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BASIC SCIENCE REVIEW

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KMT2 (MLL) family of methyltransferases in head and neck squamous cell carcinoma: A systematic review

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Abstract

Background: The involvement of the KMT2 methyltransferase family in the pathogenesis of head and neck squamous cell carcinoma (HNSCC) remains elusive.

Method: This study adhered to the PRISMA guidelines, employing a search strategy in the LIVIVO, PubMed, Scopus, Embase, Web of Science, and Google Scholar databases. The methodological quality of the studies was assessed by the Joanna Briggs Institute.

Results: A total of 33 studies involving 4294 individuals with HNSCC were included in this review. The most important alteration was the high mutational frequency in the *KMT2C* and *KMT2D* genes, with reported co-occurrence. The expression of the *KMT2D* gene exhibited considerable heterogeneity across the studies, while limited data was available for the remaining genes.

Conclusions: *KMT2C* and *KMT2D* genes seem to have tumor suppressor activities, with involvement of cell cycle inhibitors, regulating different pathways that can lead to tumor progression, disease aggressiveness, and DNA damage accumulation.

KEYWORDS

head and neck squamous cell carcinoma (HNSCC), KMT2, methyltransferases, MLL, systematic review

1 | INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a highly incident cancer worldwide, with over 800 000 new cases each year.[1] This group includes the epithelium sites of the oral cavity, tonsils, pharynx (nasopharynx, oropharynx, and hypopharynx), larynx, epiglottis, paranasal sinuses, and nasal cavity.[2,3]

The manuscript is original work and has not been published elsewhere.

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The manuscript is the result of an academic dissertation to obtain a degree from the University of Brasilia.

HNSCC carcinogenesis is a complex, multifactorial process that encompasses exposure to tobacco-derived carcinogens and excessive alcohol intake, as well as persistent human papillomavirus (HPV) infection, and its late diagnosis is highly correlated with worse patient outcomes.[3] Lately, epigenetic regulation, which is a genomic mechanism that reversibly influences gene expression without altering DNA sequences, has been shown to play key roles in the carcinogenesis of several types of cancer and has established itself as a promising therapeutic target.[4,5]

Epigenetic dysregulations affect several aspects of cancer complexity, such as the expression of oncogenes, tumor suppressors, and cell signal genes, leading to increased growth, invasion, and metastasis of cancer.[6] Therefore, there is an urgent need to determine the most relevant epigenetic alterations in HNSCC to identify diagnostic or prognostic biomarkers or targetable molecular alterations that can be used in clinical settings.

The use of epigenetic drugs as adjuvant therapy in standard cancer treatment is already a reality. To date, several targets and their combinations have been investigated to improve HNSCC treatment, as the combination of epigenetic drugs with Epidermal Growth Factor (EGF) pathway players inhibitors, chemotherapy, or immune checkpoint inhibitors,[7] such as programmed cell death protein 1 (PD1), PD1 ligand 1 (PD-L1), or cytotoxic T lymphocyte antigen 4 (CTLA4), which suppress immune inhibitory signaling leading to an efficient antitumor response.[7,8]

However, further studies are still needed to analyze the role of histone methyltransferases in modulating tumor immune responses and their potential to be used as molecular markers to predict patient response to these therapies. For example, a recent study investigated the effects of histone methyltransferases on immune response in squamous cell carcinomas, showing that NSD1 loss led to decreased interferon response and tumor immune evasion. A better understanding of such mechanisms could lead to new and more efficient treatment strategies.[9]

Among epigenetic alterations, methyltransferase proteins are responsible for post-translational alterations in proteins like histones. In histones, methylation catalyzed by these enzymes can either compact chromatin, preventing transcription or decompress it, allowing gene activity. The *MLL* (*Mixed Lineage Leukemia*) family encodes a group of five protein methyltransferases [MLL1/KMT2A (Gene ID: 4297), MLL2/KMT2D (Gene ID: 8085), MLL3/KMT2C (Gene ID: 58508), MLL4/KMT2B (Gene ID: 9757), and MLL5/KMT2E (Gene ID: 55904)—in this study, the KMT2 nomenclature was adopted to facilitate understanding] which dysregulation has been associated with several cancer types, although a clear relationship between them and HNSCC carcinogenesis has not yet been well established.

These enzymes contain DNA recognition regions, in addition to other domains, such as the catalytic SET, responsible for lysine 4 of histone 3 (H3K4) methylation, a mark related to active gene transcription; the PHD domain which provides histone recognition, binding functions, and protein interaction sites; the AT hooks domain that binds to minor groove DNA and probably contributes guiding KMT2 enzymes to specific subnuclear loci; and the CXXC domain, which binds only to unmethylated DNA, recognizing epigenetically modified DNA (CpG methylation) and contributing to target genes selection. The AT hooks and CXXC domains are present in all KMT2A fusion–related leukemias and are thought to be involved in carcinogenesis.[10–18]

Despite current knowledge about the function of these enzymes and their dysregulation in different types of cancer, their role in the pathogenesis of HNSCC still needs to be clarified. Therefore, this systematic review aims to investigate the involvement of the KMT2 methyltransferase family in the pathogenesis and progression of head and neck cancer.

2 | MATERIALS AND METHODS

2.1 | Protocol and registration

This systematic review followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[19] The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database under the number CRD42020197780.[20]

2.2 | Eligibility criteria

The review question—What is the involvement of the KMT2 (MLL) methyltransferase family in the pathogenesis of head and neck squamous cell carcinoma?—was formulated using the PICOS strategy. The inclusion and exclusion criteria were defined according to the review question and are described in detail below:

2.2.1 | Inclusion criteria

a. Studies with tissues or cells from head and neck squamous cell carcinoma patients.

b. Studies evaluating the involvement of KMT2 family members in the pathogenesis and progression of head and neck squamous cell carcinoma using patients' data.
c. In vitro studies or any study using cancer cells or tissues derived from patients with head and neck squamous cell carcinoma that present data on the involvement of KMT2 family members in this type of cancer.

2.2.2 | Exclusion criteria

a. Studies that did not define clearly the site of the tumor studied.
b. Studies that were not performed in patients, tissues, or cells from head and neck squamous cell carcinoma.
c. Studies that did not evaluate the involvement of KMT2 family members in the pathogenesis and progression of head and neck squamous cell carcinoma.
d. Inconclusive studies.
e. Studies including reviews, case reports, letters, chapters, personal opinions, and conference abstracts.
f. Studies that did not individualize data for head and neck squamous cell carcinoma.

2.3 | Information sources

The studies were identified using a custom search strategy for the following electronic databases: LIVIVO, PubMed, Scopus, Embase, and Web of Science. A gray literature search was done with Google Scholar. Duplicated references were removed by reference manager software (EndNote®, Thomson Reuters) and Rayyan.[21] Thereafter, manual searches were carried out in reference lists from the included studies, to reduce the chances of missing potential related studies during the electronic database searches. All the searches were conducted on March 7th, 2021, updated on March 31st, 2023, and can be found in Table S1.

2.4 | Study selection

The selection was conducted in two phases. In phase 1, two reviewers independently assessed the titles and abstracts of all citations identified in electronic databases. A third reviewer was involved when a final ruling was required in case of disagreement. Studies that did not meet the inclusion criteria were ruled out. In phase 2, the same selection criteria were applied to the full articles to confirm their eligibility. For this purpose, two reviewers independently assessed the full-text publications, and a third reviewer was involved whenever a final decision was required in case of disagreement. Disagreements at both stages were resolved by debate among the three reviewers. Finally, one reviewer checked the reference lists of all included articles.

2.5 | Data collection process

One reviewer gathered crucial information from each selected article. A second reviewer confirmed its reliability. Once again, any disagreement was solved by discussion to reach an agreement among the authors. For all included studies, the following information was recorded: author(s), year of publication, country, design, sample size (cases of HNSCC and non-HNSCC controls), HPV (Human papillomavirus) status, member of KMT2 methyltransferase family assessed, the primary site of the tumor, technique(s) used and results. Some studies' authors were contacted to provide unreported data or additional details.

2.6 | Risk of bias in individual studies

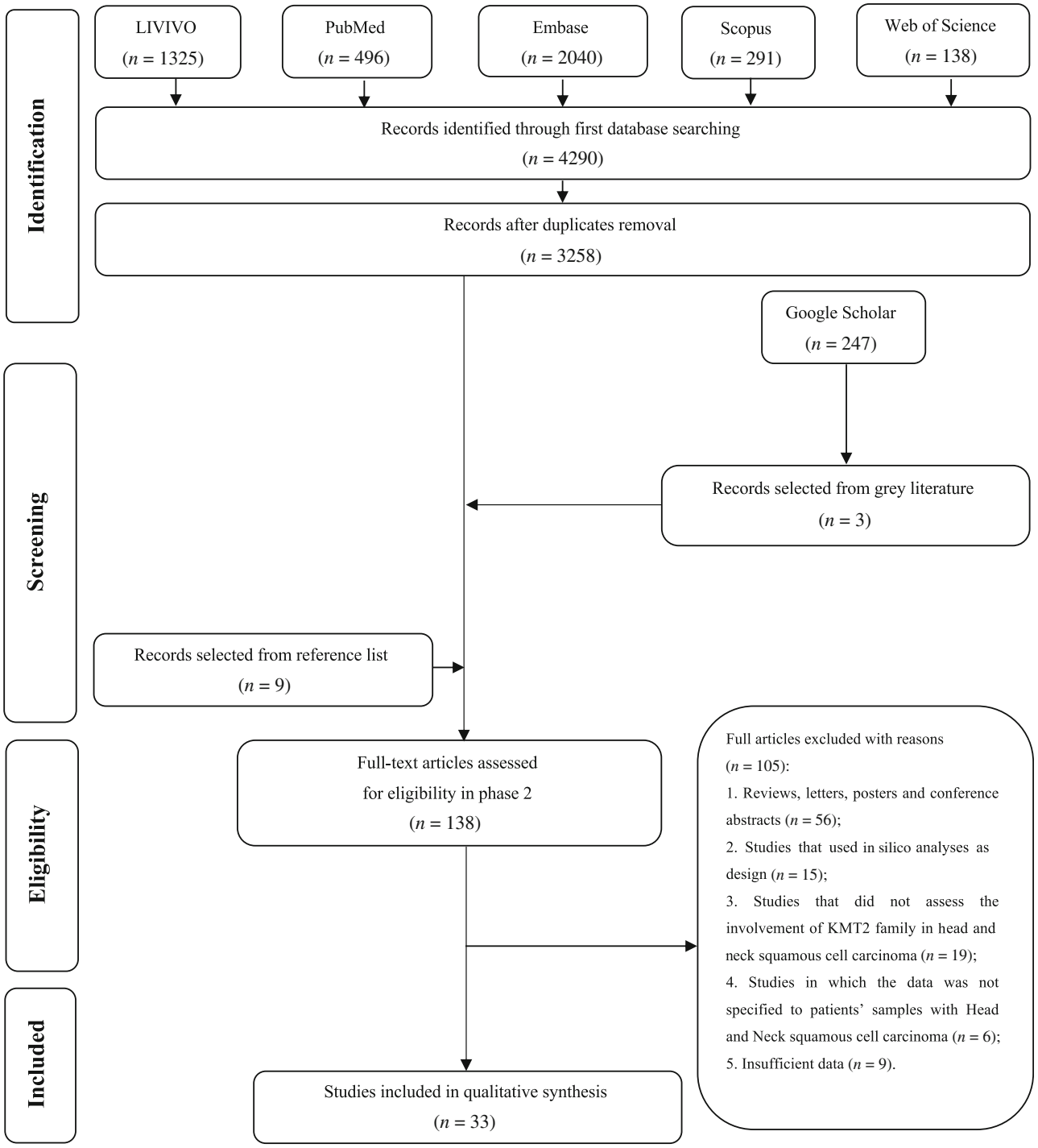
Critical assessments of the studies included were performed independently by two authors according to the checklist developed by Joanna Briggs Institute for Studies Reporting Prevalence Data.[22] Disagreements on the risk of bias evaluation were solved by debate. The risk of bias was categorized as High when the study reached up to 49% of "yes" responses; moderate, when the study reached 50% to 69% of "yes" responses; and Low, when the study reached more than 70% of "yes" responses (the "Not Applicable" items were excluded from the sum).

2.7 | Summary measures

The primary outcome of this study was to identify alterations of the KMT2 family in HNSCC, including altered RNA and protein expression or genetic and chromosomal alterations, such as Single-Nucleotide Variant (SNV), deletion, insertion, Copy Number Variation (CNV), and mutation profile.

2.8 | Synthesis of results

The whole data combination of the included studies was done by a descriptive synthesis.



†Adapted from PRISMA.

FIGURE 1 Flow diagram of literature search and selection criteria. *Source*: Adapted from PRISMA.

3 | RESULTS

3.1 | Study selection

In Phase 1 of the study selection, 4290 citations were identified in five electronic databases. After duplicate removal, 3258 citations remained. A thorough assessment of the titles and abstracts led to the exclusion of 3132 articles, with 126 articles remaining after Phase 1. The search on Google Scholar yielded 247 references, of which only 3 were included for the full-text analysis. Nine more articles were identified from the studies' reference lists.

A full-text review of the 138 articles selected in Phase 1 was conducted. This process led to the dismissal of

TABLE 1 Overview of the included studies ($n = 33$).

Author(s), year	Country	Number of HNSCC patients included	Number of controls	Site(s) of tumor	HPV status
Figueiredo et al. 2014[24]	Brazil	13	13	Larynx	NA
Bunbanjerdsuk et al. 2019[43]	Thailand	46	16	Oral cavity, oropharynx, hypopharynx, larynx, nose, and paranasal sinuses	46 p16−
Wang et al. 2022[27]	China	96	16	Oropharynx	NA
Zhu et al. *2019*[28]	China	85	36	Oral cavity	NA
Gronhoj et al. 2018[29]	Denmark	114	114	Oropharynx	HPV+
Karam et al. 2019[45]	USA	16	0	Tonsil, base of tongue, supraglottic larynx, soft palate, larynx, and other	8 p16+ and 8 p16−
Lin et al. 2021[41]	Taiwan	50	0	Gingivobuccal and other primary sites in OSCC	46 p16− 4 p16+
Lilja-Fischer et al. 2019[30]	Denmark	12	0	Tonsil, pharyngeal wall, hypopharynx, and tonsillar fossa	7 p16+ 5 p16−
Martin et al. 2014[46]	USA	22	1	Oral cavity and pharynx	NA
Fadlullah et al. 2016[38]	Malaysia	16	3	Oral squamous cell carcinoma, gingiva, buccal mucosa, and tongue	1 HPV+ (type 16 and 31) and 15 HPV−
Fan et al. 2021[42]	Taiwan	165	0	Oral cavity	NA
Ghosh et al. 2022[34]	India	28	0	Gingivobuccal	NA
Hedberg et al. 2016[47]	USA	20	20	Oral cavity, larynx, and pharynx	1 HPV+ and 22 HPV−
India project team of the International Cancer Genome Consortium, 2013[35]	India	110	0	OSCC-GB	HPV was detected in 26%
Osawa et al. 2021[37]	Japan	98	0	Oral Cavity (tongue, mandibular and maxillary gingiva, buccal mucosa, floor, and hard palate)	NA
Ghias et al. 2018[39]	Pakistan	7	0	Left buccal mucosa, right tongue, left tongue, right pyriform fossa, and lower mandible alveolus	7 HPV−
Rawal et al. 2014[36]	India	4	4	Tongue	NA
Burcher et al. 2021[55]	USA	139	0	Nasopharynx, Oropharynx, Oral cavity, larynx, sino-nasal, and unknown	HPV and/or p16− 61 p16+ 61 Not Tested 48
Day et al. 2020[25]	Canada	8	0	Base of tongue, vallecula, and tonsil	6 p16+, and 2 p16−
Haft et al. 2019[48]	USA	46	46	Oropharynx	HPV+
Machnicki et al. 2022[40]	Poland	47	0	Hypopharynx and larynx	Negative

(Continues)

TABLE 1 (Continued)

Author(s), year	Country	Number of HNSCC patients included	Number of controls	Site(s) of tumor	HPV status
Melchardt et al. 2018[23]	Austria	26	0	Larynx, oropharynx, oral cavity, and hypopharynx	1 p16+
Seiwert et al. 2015[49]	USA	120	120	Oropharynx, oral cavity, larynx, and hypopharynx	51 HPV+, and 69 HPV−
Shaikh et al. 2021[50]	USA	1667	0	Oropharynx	p16+ and HPV+ OPSCC 38% (51/134) p16+ and HPV+ non-OPSCC HNSCC 5% (10/182)
Williams et al. 2021[51]	USA	703	0	HNSCC	HPV + (634/703)
Zwirner et al. 2019[33]	Germany	20	20	Oral cavity, oropharynx, and hypopharynx.	5 HPV+, 14 HPV− and 1 unknown
Souliéres et al. 2018[31]	France	112	0	Hypopharynx, larynx, nasopharynx, oral cavity, oropharynx, other, and unknown	23 HPV+ and 89 HPV−
Mirghani et al. 2018[32]	France	71	0	Oropharynx	62 HPV+, and 9 HPV−
Pickering et al. 2015[54]	USA	44	44	Oral tongue	NA
Stransky et al. 2011[52]	USA	74	74	Oral cavity, oropharynx, hypopharynx, sinonasal, and larynx	11 HPV+ (RT-PCR)
The cancer genome atlas network, 2015[53]	USA	279	0	Oral cavity, oral pharynx, and larynx	36 HPV+ and 243 HPV−
Van Harten et al. 2019[44]	The Netherlands	24	0	Hypopharynx, base of the tongue, supraglottic larynx, floor of mouth, tonsillar fossa, tonsilla pillar, tongue, retromolar trigone, oropharynx, oral cavity, and mouth mucosa	5 HPV+, and 19 HPV−

Abbreviations: HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; NA, not available; OSCC-GB, gingivobuccal oral squamous cell carcinoma; OSCC, oral squamous cell carcinoma.

105 studies (Table S2). Finally, 33 articles were selected for descriptive analysis. A flow chart detailing the process of identifying, incorporating, and excluding studies is shown in Figure 1.

3.2 | Study characteristics

The included studies were conducted in 16 different countries, that is Austria,[23] Brazil,[24] Canada,[25,26] China,[27,28] Denmark,[29,30] France,[31,32] Germany,[33] India,[34–36] Japan,[37] Malaysia,[38] Pakistan,[39] Poland,[40] Taiwan,[41,42] Thailand,[43] the Netherlands,[44] and the United States of America.[45–55] All of them were published in English between 2011 and 2022.

The total sample size from the 33 selected studies was 4294 individuals affected by HNSCC. The individual size of samples ranged from 4 to 1667 HNSCC patients across studies.

TABLE 2 Genetic alterations in the *KMT2* family in HNSCC patients across 29 out of 33 studies included.

Author, year	*KMT2A* mutations	*KMT2B* mutations	*KMT2C* mutations	*KMT2D* mutations	*KMT2E* mutations	Techniques
Gronhoj et al. 2018[29]	+					Targeted sequencing
Karam et al. 2019[45]	+					Targeted sequencing, WTS
Lin et al. 2021[41]	+			+		WES, Sanger
Lilja-Fischer et al. 2019[30]	+		+	+		Targeted sequencing
Martin et al. 2014[46]	+		+	+		WES, WTS
Fadlullah et al. 2016[38]		+				WTS
Fan et al. 2021[42]		+				WES
Ghosh et al. 2022[34]		+				WES, WTS
Hedberg et al. 2016[47]		+		+		WES
India project team of the International Cancer Genome Consortium, 2013[35]		+		+		WES, Sanger
Osawa et al. 2021[37]		+	+	+		Targeted sequencing, Sanger
Ghias et al. 2018[39]			+			WES
Rawal et al. 2014[36]			+			Targeted sequencing
Burcher et al. 2021[55]			+			Targeted sequencing
Day et al. 2020[25]			+	+		Targeted sequencing
Haft et al. 2019[48]			+	+		WES
Machnicki et al. 2022[40]			+	+		Sanger, WES
Melchardt et al. 2018[23]			+	+		Targeted sequencing
Seiwert et al. 2015[49]			+	+		Targeted sequencing
Shaikh et al. 2021[50]			+	+		WES, WTS
Williams et al. 2021[51]			+	+		Targeted sequencing
Zwirner et al. 2019[33]			+	+		Targeted sequencing
Souliéres et al. 2018[31]				+		Targeted sequencing
Mirghani et al. 2018[32]				+		Targeted sequencing
Pickering et al. 2015[54]				+		WES
Stransky et al. 2011[52]				+		WES
The cancer genome atlas network, 2015[53]				+		WGS, WES, WTS
Van Harten et al. 2019[44]				+		WGS, Targeted sequencing, Sanger
Veeramachaneni et al. 2019[26]				+		WES, Targeted sequencing

Abbreviations: WES, whole exome sequencing; WGS, whole genome sequencing; WTS, whole transcriptome sequencing; +, present.

3.3 | Risk of bias within studies

Regarding the risk of bias evaluated by Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data, 18 studies were classified as having a low risk of bias,[27,29,34,35,37–45,49–51,54,55] and the others 15 were classified as having a moderate risk of bias.[23–26,28,30–33,36,46–48,52,53] The summary of each study's risk of bias assessment is in Table S3.

3.4 | Results of individual studies

The 33 included articles assessed one or more members of the KMT2 family in HNSCC, analyzing several aspects

(gene expression, protein expression, and genetic alterations). Features and results of the included articles are shown in Table 1.

4 | SYNTHESIS OF RESULTS

Considering the heterogeneity of the data in the included studies, they were separated into three topics: genetic, gene expression, and protein expression alterations.

4.1 | Genetic alterations

Genetic alterations within the *KMT2* family were identified in 29 out of the 33 studies encompassed in this systematic review, as presented in Table 2, involving a total cohort of 4054 HNSCC cases. Among the assessed *KMT2* methyltransferases, *KMT2D* was the most prominently affected by mutations in HNSCC samples at a rate of 6.19%, followed by *KMT2C* at 3.53%, *KMT2A* at 1.28%, and *KMT2B* at 0.54%. These studies revealed a wide array of mutations within this family, with the predominant mutation types being NS (Not Specified), missense, and truncating, as shown in Figure 2.

The most frequently altered gene, *KMT2D* (6.19%), was mentioned by 21[23,25,26,30–33,35,37,40,41,44,46–54] out of 29 studies that addressed mutational profile. In these investigations, the specimens exhibited a broad spectrum of genetic mutations within this particular gene, all with the potential to result in functional impairment. Interestingly, one study[23] detected new non-synonymous *KMT2D* mutations in metastasis of two patients who also had *TP53* mutations.

KMT2C mutations (3.53%) were identified by 14[23,25,30,33,36,37,39,40,46,48–51,55] out of 29 studies, which detected different mutations, such as single nucleotide variation, nonsense mutation, missense mutation, synonymous variation, and frameshift deletion. Curiously, one study showed that all *KMT2C* mutated cell lines also displayed *CDKN2A* promoter methylation.[46]

The *KMT2B* mutations (0.54%) were identified by 6[34,35,37,38,42,47] out of 29 studies, with the detection of single nucleotide variation, truncating mutation, nonsense mutation, and missense mutation.

The *KMT2A* mutations (1.28%) were identified by 5[29,30,41,45,46] out of 29 studies, including missense mutation, multi-hit, and frameshift insertion.

The *KMT2E* alterations were not reported within the included studies.

4.2 | *In silico* analysis of mutational profile of *KMT2* family in HNSCC samples on cBioPortal database

For a more comprehensive analysis of the mutational profiles of the *KMT2* family, as highlighted in Figure 2 and Table 2, and for comparison purposes, we conducted a supplementary *in silico* investigation using the cBioPortal for Cancer Genomics.[56–58] This platform, which is both open-source and open-access, facilitates interactive exploration of multidimensional cancer genomics datasets. Within this platform, we performed a query that

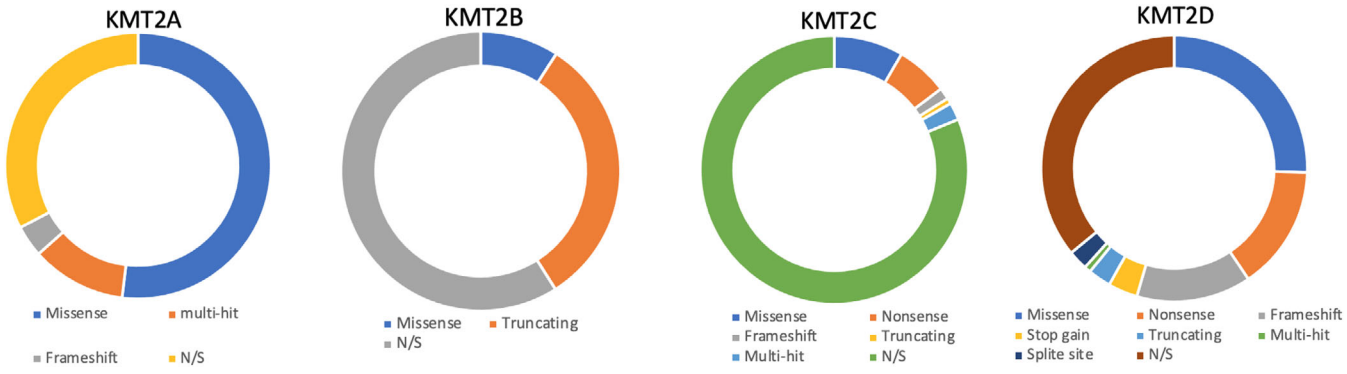


FIGURE 2 Mutational profile of the *KMT2* family in the analyzed studies. Among the samples reviewed, *KMT2D* stood out as the most frequently mutated gene, with a prevalence of 6.19%. Mutation types included 36% categorized as NS (not specified) and 25% as missense mutations. *KMT2C* ranked as the second most mutated gene, featuring in 3.53% of the samples, predominantly comprising 81% NS mutations and 8% missense mutations. The *KMT2B* exhibited the lowest mutation rate, accounting for 0.54% of cases, with a majority (59%) of NS mutations and 32% of truncation mutations. Finally, the *KMT2A*, detected in 1.28% of the samples, displayed a mutational profile consisting of 52% missense mutations and 33% NS mutations. *KMT2E* mutations were not reported within the included studies. [Color figure can be viewed at wileyonlinelibrary.com]

resulted in 5 datasets with a total of 732 HNSCC patients[52,59–61] which revealed that *KMT2D* and *KMT2C* were the most frequently mutated genes, accounting for 13% and 8% of HNSCC cases, respectively, with the majority of mutations being of the truncation type (Figure 3).

4.3 | Gene expression alterations

Gene expression of the *KMT2* methyltransferase family in HNSCC patient samples was assessed by 2 out of 33 studies using different techniques, that is real time PCR and NanoString Gene expression analysis,[24,43] as

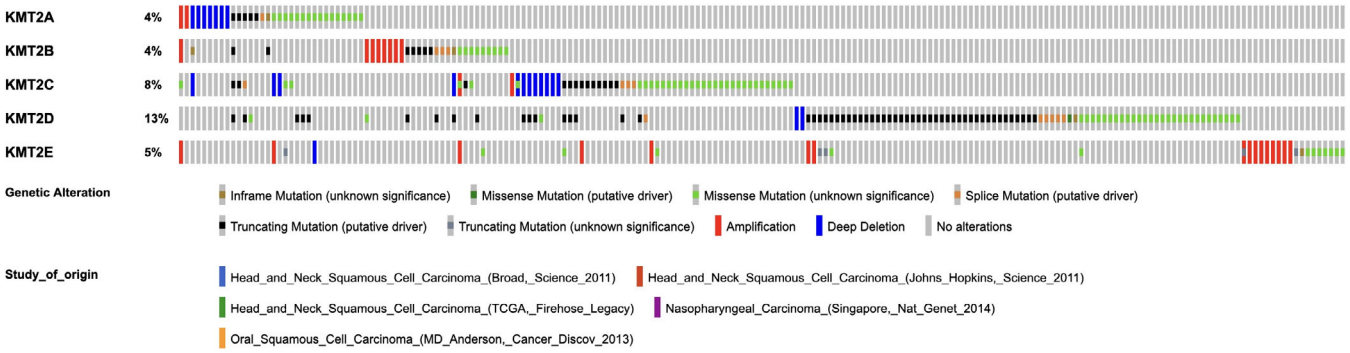


FIGURE 3 OncoPrint mutational profile using CBioPortal database.[56–58] *KMT2D* and *KMT2C* were the most frequently mutated genes, accounting for 13% and 8% of HNSCC cases, respectively. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Gene expression alterations within 2 out of 33 included studies.

Author (s), year	Number of HNSCC patients included	Number of controls	Site(s) of tumor	HPV status	*KMT2* assessed	Methods (technique used)	Result(s)
Figueiredo et al. 2014[24]	13	13	Larynx	NA	*KMT2A* *KMT2B* *KMT2C* *KMT2D* *KMT2E*	Quantitative real-time PCR (qPCR)	• Reduced expression tendency for all genes. • *KMT2C* was down-expressed ($p = 0.0199$). • All genes had a decreased expression level in advanced tumors. • Altered expression in one *KMT2* gene was correlated to a similar alteration in the other *KMT2* genes.
Bunbanjerdsuk et al. 2019[43]	46	16	Oral cavity, oropharynx, hypopharynx, larynx; nose, and Paranasal sinuses	46 p16−	*KMT2D*	NanoString gene expression analysis	• Higher expression in the group of patients who developed a second primary malignancy during the same follow-up period.

TABLE 4 Protein expression alterations within 2 out of 33 included studies.

Author (s), year	Number of HNSCC patients included	Number of controls	Site(s) of tumor	HPV status	KMT2 assessed	Methods (technique used)	Result(s)
Wang et al. 2022[27]	96	16	Oropharynx	NA	KMT2D	Immunofluorescence assay Lentiviral transfection	• Higher expression in OSCC tissues. • Silencing KMT2D in OSCC cells reduced colony formation, sphere formation, cell migration, and invasion.
Zhu et al. 2019[28]	85	36	Oral cavity	NA	KMT2D	Immunohistochemistry	• Higher expression in tumor samples. • Associated with poorer prognostic.

shown in Table 3. In such analyses, a distinct and heterogeneous profile of gene expression for the *KMT2* family was identified.

One study[24] showed a decreased expression of all *KMT2* genes (*KMT2A*, *KMT2B*, *KMT2C*, *KMT2D*, and *KMT2E*) in advanced tumors. Remarkably, only *KMT2C* was down-expressed in the tumor compared to normal samples ($p = 0.0199$). In contrast, another study[43] showed that high expression levels of *KMT2D* were associated with a second primary malignancy when compared to patients who did not develop one.

4.4 | Protein expression alterations

Only 2[27,28] out of 33 studies evaluated the KMT2 methyltransferase family's protein expression (Table 4). One study[28] showed that elevated levels of KMT2D in tumor samples were associated with poorer tumor differentiation; high histopathologic stage; worse pattern of invasion; and increased depth of invasion, suggesting that upregulation of this protein could indicate an unfavorable prognosis. The other study[27] showed that the KMT2D protein level was significantly elevated in OSCC samples compared to normal oral mucosal tissues.

5 | DISCUSSION

5.1 | Summary of evidence

At present, epigenetic events are considered an important mechanism in normal cell activities and in cancer cells processes as well. Epigenetic dysregulations influence various aspects of carcinogenesis, such as oncogenes, tumor suppressors, and signal transduction gene expression, which result in increased cancer growth, invasion, and metastasis.[6] However, there is still an urgent need to determine the most relevant epigenetic alterations in HNSCC to identify clinical useful biomarkers or actionable targets.

Among epigenetic alterations, histone lysine methyltransferases (KMTs) are enzymes responsible to transfer a methyl group from S-adenosyl methionine (SAM) to specific lysine (K) residues, forming S-adenosyl homocysteine,[62] which could lead to tightly packed chromatin or active loosened chromatin regions and consequent gene transcription.[63–69]

The *KMT2* (Mixed Lineage Leukemia) family of genes encodes 5 KMTs (MLL1-5 or KMT2A-E) which are responsible for mono-, di- or tri-methylation (H3K4me1, me2 or me3, respectively) of H3K4, marks mostly associated with loosened chromatin and gene transcription. The enzymes' function in KMT2 complexes or COMPASS (complex of proteins associated with Set1) is reported by different studies.[70–73] In addition to their function of methylating H3K4, several studies have reported other roles for these members, such as regulation of cell cycle, metabolic processes, and methylation of non-histone proteins.[74]

Recurrent alterations in *KMT2* family genes have been detected in other cancers, being associated with worse clinical outcomes,[11,75–78] but their role in the HNSCC remains unclear. Therefore, elucidation of these enzymes' role in HNSCC pathogenesis may reveal new strategies to deal with this disease.

To analyze and discuss our results properly, they were classified into two main topics, that is genetic alterations

and gene or protein expression alterations. It is noteworthy that the risk of bias within the studies included in this review was evaluated mainly as low risk, which gives greater reliability to the data synthesized in this review.

5.2 | Genetic alterations

According to our results, the majority of *KMT2* genes harbors missense, nonsense, and/or truncating mutations, except for the *KMT2E* gene. These observations suggest a potential tumor suppressive role for these genes, with particular emphasis on *KMT2D* and *KMT2C*, which were consistently the most frequently mutated genes across the studies included in our analysis. Corroborating these findings, the additional *in silico* analysis performed using five publicly accessible HNSCC studies via cBioPortal,[56–58] revealed that *KMT2D* and *KMT2C* stood out as the most commonly mutated genes, with mutation frequencies of 13% and 8%, respectively, as shown in Figure 2.

The hotspots and details of *KMT2D* and *KMT2C* mutations were not reported in the included studies, nonetheless, Liao et al. found that over 25% of *KMT2C* missense mutations were located around the first PHD finger cluster region in colorectal cancer.[79] Rhee et al. using MutClustSW analysis, identified *KMT2C* mutation in plant homeodomain 2 and in the junction of PHD3 and PHD4 of encoded protein.[80] Stroynowska-Czerwinska et al. using COSMIC data, showed that in cancer most mutations in *KMT2A-D* genes occur in the intrinsically disordered regions (IDRs), except for the *KMT2C* gene that has mutations in clustered PHDs. Some of these *KMT2A-D* mutations lead to relevant amino-acid changes in PHDs.[81] Furthermore, Rampias et al. observed *KMT2C* mutations mainly in PHD fingers 1–3 in a cohort of 72 cases diagnosed with superficial or muscle-invasive urothelial cancer after sequencing the N- and C-terminal regions of this gene.[82] Finally, in addition to the PHD domain, mutations in *KMT2C* and *KMT2D* were also identified at the amino-terminal end of the SET domain in breast cancer samples, with SET domain truncation or loss of function of these enzymes leading to tumor initiation and progression.[83]

Moreover, the co-occurrence of *KMT2D* and *KMT2C* mutations has been reported by several studies, which may suggest an important epigenetic event in HNSCC carcinogenesis.[23,25,30,33,37,40,46,48–51] In a similar study that also utilized data from the cBioPortal, a significant trend toward the co-occurrence of *KMT2C* and *KMT2D* mutations was observed in lung adenocarcinoma and squamous cell carcinoma (p-value <0.001), and these mutations were associated with driver mutations in *PIK3CA*, *PTEN*, and *ARID1A* in various cancer types.[13] Furthermore, a recent study presented these co-occurring mutations in association with Wnt signaling, ERBB2/4, TGF-β superfamily, and PI-3-kinase pathways in *KMT2*-mutant colorectal cancer.[79] Therefore, the biological significance of this co-occurrence event appears to be context-specific.

Similarly, the context-specific function attributed to this event extends to the individual functions of these genes, acting as tumor suppressors or oncogenes depending on the tissue and cancer type.[84,85] In our analysis, most mutations observed in *KMT2D* seem to have deleterious functional effects. This enzyme seems to interact with TP53, a tumor suppressor protein essential to regulate cell cycle and DNA repair, and whose inactivation is associated with a considerable proportion of human cancers.[86] The functional effects of such *KMT2D* inactivation were investigated in various tumors aiming to better understand its role. In this systematic review, we found a study that suppressed *KMT2D* in oral squamous cell carcinoma cell line using CRISPR-Cas9, which led to decreased colony formation, sphere formation, cell migration, and invasion compared to control cells.[40] In contrast, Dauch et al. showed that the loss of *KMT2D* in a cutaneous squamous cell carcinoma cell line was associated with increased proliferation, cell colony growth, migration, and accelerated progression through the cell cycle.[87] Consistently, Mll4 deficient mice (Gene ID: 381022) showed that Mll4 activates key lipoxygenase genes, such as Arachidonate 12-Lipoxygenase, 12S Type (Alox12), required for p53-mediated tumor suppression via ferroptosis, a programmed cell death, characterized by lipid peroxidation. Therefore, Mll4 deficiency results in the loss of an important tumor suppressive mechanism.[88]

Noteworthy evidence has shown that the loss of *KMT2D* is linked to several and contrasting cancer mechanisms, such as decreased expression of the tumor suppressors *PTEN* and *TP53* in bladder cancer[89]; inhibition of proliferation, migration and invasion in papillary thyroid carcinoma cells, with reduced expression of *NCOA6* and *THRB*[84]; activation of EGFR and ERBB2 with repression of multiple receptor-like protein tyrosine phosphatases (RPTPs), which activate oncogenic RTK-RAS signaling in lung squamous cell carcinoma, promoting tumor growth.[90] KMT2D also appears to play important roles in cell–cell communications via IL-6 which leads to the establishment of a tumor-supportive microenvironment in prostate cancer and may even serve as an indirect regulator of cancer cell proliferation.[91] Moreover, *KMT2D* knockout in the esophageal squamous cell carcinoma cell line prevented cell proliferation and migration, causing cell cycle arrest at G1 stage.[92]

KMT2C is a commonly mutated gene in many cancer types, which has been associated with worse outcomes. For instance, Chen et al. associated mutations in the *KMT2C* with a worse prognosis in breast cancer patients (HR), 2.00; 95% confidence interval (CI), 1.08–3.71; $p = 0.027$,[93] and Cho et al. associating pathological and clinical characteristics of 27 patients with gastric adenocarcinoma, identified that those patients with *KMT2C* mutations tend to have worse recurrence-free survival than those patients with wild *KMT2C*.[94] Several *KMT2C* mutations detected in the included studies of this systematic review seem to deactivate the protein. One of the studies identified that all cell lines that harbored *KMT2C* mutation also displayed *CDKN2A* promoter methylation.[46] *CDKN2A* encodes a cell cycle inhibitor protein, p16, (inhibitor of G1/S phase) that binds to complexes of cyclin–dependent kinases (CDK) 4 and 6, controlling the retinoblastoma (RB) protein activity. Another protein coded by CDKN2A is ARF, which prevents the degradation of p53 and allows for negative regulation of the cell cycle by binding to the MDM2. Similarly to p16, the loss of ARF leads to the progression of the cell cycle through MDM2-mediated degradation of p53.[95–97] To provide further evidence on this link, functional analysis employing in vivo CRISPR-based screening in hepatocellular carcinoma models demonstrated the essential role of *Kmt2c* (Gene ID: 231051) in co-activating the Cdkn2a locus. Its loss led to downregulation of tumor suppressor genes p16/Ink4a and p19/Arf, collaborating with Myc overexpression in carcinogenesis.[98] Consistently, another study utilizing animal model to investigate prostate cancer showed that samples with mutated *Kmt2c* displayed a Myc gene signature and loss of $p16^{INK4A}$,[99] facilitating tumor growth and metastasis. Additionally, *Kmt2c* loss in a breast cancer mice model led to Epithelial-to-Mesenchymal Transition (EMT), extracellular matrix re-organization, activation of ERK1/2, and mitochondrial dysfunction with ROS accumulation.[100] Therefore, the loss of *KMT2C* function may be an important oncogenic event that interferes with tumor suppressor mechanisms.

Furthermore, cumulative evidence has shown that KMT2D and KMT2C can act as coactivators of TP53,[101,102] therefore, loss of function of these enzymes in carcinogenesis could lead to reduced TP53 function, accumulating DNA damage due to decreased apoptosis or inefficient cell cycle regulation. The stable complex formed by KMT2C/D with ASC-2 interacts with TP53 contributing to the expression of its target genes during TP53 cellular responses.[101,103] These data suggest that *KMT2C* and *KMT2D* may have a key role in carcinogenesis, playing tumor suppressor activities.

To confirm this tumor suppressor function of the *KMT2C* gene and its involvement with other genes related to DNA repair, Rampias et al. in their RNA-seq and ChIP-seq experiment in urothelial carcinoma cells, showed that 3324 genes were affected (with up- or down-regulation) after *KMT2C* silencing using shRNAs. Of these genes, the group of down-regulated genes was enriched in the DDR and HR DNA repair pathway. Moreover, after restoring *KMT2C*, the expression of these genes was restored, suggesting that *KMT2C* performs tumor suppressor functions by regulating the expression of genes involved in the DNA repair response.[82] These findings corroborate the involvement of *KMT2C* loss in tumor formation and progression.

Although it is still too early to specify the roles of KMT2D and KMT2C in the pathogenesis of HNSCC due to the heterogeneous mechanisms played by these enzymes in different contexts, they seem to have a promising potential to be used as biomarkers to therapeutic response in immunotherapy approaches, as demonstrated in other types of cancer. Wang et al. demonstrated that *KMT2D* loss potentiates checkpoint therapy efficiency (anti-PD1, anti-PDL1, or anti-CTLA4) in bladder cancer, triple-negative breast cancer, melanoma, and lung cancer.[104] Zhang and Huang showed that mutant *KMT2* can predict the clinical benefit of immune checkpoint therapy in melanoma and non-small cell lung cancer.[105] Finally, Wang et al. showed that patients with gastric cancer harboring *KMT2* mutations may benefit from Immune Checkpoint Inhibitors (ICIs) and drugs that target DDR, MAPK/PI3K, metabolism and cell cycle pathways.[106] The potential of *KMT2C/D* mutations to predict immunotherapy response has been evaluated in other contexts with favorable results, such as in the study by Xie et al. that demonstrated *KMT2C* mutations as an independent factor capable of predicting a better response to anti-PD-1 treatments in patients with metastatic melanoma.[107] Liu et al. found that *KMT2C/D* loss-of-function variants may be a useful predictor for the effectiveness of ICIs in colorectal cancer.[108]

Additionally, other studies linked *KMT2C/D* mutation/inactivation to better response to PARP1/2 inhibitor therapy, with DNA repairing activity reported.[85,109,110] PARP1/2 inhibitors are shown to be a better approach to target specific DDR in cancer, with good clinical response in many solid tumors, including HNSCC.[111–113] Nowadays, there are some PARP1/2 inhibitors approved by the FDA for clinical use, such as Olaparib, whose combination with other anticancer therapies for HNSCC is under investigation and shows promising results.[114]

Despite the strong association of KMT2C/D members with the regulation of different pathways that could lead to tumor progression, disease aggressiveness, and DNA damage accumulation, besides its potential as a biomarker to predict immunotherapy response, further

studies are needed to better clarify such mechanisms due to the heterogeneity found in this systematic review for both genes.

5.3 | Gene or protein expression alterations

In this review we found a heterogeneous pattern of *KMT2D* gene expression but with a homogeneous protein expression pattern. As for the *KMT2C* gene, one of the included studies highlighted it as the only gene in its family to have significant down-expression in laryngeal tumors.[24] Regarding the other genes, it was not possible to conduct a critical analysis due to the insufficient number of related studies.

Confirming our findings, the heterogeneous pattern of *KMT2D* gene expression has been reported by different studies. For instance, one study shows downregulation of *KMT2D* in breast cancer ($p = 0.043$) using qPCR.[115] Sun et al. found a *KMT2D* down-expression in bladder cancer cell lines compared to normal uroepithelial cells using western blot and RT-qPCR.[89] On the other hand, high expression of *KMT2D* was documented by Abudureheman et al. 2018, both in mRNA ($p < 0.001$) and protein level ($p < 0.05$), assessed by RT-PCR and IHC, in esophageal squamous cell carcinoma[92]; by Wang et al. in papillary thyroid carcinoma cell lines using RT-PCR ($p < 0.01$) and Western blotting[84]; and also by Wang et al. in oral squamous cell carcinoma (OSCC) samples at protein level using IHC ($p < 0.01$).[27]

Considering the *KMT2C*, at present, there are studies corroborating our finding of downregulation in HNSCC, in different primary cancers. Rampias et al. in their analysis, of 104 patients with bladder cancer, showed that compared to normal tissue, *KMT2C* presented decreased expression in most of the samples, in RNA and protein levels (71/104, $p < 0.001$).[82] Sato et al. analyzed *KMT2C* mRNA from 401 patients with estrogen receptor-positive breast cancer from the CBio portal and found that patients with low expression *KMT2C* showed a worse prognosis ($p = 0.0005$).[116]

Although we did not find a correlation between prognosis for HNSCC patients with *KMT2C* and *KMT2D* gene or protein expression alterations, Abudureheman et al. observed that high expression of *KMT2D* was significantly associated with TNM stage ($p = 0.037$), tumor differentiation ($p = 0.032$) and tumor size ($p = 0.035$), and its low expression was related to better prognosis ($p = 0.011$, Log-rank test) using Kaplan–Meier survival analysis in esophageal squamous cell carcinoma.[92] Wang et al. showed an increased protein expression level as the pathological grade of patients with OSCC increased.[27] Furthermore, Rabello et al. found a decrease in *KMT2C* expression during disease progression, using qPCR in 46 patients with chronic myeloid leukemia.[117]

The distinct implications of KMT2C/D in cancer indicate a key role for them in tumor formation and progression. Furthermore, the conflicting results found in this study, as well as the heterogeneity reported in different tumor types, confirm its complex context-dependent function, as previously suggested.

6 | LIMITATIONS

A major limitation of this study was the inaccessibility to some data about mutations identified in the *KMT2* methyltransferase family by the included studies, for example, the specific type and spot of mutation, the specific amino acid altered, and the clinical parameters of patients who harbored these mutations. This data would be crucial to accurately assess the actual effect of these mutations in HNSCC. Moreover, other limitations included the insufficient number of studies that dealt with gene or protein expression of the KMT2 family in HNSCC, the lack of standardization of the nomenclature of the KMT2 (MLL) family, and the insufficient description of tumor anatomic sites in some of the studies, that made impossible the critical analysis of these data.

7 | CONCLUSIONS

The current investigation of the involvement of the KMT2 family in HNSCC showed an association between the dysregulation of these methyltransferases with carcinogenesis. Although it is not yet possible to describe the exact molecular mechanisms that these enzymes play in the pathogenesis of HNSCC, in our results, we found that the *KMT2C* and *KMT2D* genes were the most frequently altered in HNSCC among all members of their family, with a reported co-occurrence of mutation, which may indicate an important event in HNSCC carcinogenesis. However, these enzymes also perform independent activities regulating different genes and pathways involved in DNA damage response, such as *TP53*, *CDKN2A*, and NOTCH signaling pathways, among other multiple mechanisms. Therefore, alterations affecting one or both *KMT2C* and *KMT2D* genes seem to lead to tumor progression, disease aggressiveness, accumulation of DNA damage, and finally tumor immune response modulation, which emphasizes their potential to be used as

biomarkers to predict immunotherapy response. Nonetheless, novel studies are still necessary, with a larger number of HNSCC samples, including specific anatomic sites of the head and neck, as well as investigation of the multiple mechanisms affected by *KMT2C* and *KMT2D* mutations, including the response to different Immune Checkpoint Inhibitors.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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