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Research Article

# Involvement of *TP53* Mutations in the Occurrence of Oral Cavity Cancers in Senegalese Patients

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## ABSTRACT

The Head and neck cancers are a group of heterogeneous tumors, among which oral cavity cancers appear to be increasingly common. It has been reported that solid tumors are not genetically stable; accordingly, identification of the genetic factors involved in oral carcinogenesis could aid in preventing the spread of oral cancers. Therefore, in this study, we aimed to determine the involvement of *TP53* mutations in oral cavity cancers in Senegalese patients. Samples from 40 patients with cancer were included in this study. Blood samples were collected from controls. Tissues were collected from each patient during biopsy after obtaining their consent. DNA extraction, polymerase chain reaction, and sequencing were performed to obtain sequences. The chromatograms were compared to the reference sequence using Mutation Surveyor software. In addition, the COSMIC database, Mutation Taster, PolyPhen-2, and SIFT software were used to predict the pathogenicity of mutations. MEGA, BioEdit, and DnaSP software were used to analyze polymorphisms and genetic diversity. A total of 105 mutations, including 36 pathogeneses, were identified. Codon 196 was found to be under positive selection. Therefore, it was concluded that *TP53* mutations can occur early in oral carcinogenesis. This study provides valuable insights for the early detection and treatment of oral cancers in Senegalese patients.

**Keywords:** Cancer; Oral cavity; *Tp53*; Oral cancer; Head and neck cancers; Mutations

## INTRODUCTION

One Head and Neck Cancers (HNCs) comprise a group of heterogeneous tumors that are generally located in multiple anatomical sites, including the oral cavity, oropharynx, hypopharynx, and larynx. Cancers of the oral cavity are among the most common cancers [1]. In Senegal, these cancers represent 1.76% of all malignant tumors. Each year, averages of 177 Senegalese patients are diagnosed with cancer of the oral cavity and 114 die [2].

The prognosis of patients with these cancers has not improved significantly in recent years, despite the strengthening of diagnostic and therapeutic approaches. This failure is mainly due to the marked clinical heterogeneity of the biological behavior of these tumors as well as late diagnosis, lymph node metastases, and recurrences, leading to a high mortality rate for HNCs [3]. Therefore, the identification of tumor-specific vulnerabilities is a major objective of cancer research. Progress is being made owing to considerable advances in cancer genomics and an increasingly detailed knowledge of the genetic landscape of the most common tumor types. In particular, tumor suppressor genes harbor a large number of genetic events, including nonsense mutations, missense mutations, splicing mutations and indels of varying sizes scattered throughout the gene.

Some of these molecular defects involve alterations in the *TP53* gene [4], which is a tumor suppressor gene that regulates cell division. The analysis of somatic mutations in *TP53* is now widely used in clinical trials to stratify patients based on *TP53* status. In addition, new drug trials target either wild-type or mutant *TP53* to activate a *TP53* antitumor response. Therefore, *TP53* is rapidly becoming an integral part of many therapeutic and preventive strategies in clinical practice [5].

As research into the genetic origin of oral cancer has progressed, it

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has become widely accepted that solid tumors are not genetically stable. Thus, the identification of the genetic factors involved in oral carcinogenesis could provide a basis for anticipating and potentially preventing the spread of oral cancers. These observations underscore the need for a more complete understanding of *TP53* alterations. Therefore, the objective of this study was to determine the involvement of *TP53* mutations in oral cancer in Senegalese patients.

## MATERIALS AND METHODS

### Samples

This study was conducted on Senegalese patients with histologically proven oral cancer treated at the Maxillofacial and Stomatology Department of the Aristide Le Dantec Hospital in Dakar. After obtaining approval from the Research Ethics Committee of Cheikh Anta Diop University, also in the Senegalese capital, the study was conducted from February 2021 to July 2022. The inclusion criteria were as follows: a) Senegalese, b) diagnosed with cancer of the oral cavity, c) regularly followed in a hospital center, and d) signed the consent form. A total of 40 patients with oral cavity cancer and 52 controls were included in this study and numbered accordingly. Cancerous tissue was collected from each patient during biopsy. Blood samples were also collected from the control subjects.

### DNA extraction, polymerase chain reaction and sequencing

DNA was extracted from tissues and blood using the Zymo Research Kit, following the manufacturer's instructions for each biological material. The region from exon 5 to exon 6 of the gene was amplified using the following primers: Forward: 5'-GTTTCTTTGCTGCCGTCTTC-3' and Reverse: 5'-CTTAACCCCTCCTCCCAGAG-3'. A total reaction volume of 25 µl containing 12.5 µl Master Mix, 0.5 µl of each primer, 9.5 µl MilliQ water, and 2 µl cDNA was used. The polymerase chain reaction was performed under the following conditions: initial denaturation at 94°C for 7 min; 35 cycles of denaturation at 94°C for 1 min, hybridization at 64°C for 1 min, and elongation at 72°C for 1 min; final elongation step at 72°C for 10 min. After visualization under blue light, the PCR products were purified and sequenced with an ABI Big Dye Terminator Sequencing Ready Reaction Kit and an ABI PRISM 3730xl sequencer (Applied Biosystems, Foster City, CA, USA).

### Genetic analysis

**Search for mutations:** The raw sequences were submitted to Mutation Surveyor (version 5.0.1, https://softgenetics.com/products/mutation-srveyor/), which compares chromatograms with a reference sequence to determine the presence of any mutation and its position relative to the gene. The software offers excellent accuracy and sensitivity as well as low false-positive and false-negative rates in DNA analysis. In this case, we used the reference sequence of the *TP53* gene available in GENBANK (accession number NG_017013.2). The mutations found by Mutation Surveyor were submitted to the Single Nucleotide Polymorphism (dbSNP) database to determine if they had already been reported. If any variation is known and listed in this database, the position, nature, and effect on the protein of that gene are given.

The same sequences were submitted to the International Agency for Research on Cancer (IARC) database. This database is now hosted by the National Cancer Institute (NCI, IARC version R20, July 2019, available at https://*tp53*.isb-cgc.org). We considered a mutation to be previously identified if it was identified in oral cavity cancers, and all other mutations were considered new.

### Mutation prediction

The nucleotide sequences were translated into protein sequences using MEGA software version 7.0.14 [6] to predict the effect of missense mutations on the stability and functionality of the p53 protein. These protein sequences were submitted to the Catalog of Somatic Mutation in Cancer (COSMIC)[1] database, MutationTaster[2], Polymorphism Phenotyping v 2 (PolyPhen-2)[3], and Sorting Intolerant from Tolerant (SIFT)4 software.

COSMIC identifies coding variants that lead to different types of cancers and integrates all somatic coding mutations collected in the database. The mutation was predicted to be pathogenic if the score was above 0.5, and neutral if the score was below 0.5.

MutationTaster is designed to predict the functional consequences of amino acid substitutions, intronic and synonymous alterations, short insertion and/or deletion mutations, and variants that cross intron-exon boundaries. It gives a score from an amino acid substitution matrix (Grantham matrix) that takes into account the physicochemical properties of the amino acids and the score substitutions according to the degree of difference between the original and new amino acid. The scores varied from 0.0-215.

PolyPhen-2 is an automated tool for predicting the potential impact of an amino acid substitution on the structure and function of human proteins. Mutations with probability scores >0.95 are expected to be "probably damaging", while scores between 0.5 and 0.95 are expected to be "potentially damaging", and scores < 0.5 are classified as "benign".

SIFT, based on sequence homology, sorts intolerant amino acid substitutions from tolerant substitutions, and predicts whether an amino acid substitution in a protein will have a phenotypic effect. Scores lower than 0.05 are predicted to be "deleterious" and those greater than or equal to 0.05 are predicted to be "tolerant".

### Codon selection test

The sequences obtained were carefully checked, aligned, and corrected using BioEdit version 7.1.9 [7]. Alignment was used to highlight similarities between sequences by determining the positions of probable deletions or insertions. Codon selection was determined for *TP53* exons 5 and 6 in cancer tissues using MEGA version 7.0.14. Estimates of the number of synonymous (dS) and non-synonymous (dN) substitutions were calculated for each codon. The dN-dS test statistic was used to detect codons under positive selection. Significance was set at $p<0.05$.

## RESULTS

### Nature and position of mutations

The entire region, from exon 5 to exon 6, was obtained from the chromatograms. Analysis of these sequences revealed 105 mutations, of which 27 (25.71%) were already listed in the dbSNP database and 76 (73.78%) were novel (Supplementary Table). In the IARC database, 18 mutations (17.42%) have been reported in oral cavity tumors. Mutations were found in 71.42% of patients. Of the 86 mutations found in exons 5 and 6, 62 (72.09%) were missense, 22 (25.56%) were silent, and two (2.32%) were nonsense. In exon 6, two silent mutations (c.562C>T and c.672G>A) change the splice site, and the insertion of two base types (c.661_662insG and c.661_662insT) leads to a frame shift.

### Prediction of missense mutations

Table 1 shows the pathogenicity of the non-synonymous mutations. In all, 36 mutations were strictly pathogenic according to the software programs we used, six were strictly benign, and 19 did not have the same prediction according to the four software programs. However, most (c.478A>G p.160Met>Val, c.589G>A p.197Val>Met, c.589G>T p.197Val>Leu, c.604C>A p.202Arg>Ser, c.642 T>G p.214His>Gln, c.642T>A p.214His>Gln, c.645T>G p.215Ser>Arg, c.645T>A p.215Ser>Arg, c.650T>A p.217Val>Glu, c.650 T>G p.217Val>Gly, c.663G>T p.221Glu>Asp, c.663G>C p.221Glu>Asp, c.664C>A p.222Pro>Thr, and c.665C>G p.222Pro>Arg) could potentially cause disease because they were predicted to be deleterious by at least two of the four software programs. The frequency of pathogenic mutations Table 2 showed that 77.14% of the patients had a G to A substitution at positions c.638 and c.644. Furthermore, these data show that exon 6 contained more mutations than exon 5 in Senegalese patients with oral cancer.

### Codon selection test

After alignment and correction, 413 sites with 58 sequences were obtained, including 23 control sequences and 35 sequences from cancer tissues. In total, 20 sequences were eliminated because of their high diversity and ambiguity. Codon 196 (CGA), which encodes arginine, was under positive selection (p=0.009). This test indicates the superiority of missense mutations over silent mutations in this codon in patients with oral cancers. The results are presented in Table 3.

**Table 1:** Pathogenicity of mutations.

| Acid amines affected | COSMIC (score) | Mutation taster (score) | Polyphen-2 (score) | SIFT (score) |
|---|---|---|---|---|
| c.379T>C p.127Ser>Pro | pathogenic (1) | deleterious (74) | probably damaging (1) | deleterious (0.00) |
| c.451C>A p.151Pro>Thr | pathogenic (1) | deleterious (38) | potentially damaging (0.75) | deleterious (0.00) |
| c.452C>A p.151Pro>His | pathogenic (0.99) | deleterious (77) | potentially damaging (0.88) | deleterious (0.00) |
| c.463A>G p.155Thr>Ala | neutral (0.11) | deleterious (58) | benign (0.001) | tolerated (0.60) |
| c.478A>G p.160Met>Val | pathogenic (0.98) | deleterious (21) | probably damaging (0.97) | tolerated (0.25) |
| c.524G>C p.175Arg>Pro | pathogenic (0.99) | deleterious (103) | probably damaging (1) | deleterious (0.00) |
| c.526T>C p.176Cys>Arg | pathogenic (1.00) | deleterious (180) | probably damaging (1) | deleterious (0.00) |
| c.527G>A p.176Cys>Tyr | pathogenic (0.99) | deleterious (194) | probably damaging (1) | deleterious (0.00) |
| c.527G>C p.176Cys>Ser | pathogenic (1) | deleterious (112) | probably damaging (0.99) | deleterious (0.03) |
| c.550G>A p.184Asp>Asn | pathogenic (0.99) | benign (23) | benign (0.011) | tolerated (0.32) |
| c.555C>G p.185Ser>Arg | neutral (0.20) | benign (110) | potentially damaging (0.59) | tolerated (0.15) |
| c.565G>C p.189Ala>Pro | pathogenic (0.99) | deleterious (27) | probably damaging (0.99) | deleterious (0.01) |
| c.576G>C p.192Gln>His | NE | benign (24) | benign (0.021) | deleterious (0.03) |
| c.578A>C p.193His>Pro | pathogenic (0,99) | deleterious (77) | probably damaging (1) | deleterious (0.00) |
| c.578A>C p.193His>Pro | pathogenic (0,99) | deleterious (77) | probably damaging (1) | deleterious (0.00) |
| c.579T>C p.193His>Pro | pathogenic (0,99) | deleterious (77) | probably damaging (1) | deleterious (0.00) |
| c.579T>G p.193His>Gln | NE | deleterious (24) | probably damaging (1) | deleterious (0.00) |
| c.584T>A p.195Ile>Asn | pathogenic (0,99) | deleterious (194) | probably damaging (1) | deleterious (0.00) |
| c.587G>C p.196Arg>Pro | pathogenic (0,99) | deleterious (103) | probably damaging (1) | deleterious (0.00) |
| c.587G>T p.196Arg>Leu | pathogenic (0,99) | deleterious (102) | probably damaging (1) | deleterious (0.00) |
| c.589G>A p.197Val>Met | pathogenic (0,97) | deleterious (21) | probably damaging (0.98) | tolerated (0.05) |
| c.589G>T p.197Val>Leu | pathogenic (0,98) | deleterious (32) | benign (0.30) | tolerated (0.06) |
| c.595G>A p.199Gly>Arg | pathogenic (0,99) | deleterious (125) | probably damaging (1) | deleterious (0.00) |
| c.603G>T p.201Leu>Phe | neutral (0.12) | benign (22) | benign (0.021) | tolerated (0.11) |
| c.604C>A p.202Arg>Ser | NE | deleterious (110) | probably damaging (0.78) | tolerated (0.14) |
| c.605G>A p.202Arg>His | neutral (0.02) | benign (29) | benign (0.004) | tolerated (0.31) |
| c.623A>G p.208Asp>Gly | pathogenic (0.99) | deleterious (94) | probably damaging (1) | deleterious (0.01) |
| c.624C>G p.208Asp>Glu | pathogenic (0.99) | deleterious (45) | probably damaging (0.99) | deleterious (0.01) |

| | | | | |
|---|---|---|---|---|
| c.625A>G p.209Arg>Gly | neutral (0.02) | benign (125) | benign (0.008) | tolerated (0.39) |
| c.626G>A p.209Arg>Lys | neutral (0.05) | deleterious (26) | benign (00) | tolerated (0.93) |
| c.628A>G p.210Asn>Asp | neutral (0.01) | benign (23) | benign (0.42) | tolerated (0.21) |
| c.629A>T p.210Asn>Ile | neutral (0.02) | benign (149) | benign (0.001) | tolerated (0.46) |
| c.630C>A p.210Asn>Lys | neutral (0.01) | benign (94) | benign (0.56) | tolerated (0.30) |
| c.631A>C p.211Thr>Pro | pathogenic (1) | deleterious (38) | probably damaging (1) | deleterious (0.00) |
| c.632C>T p.211Thr>Ile | pathogenic (1) | deleterious (89) | probably damaging (0.99) | deleterious (0.00) |
| c.637C>G p.213Arg>Gly | pathogenic (0.96) | deleterious (125) | probably damaging (1) | deleterious (0,01) |
| c.638G>A p.213Arg>Gln | pathogenic (0.99) | deleterious (43) | probably damaging (1) | deleterious (0.01) |
| c.640C>G p.214His>Asp | pathogenic (0.90) | deleterious (81) | probably damaging (1) | deleterious (0.02) |
| c.640C>A p.214His>Asn | pathogenic (0.90) | deleterious (68) | probably damaging (1) | deleterious (0.02) |
| c.641A>G p.214His>Arg | pathogenic (0.98) | deleterious (68) | probably damaging (0.99) | deleterious (0.03) |
| c.642T>G p.214His>Gln | neutral (0.11) | deleterious (24) | probably damaging (0.96) | tolerated (0.35) |
| c.642T>A p.214His>Gln | neutral (0.23) | deleterious (24) | probably damaging (0.96) | tolerated (0.35) |
| c.643A>G p.215Ser>Gly | pathogenic (0.99) | deleterious (56) | probably damaging (1) | deleterious (0.00) |
| c.644G>A p.215Ser>Asn | pathogenic (0.99) | deleterious (46) | probably damaging (1) | deleterious (0.00) |
| c.645T>G p.215Ser>Arg | neutral (0.45) | deleterious (110) | probably damaging (1) | deleterious (0.00) |
| c.645T>A p.215Ser>Arg | neutral (0.40) | deleterious (110) | probably damaging (1) | deleterious (0.00) |
| c.647T>G p.216Val>Gly | pathogenic (1) | deleterious (109) | probably damaging (1) | deleterious (0.00) |
| c.650T>A p.217Val>Glu | neutral (0.45) | deleterious (121) | probably damaging (0.99) | deleterious (0.01) |
| c.650T>G p.217Val>Gly | neutral (0.41) | deleterious (109) | probably damaging (0.51) | deleterious (0.00) |
| c.652G>A p.218Val>Met | pathogenic (0.98) | deleterious (21) | probably damaging (0.99) | deleterious (0.00) |
| c.653T>G p.218Val>Gly | pathogenic (0.99) | deleterious (109) | probably damaging (1) | deleterious (0.00) |
| c.655C>G p.219Pro>Ala | pathogenic (0.99) | deleterious (27) | probably damaging (1) | deleterious (0,00) |
| c.658T>G p.220Tyr>Asp | pathogenic (0.99) | deleterious (160) | probably damaging (1) | deleterious (0.00) |
| c.658T>C p.220Tyr>His | pathogenic (0.99) | deleterious (83) | probably damaging (1) | deleterious (0.00) |
| c.661G>A p.221Glu>Lys | pathogenic (0.99) | deleterious (56) | probably damaging (0.97) | deleterious (0.00) |
| c.662A>T p.221Glu>Val | pathogenic (0.99) | deleterious (121) | probably damaging (0.95) | deleterious (0.00) |
| c.662A>C p.221Glu>Ala | pathogenic (0.99) | deleterious (107) | probably damaging (0.98) | deleterious (0.00) |
| c.663G>T p.221Glu>Asp | pathogenic (0.80) | deleterious (45) | benign (0.13) | deleterious (0.00) |
| c.663G>C p.221Glu>Asp | pathogenic (0.80) | deleterious (45) | benign (0.13) | deleterious (0.00) |
| c.664C>A p.222Pro>Thr | pathogenic (0.90) | deleterious (38) | benign (0.10) | deleterious (0.02) |
| c.665C>G p.222Pro>Arg | NE | deleterious (103) | potentially damaging (0.70) | tolerated (0.09) |
| c.667C>G p.223Pro>Ala | pathogenic (0.99) | deleterious (27) | probably damaging (0.99) | deleterious (0.00) |
| c.670G>C p.224Glu>Gln | neutral (0.5) | benign (29) | benign (0.04) | tolerated (1) |

**Table 2:** Frequency of deleterious mutations.

| Mutations | % Patients |
|---|---|
| c.379T>C p.127Ser>Pro | 2.85 |
| c.451C>A p.151Pro>Thr | 2.85 |
| c.452C>A p.151Pro>His | 2.85 |
| c.524G>C p.175Arg>Pro | 2.85 |
| c.526T>C p.176Cys>Arg | 5.71 |
| c.527G>A p.176Cys>Tyr | 5.71 |
| c.527G>C p.176Cys>Ser | 2.85 |
| c.565G>C p.189Ala>Pro | 11.42 |
| c.578A>C p.193His>Pro | 8.57 |
| c.579T>C p.193His>Pro | 11.42 |
| c.579T>G p.193His>Gln | 8.54 |
| c.584T>A p.195Ile>Asn | 5.71 |
| c.587G>C p.196Arg>Pro | 20 |

| | |
|---|---|
| c.587G>T p.196Arg>Leu | 8.57 |
| c.595G>A p.199Gly>Arg | 14.28 |
| c.623A>G p.208Asp>Gly | 40 |
| c.624C>G p.208Asp>Glu | 48.57 |
| c.631A>C p.211Thr>Pro | 45.71 |
| c.632C>T p.211Thr>Ile | 31.42 |
| c.637C>G p.213Arg>Gly | 37.14 |
| c.638G>A p.213Arg>Gln | 77.14 |
| c.640C>G p.214His>Asp | 65.71 |
| c.640C>A p.214His>Asn | 11.42 |
| c.641A>G p.214His>Arg | 14.28 |
| c.641A>G p.214His>Arg | 14.28 |
| c.643A>G p.215Ser>Gly | 65.71 |
| c.644G>A p.215Ser>Asn | 77.14 |
| c.647T>G p.216Val>Gly | 68.57 |
| c.652G>A p.218Val>Met | 34.28 |
| c.653T>G p.218Val>Gly | 54.28 |
| c.655C>G p.219Pro>Ala | 54.28 |
| c.658T>G p.220Tyr>Asp | 25.71 |
| c.658T>C p.220Tyr>His | 45.71 |
| c.661G>A p.221Glu>Lys | 57.71 |
| c.662A>T p.221Glu>Val | 60 |
| c.662A>C p.221Glu>Ala | 8.57 |
| c.667C>G p.223Pro>Ala | 48.55 |

**Table 3:** Codon selection test.

| Codon | Triplet | dS-dN | P-value |
|---|---|---|---|
| 196 | CGA | 5.220384 | 0.009636 |

## DISCUSSION

The objective of this study was to determine the involvement of the *TP53* mutations in oral cancers in Senegal. A total of 105 mutations were found in 40 patients, of which 76 were novel and 27 were previously reported in the databases. This number is higher than the 36 mutations found by Manoharan et al., [8] in a study of 44 patients from Sri Lanka, India. Polten et al., [9] found 39 mutations in 26 patients. Therefore, the reported frequency of *TP53* mutations in HNCs varies considerably. This may reflect the techniques used to detect mutations, the regions of *TP53* analyzed, and differences in mutation rates between anatomical sites [10]. In our study, 72.09% of the mutations were missense mutations, 25.56% were silent mutations, and 2.32% were mutations that led to a stop codon. In exon 6, two silent mutations altered the splice site, and one insertion resulted in a reading frame shift. These results are consistent with Olivier et al., [11], who found that 75% of *TP53* mutations are missense substitutions. They also found other alterations including insertions and frame shifts (9%), nonsense mutations (7%), and silent mutations (5%). Our results are also consistent with Bouaoum et al., [12], who found that 73.16% of *TP53* mutations are missense mutations, 9.06% are frameshift mutations, 8.17% are nonsense mutations, 3.62% are silent mutations, and 2.4% are splice mutations.

These results also concur with Bennett et al., [13], who found that missense mutations are common in *TP53* tumor suppressor genes. Most of these mutations are clustered in the DNA-binding domain of the protein, encompassing exons 5 and 6. The most frequent mutations are known to cooperate with oncogenes for cell transformation [14]. These observations have led to the hypothesis that missense mutations are preferentially selected in cancers because they carry specific pro-oncogenic functions [15]. However, the mutation spectrum is heterogeneous with a mixture of missense, nonsense, frameshift and splice mutations. Truncating mutations result in non-functional proteins and are caused by nonsense and splice site mutations, as well as frameshift deletions and insertions. Recently, truncation mutations in exon 6, observed at higher than expected frequencies in several cancer types, have been validated to confer gainof function by promoting metastasis [16].

Interestingly, frameshift mutations are significantly more frequent in HNCs than in other cancers [11]. Immunohistochemical analyses performed on tumors as well as cell lines show that these mutations lead to a complete absence of p53 protein expression and can therefore be considered p53-null [17]. Similarly, exonic mutations near intron/exon boundaries can interfere with the proper splicing of a gene. Cells use this splicing phenomenon to produce more than one protein from a single transcribed gene

with different and often opposing functions [18]. The diverse repertoire of proteins in a mammalian cell far exceeds the total number of genes in the genome, challenging the classical "one gene, one enzyme" hypothesis [19].

The involvement of the *TP53* gene in oral cancer was further confirmed by the large number (71.42%) of patients with at least one mutation in this gene and the pathogenicity of 36 different mutations. Mutation frequencies ranging from 2.85-77.14% were observed in patients with a higher mutation frequency in exon 6. These results are consistent with those of Zhou et al., [20], who found *TP53* mutations in 75.6% of patients with oral cancer. Shi et al., [21] identified 36 point mutations in *TP53* and 25 of those mutations abolished the normal function of p53. Thus, these pathogenic mutations may impair the ability of p53 to bind with a high affinity to its cognate DNA-binding sites.

The higher mutability in exon 6 may be due to differences in geographic or ethnic origins. For example, in the United States, most mutations are detected in exon 7, whereas in Sweden and Japan, they are detected in exon 8 [22,23]. Thus, distinguishing driver mutations from random transient mutations is a major challenge. In contrast, frequent mutations are undeniably driver mutations that are selected during neoplasia [17]. The wide distribution of non-functional p53-related point mutations, as well as stop codon mutations and frameshift insertions in the *TP53* gene in oral cells, emphasizes the critical role of p53 in carcinogenesis [21]. As a result, the selection for nonsense mutations is stronger than that for missense mutations in cancer, while silent mutations are counter-selected [14]. In our study, truncation mutations such as p.213R>* and p.220Tyr>*, and frameshift mutations such as p.221Glu>Glyfs*4 and p.221Glu>Valfs*4, which lead to loss of protein function, could be important selection factors in oral carcinogenesis.

The selection of codon 196 in Senegalese patients confirms the essential role of p53 in carcinogenesis. We noted that 28.57% of our patients had either an arginine-to-proline change or an arginine-to-leucine change at this codon. This suggests that all mutations occurring in this codon are missense mutations, resulting in the substitution of arginine by another codon. Although codon 175 (p.176Cys>Arg) is a *TP53* hotspot, it was found in only one of our patients, conferring a selection advantage to codon 196. An imbalance in codon 196 (p.Arg196 * and p.Arg196Gln) was observed by Leroy et al., [17]. These differing results could be explained by the fact that cancer development could be attributed to the disruption of critical pathways that are not common to all neoplasms [24]. However, the mutational status of *TP53* in tumors suggests a preference for mutations in the Arg allele [25,26]. Thus, mutations at codon 196 could be considered penetrant mutations in oral carcinogenesis in Senegalese patients. With this set of variants in hand, it might be possible to paint a precise landscape of *TP53* residues that are essential for the tumor suppressor effect of this gene.

## CONCLUSION

This study provides a molecular basis for the early detection and treatment of oral cancers in Senegalese patients, although it is limited by a small sample size and some sequencing errors in the laboratory. However, further studies with larger sample sizes and more accurate laboratory techniques are required to confirm our results. Multiple alterations in the *TP53* gene were detected in this study, suggesting that *TP53* may be a useful molecular marker in oral cancers. Many of these alterations are pathogenic and may lead to cancer. We are moving toward molecular medicine, where specific mutations in *TP53*, in combination with other tumor characteristics, will determine the clinical response. Thus, exploring the contributions of different variants will be very laborious, but certainly essential, to understand how each mutation affects the function of the protein. Therefore, the focus should be on developing studies on *TP53* mutations in clinical trials. Large trials with hundreds of patients randomized to different treatment protocols are required. This could provide sufficient statistical power to determine the exact impact of mutations in this gene on the response to treatment as well as overall survival. These data will also help to define the tumors and clinical settings in which *TP53* may be a specific target for new therapeutic approaches.

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