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Review

TERT promoter mutations in head and neck squamous cell carcinoma: A systematic review and meta-analysis on prevalence and prognostic significance

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ABSTRACT

Objectives: To estimate the prevalence of two most common and mutually exclusive −124 C > T and −146 C > T *TERT* promoter mutations in HNSCC and analyse their prognostic role.
Materials and methods: The databases Medline (via Ovid), Embase (via Ovid), Cochrane Library, Scopus, and Web of Science (Core Collection) were searched from inception to December 2022 to identify studies analysing *TERT* promoter mutations in HNSCC. Pooled prevalence of *TERT* promoter mutations and hazard ratio (sHR) of death/progression, with corresponding confidence intervals (CI), were estimated.
Results: The initial search returned 6416 articles, of which 17 studies, including 1830 patients, met the criteria for prevalence meta-analysis. Among them, 8 studies fitted the inclusion criterion to analyse the prognostic impact of *TERT* promoter mutations. Overall, 21% (95% CI: 12%-31%) of HNSCCs harboured *TERT* promoter mutation. *TERT* promoter mutations were more commonly found in oral cavity cancer (prevalence = 47%, 95% CI: 33%–61%), followed by laryngeal/hypopharyngeal cancer (prevalence = 12%, 95% CI: 4%-25%), while they were quite rare in oropharyngeal cancer (prevalence = 1%, 95% CI: 0%-4%). *TERT* promoter mutation −124 C > T was associated with a higher risk of death (sHR = 2.01, 95% CI: 1.25–3.23) and progression (sHR = 2.79, 95% CI: 1.77–4.40), while −146 C > T *TERT* promoter mutation did not show any significant correlation neither to overall nor progression-free survival.
Conclusion: *TERT* promoter mutations were mainly topographically restricted to oral cavity cancer. −124 C > T was the most common *TERT* promoter mutation and was significantly associated to worse outcome in HNSCC.

Introduction

Worldwide, approximately 880,000 people are diagnosed with a head and neck cancer each year [1]. The majority of these (i.e., about 375,000) are localized in the oral cavity, with about 185,000 in the larynx, 185,000 in the oropharynx and hypopharynx, and 135,000 in the nasopharynx. Most of head and neck cancers are head and neck squamous cell carcinomas (HNSCC) [2].

Tobacco smoking and alcohol abuse are the major established risk factors for HNSCC [1]. The role of high-risk alpha human papillomaviruses (HR α-HPVs) infection in the aetiology of oropharyngeal SCC has increased in the last decades, being the strongest independent prognostic factor for this subset of cancers [3,4]. Conversely, no robust biological biomarkers are available for HNSCC arising from other head and neck sites including oral cavity and larynx [1]. There is, therefore, a strong need to have biomarkers able to stratify the risk in these patients.

Among molecular markers proposed for risk stratification of HNSCC patients, the detection of telomerase reverse transcriptase (*TERT*)

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promoter mutations has attracted considerable interest given that most HNSCCs express high levels of *TERT* transcripts that have proved to be associated with worse responses to treatment and high risk of progression [5,6]. Telomerase is an enzyme that avoids the loss of telomeres at each cell replication. It presents a catalytic subunit with reverse transcriptase activity, *TERT*, and an RNA component which primes DNA synthesis from telomere repeats (TERC, telomerase RNA component). Somatic cells shorten the telomeres each cell cycle; this prevents the unlimited cell division, whereas cells that have active telomerase possess unlimited proliferative potential [7]. Since the acquisition of unlimited proliferation capacity represents a critical hallmark required for cell malignant transformation, telomere/telomerase complex is a pivotal component in the neoplastic process [8]. *TERT* is normally expressed in adult humans only in germ cells, transit-amplifying stem-like cells, and proliferating/stimulated B and T cells, but it is estimated that in 85% of cancers telomerase are reactivated during the process of carcinogenesis [9]. While the mechanisms that lead *TERT* to be reactivated in cancer cells have still to be completely understood, *TERT* reactivation can be explained by the genetic and epigenetic factors, such as *TERT* amplifications, *TERT* structural variants, rearrangements, promoter methylation, and mutations within *TERT* promoter region [9,10]. *TERT* promoter mutations mainly appear at nucleotides 1,295,228 (−124 C > T) and 1,295,250 (−146 C > T) and represent the most common non-coding mutations in solid tumours being recorded in several cancer types with a broad spectrum of prevalence [11–15]. These two mutations occur in a mutually exclusive manner, with the −124 C > T showing greater prevalence than −146 C > T. Both mutations increase *TERT* promoter activity and augment *TERT* gene transcription by creating *de novo* binding sites for E-twenty-six (ETS) transcription factors family [11,12,16]. In addition, recent evidence suggests that *TERT* polymorphisms can also play an important role in oncogenesis [17–20]. By interacting with the Wnt/β-catenin and the NF-kB signalling pathways, telomerase may play non-canonical functions directly linked with tumour progression, making it a possibly appealing prognostic marker [21,22]. Recently, *TERT* promoter mutations were observed to be associated with a highly aggressive behaviour in several cancers including thyroid, bladder, and melanoma skin cancers [23–25]. However, results from studies evaluating the association between *TERT* promoter mutations, cancer biology, and outcome in HNSCC were often inconsistent [17,26–29].

Thus, the aim of this systematic review and meta-analysis was to estimate the pooled prevalence of *TERT* promoter mutations in HNSCC and to investigate their prognostic significance.

Materials and methods

Search strategy

The protocol for this systematic review was registered with PROSPERO with the code CRD42022338251. The databases Medline (via Ovid), Embase (via Ovid), Cochrane Library, Scopus, and Web of Science (Core Collection) were searched from inception to December 2022. The search strategy was developed in consultation with an experienced medical librarian (VP) using the PRESS checklist and reported according to the PRISMA-S guidelines [30,31] (**eMethod in Supplementary Online Content**). Databases were searched separately, rather than multiple databases being searched simultaneously on the same platform. The search syntax was adapted for each database to account for variation between thesaurus terms/controlled vocabulary across each database. Exact search terms used in each database are present in supplementary materials. Results were deduplicated using Endnote 20 software. Endnote was set to identify articles as duplicates if they matched in the Author, Year, Title, Short Title, and Reference Type fields, and was also set to ignore differences in item record spacing and punctuation in these fields when identifying duplicates. The reference lists of articles included in this review, as well as narrative reviews published in the last 10 years, were also manually searched to minimize the risk of missing data. Two authors (PBR, ES) independently screened all titles and abstracts generated by the search and then evaluated the full texts of all the relevant articles identified against the inclusion criteria (Fig. 1); a third author (DB) settled discordances when present.

Outcome measures

The primary outcome of this meta-analysis was the prevalence of *TERT* promoter mutations in HNSCCs, measured as the proportion of patients carrying the mutation, distinguishing between −124 C > T, −146 C > T, or other non-specified *TERT* promoter mutations. The secondary outcome was the influence of *TERT* promoter mutations on overall survival (OS) or progression-free survival (PFS); the measure effect of the secondary outcome was the hazard ratio (HR) of death or recurrence, according to the mutational status.

Selection criteria

All observational studies that analysed *TERT* promoter mutations in HNSCCs were included. Research letters were also considered. Inclusion criteria were: 1) studies investigating *TERT* promoter mutations in patients with HNSCC; 2) patients who underwent treatment with curative intent (i.e., surgery and/or chemo/radiotherapy); 3) studies reporting *TERT* promoter mutation prevalence or HR for death or progression, with corresponding confidence interval; 4) studies evaluating *TERT* promoter mutation in surgical specimen.

The exclusion criteria were: 1) reviews and editorials; 2) studies with fewer than five patients; 3) non-English language studies; 4) studies containing aggregated and non-extractable data, or duplicated data from previously published work; 5) studies including distant metastatic cancers.

Data extraction

An electronic data-collection form was used to extract the following data: 1) study information: first author, year of publication, cohort characteristics, total number of patients; 2) clinical data: cancer site and subsite, number of cancers for each location, pathology stage, treatment; 3) analysis of the mutations: total number of *TERT* mutations, number of wild typed cancer, number of the single mutations studied; 4) data on prognosis, when available: HR of PFS and/or OS with corresponding 95% CI. Two authors (JP, ES) independently assessed the quality of the included studies with the Newcastle-Ottawa Scale (NOS) [32].

Statistical analysis

The number of total cases and of those carrying *TERT* promoter mutation, HR of death/progression and corresponding 95% CI were extracted from each study; the standard error of the log HR was derived from the log CIs. Summary estimate of *TERT* mutation prevalence and hazard ratio (sHR), with corresponding 95% CI, were calculated according to random-effects models of DerSimonian and Laird [33] as a weighted average, giving each study a weight proportional to its precision and incorporating both within-and between-study variability. Analyses were conducted separately for *TERT* promoter mutations −124 C > T and −146 C > T and for non-specified *TERT* promoter mutations. Statistical heterogeneity among studies was evaluated using the I^2 and τ^2 statistics.[33] Prevalence analysis were stratified by cancer site and, for oral cancer, by cancer subsite. Influence analysis was performed when summary estimate was estimated from five or more studies: summary estimate was calculated by omitting one study at a time. Publication bias was assessed through a funnel plot [34]. The results of the meta-analysis were presented graphically using forest plots, reporting the estimates from individual studies, the summary estimates and corresponding 95% CI. Statistical significance was claimed for $p < 0.05$.

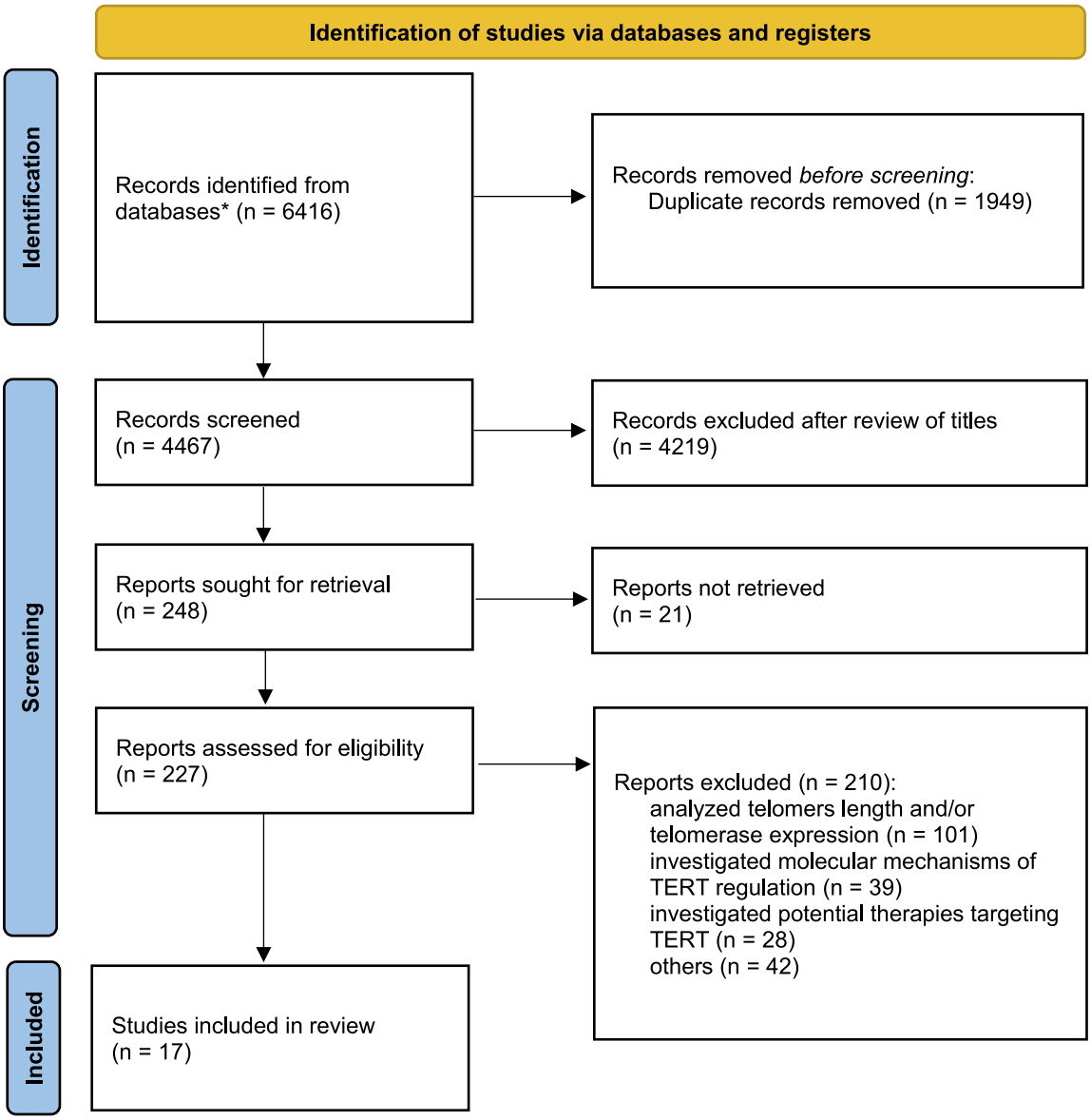

Fig. 1. PRISMA flow chart of study selection process.

Results

Search results and study selection

Once the duplicates were eliminated, 4467 items were screened, excluding 4219 articles based on the title. The full text of the remaining 248 articles was further reviewed, and 17 articles met the inclusion criteria for the metanalysis, as described in Fig. 1. The included articles [13–15,17,26–29,35–43] involved 1830 patients and were published between 2013 and 2021 (**eTable in Supplementary Online Content**). As data for oral cavity cancers were included in Giunco *et al.* [17], only findings for non-oral cavity HNSCCs by Boscolo-Rizzo *et al.* [35] were included in the meta-analysis. Among the selected studies, 11 articles evaluated the prognostic role of *TERT* promoter mutations in HNSCC, but only 8 of those, including 1102 patients, reported HR with 95% CI that fitted the requirements for this meta-analysis.

Characteristics and quality of the included studies

Nine articles focused on the prevalence of *TERT* promoter mutations in a specific head and neck site [15,17,26,27,36–39,43]. In the seven studies that included subjects with oropharyngeal SCC [13,14,28,29,35,37,43], HPV status was available in 287 out of 311 cases (92.3%). Among them, 155 cases (54.0%) were HPV-positive based on p16 overexpression and/or presence of HPV-DNA. Among the 11 studies that analysed prognostic impact of *TERT* promoter mutations, seven studies analysed the OS [17,26,28,29,35,37,39] and four studies focused on the PFS [17,29,37,39]. Overall, quality was satisfactory, with seven out of eight studies investigating OS or PFS with NOS ≥ 7 (**eTable in Supplementary Online Content**).

Prevalence of TERT promoter mutations in HNSCC

Overall, 21% (95% CI: 12%-31%) of HNSCCs harboured *TERT* promoter mutation (Fig. 2). Differences emerged according to cancer site ($p < 0.01$): *TERT* promoter mutations were more frequent for cancer arising in the oral cavity (47%, 95% CI: 33%-61%) and larynx/hypopharynx (12%, 95% CI: 4%-25%), while *TERT* promoter mutations were quite uncommon in oropharyngeal SCC (1%, 95% CI: 0%-4%). HNSCCs harbouring −124 C > T *TERT* promoter mutation (12%; 95% CI: 4%–23%; Fig. 3**A**) were twice as frequent than those carrying −146 C > T *TERT* promoter mutation (6%; 95% CI: 2%-13%; Fig. 3**B**). Prevalence of

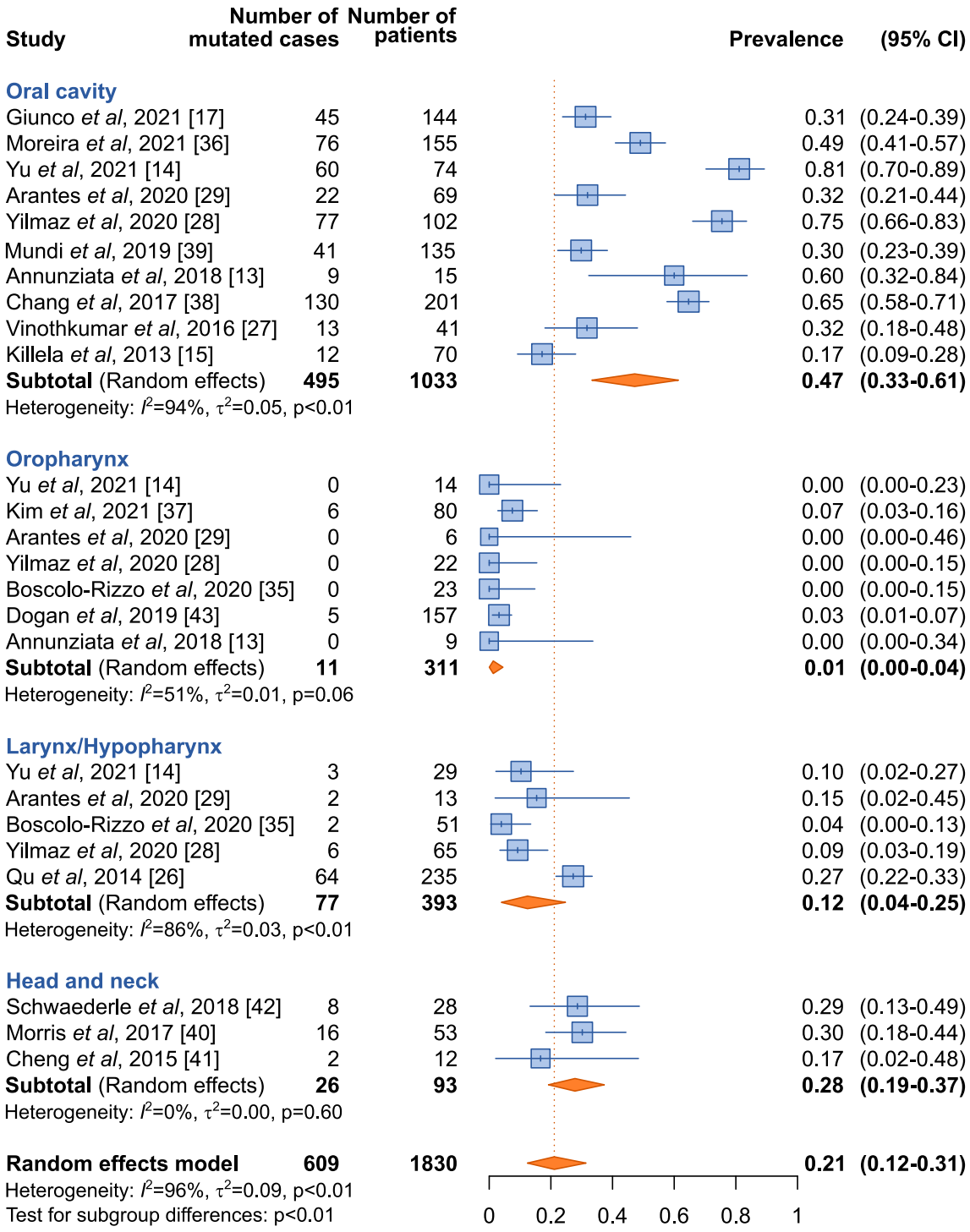

Fig. 2. Forest plot of prevalence of any *TERT* promoter mutations according to the site of HNSCC.

TERT promoter mutations was not statistically significant across different oral cavity subsites (p = 0.64), though *TERT* promoter mutations were more frequent in cancers of the buccal mucosa (62%; 95% CI: 21%-95%) and tongue (49%; 95% CI: 24%-74%; Fig. 4).

An analysis of *TERT* promoter mutations in oral cavity SCC was conducted differentiating the studies based on the geographic area of the included patients (**eFigure in Supplementary Online Content**). The meta-analysis indicated that *TERT* promoter mutations were more frequently reported in studies from Asia (59%, 95% CI 38%-77%), followed by North America (49%, 95% CI 1%-99%), Europe (40%, 95% CI 28%-52%), and South America (32%, 95% CI 21%-44%). A significant heterogeneity was found among the articles (I^2 = 95%; p < 0.01).

Prognostic significance of TERT promoter mutations

TERT promoter mutation −124C > T was associated with a significant higher risk of death (sHR = 2.01, 95% CI 1.25–3.23 – Fig. 5**A**) and progression (sHR = 2.79, 95% CI 1.77–4.40 – Fig. 5**B**). Conversely, −146 C > T *TERT* promoter mutation did not show a significant association with outcomes.

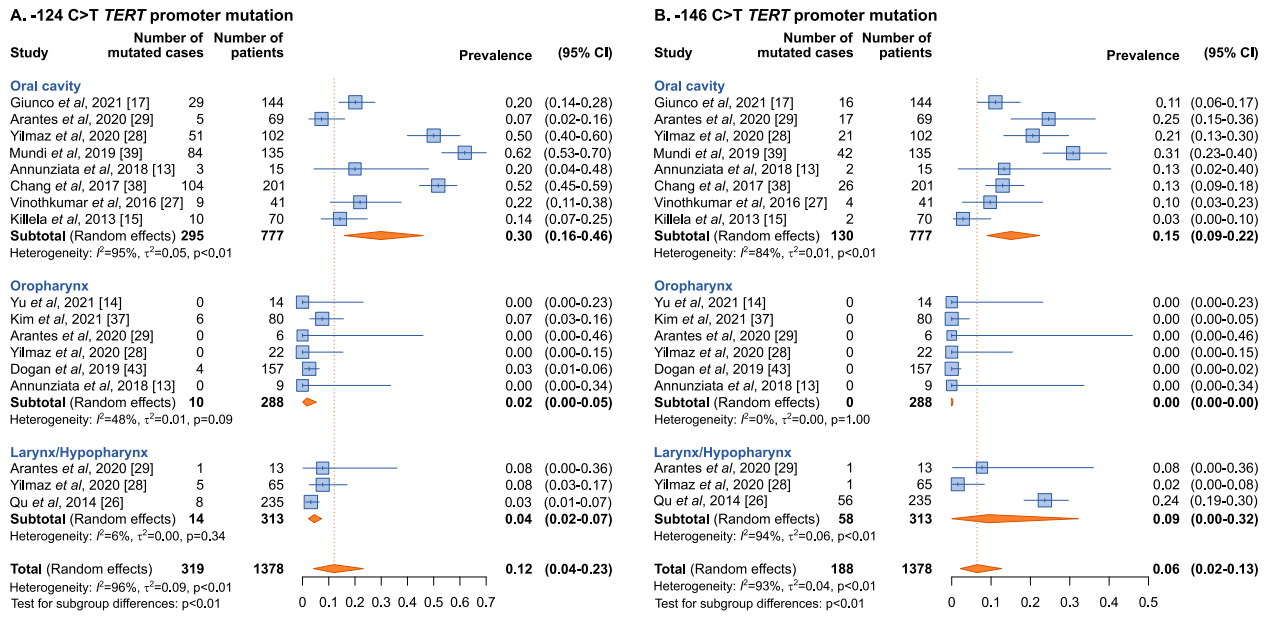

Fig. 3. Forest plot of prevalence of mutation −124 C > T (A) and 146 C > T *TERT* promoter mutations (B) according to cancer site.

Discussion

From this systematic review and meta-analysis, it emerged that *TERT* promoter mutations were identified in 21% of HNSCCs. However, these mutually exclusive mutations, i.e. −124 C > T and −146 C > T, were by far more prevalent in SCCs of the oral cavity compared to the other head and neck sites. Nearly half of oral cavity carcinomas harboured, indeed, mutations in the *TERT* promoter. Conversely, only 1% and 12% of the SCCs arising from the oropharynx and larynx/hypopharynx harboured *TERT* mutations respectively. Confirming data collected by analysing different types of malignancies and human cell lines [44], −124 C > T mutation was the most commonly found. Despite the high heterogeneity across studies, all but one of the those analysing the prevalence of *TERT* mutations in the oral cavity consistently report rates ≥ 30%, while all but one of the studies investigating the prevalence of these mutations in laryngeal/hypopharyngeal cancers reported prevalence rates ≤ 15%. No heterogeneity was observed in prevalence data of *TERT* promoter mutations in oropharyngeal cancers with most of studies consistently reporting no mutations in these malignancies. Furthermore, although not statistically significant, we observed differences in prevalence according to the subsite of origin of the tumour within the oral cavity and according to the geographical area.

We have no mechanistic explanations for these observations. Reactivation of *TERT* through promoter mutations has been observed to occur more frequently in tumours originating from tissues with relative low rates of self-renewal [15,44]. Highly proliferative tissues retain, indeed, a pool of telomerase positive cells necessary for their long-term self-renewal capacity and ability to maintain tissue homeostasis [45]. It has been postulated that mutations of the *TERT* promoter in tumours originating from these tissues do not confer a direct proliferative benefit as telomeres are still long enough or telomerase is active [45]. However, the epithelium lining the oral cavity has a relatively high rate of self-renewal with this rate being not significantly different from that of other head and neck sites [46] and is provided by a compartment of stem cells expressing detectable levels of *TERT* mRNA [47].

Another hypothesis is that exposure to particular risk factors may account for sites and subsites topographic differences, as well as for geographical differences. A substantial and increasing proportion of oropharyngeal SCCs, around 60%-70% in Western populations [48,49], are known to be caused by transforming HR α-HPV infection, mainly HPV type 16 [50]. HR α-HPV-driven oropharyngeal SCCs express very high *TERT* levels [47]. It has been demonstrated that HPV16 E6 oncoprotein physically and functionally interacts with telomerase complex and increases *TERT* catalytic activity, thus contributing to cell immortalization and transformation [51]. Consequently, in HPV-driven oropharyngeal SCCs, *TERT* reactivation mechanisms would be independent of the promoter mutations and there would be no selective pressure towards neoplastic clones possibly harbouring *TERT* promoter mutations. Most of cases of oropharyngeal SCC included in the present series were tested for HPV with 54% of them being HPV-positive Thus, HPV-induced carcinogenesis in the oropharynx may only partially explain the very low rate of *TERT* promoter mutations found in these tumours.

While laryngeal, hypopharyngeal, and non-HPV-driven oropharyngeal SCCs are mostly attributable to exposure to tobacco smoke and alcohol, several other risk factors, including smokeless tobacco, betel quid chewing, areca nut, poor dentition, poor oral health, and trauma due to sharp or broken tooth were described for cancer of the oral cavity [1]. Interestingly, a study conducted in a case series from Taiwan identified betel nut chewing as the main risk factor for *TERT* promoter mutations [38]. Thus, specific risk factors could target the *TERT* promoter region and explain both the peculiar topographical restriction and the different geographic distribution of tumours harbouring *TERT* promoter mutations. An indirect support to this hypothesis also comes from the observation that, while skin melanomas frequently harbour mutations of the *TERT* promoter, uveal melanomas do not show such mutations [44]. As an alternative explanation, retaining a sufficient proliferative capability in response to chronic damage related to persistent exposure to particular risk factors could be challenged by the telomere-dependent proliferative barrier, despite the high rate of self-renewal of the oral cavity epithelium. In this context, *TERT* promoter mutations could provide an immediate and strong proliferative advantage on these cells [45].

With respect to oncological outcomes, an important finding emerging from this meta-analysis is that HNSCC patients with tumours harbouring −124 C > T *TERT* promoter mutation had a poor prognosis showing a more than doubled risk of death and progression than patients with tumours not harbouring this mutation. To our knowledge, this is the first meta-analysis that has attempted to summarize the scientific evidence on the association between the presence of a specific somatic mutation of the TERT gene promoter, i.e., −124 C > T, and the oncological clinical outcomes in HNSCC patients. The importance of our

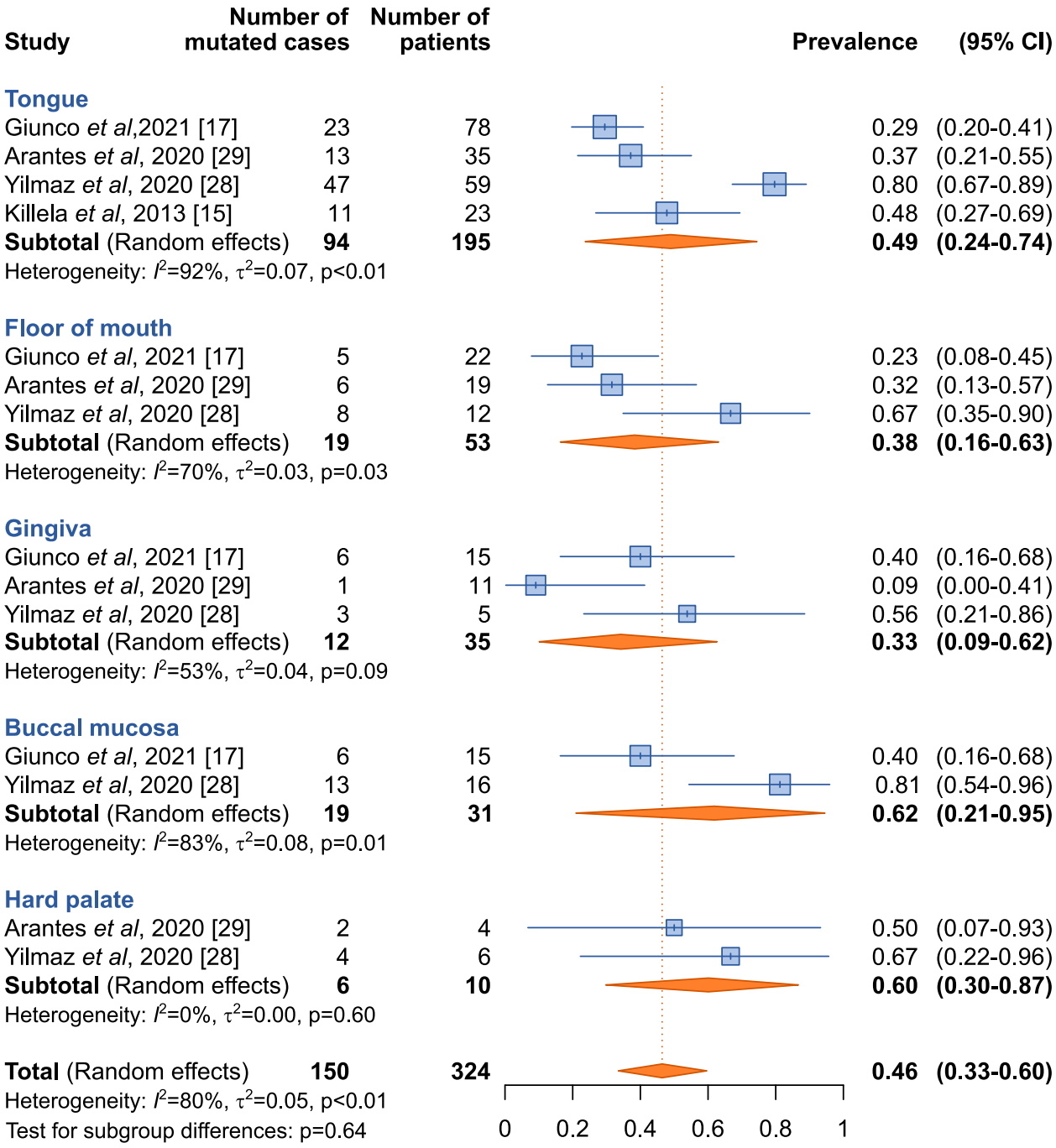

Fig. 4. Forest plot of prevalence of any *TERT* promoter mutations in oral SCC according to oral cavity subsite.

finding emerged when the association analyses were carried out considering non-specified *TERT* promoter mutations. In this case, the presence of −124 C > T, or −146 C > T or other less frequent and functionally not characterized *TERT* promoter mutations, such as −124 C > A [28], had no predictive effect on OS and PFS, highlighting the relevance of evaluating the impact of −124 C > T *TERT* hotspot on clinical behaviour independently.

Through the creation *de novo* binding site for transcription factors, the presence of −124 C > T or −146 C > T *TERT* promoter mutations is considered a reliable indicator of sustained telomerase expression that drives cancer cell immortalization and progression and has been proposed as a potential biomarker for cancer prognosis associated with clinically aggressive behaviour [16]. However, the clinicopathological association of *TERT* promoter mutations is cancer-dependent, and studies on different tumour types, including head and neck cancers, have reported contradicting clinical effects of *TERT* promoter mutations, ranging from poorer survival associated with the −124 C > T or −146 C > T TERT promoter mutation to unchanged clinical outcome [17,25–29,38]. Although both the −124 C > T and −146 C > T mutations create an identical 11-base sequence for binding the ETS transcription factor, promoting a similar increase of *TERT* transcription *in vitro* [52,53], previous reports demonstrated that these mutations are functionally distinct. The −146 C > T mutation, unlike −124 C > T, activated *TERT* transcription by binding the p52/ETS complex, thereby stimulating *TERT* expression via non-canonical NF-kB signalling [54,55]. In addition, *in vivo*, the −124 C > T mutation was associated with higher *TERT* expression/telomerase activity compared to −146 C > T [53,56]. These functional differences might partially account for the

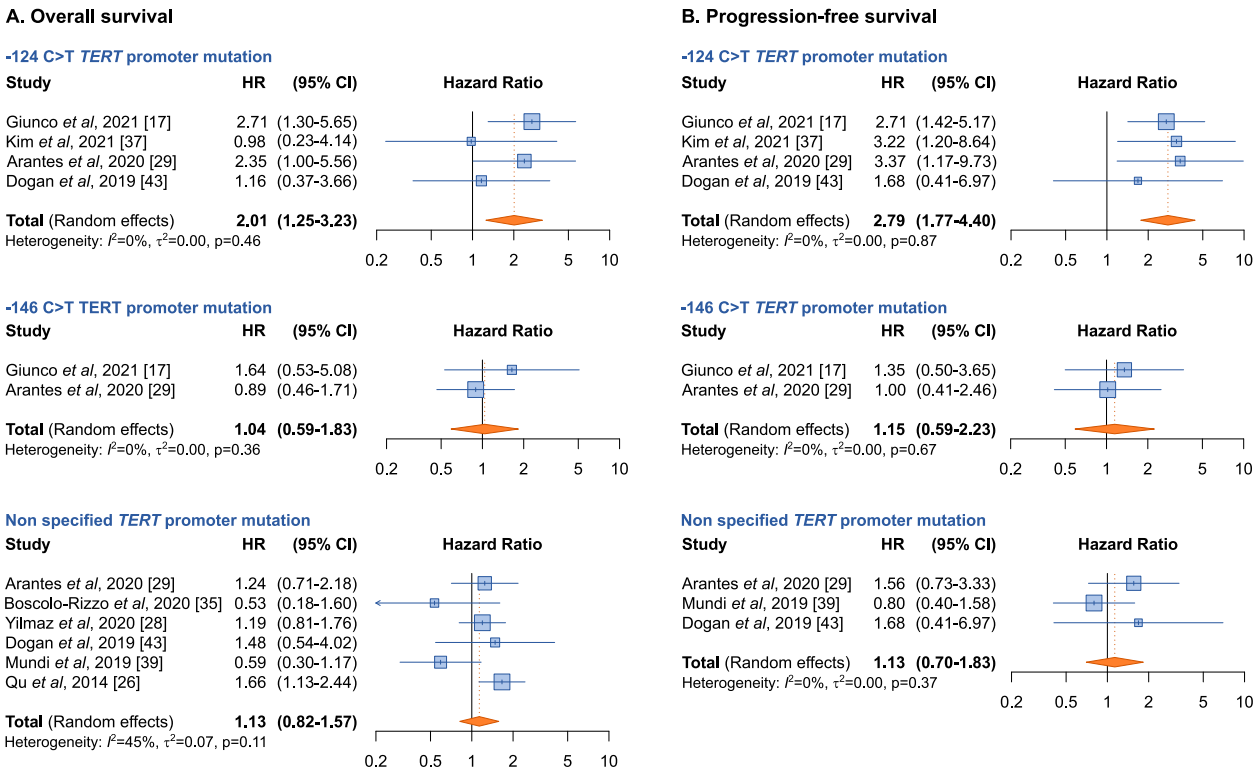

Fig. 5. Forest plot visualizing the association of *TERT* promoter mutations and overall survival (A) and progression-free survival (B).

conflicting results obtained when specified or non-specified *TERT* promoter mutations were evaluated in clinicopathological associations. In particular, the higher *TERT* expression conferred by the −124 C > T *TERT* promoter mutation could explain its association with the more aggressive clinical phenotype that emerged from this meta-analysis, considering that consistent evidence stemming from a substantial number of studies reports worse clinical course and/or shorter survival of HNSCC patients with high tumour *TERT* mRNA expression and/or telomerase activity [5,6,35,47]. In accordance with this line of reasoning, it is not surprising that a recent meta-analysis evaluating the prognostic role of TERT upregulation alterations in patients with SCC of the oral cavity failed to find any association between non-specified *TERT* promoter mutations with OS or PFS while TERT protein overexpression resulted as a prognostic indicator of poor survival in these patients [57].

The biological bridge between high TERT/telomerase expression and the more aggressive tumour phenotype is still partially unidentified and seems not to be attributable only to TERT's ability to maintain telomere length. Indeed, growing evidence indicates that TERT may promote tumorigenesis through telomere-length independent functions, including enhancement of proliferation, resistance to apoptosis, inflammation, invasion and metastasis [22,58] ultimately contributing to all the major characteristics of the cancer phenotype [8].

Finally, it must also be kept in mind that the effect of the *TERT* promoter mutations on *TERT* expression, and in turn on clinical prognosis, may be further complicated by the presence of the rs2853669 single nucleotide polymorphism (SNP). The minor C-variant allele disrupts a pre-existing ETS binding site at −245 bp in the *TERT* promoter region, thus counteracting the transactivation activity of the *TERT* hotspots [59,60]. Only one study among those included in our meta-analysis examined the clinical impact and the prognostic importance of the rs2853669 polymorphism [17]. The results of this study showed that patients' non-carrier of the SNP had an increased risk of disease progression and the coexistence of the T/T genotype of rs2853669 and the −124 C > T *TERT* promoter mutation increased the risk of adverse clinical outcome conferred by this mutation [17]. Greater attention to this SNP might substantially improve the HNSCC patients' risk stratification allowing a greater personalization of care for these patients in terms of planning follow-up protocols and selecting patients more at risk of disease progression.

Conclusion

In conclusion, *TERT* promoter mutations are mainly topographically restricted to oral cavity SCC. The presence of −124 C > T mutation in the *TERT* gene promoter is associated with a worse prognosis of HNSCC patients. Therefore, this mutation appears as a promising biomarker to stratify the prognosis in patients with oral SCC for whom no other biomarkers capable of predicting outcome are currently available. However, these findings should be taken with caution as they are based on a still limited number of studies. We recommend that future research focuses on the different prognostic impact of each *TERT* promoter mutations to obtain a more precise risk stratification in HNSCC, thus helping clinicians to better predict patient outcome and consequently tailor treatment decisions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.oraloncology.2023.106398.

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