 associated mutation which is most likely responsible for the development of this TSCC.

The complete deletion of GSTT1 belonging to glutathione S-transferases family of enzymes that play a functional role in detoxifying harmful products in tobacco smoke such as hydrocarbons, may have served as a driving factor as it increases the individual's susceptibility to developing TSCC in cases where the patient was exposed to environmental smoke. Gains on 3q, 8q, and 11q as well as a loss on 3p in the genome of this tumor are some of the genetic changes which are consistent with HNSCC<sup>27</sup>. Strikingly, there was no deletion in the 9p21 region containing the CDKN2A gene (p16). This observation has also been reported in other younger patients with HNSCC, despite this region being consistently altered in older cohorts.<sup>28</sup> However, reports have suggested that it is rather p16 methylation than deletion which is more common in HNSCC patients younger than 40 years.<sup>28</sup>

The amplification in this patient's tumor of PIK3CA, CARD11, and SRSF2 and the loss of SMAD2 are mutations that have been reported in other cases of TSCC.<sup>13</sup> Furthermore, amplification of 3q in the region containing the squamous lineage transcription factors TP63 and SOX2, and the oncogene PIK3CA are some of the molecular changes reported in HNSCC which may contribute to the development of the tumor in this patient. Amplification of MYC and JUN oncogenes may also play a contributing role in the development of this tumor because JUN is known to increase the activity of cdk2 kinase and G1/S cell cycle progression independent of mitogen.<sup>11</sup> Conversely, LOH in TSGs, which is also considered a key event in the development of cancer, was detected in APC, RAF1, SLC2A2, and TOB1. LOH events on these genes have been previously reported to occur at higher frequencies of 73%, 71%, 41%, and 75%, respectively, in oral cancer cases.<sup>29</sup>

This patient showed no detectable aberrations in the NOTCH (NOTCH1, NOTCH2, and NOTCH3), EGFR, RAS (HRAS, KRAS, and NRAS), FADD/CCND1, FAT1, PTEN, and CDKN2A genes which are commonly mutated in HNSCC, although mutations in some of these genes have been detected in other young nonsmoking adults with TSCC.<sup>29</sup> For example, a significantly high copy number of the EGFR gene was detected in patients with TSCC with the majority of them being nonsmokers.<sup>28,30</sup> The absence of an EGFR mutation in this case may suggest that this is not necessary for TSCC formation and therefore, the genetic alterations in this case differ from the classical progression model of HNSCC development in nonsmoking young adults as proposed by Toner and O'Regan.<sup>28</sup> This progression model proposed that an amplification of EGFR as a first event in a normal mucosa will lead to hyperplasia, followed by a p53 mutation to cause dysplasia. CCND1 gain in the

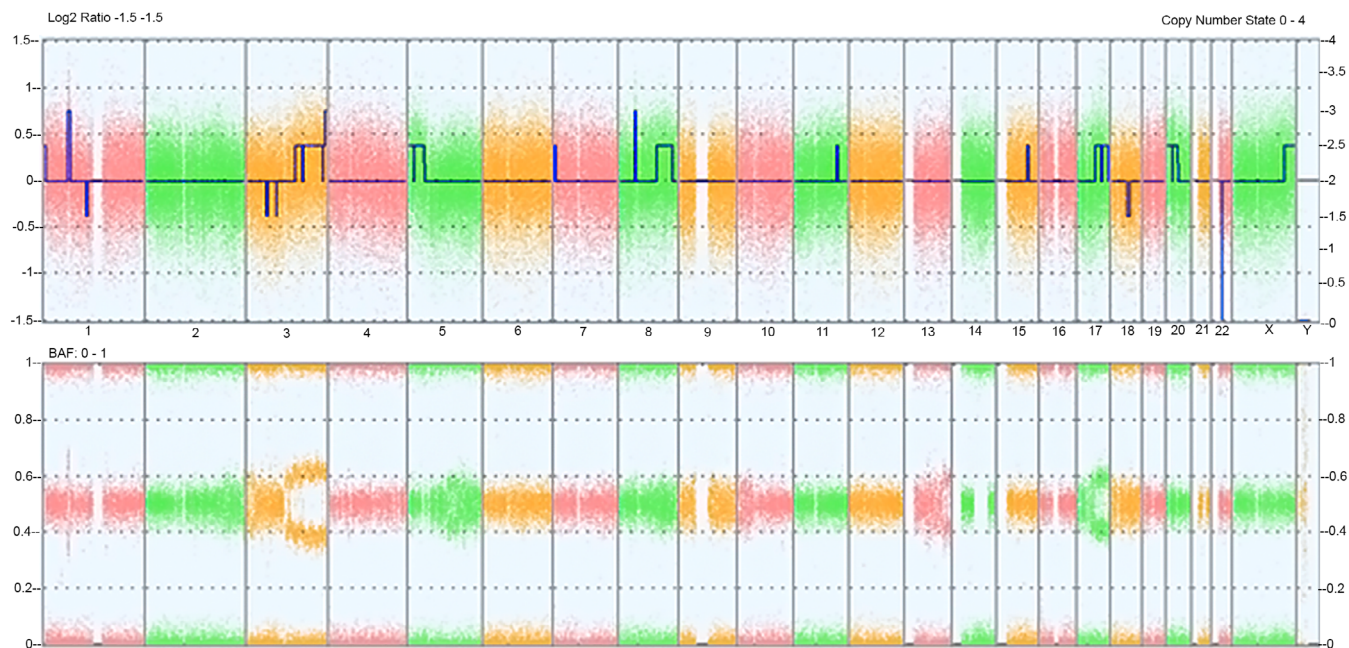


Fig. 3. OncoScan Whole Genome View of the tumor. Log2 ratio is used for interpretation of copy number state (CN) while B-allele frequency (BAF) together with log2 ratio are used to interpret loss of heterozygosity (LOH). A CN state of 2 (diploid) is represented by a straight line along a log2 ratio of zero, while log2 values above and below zero represented by the blue lines denote gain (CN > 2) and loss (CN < 2), respectively, in that region. The three distinct tracks at 1, 0.5, and 0 in BAF represent the BB, AB, and AA allele, respectively. Lack of AB calls (absence of track at 0.5) represents a LOH in that region.

dysplastic lesion then causes carcinoma in situ (CIS) and a loss of PTEN in CIS leads to the development of squamous cell carcinoma in a young nonsmoking adult.<sup>28</sup>

In summary, the TP53:p.R213\*:c.637C>T mutation reported here is the first to be described in TSCC. This mutation is known to be present in individuals with Li-Fraumeni syndrome with a high-risk genetic susceptibility for developing cancer. Alternatively, acetaldehyde, a metabolite of alcohol, is also known to primarily cause this C>T mutation,<sup>31</sup> which is a common mutation in many cancers.<sup>29</sup> A complete deletion of GSTT1 involved in the

detoxification of harmful tobacco smoke products may have contributed to the development of this tumor if the patient was exposed to environmental smoke. Also, LOH in APC, RAF1, and TOB1, with a reportedly higher frequency of occurrence in oral cancer, may also have initiated or promoted the development of this tumor. Furthermore, amplification of the JUN oncogene known to stimulate G1/S cell cycle progression independent of mitogen may also have contributed in the development of this TSCC. Hence, even though all the above aberrations, which are key events in cancer development, could potentially have played an important role in the onset and progression of this tumor, further studies should be performed to confirm the current findings.

TABLE I.

The Mutational Landscape of Some Known Cancer Associated Genes Detected in This Tumor.

Chromosomal Arm with Aberrations	Affected Genes with Possible Role in Oral Cancer
1p	JUN (gain) <sup>11</sup>
3p	RAF1 (Cn-LOH) <sup>12</sup>
3q	Gain (TP63, SOX2, and PIK3CA <sup>13</sup> ), SLC2A2 (CNG-LOH) <sup>12</sup>
5q	APC (Cn-LOH) <sup>12</sup>
7p	CARD11 (gain) <sup>13</sup>
8q	MYC (gain) <sup>14,15</sup>
17p	TP53:p.R213*:c.637C>T mutation <sup>16-23</sup>
17q	SRSF2 (gain) <sup>13</sup> , TOB1 (CNG-LOH) <sup>12</sup>
18q	SMAD2 (loss) <sup>13</sup>
22q	GSTT1 (loss) <sup>24-26</sup>

Cn-LOH and CNG-LOH represent copy number neutral-LOH and copy number gain-LOH, respectively.  
LOH = loss of heterozygosity.

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