Universidad Europea

# GRADUATION PROJECT

## *Degree in* Dentistry

# PHYSIOLOGICAL EXPRESSION IN THE ORAL CAVITY OF THE MOST COMMON MUTATED GENES IN ORAL CANCER

**Madrid, academic year 2022/2023**

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**ABSTRACT:**

**Introduction**: Cancer is a life-threatening disease. It's the second cause of mortality. It's an international problem, and because of that, it's one of the most researched topics. In dentistry, cancer is the most serious disease. Oral cancer mostly presents as oral squamous cell carcinoma (OSCC) and affects the cells of the epithelial tissue. Early diagnosis and staging are essential. We wanted to study the physiological expression of the most common mutated genes in oral cancer to establish a relationship between gene expression and the disease. **Objectives**: The main objective of this research is to make dentists aware that they are the first to identify a potentially malignant lesion. Our secondary objective is to highlight the use of informatics data. Our tertiary objective is to know if the main mutated gene is expressed in healthy tissue. **Materials and methods**: Variable sources: the Catalogue of Somatic Mutations in Cancer (COSMIC), the ICGC Data Portal, the cBioPortal for Cancer Genomics, the National Cancer Institute: GDC Data Portal, the Haniffa Lab, and the human protein data base. **Results**: Dentists have a major role; they are the first to diagnose patients with potential oral cancer. Informatic data sharing and access to the history of cases permit advances and enhance survival. Without informatics, our research couldn't take place. The most common mutated gene in oral cancer is TP53, but the highest expression of TP53 takes place in the keratinocytes (not in epithelial cells). Additionally, the expression of TP53 was similar in both healthy and pathological samples. **Conclusion**: The most common mutated gene was TP53. Other genes, such as PIK3CA, NOTCH1, CDKN2A, TTN, FAT1, and CASP8, were sometimes mutated, but the impact of these mutations on oral cancer remains uncertain. More research needs to be conducted.

**KEYWORDS:** dentistry; cancer; OSCC; genes; TP53

**RESUMEN:**

**Introducción**: El cáncer es una enfermedad potencialmente mortal. Es la segunda causa de mortalidad. Es un problema internacional y por eso uno de los temas más investigados. En odontología, el cáncer es la enfermedad más grave. El cáncer oral se presenta sobre todo como carcinoma oral de células escamosas (CCEO) y afecta a las células del tejido epitelial. El diagnóstico precoz y la estadificación son esenciales. Queríamos estudiar la expresión fisiológica de los genes mutados más comunes en el cáncer oral para establecer una relación entre la expresión génica y la enfermedad. **Objetivos**: El objetivo principal de esta investigación es concienciar a los odontólogos de que son los primeros en identificar una lesión potencialmente maligna. Nuestro objetivo secundario es destacar el uso de los datos informáticos. Nuestro objetivo terciario es saber si el principal gen mutado se expresa en el tejido sano. **Materiales y métodos**: Fuentes variables: COSMIC, ICGC Data Portal, cBioPortal, GDC Data Portal, Haniffa Lab y el portal de datos de proteínas humanas. **Resultados**: Los dentistas desempeñan un papel fundamental; son los primeros en diagnosticar a los pacientes con un posible cáncer oral. El intercambio de datos informáticos y acceso al historial de los casos permiten avanzar y mejorar la supervivencia. Sin informática, nuestra investigación no podría llevarse a cabo. El gen más mutado en cáncer oral es el TP53, pero la mayor expresión de TP53 tiene lugar en los queratinocitos (no en las células epiteliales). Además, la expresión de TP53 era similar tanto en las muestras sanas como en las patológicas. **Conclusiones**: El gen mutado más común fue el TP53. Otros genes como PIK3CA, NOTCH1, CDKN2A, TTN, FAT1 y CASP8, estaban mutados en ocasiones, pero las repercusiones sobre cáncer oral siguen siendo inciertas. Es necesario realizar más investigaciones.

**PALABRAS CLAVE:** odontología; cáncer; OSCC; genes; TP53

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## 1. INTRODUCTION:

### 1.1. Cancer is a lethal disease

Cancer is a life-threatening disease; it's the 2nd leading cause of death around the world. It is known that there are 277 different forms of cancer, which makes it difficult to manage (1, 2).

The prevalence of cancer is continuously increasing (1, 2, 3). It is a major health problem as it affects a large portion of the population (1, 2). 1/3 of people will suffer from cancer, so it is a big challenge in the field of medicine (1, 2). It requires a concise diagnosis to permit effective treatment (1). The management of oral cancer is really complex as it involves aesthetic and functional criteria (2). The disease is really hard to manage due to its presentation (4).

In women, the form of cancer that take place with the highest prevalence is breast cancer, followed by cancers of the airways (lungs and bronchi), digestive system (colon and rectum), uterus, and thyroid glands (1); in men, the biggest percentage of cancers occur in the prostatic gland, followed by those of the airways (lung and bronchi), digestive system (colon and rectum), urinary tract, and thyroid gland.(1) This disease is also very unfair as it can attack young people (1,7). The most common type in children is blood cancer (leukemia), followed by lymphatic system cancers and brain cancers (1). Chemical components in our daily lives as well as during pregnancy have a major role and impact in cancer as they can influence the formation of cancerous cells (1, 3, 5).

We can affirm that some substances, such as alcohol or tobacco, increase the risk of cancer occurring (1, 3, 6, 7, 8, 16). Genetics also have a huge place in the course of the disease (9-13). In some countries, consumption of areca nut products is also contributing to the incidence of the disease (7, 14, 15, 16).

In addition, it is known that some microorganisms can interact with the human body, such as viruses (EBV, HIV, and HPV, especially types 16 and 18) and bacteria that could influence cancer formation (1, 3, 6).

Statistically, in the USA, 39.5% of men and women will be diagnosed with cancer at some point during their lifetimes (based on 2015–2017 data). (1).

« In 2020, an estimated 1,806,590 new cases of cancer will be diagnosed in the United States and 606,520 people will die from the disease » (1, 7).

Looking at these numbers (based on one year), we can think that around 30% of the affected people will die of cancer, but in reality, it is much more complex to evaluate the numbers since many types of cancer occur over several years (2, 4). Graphic shows Incidence rate in the oral cavity, per 100 000 (Annex 1) (15).

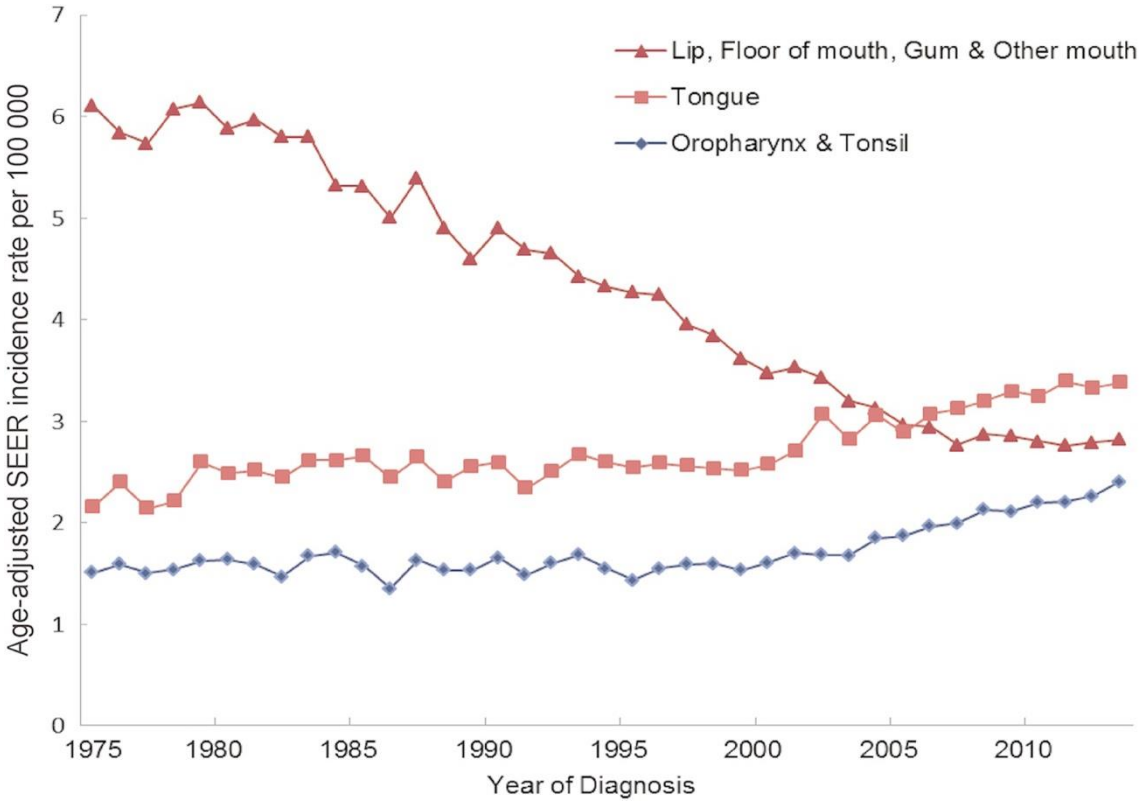


***Figure 1**: Age-adjusted incidence site of oral cancer per 100 000, Update on oral and oropharyngeal cancer staging*

### 1.2. Oral cavity anatomy

The mouth, or oral cavity, is an organ defined as the space that extends from the anterior part of the lips to the oropharyngeal isthmus; it extends from the lips anteriorly to the faucial pillars posteriorly (5). It is delimited by the cheeks laterally, the palate superiorly, and the floor of the tongue inferiorly (5, 6).

The functions of the oral cavity are vital to our health since they enable us to perform breathing, eating, and swallowing (5). It also plays an important role in phonation (1, 5). The oral cavity is the first part of the digestive system and contains structures such as salivary glands that permit the lubricating of food and easiest digestion (5).

The tongue is a major organ of the oral cavity; it's a muscular structure that is attached to the floor of the mouth; the apex of the tongue is mobile and allows for the production of sounds while speaking (5). The tongue also contains taste receptors. It is an organ of relevance to our topic as it is one of the most affected areas in the oral cavity by oral squamous cell carcinoma (OSCC) (3, 5).

### 1.3. Oral cancer definition

Oral cancer is a type of cancer that develops in the spaces of the head and neck (4). It's a cancer that forms in the tissues of the oral cavity (the mouth) or the oropharynx (the part of the throat at the back of the mouth) (2, 4). Oral cancer is the 6$^{th}$ most common cancer worldwide, and 9/10 are oral squamous cell carcinomas (6, 8). It is diagnosed by a dentist or a doctor who examines patients for signs and symptoms of precancerous and/or cancerous lesions (2, 4). It produces different symptoms depending on the individual (2, 6). In the majority of cases, cancer is present as a plaque, a patch, or an ulcerous lesion that doesn't heal for 14 days (2, 6, 16). Most rarely, it can be present as a cyst (2). In addition to this, patients could experience pain while eating, chewing, swallowing, and speaking (6). Patient might refer a sudden weight loss (6). Most cancers that develop in the mouth develop in the epidermis as oral squamous cell carcinomas (OSCC) (7, 16). The severity of the disease and prognosis are associated with the stage of cancer when the diagnosis is made (7).

Staging must be made using the TNM system (2, 6, 16):

Stage 0: Carcinoma in situ

Stage 1: A smaller tumor that has not grown out of the organ in which it began.

Stages 2 and 3: A larger tumor that has grown outside the organ in which it began to extend to the nearby tissues

Stage 4: Spread of cancer to distant areas of the body via the blood or lymphatic system (metastatic spread) » (6, 7; 16).

A biopsy should be required to confirm the final diagnosis of cancer and to allow for staging and reporting (7). Other types of oral cancer exist, but they occur infrequently in comparison to OSCC (7-9). It is really common in oral cancer to find as a sign of the disease the presence of a fissure, an indurated mass, a red or white lesion, or a chronic ulceration associated or not with lymph node enlargement (7, 9, 16). Carcinomas develop in any part of the mouth, with a higher frequency on the posterior lateral border of the tongue as well as on the floor of the mouth (7, 16). It is also commonly found in the lower lip space as an ulceration or a crust (7, 16).

Oral cancer is treated in different ways, such as with irradiation or surgery, or with immunotherapy and chemotherapy, which are given when the stage of the disease is advanced, when metastasis is present, or to prevent the spread of the disease to distant organs (6, 7, 8, 16).

### 1.4. Genes mutations accumulation

In 1957, Francis Crick established the so-called "dogma of molecular biology": the DNA is the molecule from which an RNA is synthesized (a process known as transcription), and from the RNA a protein is synthesized (a process known as translation) (17). Therefore, DNA is the molecule that carries the genetic information that is transmitted to the offspring of an organism (or from cells to daughter cells inside the same organism) (17). Proteins are, on the other hand, the functional units inside a cell. From the "dogma of molecular biology, it is easy to understand that mutations in DNA may affect the formation of proteins (17). However, it is important for this work to understand that not all DNA mutations affect the formation of proteins. Indeed, most of the mutations in the genome are neutral for protein function (17).

A gene is the functional basic unit of DNA, and gene mutation is essential for life, as is the force of evolution or organism variation among species. However, gene mutation is also responsible for the rise of many diseases, including carcinogenesis (6–9). The impact of the environmental factors (alcohol, tobacco, sun, etc.) may increase the inhibition of some defenses of the organism, such as tumor suppressor genes (6,9,10). « Cancer occurs due to the progressive accumulation of abnormalities in cellular DNA, which, in growth, give advantage to cancer cells and facilitate metastatic dissemination » (11). Oral cancer is mostly present as an oral squamous cell carcinoma (OSCC) (11, 12, 14, 16, 19). In order to take place, cancer has to go through multiple phases of alterations. The biggest alterations occur in 2 groups: tumor suppressor genes and oncogenes (11, 16). Cancer is known to take place due to an accumulation of abnormalities in cellular DNA (11, 16). They can either be overexpressed or deactivated through deletions, mutations, or methylation (12, 13). There is new research that is conducted every year in molecular biology to help with diagnosis and personalized treatment (12). The success of treatment mostly depends on the type of mutation that is present in the cancerous cells (6). A number of particular genes are yet known to be involved in the process of carcinogenesis, but a lot of them remain to be studied and are of unknown impact (12). In order to discover new correlations, researchers often compare normal and tumoral tissue (12). One of the most common and important tumor suppressor genes to be commonly mutated in various cancer types is TP53, but many other individual factors interact differently in each cancer's history (1, 6, 12, 13, 19).

### 1.5. Oral squamous cell carcinoma (OSCC)

OSCC is the most common type of cancer to occur in the oral cavity (6, 8, 11, 12, 14, and 18). Oral squamous cell carcinoma is a lethal pathology of the epithelium of the mouth and the most common type of all oral cancers (6, 11, 12, 14, 18 and 19). There is a mean of 350.000 cases diagnosed each year all over the world (11, 14).

Unfortunately, the number of people affected and dying from it hasn’t been decreasing much, no matter the research and advances in cancer management (14). The big

problem with OSCC is that in the initial phase of the disease, it goes unnoticed most of the time and is discovered later on with the complications that come with it (advanced stage, metastasis) (14, 16). For that, performing regular screening and adequate exploration of the most common area to be affected (lips, tongue, floor of the mouth) by a dentist or maxillofacial surgeon should be performed in order to catch the disease in the earliest period and offer the best treatment (14, 16). Oral squamous cell carcinoma can be accompanied by or start with a precancerous lesion in the form of leukoplakia (Annex 2 shows leukoplakia undergoing transformation into a malignant lesion) or erythroplakia (14, 16). Depending on the extent, cancer could extend to the cervical lymph node or to distant areas (6, 14). The survival rate of oral squamous cell carcinoma in a 5-year window is between 40 and 50%. Additionally, the prognosis could depend on the patient's age and the presence of other chronic conditions, as well as the lymph node involvement, size, and location of the tumor (6, 16). Asia is the continent with the highest incidence of oral cavity and oropharyngeal cancers and the highest mortality rate (8).

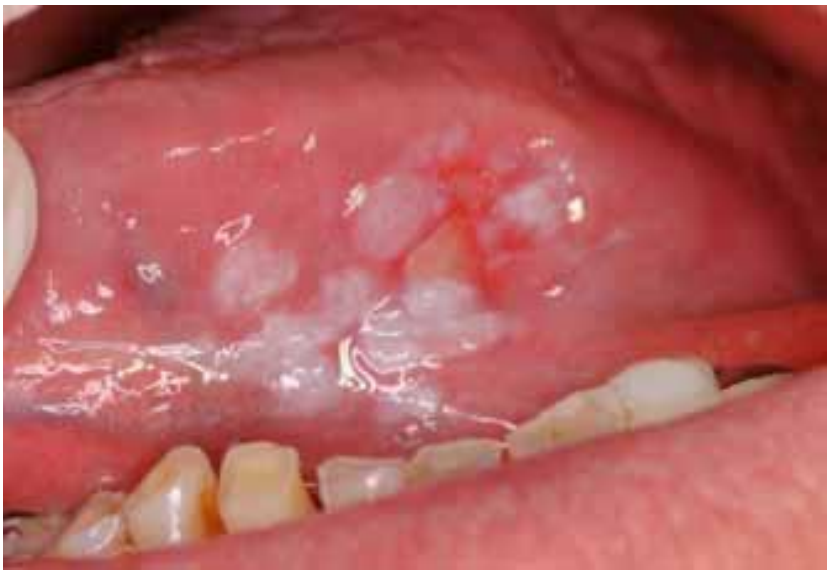

***Figure 2:** Leukoplakia of the lateral part of the tongue, undergoing into malignant transformation*

In our research, we are going to analyze different genes and understand their prevalence and impact on oral cancer, particularly in the case of oral squamous cell carcinoma.

## 2. OBJECTIVES:

1) Make dentists aware that they are the first to identify a potentially malignant lesion and understand that early diagnosis is the key to an improved prognosis.

2) Highlight the use of informatics data in cancer research; in our research, which are the main mutations implicated in oral cancer?

3) Understand whether the main mutated gene is expressed or not in oral healthy tissue.

Hypothesis: The most common mutated gene in OSCC is TP53, and it is expressed mostly in non-healthy tissue.

## 3. MATERIALS & METHODS:

Articles have been researched in Mendeley, finding the same or similar articles. Understanding the relevance to our topic, additional sources of material and information have been added from data-bank websites.

### 3.1. The most common mutated genes in OSCC (head and neck/oral cavity):

#### 3.1.1. Data from the catalogue of somatic mutation in cancer (COSMIC):

COSMIC is the largest and most understandable support for seeking the influence of genetic mutation in cancer. The sources are of two types: expert curation data and genome-wide screen data.

To know the most common mutated genes in OSCC, the selection of "tools" to access the "cancer browser" was performed to start conducting our research. In order to narrow our study to the oral cavity only, and particularly to the OSCC type of cancer, the following filters were used: 1) Tissue selection: "Upper aerodigestive tract"; 2) Sub-tissue selection: "Head and neck"; 3) Histology selection: "Carcinoma"; 4) Sub-histology selection: "squamous cell carcinoma".

1. First, we looked in the “Genes” section. Results of 2121 samples were obtained. This gives rise to the most common mutated genes in oral squamous cell carcinoma.
2. Then, we looked in the “Distribution section/Summary”. Results for 2115 samples were obtained. This allows us to specify the types of mutations OSCC presents in the genes that are mutated.
3. As we advanced in our research we found out that TP53 was the most commonly mutated gene, so we wanted to particularly understand the type of mutation this gene presents.

- We looked in the “Genes” section. Results of 2121 samples were obtained. We then entered the TP53 section to see details only for this gene. (All genes)
- We looked in the “Distribution section/Summary”. Results for 1445 samples were obtained. (TP53 only)

### 3.1.2. Data from the ICGC Data Portal:

The international cancer genome consortium data portal permits access to many tools related to cancer data, such as searching, visualizing, or downloading information; it is open-source and constituted by an active community.
In the quick search area, "TP53" was written. 1) Selecting "mutations" gave us access to cancer distribution in the 6575 donors affected (all types of cancer). 2) In the filter table, "head and neck" was typed to reduce it to the tissue of interest for our research; 3) "HNSC-US" selection to obtain data for squamous cell carcinoma only (528 donors). 4) Selection of "mutations" (between the 3 sections: summary, donors and mutations) to access the most frequently mutated genes in OSCC.

### 3.1.3. Data from the cBioPortal for Cancer Genomics:

The software is based in the Center for Molecular Oncology at the Memorial Sloan Kettering Cancer Center (MSK). It stores various types of data, such as mutations, DNA/RNA/mRNA/microRNA, protein and phosphoprotein levels, DNA methylation, etc.
In the section: select studies for visualization and analysis, we typed 1) "head and neck". 2) "Head and neck squamous cell carcinoma: oral squamous cell carcinoma" (last on the list). 3) "Explore selected studies". This will give us access to the mutated genes in 40 samples.

### 3.1.4. Data from the National Cancer Institute: GDC Data Portal

It is a strong platform that allows research and bioinformatics in the field of cancer by providing a source for the analysis of data. Of the techniques used, we can find enrichment analysis, cohort comparison, set operations, and oncogrid.
In the search section (e.g., BRAF...), we typed "TP53" and selected 1) "GN TP53". In the cancer distribution area, we selected: 2) "Open to Exploration" (on the right). It gives us access to the "Search Cases" section with the possibility of selecting one or multiple sites. 3) Primary site" (45 more): we selected the areas of interest that are present in the oral cavity: "other and unspecified parts of the tongue", "other and ill-defined sites in the lip, oral cavity, and pharynx", "floor of the mouth", "other and unspecified parts of

the mouth", "tonsil", "base of the tongue", "oropharynx", "gum", "lip" and "palate". It includes a total of 284 cases.

### 3.1.5. Data from intOgen: Integrative onco genomics:

It is a foundation that is based on the extirpation of mutational sequences from donor patients. Of the methods used, we can find mutational count, clustering of mutations, protein sequencing and structuring, functional domain identification...
We searched HNSC for: 1) Head and neck squamous cell carcinoma. 2) Selection of mutational cancer driver: "Plot". We had access to the most recurrently mutated gene in the head and neck of 397 samples.

### 3.1.6. Data from the gingiva health disease: Haniffa lab

It's a data base that references the cells of the human body. A healthy reference helps diagnose pathological conditions. It also applies diverse techniques to understand how the immune system works and maintains health.
1. On the home page, we selected: 1) "gingiva_health-disease.H5AD". 2) "Paper Labels" selection. Analyzed with a scatterplot technique.
2. In the search section, the following are entered: "TP53"; "KRT14"; "KRT6A"; "CDKN1A"; "NOTCH1"; "PIK3CA"; the technique used is a scatterplot. Each gene is analyzed independently to see their interactions in a diseased tissue.

## 3.2. <u>The expression of proteins in the oral tissue (normal tissue):</u>

The human protein database was used in order to extract data from the knowledge resource. We searched for information about TP53 using the following steps: 1) "TP53: tumor protein p53" selection; 2) "tissue" selection; 3) "ASSAYS: protein expression" selection; 4) "oral mucosa" selection. Another step was to find new findings for tp53 using the following steps: 1) "TP53: tumor protein p53" selection; 2) "RNA tissue" selection.

## 4. RESULTS:

Oral cancer is a deadly disease that affects people all over the world. As a result of this, understanding the different factors that interact in the genetics of cancer is primordial. Among the oral cavity, OSCC is the most relevant malignant disease and the most common type of cancer in the oral cavity. We wanted to know which genes are most commonly mutated in oral cancer. Bioinformatic analysis has become a very powerful tool in the research field. For this purpose, we decided to use common bioinformatic tools to answer our research question. We started our research by using COSMIC resources (see materials and methods). Cosmic is the largest catalogue of somatic mutations in cancer. This tool has been developed by the Sanger Institute for Cancer Research.

### 4.1. The most common mutated genes in OSCC

#### 4.1.1.1. The most common mutated genes in OSCC (COSMIC)

We began our research with the use of the COSMIC database, for which we opened the cancer browser and came across the finding of the most common mutated gene in oral squamous cell carcinoma (for a more detailed description of the filters applied, see materials and methods). The results obtained are shown in Figure 3.

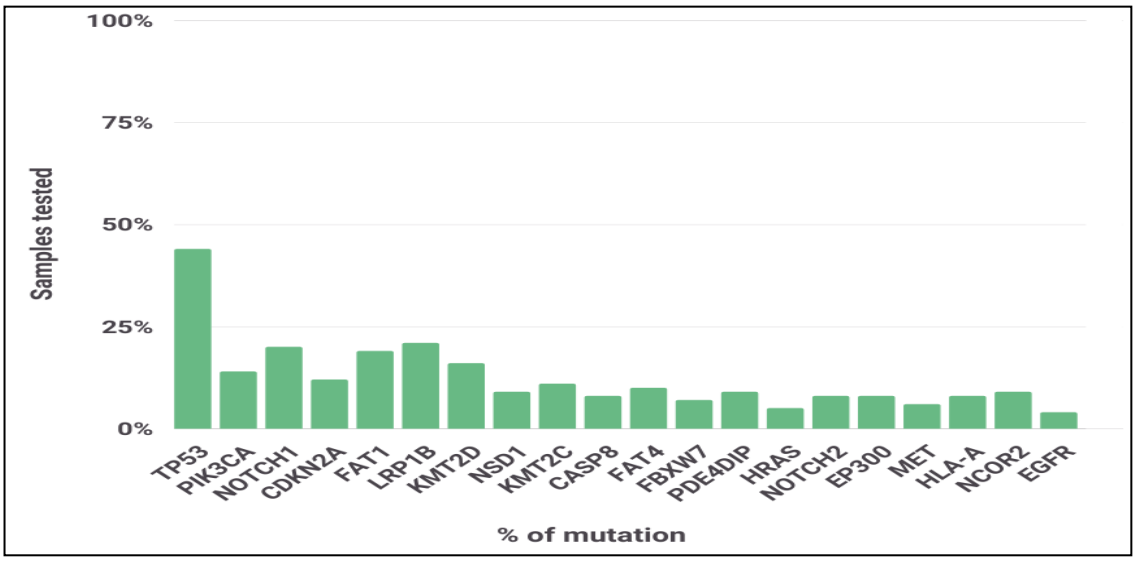


***Figure 3*: The most common mutated genes in OSCC**. *COSMIC (2022) cancer browser*. *Green bars represent the percentage of cancer samples that has a mutation for different genes annotated in the X axis.*

Using the COSMIC browser, we found that among the most common mutated genes in OSCC were those illustrated in the graph table above. (Figure 3): 21% of the cases were mutated for the LRP1B gene; 20% of the cases presented a mutation for the NOTCH1 gene; the KMT2C gene was mutated in 16% of the cases; but incontestably, the TP53 gene was the gene that was most commonly mutated, being present in 44% of the cases, almost in half of the cases, which is coherent with the findings of the authors of the articles we read to introduce the topic (6, 12, 13, 19, 20) (Figure 3).

#### 4.1.1.2 The most common types of mutations in OSCC (COSMIC)

We then found it interesting to know what types of mutations were present in the most common mutated genes in oral cancer; and we used another type of data from the COSMIC database. Results are illustrated in Figure 4.

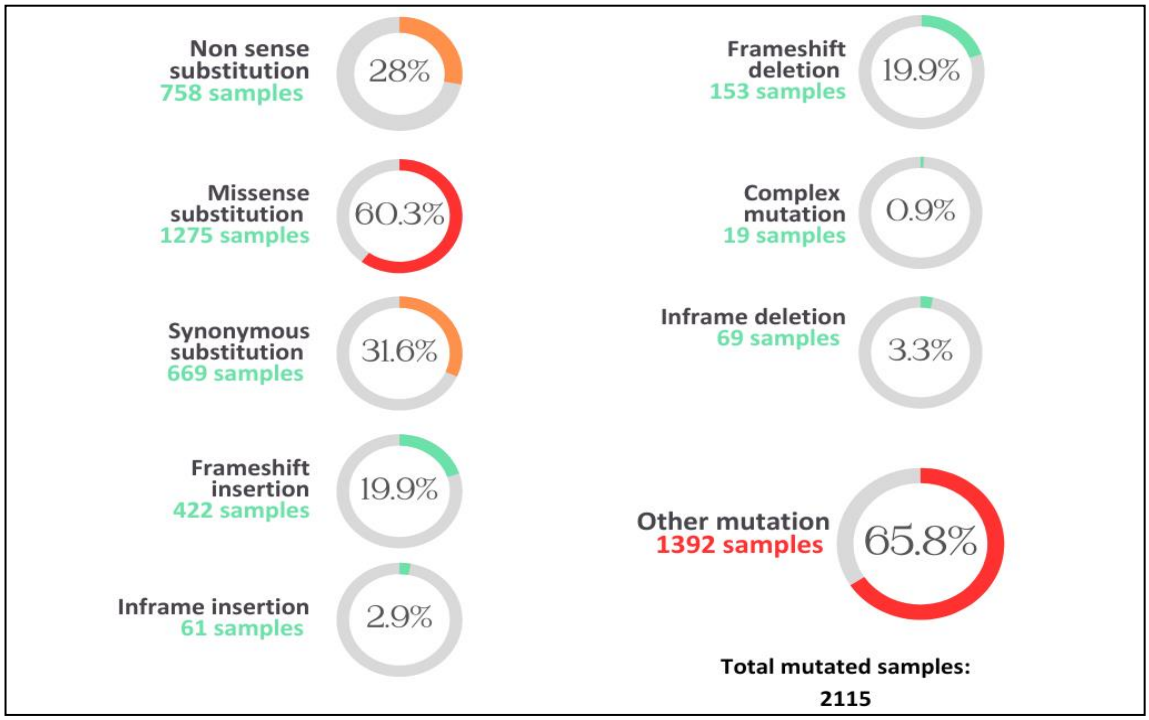


***Figure 4*: The different types of mutation in the most common mutated genes in OSCC.** *COSMIC cancer browser. Higher type represented in a red section, medium type in orange section and low type in green section.*

The most common type of mutation encountered in samples with OSCC was of the missense substitution type (60.3%), and they represented more than half of the mutation that were able to be identified. Other mutations present in medium amounts

were of the synonymous substitution type (31.6%) and 65.8% of the mutations weren't identified. Some samples present an accumulation of mutations, which is why the total of mutated samples doesn't correspond with the total number of the types of mutations that exist. These results are simplified in Figure 4 above.

### 4.1.2. The most common mutated genes in OSCC (ICGC Data Portal)

Continuing our research, we decided to confirm the data obtained with the Cosmic Database about the most frequently mutated genes in OSCC. For this purpose, we moved to and used data from the ICGC Data Portal. The International Cancer Genome Consortium (ICGC) is an international consortium that provides tools to analyze cancer genomes. It is probably the most ambitious research project since the human genome project.

We repeated our search in the portal (for a complete description of the filters applied, see materials and methods), and the results obtained are shown in Figure 5.

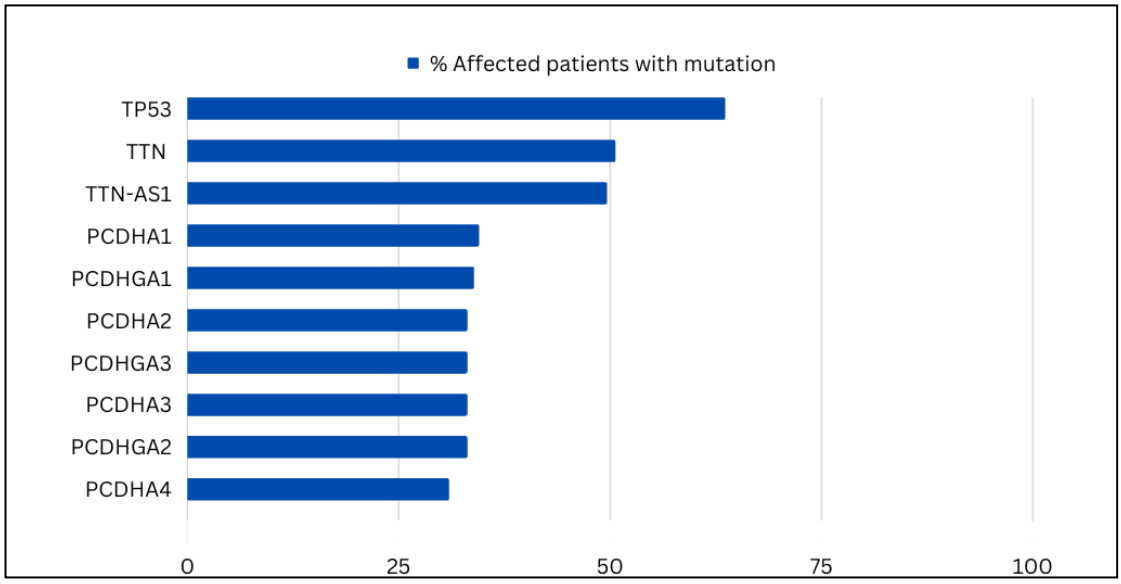


***Figure 5*: The most common mutated genes in OSCC.** *ICGC Data Portal. Blue bars represent again the percentage of samples showing a mutation for genes annotated in the Y axis.*

This bar chart represents the most common mutated genes we found in oral squamous cell carcinomas from 508 donors. It includes a scale from 0 to 100. The bars in blue represent the percentage of affected cases for a specific mutation.
We founded as a result of the most common mutated genes in OSCC: the TTN gene being mutated in 50.59% of the samples, the gene TTN-AS1 being mutated in 49.61% of the

donors, and the PCDAH1 gene being mutated in 34.45% of the cases, but undoubtedly, the TP53 gene was again the most common mutated gene, being a mutation is present in 63.58% of the cases, giving another conformation to the authors affirmations (6, 12, 13, and 19) (Figures 4 and 5).

### 4.1.3. The most common mutated genes in OSCC (cBioPortal)

A similar approach has been taken using other bioinformatic tools like the cBioPortal for Cancer Genomics (Figure 6), the GDC data portal for cancer (Figure 7), and the IntOgen portal (Figure 8). All these websites are widely used in the cancer research field.

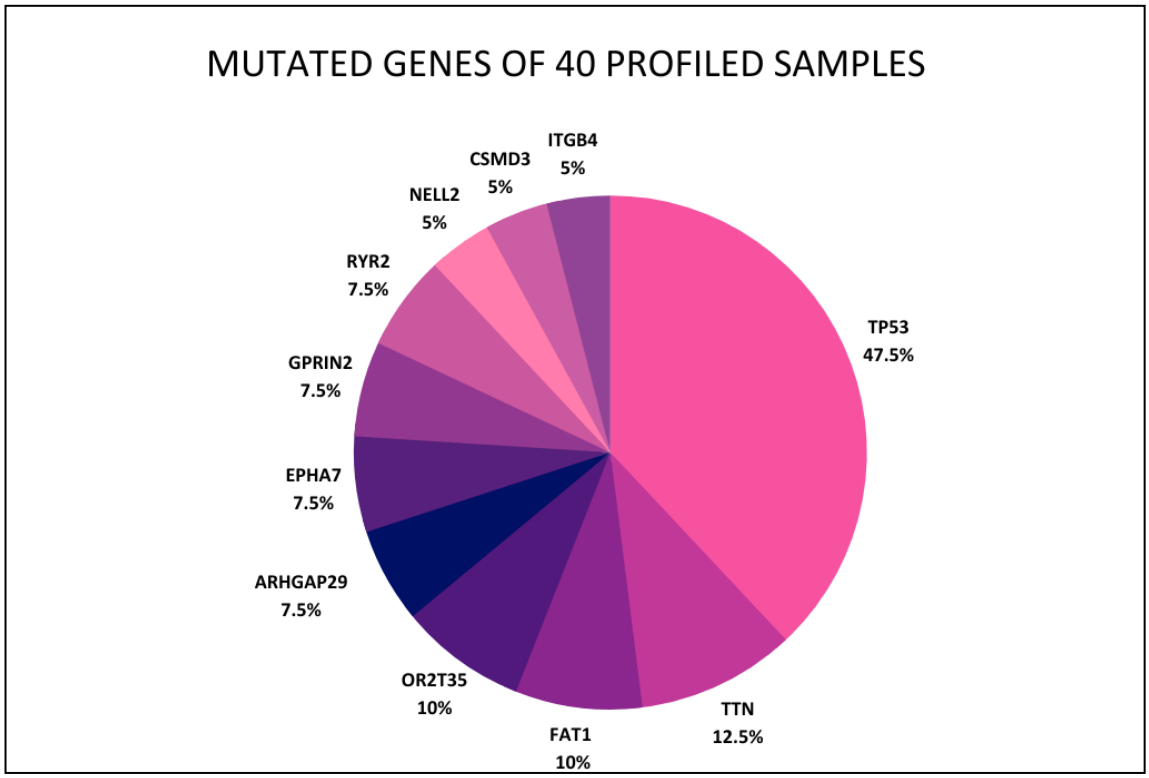


***Figure 6**: The most common mutated genes in OSCC from the CBioPortal for Cancer Genomics*. *Circle diagram showing the most common mutations in OSCC.*

In accordance with previous analysis (Figures 4 and 5), in all the following databases, we found out that TP53 was the most common mutation in OSCC (Figures 6, 7 and 8).

### 4.1.4. The most common mutated genes in OSCC (GDC Portal)

As we wanted to confirm that TP53 was the most common mutated gene, we continued our research with the help of the GDC portal, and we obtained the result illustrated in figure 7:

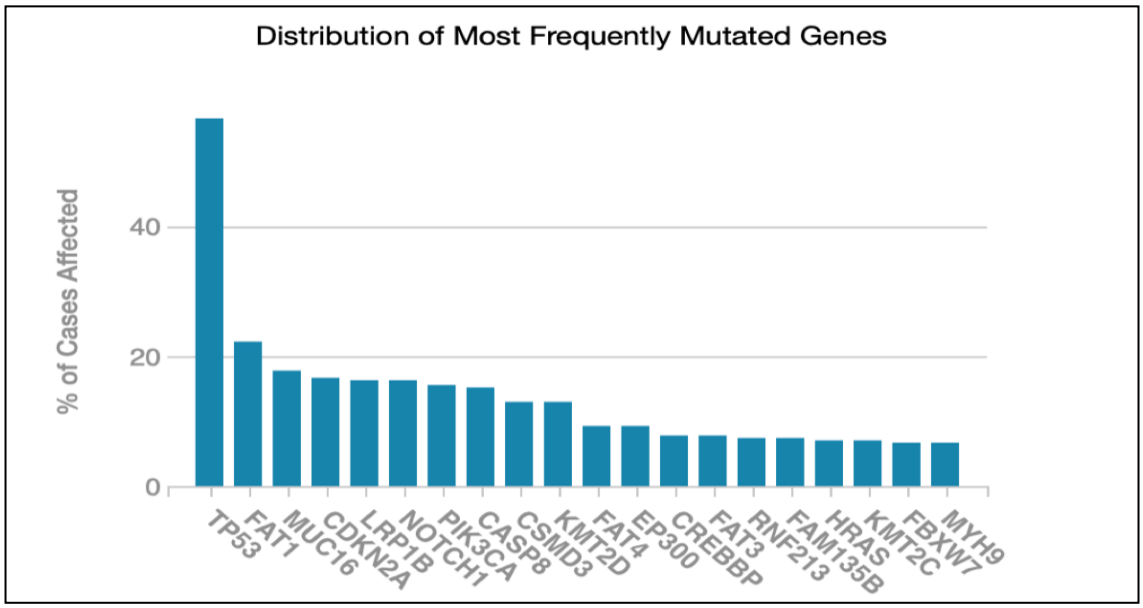


***Figure 7:* Distribution of most frequently mutated genes.** *GDC Portal. Blue bars represent the percentage of samples showing a mutation for genes annotated in the X axis.*

### 4.1.5. The most common mutated genes in OSCC (IntOgen Portal)

Interestingly, while TP53 is the most common mutation, other genes found mutated in OSCC samples appear variable depending on the used tool. This may reflect how variable tumors are, even if all the analyzed samples are OSCC. Illustration in Figure 8.

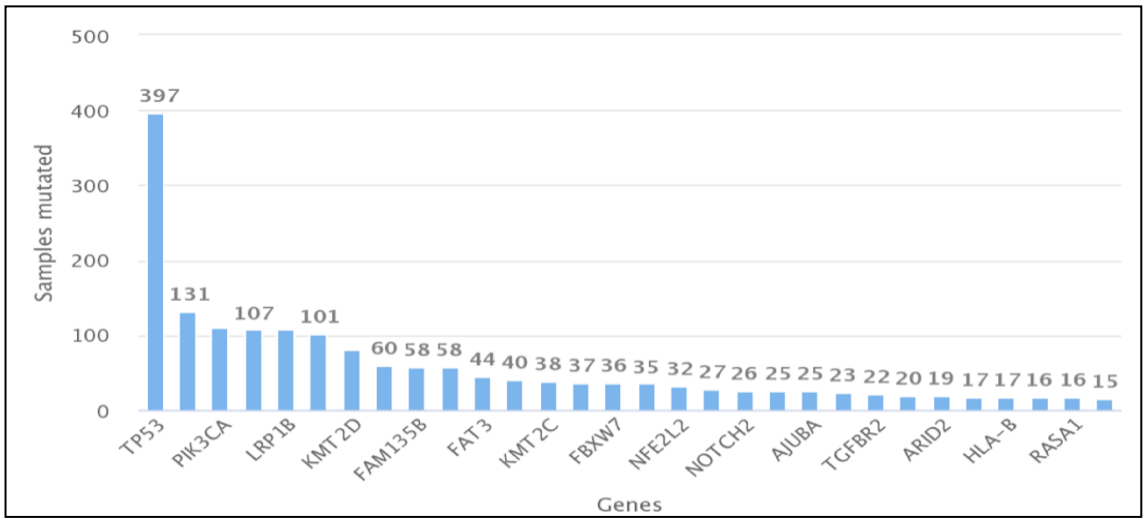


***Figure 8:* The most common mutated gene in head and neck squamous cell carcinoma.** *IntOgen Portal. Blue bars represent the percentage of samples showing a mutation for genes annotated in the X axis.*

Altogether, the data obtained from the different databases show that TP53 is the most common mutation in OSCC (Figures 3 and 5-8). In varying proportions depending on the different groups, PIK3CA (Figures 3,7 and 8), NOTCH1 (Figures 3 and 7), CDKN2A (Figures 3 and 7), TTN (Figures 5 and 6), FAT1 (Figures 3,6 and 7), and CASP8 (Figures 3 and 7) were also found to be mutated, but in a nonuniform distribution among the different groups.

### 4.1.6. The most common types of mutations in gene TP53

As a fact that TP53 was confirmed to be the most common mutated gene in oral squamous cell carcinoma, we wanted to see which were the most common types of mutation present in gene TP53 in order to compare them with the mean result from the totality of mutated genes (Figure 4). The results are illustrated in Figure 8.

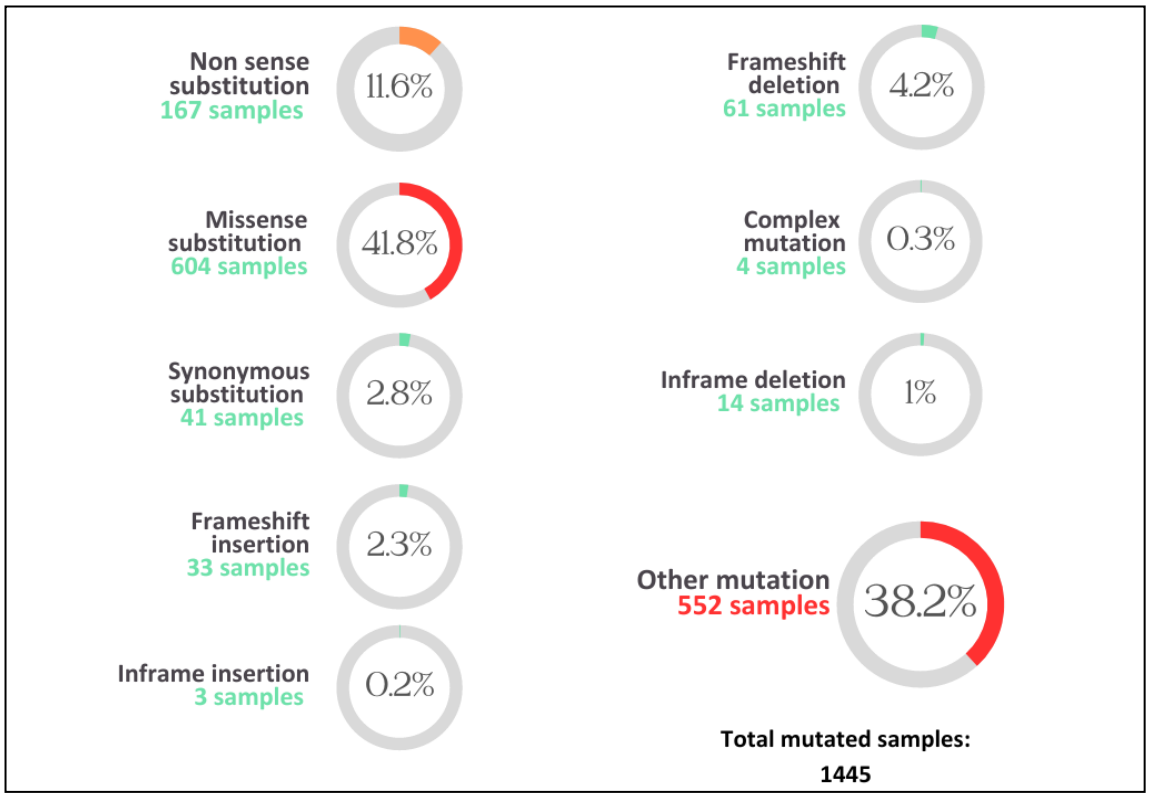


***Figure 9*: The different types of mutations present in the gene TP53 in the OSCC.** COSMIC database**.** *Higher type is represented in a red section, medium type in an orange section and low type in a green section.*

The most common type of mutation to be identified in gene TP53 is of the missense substitution type (41.8%) (Figure 9). A small amount of non-sense substation type of mutation were found (11.6 %) among the samples and 1/3 of mutation types weren't

identified (38.2%) (Figure 9). Some samples present an accumulation of mutations, and this is why the total of mutated samples doesn't correspond with the total of the type of mutations that exist. By comparing the results in Figures 4 and 9, we can confirm that the most common type of identified mutation is of the missense substitution type in both the most commonly mutated genes (all of them) and in TP53 (specifically). Also, in both (Figures 4 and 9), a large amount of an unidentified type of mutation seemed to take place, indicating that much more research needs to be conducted.

#### 4.1.7. Cellular expression of TP53

Since we found as a result that gene TP53 is the most expressed mutated gene in oral cancer, we asked ourselves if this mutated gene was present in normal tissue or if it was a malignant gene present only in OSCC. It is important to understand that the mere presence of a mutation does not mean that it is harmful by itself. Moreover, the mutation can be the cause of the cancer or a consequence of the sickness. This is especially true when dealing with tumor suppressor genes like TP53.

In order to clarify the topic, we address the question of which cells of the oral cavity express TP53. We have to keep in mind that oral squamous cell carcinoma develops from the epithelial tissue of the oral cavity, so we should expect that TP53 is expressed in epithelial cells. For this purpose, we used, data from the "Gingiva health disease" from the Haniffa lab. In this web site, we find a recent tool for integrating single-cell sequencing data from an oral sample (37). Very briefly, in genome sequencing, DNA is extracted from tumor samples, and all the DNA from the sample is sequenced independently of which cells it derives from. On the other hand, in single-cell sequencing, samples are disaggregated into single cells, and RNA from single cells is sequenced. This kind of approach allows clustering cells according to the genes they express.

#### 4.1.8. Separation of cellular population

We start our approach by identifying genes expressed in the gingival diseased tissue, and for that, we used a program that clustered cells using a different color for each

cluster (Figure 10). In the table in Figure 10, the different genes and their associated colors are defined. It includes a variety of 29 genes (variables). The epithelium being the tissue of interest in our research, we focused on P. epithelium 1 (orange), P. epithelium 2 (yellow), and P. epithelium 3 (green), as indicated by the red arrow. A scatterplot was used to separate the different elements (variables). A scatterplot is a mathematical material that displays values for different variables (here the variables are the different genes, represented in 29 distinct colors).

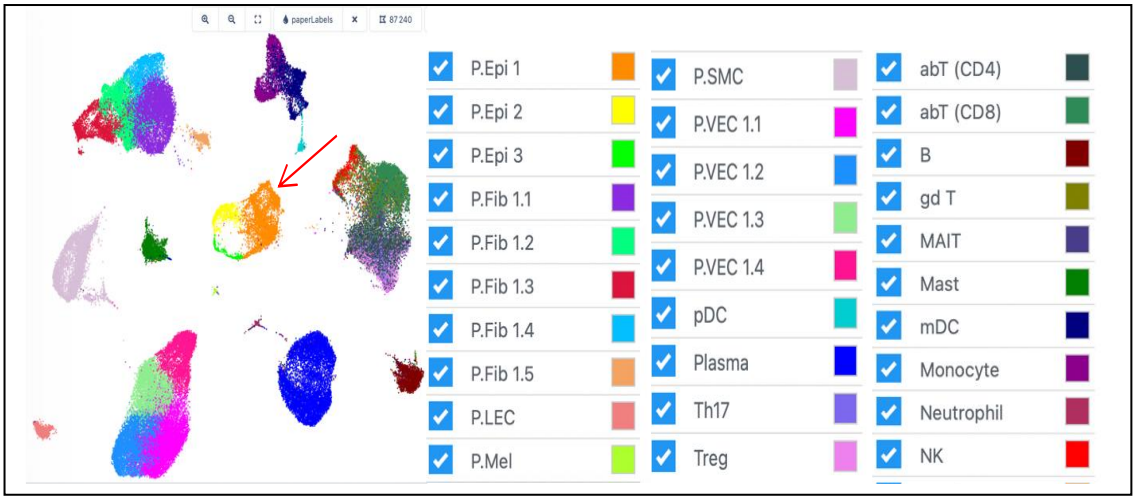


***Figure 10*: Scatterplot in Embedding Basis: Gingiva: Health and Disease.** *Data from the Haniffa Lab. Cell clusters are shown in different colors and epithelial tissue has populations in yellow, orange, and green. A red arrow indicates the population of interest.*

### 4.1.9. Identification of TP53 expression in population of interest (epithelial tissue)

Of the genes we had encountered, we wanted to understand at what level they are expressed in a diseased gingiva to understand their relevance to our topic. A scatterplot analysis was performed to obtain results. A scale of colors was used to grade the presence of mutations. Scale from 0 to 4: Dark Blue (0-1); Green (1-2); Yellow (3-4). They are illustrated in Figure 11.

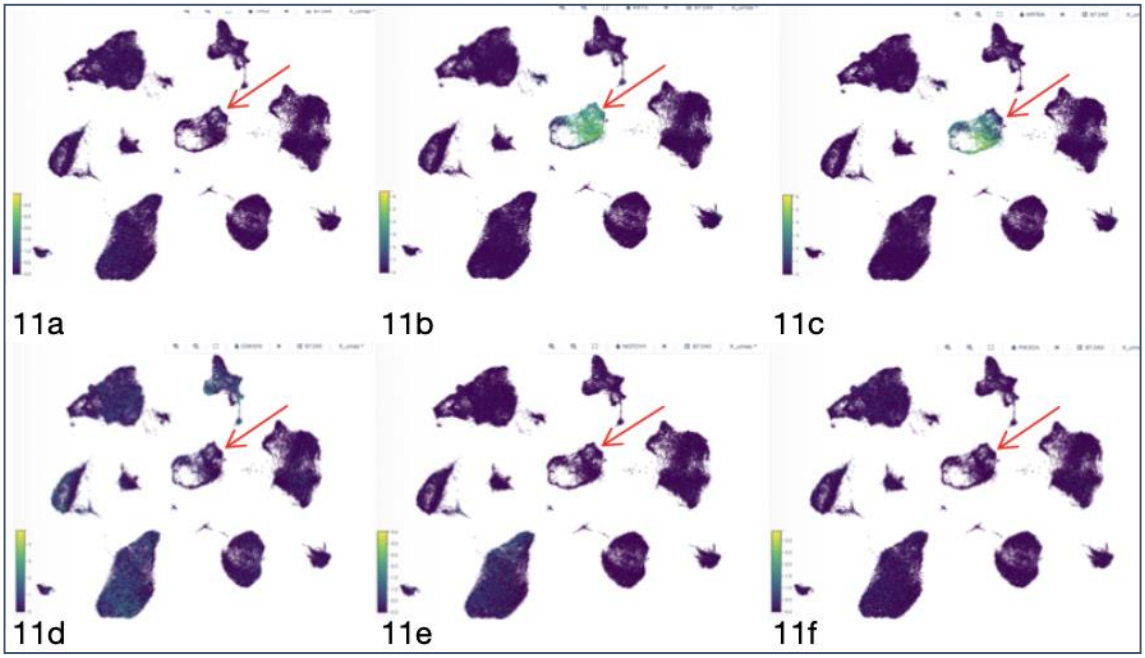


***Figure 11 (a, b, c, d, e, f):* Scatterplot in embedding basis: Expression level of genes involved in OSCC.** *Data from the Haniffa lab. The red arrow indicates the population of interest (epithelial tissue). The program uses a scale of different colors from high expression (green) to low expression (dark purple).*

The scatterplot technique was used to research the gene expression of the following genes; each of them is represented in its corresponding scatterplot (11: a–f).

In total, 6 genes (of the most common mutated genes encountered in Figures 3, 5, 6, 7 and 8) were analyzed in order to see if they happened to be mutated in the epithelial tissues (red arrow) and are represented in Figure 11.

- 11a: TP53 gene expression
- 11b: KRT5 gene expression (keratin)
- 11c: KRT6A gene expression (keratin)
- 11d: CDKN1A gene expression
- 11e: NOTCH1 gene expression
- 11f: PIK3CA gene expression

As the colors give us a scale order. We then concluded that the genes with the highest expression were KRT5 (11b) and KRT6 (11c). This is the first time that we have encountered the fact that TP53 isn't the gene expressed in the highest amount. Here, the most mutated genes in the epithelium of a diseased gingiva were those that are associated with the keratinocytes.

## 4.2. Gene expression in normal tissue:

### 4.2.1. TP53 expression in normal healthy tissue:

Since we found that TP53 is the most common mutation in OSCC (Figures 3 and 5-8) but that TP53 is mainly expressed in keratinocytes (Figures 10 and 11), it made us question whether TP53 is expressed in healthy normal oral tissue.

For this purpose, we use the human protein database (38), where, among other functions, it is possible to obtain data from staining with antibodies know as immunohistochemistry. In these stainings, we are seeing the protein expression inside a tissue (in our case, the oral epithelium. Moreover, it is possible to compare normal tissue (healthy squamous epithelial cells) with abnormal tissue (oral squamous cell carcinoma). For our research, we compared normal tissue with abnormal tissue for the expression of TP53 (Figures 12 and 13).

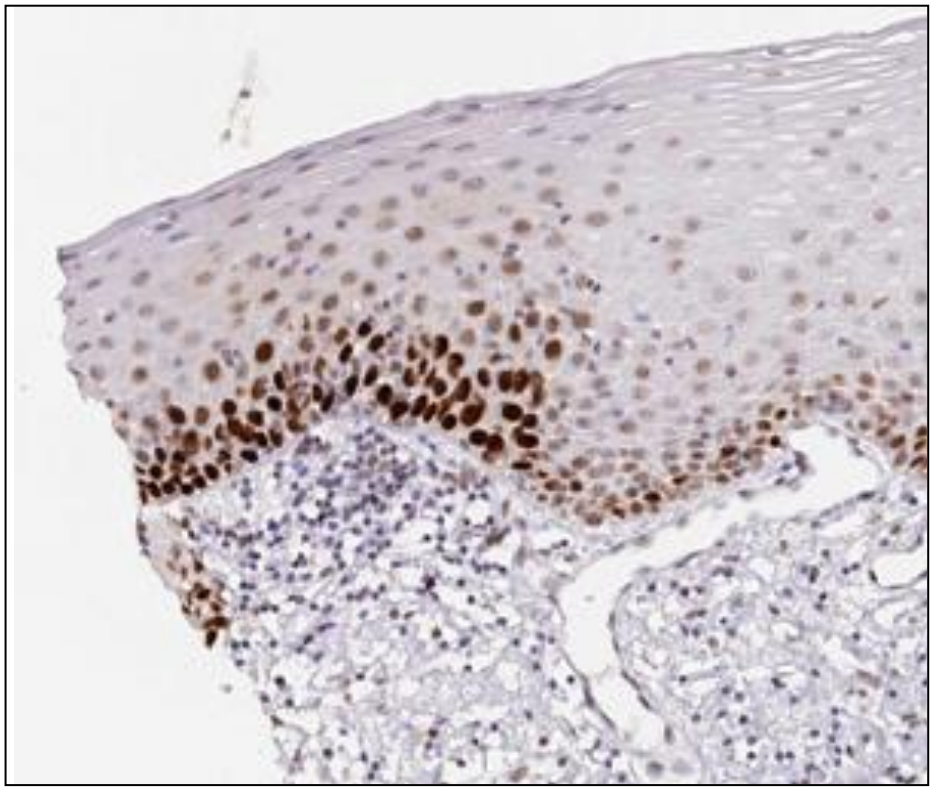

***Figure 12:*** ***Antibody staining: oral mucosa. Age of patient: 74. Normal tissue: squamous epithelial cells. Antibody staining for TP53: High staining****. Data from the human protein data base. Positive staining is represented in brown. A scale of intensity ranges from light coloration to high coloration of brown.*

### 4.2.2. TP53 expression in oral squamous cell carcinoma:

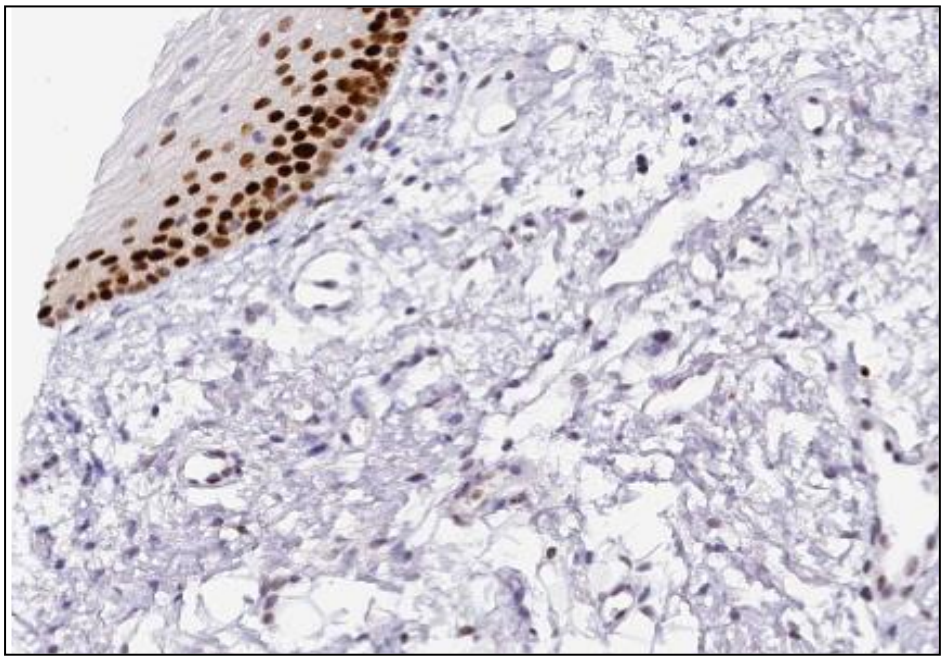

***Figure 13:*** ***Antibody staining: oral mucosa. Age of patient: 68. Oral tissue: squamous cell carcinoma. Antibody staining for TP53: high staining.*** *Data from the human protein data base. Positive staining is represented in brown. A scale of intensity ranges from light coloration to high coloration of brown.*

By comparing the staining results obtained from the two samples, oral normal tissue (Figure 12) and oral squamous cell carcinoma (Figure 13), we can observe a high level of staining. Both samples show high similarity in TP53 antibody staining. TP53 gene being present in both healthy and pathological tissue, we then understand that the presence of this gene, mutated or not, is related to normal function but also to pathological function. In order to understand more about the role of gene TP53 and why it is always present, we are going to define it later on.

## 5. DISCUSSION:

### 5.1. Dentists have a key role in oral cancer diagnosis and research:

The main objective of this research is to make dentists aware that they are the first to identify a potentially premalignant or malignant oral lesion. (14,16) Early diagnosis and adequate treatment promote a higher survival rate and enhance the quality of life of affected patients. (1, 14)

To catch the disease in its earliest phase, as dentists, we should consider the mouth in its entirety and give the tissues the same level of importance as the teeth. (1, 14, 16) Doing our best to save a patient from oral cancer is much more important than anything else at our level. Oral cancer, and, most specifically, oral squamous cell carcinoma, occur in the epithelial cells of the mouth. (14) For that reason, inspecting the oral cavity for any type of suspicious lesion should be part of our routine practice and be included in all our diagnoses, offering the patient the best treatment outcomes. Having strong knowledge of the different lesions and appropriate considerations for them is of high importance to knowing when it is necessary to perform a biopsy (removing a sample of the lesion to be identified with a microscope by a pathologist) or not. (7) Clinical training during our entire career and studying of lesions to be able to identify them should be done by all dentists to stay updated each year (8).

In the field of dentistry, the role of the dentist is of the utmost importance, as they are the healthcare professionals who are responsible for identifying any suspicious lesion of the oral cavity and deciding whether it is necessary to perform a biopsy. (2) They play a crucial role in initiating the process of diagnosing and studying any potentially cancerous lesion. The early detection of such lesions can significantly improve the chances of successful treatment and recovery (7). Therefore, the dentist's position is in the first line of detection of oral cancer, which is crucial to oral health. Dentists should suspect any lesion that has been present for more than 3 consecutive weeks, and a biopsy should be performed to confirm any presumptive diagnosis. (7)

**5.2 As a result of available bioinformatic tools, TP53 was identified to be the most common mutated gene in OSCC:**

Bioinformatic analysis is becoming a powerful approach in many fields of research, and in particular in cancer research (1, 3, 11, 19, 20, 22, 23). In this work, we have tried to approach a simple objective: what the most common mutated genes in OSCC are. For that, we've been using the most common bioinformatic tools available. Researchers often use the same sources that we selected (1, 3, 11, 19, 20, 22, 23). We have done this research also with a pedagogic aim, trying to illustrate to odontologists how this kind of approach is routinely done in research labs all over the world. Incontestably, the use of informatics in our research was essential. Recorded cases with open access gave us the opportunity to bring out new ideas. All of this is made possible thanks to the cooperation of numerous scientific domains; informatics has given a new perspective on the subject of medicine by giving the opportunity to share information, contents, and discoveries in the simplest and fastest way possible, and this has led to increasing the effectiveness of cancer research in many types of cancer (30).

In order to answer the main question from this research (which are the main mutated genes in oral cancer), we have used the Cosmic Web site (see materials and methods and Figure 3) or the ICGC portal (see materials and methods and Figure 5). Both tools are based on information about DNA mutations sequenced directly from patients. To understand the importance of this tool, we want to underline that the ICGC portal has been defined as the most ambitious project since the human genome project, or that the Sanger Institute is probably one of the most important research centers in the field of cancer investigation. We have also used the cBioPortal for Cancer Genomics (Figure 6), the GDC data portal for cancer (Figure 7), and the IntOgen portal (Figure 8). All these databases are based on the presence of DNA mutations, but they are different among them and offer different analysis tools. However, a more complex use of available programs as well as trying to explain the differences among them is far beyond the aim of this work.

All the above databases are based on DNA sequencing and describe the presence of DNA mutations. However, the presence of a DNA mutation does not mean a harmful event (see introduction). To underline this concept, we have used two different tools that are

not based on genome sequencing. The first is the Haniffa Lab Project, an ambitious project collecting single-cell data from oral tissue. The second is the human protein database, where, among other functions, it is possible to obtain a scale of staining showing the presence of proteins in healthy and pathological tissues (See figures 12 and 13). In the results, we have explained a little bit the base of these tools, but once again, the complex use of these tools is not the aim of this work.

Altogether, we can conclude that bioinformatics is becoming more important in the research field.

While performing a biopsy gives us a conclusion about the type of lesion we are facing, understanding the underlying mechanisms of cancer is of high importance to research the best treatment and enhance the prognosis (12, 23, 31). From that point and at our level, we wanted to understand the relationship between the genetic mutation of some genes and their physiological expression in oral cancer. We wanted to understand if the genes that showed to be expressed in the largest amount are expressed because of their initial function in our body or if they are present as an aggravating factor in oral squamous cell carcinoma. Because of the complexity of genetics, we targeted a specific tissue of interest: epithelial tissue, where oral squamous cell carcinoma is known to originate (Figures 10 and 11).

We used a type of investigation that was based on the analysis of informatics data from registered cases of oral cancer (see materials and methods). The data that were primarily analyzed were the most common mutated genes in oral squamous cell carcinoma (Figure 3 and 5-8).

All of our data gave rise to the same result as the majority of publications from authors: TP53 is the gene that is the most commonly mutated in oral cancer (6, 12, 13, 19, 23). Other genes were seen to be as well mutated in multiple samples: PIK3CA, NOTCH1, CDKN2A, TTN, FAT1, and CASP8 (Figure 3 and 5-8); but as a comparison to TP53 percentages, the amount of mutation of these genes was expressed in a much smaller number when we compared and studied the results obtained in all our different groups. We thought it would be interesting to see which type of mutation occurs in the most commonly mutated gene, and we obtained the results that are illustrated in figure 4. The results confirm how important bioinformatic is. It also shows that we still lack many

knowledges in the topic, and we agree with researchers who have come to the same conclusion than us. (1, 3, 11 and 19-23)

To spread out our research, we needed to analyze in which tissues TP53 is most expressed (as it is the gene most mutated in oral squamous cell carcinoma). We know that oral squamous cell carcinoma attacks the cells of the epithelial tissue, so we logically thought at first that we would find TP53 mostly present in the epithelial cells. In order to see if our thoughts were justified, we used data from the Haniffa lab and obtained the results that are illustrated in figures 10 and 11. Surprisingly, when we isolated the different tissues and the different cells that form the samples, we found out that the expression of TP53 was present in a bigger amount in the keratinocytes and in a really low amount in the epithelial cells (see figures 10 and 11). As this created a doubt, we then found that it was a necessity to know if the expression of TP53 was also present in normal tissue or not.

**5.3 The expression of gene in normal tissue compared to pathological tissue:**

To understand if TP53 was expressed in healthy tissue, we used information from the protein database and did a comparison between epithelial cells of the oral cavity in a normal (healthy) sample and a cancerous sample. We obtained the results that are illustrated in Figures 12 and 13. We curiously found a really high antibody staining for TP53 in both the healthy sample and the oral squamous cell carcinoma sample, while we were expecting to find a really high antibody staining in the diseased sample and a really low or nonexistent staining in the healthy sample. We then tried to link our findings with our knowledge, but we started doubting other people's affirmations (3, 6, 10, 19, 20, 22, 31). As TP53 is a tumor suppressor gene, we were uncertain, based on the conclusions of some authors, of the function of this gene when present and/or mutated (3, 19, 31). A confusion was raised: this gene is present to eliminate a potentially malignant cell that will give rise to a tumor (as it has the role of a tumor suppressor gene), or this gene is present to potentially participate in the rise of a malignant cell. Another doubt was weather TP53 is mutated in a higher amount because the tumor will send a signal that will disrupt the function of this gene, or if the tumor itself is responsible for influencing the mutation of the genes.

We found out that the TP53 gene wasn't the most commonly expressed gene in the epithelial cells (see figure 10 and 11). We discussed that the genes that were present in higher quantity were those that express in the keratinocytes: gene KRT5 and gene KRT6A (see figure 11), and as oral squamous cell carcinoma takes place in the epithelial tissue, it gave rise to a discordance between our thoughts and the reality (3, 6, 19, 20, 21, 22, 31).

TP53 can be the most commonly mutated gene, but this doesn't mean with certainty that it is the gene with the biggest impact in oral squamous cell carcinoma. (Figures 12 and 13)

The fact that TP53 is expressed at high levels in all cancer histories, as our research showed in OSCC, is most probably primarily due to its fundamental role as a tumor suppressor gene, but the impact and role of this gene remain uncertain. New findings in cancer research can help treatment outcomes and decisions, but it remains very difficult to generalize (3, 4, 5).

It would be interesting for new discoveries on how to manage oral cancer to study the role of the keratinocytes in oral squamous cell carcinoma and how they interact with the TP53 gene and all the other genes (PIK3CA, NOTCH1, CDKN2A, TTN, FAT1, and CASP8) (See figures 3, 5, 6, 7 and 8). We can say with certainty that much more studies need to be conducted in order to understand the role of each gene in oral cancer and to properly identify them (Figures 4 and 9) (21).

One thing is confirming which gene is the most present, and another thing is confirming with certainty the role it has in the course of the disease...

Gene TP53 presence was known to be an aggravating factor in cancer, impacting the occurrence of earlier onset of cancers and the survival rate being significantly shorter in patients that present with TP53 gene mutation (22, 27, 28). While we start to understand better after each research study, the role of TP53, we still can't categorize the entirety of the mutations TP53 can present, and because of this, it directly influences the complexity of managing all the types of oral cancer. (Figure 9)

Genetics is one of the hardest and most complex topics (22, 23). More studies need to take place, and focusing on the percentage of mutation shouldn't be the only thing to consider when we want to enhance the survival rate and understand the mechanisms of cancer (21). Understanding the function of each gene and how they interact with the

cells and tissues present in a type of cancer must be of high importance in order to give rise to any affirmations. (See figure 10, 11)

It is primordial to analyze each case independently (5,6). Additional factors such as lifestyle and habits have a high impact on gene behavior; it is still very important to perform prevention on patients that expose themselves to risk factors, and it is essential that each case be studied individually to achieve a successful treatment (6, 23).

## 6. CONCLUSION:

1) The primary aim of cancer research is to improve the survival rates of patients diagnosed with cancer. Dentists play a crucial role in detecting and diagnosing oral cancer in its early stages. The genetic expression of a patient's cancer history plays a significant role in the incidence and prognosis of the disease. It is therefore imperative for dentists to remain vigilant in their assessments and examinations, as the early detection of oral cancer can lead to better treatment outcomes and enhance the survival rate of patients. When a dentist performs a biopsy, it gives researchers the opportunity to study a lesion genetically. For that reason, dentists are on the front lines of oral cancer research.

2) The field of informatics has had a significant impact on the accessibility of data for professionals conducting research. It is particularly evident in the field of genetic cancer research. In this work, we have illustrated a simple bioinformatic approach with the pedagogic aim of showing to odontologists how big data analysis is routinely used in the research of cancer. The most common mutated gene in oral cancer occurred incontrovertibly to develop in greater proportions in the gene TP53. While other genes such as PIK3CA, NOTCH1, CDKN2A, TTN, FAT1, and CASP8, were also present mutated in some samples, TP53 was the most mutated gene, present in all the analyzed groups. Also, mutated TP53 was more present in the keratinocytes than in the epithelial cells (where OSCC takes place).

3) While TP53 is a tumor suppressor gene, it is expressed in the same amount in healthy tissue and pathological tissue of the oral cavity.

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