**RAS Mutations in Head and Neck Cancer: A Systemic Review and Meta-Analysis**

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**Abstract:**

Herein, a systematic review and meta-analysis were conducted to establish the prevalence of HRAS, KRAS, and NRAS mutations in head and neck cancer (HNC) patients. Overall, 149 studies from the past 20 years comprising over 8500 patients were selected and integrated for this analysis. The available data were stratified according to geographical region, clinical features, and tumor characteristics, including human papillomavirus (HPV) infection status, tumor stage, and tumor grade. The estimated global HRAS mutation prevalence was 7% (95 % CI = 5.38-9.06, p<0.01, I2 = 87%), but this rate was more than twice as high in South Asia (15.28% ,95 % CI = 12.34-18.77, p=0.13, I2 = 39%). KRAS and NRAS estimated mutation prevalence rates were 2.89% (95 % CI = 2.19-3.80, p<0.01, I2 = 67%) and 2.20% (95 % CI = 1.86-2.59, p<0.01, I2 = 29%) respectively. Odds ratio (OR) analyses revealed a significant association between HRAS mutation and high tumor stage or grade (OR = 3.63; 95% CI = 1.53-8.64). Additionally, a significant association was found between HPV-positive status and KRAS mutation (OR=2.09, 95% CI = 1.01-4.31). In addition, the distribution of codon substitutions in HRAS, KRAS, and NRAS associated with different HNC anatomical sites was assessed. Overall, this comprehensive meta-analysis offers insight into the worldwide prevalence of RAS family mutations and reinforces their promise as viable therapeutic targets in HNC patients

**1. Introduction:**

Head and neck cancer (HNC) includes neoplasms that arise in the oral cavity, pharynx, larynx, sinuses, nasal cavity, and salivary glands (1). The main risk factors associated with HNC incidence include tobacco smoking, alcohol abuse, and human papillomavirus (HPV) infection. Other risk factors include wood and leather dust exposure, Epstein Barr Virus (EBV) infection, and betel nut chewing (2). Intensive research in recent decades has confirmed that this disease is exceptionally heterogeneous at the molecular level, and there is no single genetic alteration or a unique dysregulated molecular pathway responsible for its development and progression (3). This heterogeneity may explain the limited efficiency of current systemic therapies for HNCs, emphasizing the need to study specific and less common genetic alterations that may affect disease characteristics and clinical outcomes in HNC patients.

Ras GTPase family proteins are crucial players in many signaling networks, controlling cell proliferation, differentiation, and survival (4). HRAS, KRAS, and NRAS share significant sequence homology and largely overlapping functions (5). Mutations in RAS family members are well-established drivers of cancer. Gain-of-function mutations in RAS genes are found in ∼19% of human cancers (6), prompting interest in identifying anti-RAS therapeutic strategies for cancer treatment. The immense effort put towards the development of RAS inhibitors has led to several breakthroughs in recent years, allowing for the targeted treatment of patients with alterations in these RAS genes (7)(8).

Many studies have reported the prevalence of HRAS, KRAS, and NRAS mutational status in HPV-positive and HPV-negative HNC patients. Although mutations in these RAS family genes are seemingly rare in some large cohorts, a broader analysis of the data reveals considerable variation among studies. A broad analysis of the prevalence of mutations in specific RAS family members thus has the potential to better characterize the HNC landscape and the potential for innovation in personalized treatments. Therefore, the purpose of this study was to conduct the first systematic review and meta-analysis evaluating the prevalence of mutations in RAS genes in HNC. In addition, this analysis examined the relationship between these mutations and tumor anatomical sites, geographical regions, and patient clinical features.

**2. Methods**

This systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Checklist (9)

**2.1 Study design**We evaluated the prevalence of mutations in the HRAS, KRAS, and NRAS genes in patients with head and neck squamous cell carcinoma.

**2.2 Search Strategy**

A systematic literature review was conducted by searching the PubMed, Embase, Web of Science, and Cochrane Central Register of Controlled Trials databases in June 2021 for studies published in the English language since 1 January 2000. The search string included 'RAS' and ‘mutation’ and one of the following terms: ‘Head and neck cancer’, ‘Head and neck squamous cell carcinoma’, ‘Oral cancer’, ‘oral squamous cell carcinoma’, ‘tongue’, ‘lips’, ‘nasopharyngeal’ /’nasopharynx’, ‘pharyngeal’/’pharynx’, ‘laryngeal’/’larynx’, ‘oropharyngeal’/’oropharynx’, ‘Salivary gland’, ‘sinonasal’/’nasal’/’sinus’, ‘oropharyngeal’/’oropharynx’, ‘hypopharyngeal’/’hypopharynx’, or ‘tonsil’. The bibliographies of retrieved studies and systematic reviews identified in the search were screened for relevant references. Publicly available databases were screened for unpublished data.

**2.3 Selection Criteria**

To be eligible for inclusion in this meta-analysis, studies had to include a mutational analysis of at least one of the target genes (HRAS KRAS, NRAS) and report the prevalence and frequency of mutations as an outcome measure. Exclusion criteria were defined as: (1) Studies displaying results from patients with tumors other than HNC or mutations other than those in the target genes; (2) Studies that did not report data related to the prevalence or frequency of the target genes mutations; (3) studies that did not evaluate target genes for somatic mutations; (4) Studies published before January 1, 2000; (5) studies that were conducted using cell lines or animal models; (6) studies of pediatric populations; (7) review articles, letters, personal opinions, book chapters, or conference abstracts; (8) studies that contained data included in other studies or studies in which it was not possible to determine whether duplicate data were included; and (9) studies enrolling fewer than 10 patients.

**2.4 Data extraction**

Two reviewers (SJ, ON) screened the studies at the title and abstract level, followed by a full-text review. Disagreements over inclusion were resolved by consensus adjudication, and studies were extracted into a standardized extraction database. Extracted variables included study cohort size, number of mutated cases for each RAS family gene, primary tumor location, tumor grade or stage, geographical origin of studied patients, mutation assessment method, mutated codon, HPV-status, and biopsy type, if reported.

**2.5 Evaluation of Quality and Risk of Bias**

Our study selection process excluded individuals case reports and cohorts of < 10 patients due to the risk of bias. All papers considered after initial screening were reviewed and scored for risk of bias according to the Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data (10) (Supplementary Table 1). Studies that did not evaluate all three RAS family members were considered more prone to the risk of bias and were not included in the general prevalence analysis**.** In addition, publication bias and heterogeneity were assessed by visual inspection of funnel plots and via Egger's regression test (11) (Supplementary figure 1).

**2.6 Statistical analysis**

Pooled prevalence rates, pooled odds ratios, and forest plots were generated using the R Meta and MetaFor Packages (12,13). The Cochrane Q chi-squared test and I2 statistic were used to examine the heterogeneity across studies. Fixed-effects models were used to assess the pooled prevalence of genes for results with low heterogeneity (I2 ≤ 50%). Otherwise, random-effects models were used for conducted analyses. A sensitivity analysis using a "leave-one-out" paradigm from the built-in function in MetaFor as proposed by Wang et al. (14) was used to assess each study's effect on the overall pooled prevalence and detected outliers (14). First, the pooled overall prevalence of mutations in the three different target genes (KRAS, HRAS, NRAS) was calculated with a corresponding 95% confidence interval (95% CI). Next, we performed subgroup analyses according to geographical region and anatomical site. Finally, we assessed the association between the RAS gene mutational status and HPV status or tumor grade using the R MetaBin function.

**3. Results**

**3.1 Study selection and characteristics**

The flow diagram shown in Figure 1 depicts the search strategy, study selection process, and obtained results for the present. A total of 867 studies were retrieved from four electronic databases (Pubmed, Scopus, Web of Science, and Cochrane) and a bibliography screen. After the removal of duplicates, 375 studies were considered potentially eligible for evaluation, of which 217 did not meet all inclusion criteria, leaving a final sample of 158 studies. The references for the studies included in the meta-analysis are listed in Supplementary Materials 2. Nine additional studies were excluded due to the high risk of bias according to the Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data. To reduce the risk of bias, only papers with the highest grade (n=85) were included for pooled analyses of overall mutation prevalence.

**3.2 Study characteristics**

Detailed characterization of the studies is provided in Supplementary Table 3. Of the 149 records included in this analysis, 112, 130, and 93 included data pertaining to HRAS, KRAS, and NRAS, respectively, with 85 studies including analyses of all three RAS family genes.

All 149 included studies were published in English between the years 2000 and 2021. The studies were conducted in 29 different countries: Australia, Belgium, Brazil, Bulgaria, Canada, China, the Czech Republic, Denmark, France, Germany, Greece, Hungary, India, Israel, Italy, Japan, Korea, Malaysia, Mexico, the Netherlands, Serbia, Singapore, Spain, Sweden, Taiwan, the United Kingdom, the United States of America, Vietnam, and Yemen. Four studies included a mixed population from various geographical regions. In total, 148 of the included studies were cohort studies, while one was a phase 1 clinical trial. Forty-seven studies used targeted next-generation sequencing (NGS), 46 utilized Sanger sequencing, 23 employed whole-exome sequencing, 9 conducted Mass Array analysis, 4 used whole-genome analysis, and 20 employed other or mixed analysis methods.

The anatomical location of the tumors in the included study cohorts was predominantly in the oral cavity (HRAS n=3102 [33.9%]; KRAS n=2949 [32.2%]; NRAS n=2113 [23.1%]) followed by the salivary glands (HRAS n=2478 [27.1%]; KRAS n=2352 [25.7%]; NRAS n=2199 [24%]). Additional sites included the sinonasal region (HRAS n=233 [2.5%]; KRAS n=654 [7.1%]; NRAS n=168 [1.8%]), nasopharynx (HRAS n=687 [7.5%]; KRAS n=702 [7.7%]; NRAS n=587 [6.4%]), oropharynx (HRAS n=1428 [15.6%]; KRAS n=1695 [18.5%]; NRAS n=1243 [13.6%]), hypopharynx (HRAS n=228 [2.5%]; KRAS n=293 [3.2%]; NRAS n=113 [1.2%]), larynx (HRAS n=753 [8.2%]; KRAS n=786 [8.6%]; NRAS n=518 [5.6%]), and other sites (HRAS n=233 [2.5%]; KRAS n=285 [3.1%]; NRAS n=244 [2.6%]).

**3.3 Risk of bias within studies**

With respect to the risk of bias identified using the Joanna Briggs Institute Critical Appraisal Checklist for Studies Reporting Prevalence Data scoring, two studies (15)(16) were classified as having a high risk of bias and were therefore excluded from this meta-analysis. Eleven studies were classified as having a moderate risk of bias due to a small cohort size, while 57 studies were classified as having a moderate risk of bias due to their having only analyzed one of the three RAS family target genes. The remaining 85 studies were classified as having a low risk of bias and were used in the general prevalence analysis. All low and moderate risk studies were used in prevalence analyses pertaining to tumor anatomical sites, mutated codons, and the association between RAS mutations and patient clinical features. A summary of the risk of bias assessment for each study can be found in Supplementary Table 1.

**3.4 Prevalence of RAS mutations**

*HRAS mutations* were identified in 564 tumors from 8501 patients. The mean prevalence of HRAS mutations was 7% (95% CI = 5.38-9.06, p<0.01, I2 = 87%) (Fig. 2A). Geographical region-specific analyses revealed significant differences in these rates in different regions of the world (Q=22.51, Pv<0.0001). The mean frequency of HRAS mutations in South Asia was 15.28% (95 % CI = 12.34-18.77, p=0.13, I2 = 39%), with this rate being higher than in other geographical regions including East Asia (5.07%; 95% CI = 1.99-12.31, p<0.01, I2 = 93%), Europe (4.65%; 95% CI = 2.57-8.28, p<0.01, I2 = 80%), and North America (6.87%; 95% CI =4.77-9.79, p<0.01, I2 = 76%). (Fig. 3A, Supp Figure 2).

*KRAS mutations* were identified in 188 tumors from 8631 patients. The mean prevalence of KRAS mutations was 2.89% (95% CI = 2.19-3.80, p<0.0.1, I2 = 67%) (Fig. 2B), with no significant difference in prevalence between analyzed geographical regions (Q=1.41, Pv=0.7). The mean frequency of KRAS mutations in Europe was 3.54% (95% CI = 2.18-5.17, p<0.01, I2 = 58%), which was slightly but not significantly higher than rates in other geographical regions including East Asia (2.20%; 95% CI = 1.09-4.42, p<0.01, I2 = 70%), South Asia (2.95%; 95% CI = 0.76-10.77, p<0.01, I2 = 72%), North America (2.61%; 95% CI =1.60-4.23, p<0.01, I2 = 65%). (Fig. 3B, Supplementary Figure 2).

*NRAS mutations* were identified in 113 tumors from 8512 patients. The mean prevalence of NRAS mutations was 2.20% (95 % CI = 1.86-2.59, p<0.01, I2 = 29%) (Fig. 2C). No significant differences in these rates were observed among different parts of the world (Q=3.32, Pv=0.34). The mean frequency of NRAS mutations in South Asia was 1.11% (95 % CI = 2.18-5.17, p=0.98, I2 = 0%) while in East Asia this rate was 1.66% (95% CI 1.05-2.60, p=0.27, I2 = 0%) which was slightly, but not significantly, lower than the rates in Europe (2.72%; 95% CI=1.88-3.92, p=0.02, I2 = 46%) and North America (2.45%; 95 % CI =1.82-3.28, p=0.29, I2 = 10%) (Fig. 3C, Supplementary Figure 2).

**3.5 Anatomical site**

As HNC includes tumors that arise from a wide range of anatomical sites and sub-sites, an analysis of the frequency of mutations in these three RAS genes was performed for seven major anatomical areas. A summary of these analyses is presented in Figure 4A and Supplementary Figure 3).

*HRAS mutation -* A significant difference in the prevalence of HRAS mutations was detected between anatomical sites (Q=67.96, Pv<0.0001). HRAS mutations were more frequently found in tumors arising from the salivary gland (10.37%; 95% CI=7.18-14.06) and oral cavity (7.36%; 95% CI=5.39-9.76) as compared to tumors arising from the sinonasal cavity (1.2%; 95% CI=0.2-3), oropharynx (2.6%; 95% CI=1.12-4.56), nasopharynx (0.68%; 95% CI=0-4.06), larynx (2.76%; 95% CI=0.99-5.38) and hypopharynx (0.12%; 95% CI=0-0.04).

*KRAS mutation –* A trend towards more frequent KRAS mutations was observed tumors arising from the sinonasal cavity (5.67%; 95% CI=1.33-12.74) as compared to tumros arising from the salivary glands (0.98%; 95% CI=0.33-1.96), oral cavity (0.7%; 95% CI=0.17-1.59), oropharynx (1.49%; 95% CI=0.6-2.77), nasopharynx (0.83%; 95% CI=0.29-1.63), larynx (1.43%; 95% CI=0.34-3.25) and hypopharynx (0.84%; 95% CI=0-3.18). However, these differences were not robust (Pv=0.29 and Q=8.5).

*NRAS mutation –* A significant difference between anatomical sites was also seen for NRAS mutations (Q=18.37, Pv 0.01), with a rate of 1.85% (95% CI=0.92-3.1) in the nasopharynx as compared to lower rates in tumors arising from the salivary glands (0.51%; 95% CI=0.11-1.22), oral cavity (0.3%; 95% CI=0.11-0.58), sinonasal cavity (0.28%; 95% CI=0-1.65), oropharynx (0.65%; 95% CI=0.28-1.16), larynx (0.16%; 95% CI=0-0.68), and hypopharynx (0%; 95% CI=0-0.85).

**3.6 Amino acid substitution**

*HRAS mutation -* in an analysis of all cases with HRAS mutations, 24%, 20%, and 39% were in codons 12, 13, and 61, respectively. Salivary gland tumors exhibited a higher frequency of mutations in codon 61 (67%), while in tumors of the oral cavity, mutations in codon 12 were the most frequent (50%) (Figure 4B left panel). Mutations in codon 12 were mostly G12S (56.3%), while mutations in codon 13 were primarily G13R (46.8%). Lastly, mutations found in Q61 were primarily Q61R (49.2%), Q61K (26.4%), and Q61L (22.2%) (Supplementary Figure 4). Mutations in other codons accounted for 16% of overall HRAS mutations.

*KRAS mutation -* mutations in codon 12 were the most frequent across all anatomic sites, followed by codon 13. Mutations in codon 61 were primarily detected in tumors arising from the oropharynx (17%) (Figure 4B middle panel). Among codon 12 mutations, the most common amino acid substitution was G12D (51%), followed by G12V (16.3%), G12C (12.9%). Other substitutions included G12A (7.5%), G12R (6.1%), and G12S (5.4%) (Supplementary Figure 4). Mutations in other codons accounted for 19% of overall KRAS mutations.

*NRAS mutation –* NRAS mutations were more evenly distributed among codons (Figure 4B right panel). Site-specific analyses should be interpreted with caution owing to the limited number of mutated cases. Mutations in other codons accounted for 33% of overall NRAS mutations.

**3.7 Association between RAS mutations and patient clinical features**

**3.7.1 RAS mutation and disease stage/grade**

Tumor grade and stage are well-studied prognostic factors for HNC (17). In total, 44 studies reported details regarding the tumor stage or grade of patients along with mutation status. Tumors with stage or grade of 1 and 2 were defined as low-grade tumors, while those with a stage or grade of 3 and 4 were categorized as high-grade tumors. An odds ratio analysis revealed a significant association between HRAS mutation and advanced stage (OR = 3.63; 95% CI = 1.53-8.64) (Figure 5). KRAS (OR = 2.41; 95% CI = 0.85-6.86) and NRAS (OR = 1.52; 95% CI = 0.68-3.41) mutations were both associated with an OR>1, but did not reach statistical significance (Supplementary Figure 5)

**3.7.2 RAS mutations and HPV status**

Of the 38 cohort studies that reported on the HPV status of patients, only 25 provided specific patient data, and of these, 17 included both HPV-negative and HPV-positive patients allowing for an odds ratio analysis. This analysis revealed a significant association between HPV-positive status and KRAS mutations with an OR of 2.09 (95% CI = 1.01-4.31) (Figure 6), but no significant correlation between HPV-positive status and HRAS or NRAS mutation (Supplementary Figure 6).

**4. Discussion**

The current low survival rates of patients with advanced and metastatic HNC highlights the need for improvements in the personalized treatment of affected individuals (18). The RAS proteins are the most common targets of oncogenic mutations across cancer types (4). After years of extensive research, new strategies have emerged allowing for the targeting of the RAS-MAPK pathway, opening new therapeutic options to affected patients (7). While RAS mutations are not as prevalent in HNC as in other cancer types, many studies on RAS mutational prevalence in HNC have been conducted over the years. These studies have led to various and sometimes contradictory conclusions pertaining to RAS mutation prevalence and the association between such mutations and prognosis and risk factors. This meta-analysis integrates findings from the past 20 years and provides updated insight into the global prevalence of mutations in RAS family genes, underscoring their promise as potential therapeutic targets in HNC patients.

The prevalence of mutations was highest for the HRAS gene, following by KRAS and NRAS. This aligns with previous reports on the higher frequency of HRAS mutation in HNC as compared to its frequency in other cancer types in which KRAS mutations are most prevalent, followed by NRAS mutations (6). The results of our prevalence analysis exhibit some divergence from the results of The Cancer Genome Atlas (19,20), one of the most significant studies carried out on an HNC patient population. Our data suggest a slightly higher incidence of HRAS (7% compared to 6.25%) and KRAS (2.89% compared to 1.65%) mutations and a somewhat lower prevalence of NRAS mutations (2.2% compared to 2.65%). These slight differences may be due to the more heterogeneous population of patients from diverse geographical regions, disease stages, and detection methods included in our analysis.

HNC is a heterogeneous disease spanning many anatomical sites. Our analyses revealed that there were certain differences in RAS mutational prevalence according to the anatomical site, which may account for some of the heterogeneity between cohorts in the overall prevalence analysis. HRAS mutations were more prevalent in the oral cavity and salivary gland tumors. In contrast, KRAS mutations were more frequent in sinonasal tumors, and NRAS mutations were found chiefly in tumors of the nasopharynx. This variation in frequency between tissue types may be due to the differences in baseline expression and activity of RAS in different anatomical sites that may affect cellular reprogramming and tumor formation (6). An additional explanation may be differences in quality and quantity of exposure to risk factors (21). For example, the higher frequency of HRAS mutation in the salivary gland and oral cavity tumors might be due to their exposure to volatile nitrosamines through the mouth and nose (22).

These findings emphasize the importance of considering the anatomical site of the tumor in order to achieve a more accurate assessment of RAS mutation frequencies. In addition, the site-specific prevalence analysis hints at mutual exclusivity between the RAS genes as each gene is most prevalent in a different anatomical region in which the other RAS family members are rare. Mutual exclusivity between RAS family members is expected, as co-expression leads to oncogene-induced senescence (23).

Our data reveal a significantly higher prevalence of HRAS mutations in South Asia (above 15% as compared to 7% worldwide). These findings corroborate previous studies (24–27). Studies conducted assessing oral cancer in India have identified region-specific risk factors, such as smoking bidis (cigarettes wrapped in a tendu or temburni leaf) (28,29), chewing betelnut (30), and oral hygiene (31). These risk factors contribute, separately and synergistically, to the development of tumors specifically within the oral cavity (32–35). Indeed, in our database, 86% of the samples from South Asia were oral cancer patients, as opposed to other primary tumor sites. As noted above, HRAS mutation frequency is higher in oral cancer worldwide. Thus, further studies need to be done to determine whether these risk factors directly cause mutation in HRAS upon exposure or whether these factors increase the odds of tumors developing in the oral cavity, in which the prevalence of HRAS mutations is high.

Recent studies have shown that different amino acid substitutions in KRAS can result in distinct oncogenic effects (36,37). In addition, mutant-specific KRAS inhibitors, namely G12C and G12D, are in various stages of development (38–40). We found that the most frequent amino acid substitution in codon 12 of KRAS was G12D (51%), followed by G12V (16.3%) and G12C (12.9%). These alterations in the KRAS G12 codon are likely associated with patient smoking habits, as described by Dogan et al. in the context of lung cancer (41).

A considerable percentage of HRAS mutations were present in codon 61, particularly in salivary gland tumors. Limited studies to date have been performed to understand the etiology of these specific alterations, but recent analyses in salivary gland cancer patients have noted the diagnostic significance of these mutations (42). Clinicians have thus started to target HRAS for the treatment of HRAS-driven salivary gland tumors (43). These findings may help evaluate the size of the subpopulations that may benefit from a particular treatment.

Around 20% of HRAS and KRAS mutations were in sites other than codons 12, 13, and 61. For NRAS, this number was even higher (33%). These data emphasize the importance of unbiased gene sequencing and the possibility of bias in studies that use codon-specific analysis methods.

Tumor stage is a well-identified prognostic factor for HNC (17). Data regarding the association between RAS gene mutations and prognosis in HNC, however, are contradictory. Some studies link RAS mutation with stage and disease recurrence (44–47), while others predict better prognosis and overall survival (48–50). Our meta-analysis found that mutations in HRAS are significantly associated with high stage/grade scores, emphasizing the importance of considering RAS mutational status when gauging patient prognosis. KRAS and NRAS mutations exhibited a trend towards being associated with high stage/grade scores. The lower number of mutated cases available for OR analysis for KRAS and particularly NRAS may account for the observed lack of statistical significance.

An association between RAS mutations and HPV status in HNC has been suggested previously (46,51). In accordance with these prior findings, our data reveal a significant association between HPV-positive status and KRAS mutations. Other HPV-related cancers also exhibited a similar association. For instance, HRAS, KRAS, and NRAS expression levels among cervical cancer patients were higher in HPV-positive cases relative to HPV-negative samples (52). *In vitro* studies have shown that the mechanistic basis for HPV-induced tumorigenesis employs RAS activation and MAPK signaling (53). In addition, transduction of HRAS on the background of E6E7 expression causes oncogenic transformation (54). Notably, KRAS mutations, HRAS mutations, and HPV infection were mutually exclusive in benign neoplasms of the head and neck (15). These findings suggest that RAS mutations in the context of HPV infection contribute to carcinogenesis transformation. In contrast to the significant association between KRAS mutational status and HPV status, this meta-analysis did not detect a substantial difference when comparing the relationship between HPV status and the prevalence of HRAS and NRAS mutations.

Several agents are currently under evaluation for RAS-MAPK pathway inhibition in various cancers (reviewed in (7)). HNC patients are currently included in some of these clinical trials, which include ERK and MEK (NCT02465060, NCT03264066), G12C KRAS (NCT04185883), HRAS (NCT03719690, NCT02383927, NCT04997902), SHP2 (NCT04721223, NCT04000529, NCT03634982), and SOS1 (NCT04111458) inhibitors. Knowledge regarding the prevalence of RAS family mutations and associated characteristics in HNC may enable researchers to better assess the need and potential of trials with molecularly relevant targeted therapeutics.

The major strength of this work is the large number and range of patients included to estimate the prevalence of HRAS, KRAS, and NRAS mutations. However, certain methodological limitations of this review should be considered. First, even after selecting only those studies with a low ‘risk of bias score’, the heterogeneity between studies remained high. We believe that this is due to the heterogeneous nature of HNC, which includes a wide range of anatomical sites and etiologies. We attempted to address this issue by conducting additional sub-group analyses, which consistently revealed significant differences between groups. A second limitation of this analysis is the differences in the sequencing methods used among studies, which may have influenced overall pooled results by interfering with the accuracy and precision of pooled estimates. Third, we did not analyze sufficient data pertaining to patient-specific risk factor exposure. Such data could potentially have strengthened the observed associations in this study and provided additional corresponding insights. Lastly, we did not present quantitative data regarding the mutual exclusivity of mutations in RAS family genes. Despite these limitations, this is the most extensive analysis and the first meta-analysis conducted to date to have assessed the prevalence and characteristics of mutations in RAS family genes among HNC patients.

In conclusion, this study highlights RAS as a potential therapeutic target in a subset of HNC patients, shedding light on differences in mutational prevalence rates according to geographical region and tumor anatomical site. In addition, this analysis demonstrates that RAS mutations are associated with tumor stage and HPV status.

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**Conflict of Interest**

The authors declare no conflict of interest.

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