Genetic Mutations in Young Nonsmoking Patients With Oral Cavity Cancer: A Systematic Review

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Abstract

Objective. This investigation aims to review the known genetic mutations associated with oral cavity squamous cell carcinoma (OCSCC) in young adults with limited environmental risk factors (YLERs).

Data Sources. A comprehensive search strategy was designed to identify studies in MEDLINE (Ovid), Embase (Ovid), and Scopus from database inception to May 2017 that included adults $\leq$50 years of age with OCSCC and minimal tobacco use history ($\leq$10 pack-years) who had their tumors genetically sequenced or mutational profiles analyzed.

Review Methods. Identified articles were screened by 2 reviewers. Quality of evidence was graded by the MINORS criteria for case-control studies; other studies were graded by assigning a level of evidence for gene mutation literature.

Results. Thirteen studies met our inclusion criteria, and 130 patients met our criteria for age and tobacco history. *TP53* was the most commonly evaluated gene (10 of 13 studies) and the most frequently observed mutation. One study reported that nonsmokers had significantly fewer *TP53* mutations, while 9 studies found no difference in the prevalence of *TP53* mutations. No other mutations were found specific to this cohort.

Conclusions. *TP53* mutations may occur at a similar rate in YLERs with OCSCC as compared with older patients or those with risk factors. However, few studies have aimed to characterize the genetic landscape of oral cavity tumors in this population, often with small sample sizes. Future studies are needed to explore unidentified genetic alterations leading to tumor susceptibility or alternative mechanisms of carcinogenesis.

Traditionally, oral cavity squamous cell carcinoma (OCSCC) has been observed in older men with a history of environmental risk factors, such as tobacco and alcohol use. While the incidence of oropharyngeal cancer has been increasing due to carcinogenic strains of human papillomavirus (HPV), the overall incidence of squamous cell carcinoma seen in the oral cavity has decreased.[1,2] It is hypothesized that this is due to the declining rates of tobacco use in younger patient populations.[3] While the overall incidence of OCSCC is decreasing, the frequency of oral tongue squamous cell carcinoma (OTSCC) has been observed to be increasing, particularly in this younger cohort of patients.[4,5] Specifically, a higher rate has been reported among young White individuals (<50 years) without traditional risk factors and predominantly

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among women.[6-8] Tobacco use in this young cohort with oral tongue cancer has been reported to range between 10% and 50%.[7,9] Furthermore, this increase in OTSCC has been observed among the same birth cohorts in White men and women, particularly those born after the 1940s.[10] Strikingly, this same increase in incidence has not been observed among other subsites of the oral cavity, such as the buccal mucosa, upper and lower alveolus and gingiva, hard palate, or floor of mouth.[11]

To date, there has been no definitive etiology proposed for the increasing incidence of OTSCC, despite the suggestion that a combination of genetic predisposition and environmental exposures may portend an elevated risk.[12] As the application of next-generation sequencing technologies continue to rapidly develop, identifying genetic mutations present in this young OCSCC population with low environmental risk may provide insight into causality and/or potential targeted therapies. This article aims to review the known mutational landscape of OCSCC, particularly of the oral tongue subsite, in young adults with low environmental risk (YLERs).

Methods

This systematic review was conducted in accordance with the recommendations of the PRISMA statement (Preferred Reporting Items for Systematic Reviews and Meta-analyses).[13]

Search Strategy

A comprehensive search strategy was designed and executed by R.P. in MEDLINE (Ovid), Embase (Ovid), and Scopus for results published from database inception through May 17, 2017. The search strategy utilized all relevant controlled vocabulary and keyword terms in an effort to yield all results with data pertaining to the mutational landscape of OCSCC in adults with a tobacco use history ≤10 pack-years.

Eligibility Criteria and Study Selection

Studies in the English literature were included that pertained to adults ≤50 years old ("young") who had OCSCC, a smoking history ≤10 pack-years ("nonsmoking"), and their tumors genetically sequenced or mutational profiles analyzed. Ten pack-years was chosen as the cutoff for smoking history due to a previous analysis showing significant escalation in the risk of OCSCC development above this amount for adults of all ages.[4] Studies without information pertaining to genetic mutations were excluded. While OTSCC was the primary focus of our review, given the low number of relevant studies, all oral cavity subsites were included in the eligibility criteria to capture the maximum number of cases. For the same reason, records of alcohol and smokeless tobacco habits were not factored into the eligibility criteria. Study selection was conducted independently by 2 reviews (K.O.S. and R.R.B.) in 2 successive rounds. Records that appeared to meet eligibility criteria or could not be definitively excluded were advanced to the second round, wherein they were screened on the basis of full text.

The articles describing gene mutations discovered through our search strategy were reviewed and assigned a level of evidence for gene mutation literature, as adapted to our study from a previous study of genetic mutations in pulmonary artery hypertension.[14] Case-control studies were graded according to the MINORS criteria (Methodological Index for Non-randomized Studies; **Table 1**).[15]

Results

Literature Overview and Study Selection

Our search yielded a total of 187 records across all databases. After deduplication, 181 unique records remained. The PRISMA flow diagram in **Figure 1** summarizes the results of the study selection process. Thirteen articles met the inclusion criteria: 8 case series, 3 case-control studies, and 2 case reports.

Risk-of-Bias Assessment

Ten studies showed negative evidence for increased *TP53* gene mutations in YLERs with OCSCC; 1 showed experimental evidence for other genetic alterations in YLERs with OCSCC; and 2 had genetic evidence in OCSCC-related disease. The MINORS criteria scores for the 3 case-control studies[16-18] ranged from 3 to 5 (out of 7). The levels of evidence and MINORS criteria scores are listed in **Table 1**.

Patterns of TP53 Mutation and Expression

TP53 was the most commonly evaluated gene, analyzed in 10 of the 13 studies. Nine studies revealed no difference in *TP53* mutation rates between YLERs and their counterparts with OCSCC, though the types of mutations and expression rates varied.[16,17,19-25] Two studies revealed that YLERs did not have mutations in exons 5 to 9 of *TP53*, whereas older smokers had mutations in this region.[17,23] However, 2 studies identified mutations within this region of *TP53*. Vettore et al found no mutations unique to younger patients or nonsmokers and that the types of base changes were similar between cohorts.[25] Tan et al identified no significant correlation regarding mutational status in genes tested in a panel for lung cancer, which included *TP53* and smoking history, with the caveat that a low number of YLERs (n = 2) were included in their cohort.[24] Krishnan et al reported that *TP53* mutations were similar among YLERs and their counterparts, but they reported a 20% lower *TP53* mutational frequency (somatic mutation frequency per megabase) in the nonsmoking group across all ages.[20] However, Pickering et al and Braakhuis et al noted that *TP53* was more typically inactivated in younger patients, though this was not statistically significant.[16,21] When comparing expression of p53 with mutations in *TP53*, 1 study noted that 81% of YLERs with OCSCC overexpressed p53 by immunohistochemistry and that the pattern of overexpression was highly correlated with the histologic grade of the tumor, despite not finding any *TP53* mutations in the targeted region, exons 5 to 9, where 92% of *TP53* mutations typically occur.[22,26] This reported rate of p53 overexpression was slightly higher than

Table I. Summary of the Included Articles and Mutations.

Study	LOE [a]	Type	Methods used	NS criteria	Smokeless tobacco use	Alcohol use	No. meeting criteria	OCSCC locations	Study location	Mutations in YLERs
Atula (1996)[19]	iii	Case series	IHC: p53 and Bcl-2 proteins. PCR-SSCP: *TP53* (exons 5-9)	No lifetime smoking history or quit ≥5 y previously	NA	All were "moderate / social" consumers	8	Oral tongue	Finland	*TP53*
Böckle (2010)[29]	vii	Case report	NA	No reported smoking history	NA	Never used	1	Cheek	Austria	*AIRE*
Braakhuis (2014) [b,16]	iii	Case-control	Sanger sequencing: *TP53* (exons 5-9). HPV: p16-IHC followed by HPV-DNA GP5+/6+ PCR	≤10 pack-year smoking history	NA	11 patients had > 10 unit-years	19	Oral tongue, retromolar trigone, cheek, floor of mouth	Netherlands	*TP53*
Li (2015) [b,18]	iii	Case-control	WES. Oncogenic viruses: RNA massively parallel sequencing	No history of tobacco smoking	No history of tobacco chewing	None provided	6	Oral tongue	USA	*TP53, CTNNA3, EIF3A, EP300, FXR1, NEK8, NOTCH1, NOTCH2, NOTCH3, PIK3CA, PKHD1L1, PTCHD2, RALGAPB, SPEN, UBR4*
Lingen (2000)[22]	iii	Case series	IHC: p53 protein. PCR-SSCP, direct sequencing: *TP53* (exons 5-9)	No reported direct exposure	No reported direct exposure	≤3 drinks/wk	21	Oral tongue	USA	No YLERs had *TP53* mutations
Krishnan (2015)[20]	iii	Case series	WES. CNA. HPV: type specific. qPCR or HPV16 digital PCR	Not explicitly defined	Not explicitly defined	Not explicitly defined	20	Oral tongue	India	*TP53, CDKN2A, CASP-8, NOTCH1, DMD, U2AF1, OBSCN*
Pickering (2014)[21]	iii	Case series	WES. CNA	<1 pack-year	Not provided	Not provided	16	Oral tongue	USA	*TP53, FAT1, CDKN2A, CASP8, NOTCH1, MLL2, PIK3CA*
Singh (2016) [c,17]	iii	Case-control	PCR-SSCP, direct sequencing: *TP53* (exons 4-9)	No reported use	No reported use	Not included	2	Alveolus, oral tongue	India	*TP53*
Sorensen (1997)[23]	iii	Case Series	IHC: p53 protein. PCR-SSCP, direct sequencing: *TP53* (exons 4-10)	No reported smoking history	NA	No reported alcohol use	6	Oral tongue	USA	No YLERs had *TP53* mutations
Tan (2014)[24]	iii	Case series	Sequenom multiplexed LungCarta panel 1.0	No reported smoking history	NA	NA	2	Oral tongue	Singapore	*STK-11, BRAF*
Tong (2004)[31]	vi	Case series	HuSNP assay. Microsatellite analysis: chromosome 3p, 6q, 9. HPV: qPCR of E6/E7 regions of HPV16. *FANC-C* gene sequencing; IVSF-4+ locus	No reported history	NA	No reported history	16	Oral tongue, front of mouth, mandible	USA	At: 3p, 6q, 9p, 9q. No YLERs had mutations in IVSF-4+
Vettore (2015)[25]	iii	Case series	WES and targeted deep sequencing	<1 y	<1 y	NA	18	Oral tongue	Singapore	*TP53, DST, USH2A, PRKDC, LRP1B, MACF1, RNF213, MLL3/KMT2C, COL6A6, PKHD1L1, VWA8, PLEC, SYNE1, BAI3, FN1, ZFHX4, PKHD1, KIAA1731,*

(continued)

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Table I. (continued)

Study	LOE[a]	Type	Methods used	NS criteria	Smokeless tobacco use	Alcohol use	No. meeting criteria	OCSCC locations	Study location	Mutations in YLERs
										APC, DLEC1, LILRB1, SYNE2, FAT1, FAT2, FAT3, FAT4, PDE4DIP, RYR2, DNHD1, CASP8, ZSWIM5, COL27A1
Vinarsky (2009)[30]	vii	Case report	CDKN2A sequencing: INK4a locus	<2 pack-years	NA	No reported history	1	Oral tongue	USA	INK4a

Abbreviations: AI, allelic imbalance; CNA, copy number alteration; IHC, immunohistochemistry; LOE, level of evidence; MINORS, Methodological Index for Nonrandomized Studies; NA, not available; NS, nonsmoking; OCSCC, oral cavity squamous cell carcinoma; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; SSCP, single-strand conformation polymorphism; WES, whole-exome sequencing; YLER, young adults with low environmental risk.

[a]The levels included (i) annotation error, (ii) unrelated, (iii) negative evidence, (iv) related but not mutated, (v) mutation evidence in OCSCC, (vi) other genetic alterations in OCSCC, and (vii) genetic evidence in OCSCC-related disease.

[b]MINORS: 3 out of 7.

[c]MINORS: 5 out of 7.

previously reported rates in ''classical'' squamous cell carcinoma of the head and neck, in contrast to previous studies showing a strong correlation between p53 overexpression and tobacco and alcohol usage.[22,27,28]

One study revealed that nonsmokers had significantly fewer *TP53* mutations when compared with smokers. Li et al performed exomic sequencing on samples from 6 never-smokers with OTSCC and compared them with 5 smokers elected as controls.[18] We were able to obtain demographic data on the 6 never-smokers: 5 of the 6 were ≤50 years old, 1 of whom had a *TP53* mutation. In contrast, all 5 sequenced smokers had *TP53* mutations. Never-smokers had significantly fewer *TP53* mutations than smokers.

Other Mutational Differences

Other mutations unique to the population of YLERs were *CDKN2A, RASA1, STK-11,* and *BRAF,* though none were specific for this population.[20,24] The 2 case reports identified *INK4a* and *AIRE* as mutated genes in YLERs with coexisting conditions who developed OCSCC.[29,30] Other genetic perturbances were found by Tong et al, who discovered an elevated frequency of allelic loss in a novel region on chromosome 6q, though there was otherwise no difference between young nonsmokers and their counterparts.[31] Chromosome 9q had a high rate of loss of heterozygosity (LOH) and allelic imbalance. The authors found no evidence of HPV-16 infection or *FANC-C* IVSF-4+ mutations in any of the nonsmoker samples. Li et al reported distinct mutational spectrums between the cohorts, with a greater proportion of C:G>G:C transversions in cancers from nonsmokers ($P < .0001$) and A:T>G:C ($P < .0001$) or A:T>T:A substitutions ($P < .0001$) among smokers.[18] Three studies tested for presence of HPV; however, none of the samples from nonsmoking patients contained evidence of HPV.

Discussion

While traditional environmental risk considerations regarding the development of OTSCC have largely implicated tobacco use, OTSCC in young nonsmokers is becoming increasingly prevalent despite declining smoking rates worldwide.[5] Interestingly, the rate is decreasing among non-Whites and non-Hispanics, as well as in those >45 years of age across all ethnicities.[6] Though OTSCC in YLERs has emerged as a distinct clinical entity, studies to date have failed to identify a clear etiology. Our review attempts to synthesize our existing knowledge of the genetic changes underlying carcinogenesis in this unique cohort.

TP53 Mutations

The rate of *TP53* mutations is overall similar between YLERs with OTSCC and older smoking patients with OTSCC. Our findings do differ somewhat from prior studies indicating that *TP53* mutations may be less predominant among nonsmokers with head and neck squamous cell carcinoma (HNSCC).[32,33] Of 10 studies evaluating *TP53* mutations in this population, only 1 reported significantly fewer
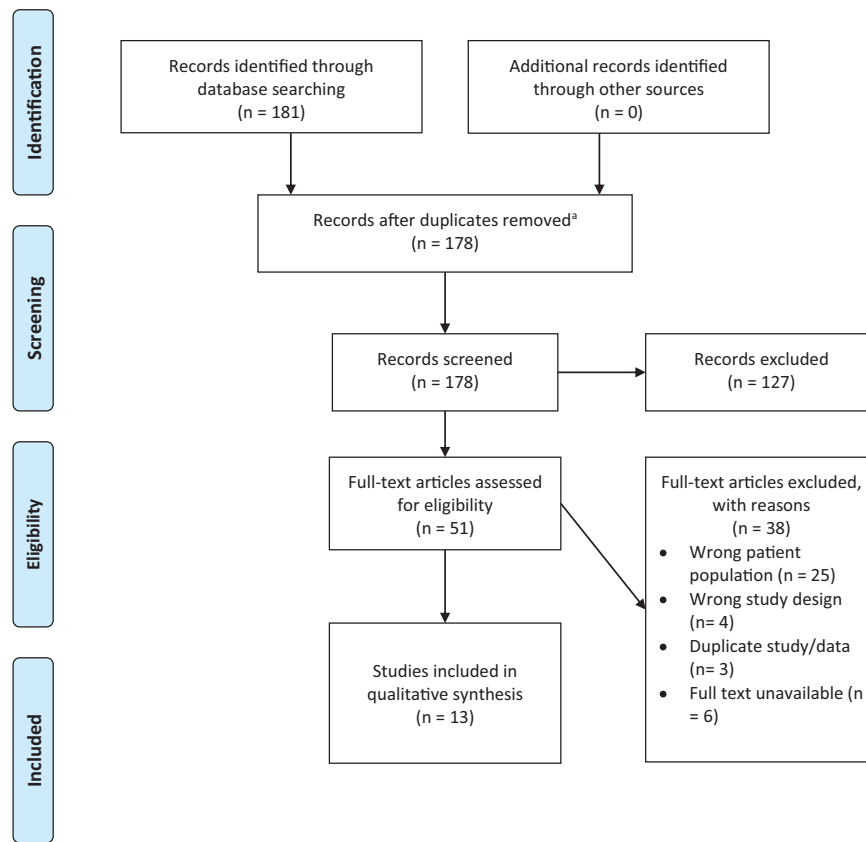
Figure 1. PRISMA flow diagram illustrating the selection process of the included articles.

TP53 mutations among never-smokers.[18] Some of these differences may be attributable to small sample sizes and variations in sequencing methods, which limits the ability to form definitive conclusions regarding mutational differences. However, it is also possible that the *TP53* mutations found in this cohort differ from those typically seen in other HNSCCs. For instance, several studies suggest that *TP53* mutations in this population may be occurring in locations other than those traditionally seen in HNSCC. Although it was previously shown that almost all *TP53* mutations seen in HNSCC occur within exons 5 to 9, multiple studies included in our review found mutations either to be absent or outside this region.[17,22,23] As a number of studies sequenced limited high-yield exons or focused on previously determined hotspot mutations, more widely distributed inactivating mutations or those in other exons may have gone undetected.[17,23,24] In fact, Singh and colleagues found that exon 4 was the most commonly mutated exon, and they identified several novel *TP53* mutations, many occurring outside predetermined ''hot spots.''[17] Furthermore, the type of *TP53* mutation, rather than the frequency or location, may play a role. One study reported a higher rate of truncating-type *TP53* mutations in younger patients.[16] Truncating mutations have been associated with poorer prognoses in HNSCC,[34] which may explain why some studies suggest poorer outcomes in YLERs with OTSCC. Moreover, there appears to be no predictable relationship

between *TP53* and p53 protein expression, as reflected in many of the included studies.[19,22,23]

Truncation events may lead to lack of expression or inability to detect the truncated protein through available methods, thus making it challenging to define the affiliation between mutation and expression. Importantly, these mutational differences may have implications in terms of prognosis and effectiveness of potential therapies in this population. Overall, it appears that *TP53* mutations are present at similar rates in YLERs with OTSCC, though clinical history and associated mutational spectra suggest an underlying mechanism unrelated to direct carcinogen exposure that has yet to be ascertained.

Other Potentially Implicated Genes

The pathogenesis of OTSCC in YLERs is likely to be multifactorial, and *TP53* is seemingly one of the few implicated genetic drivers in this population. Though no other gene mutations were found to be specific to this patient population, several genes were differentially mutated according to age and risk factors. One study found the *CDKN2A* gene, previously noted to be present in OCSCC, to be mutated exclusively in nonsmokers and past-smokers.[20,35] It also noted a higher rate of mutations in the arachidonic acid and Toll-like receptor pathways in the nonsmoking population.[20] *RASA1* may play a role in a subpopulation of patients based on exposure, as it was found to be specific to patients who

had never chewed tobacco, regardless of smoking history.[20] The roles of other commonly altered genes—such as *CASP-8, NOTCH-1, FAT1,* and *PIK3CA,* which were found to be less frequently mutated in YLERs—remain in question but may be involved in the regulation of key signaling pathways involved in tumorigenesis.[32] *INK4a,* often mutated in FAMMM, may be involved in the development of recurrent HNSCC, whether as part of a familial cancer syndrome or as an isolated genetic marker for the development of HNSCC in young adults.[30,36] Other coexisting genetic conditions, such as Fanconi anemia, may have some significance, but this role remains to be clearly defined. Overall, our review did not identify HPV to be associated with malignancy in this group of patients, which follows the general consensus suggesting that HPV does not play a major role in the development of OCSCC/OTSCC in this young population.[37-39]

Differing Mutational Landscapes and Genetic Contexts

The genome-wide mutational landscape may be different in YLERs with OCSCC. For instance, one study in our review found elevated rates of allelic imbalance in chromosomes 6q and 9q in nonsmokers.[31] A study not included in our review found increased rates of LOH in distal regions of chromosome 17p, home to *TP53,* and other loci in YLERs,[40] which have been linked to driving the transition from hyperplasia to dysplasia in HNSCC.[32,40] While it found the degree of LOH at chromosomes 3, 9, and 17p to be similar among younger and older patients with OCSCC, the evaluation of additional chromosomes may reveal yet unknown molecular characteristics in this population.[40] Of note, a prior study evaluating lung cancer identified a region on chromosome 6q that conferred an increased risk of cancer in never-smokers and light smokers (with ''light'' defined as <20 pack-years) who had a particular ''risk haplotype.''[41] Although the reviewed study identified regions of loss without distinct haplotypes,[31] further investigation in this and other areas of the genome may provide insight into potential susceptibility loci.

It should be noted that race and sex may affect the genetic changes underlying tumorigenesis in particular populations, and it has been demonstrated that certain genes are differentially mutated in OTSCC across ethnicities. For instance, Asian patients with OTSCC were found to have mutations in genes such as *DST, RNF213, STK-11,* and *BRAF,* whereas the largely North American cohort represented in The Cancer Genome Atlas had more frequent mutations in *TP53, CDKN2A,* and *NOTCH1.*[24,25] These biologic differences should not be overlooked as potential contributing factors, and further investigation into this aspect of OTSCC may provide important information regarding underlying etiology and perhaps even prognosis.

Finally, while OTSCC in YLERs exists as a distinct clinical and epidemiologic entity, studies to date have yet to identify any genomic or etiologic factors unique to this subpopulation. Furthermore, often contrasting observations related to *TP53* and other potential genetic drivers are difficult to synthesize in light of the limitations accompanying such studies. Additional factors, such as the oral microbiome and immune microenvironment, have been suggested to play a role in the pathogenesis of OTSCC in YLERs. Similar to the role of the gut microbiome in gastrointestinal cancers, the disruption of the normal and potentially ''protective'' oral microbiome may be associated with the development of OCSCC.[42,43] Specifically, there may be a role of oral candidiasis, as we identified a case report of a patient with chronic mucocutaneous candidiasis who developed recurring OCSCC.[29] The disruption of the normal oral microbiome by some *Candida* species may promote carcinogenesis, possibly through production of carcinogenic factors, and a similar pattern could be the case in YLER patients with OTSCC.[44,45]

Emerging research suggests that the primary difference in OCSCC between smokers and nonsmokers lies in the immune microenvironment, particularly in the PD-1 and PDL-1 pathways.[46,47] Despite having similar or higher levels of inflammatory infiltrates as compared with older smokers, the immune response in YLERs may be attenuated, particularly with regard to immune activation and immune exhaustion.[47] These findings elicit promising therapeutic considerations, particularly with regard to immunotherapies, warranting further genetic analysis to potentially direct future management strategies.

Limitations

Overall, few studies have been conducted on genetic mutations in the young nonsmoking population with OCSCC. Furthermore, most are restricted by small sample sizes and are case reports, case studies, or case-control studies, ultimately limiting the applicability of these results. Moreover, inadequate sample sizes prevent appropriate stratification by age, sex, and risk habits. Our analysis was also constrained by discrepancies in the annotation of environmental risk factors. To capture all relevant studies, the use of smokeless (chewing) tobacco was not included in our selection criteria due to its inconsistent incorporation among studies. However, it is an important consideration in this context given that it may reflect tobacco habits across different populations and is a known risk factor for the development of OCSCC. Alcohol use was also not consistently reported, though it is a well-documented risk factor for the development of OCSCC.[48] In addition, the definition of nonsmoking varies considerably among studies, and while all studies met our criteria of a ≤10–pack-year history, specific criteria among individual studies differed. The variations in age and tobacco criteria used among studies hinder the interpretation of individual findings and limit our ability to aggregate data. Last, comprehensive mutational analysis requires significant resources, which often limits the scope of such studies. Significant variation in the methods used to perform mutational analysis complicates the comparison of findings among studies. The relative rarity of OTSCC in our targeted cohort makes it difficult to appropriately power these studies and draw accurate conclusions. While it is apparent that

further study is needed to gain meaningful insight into this unique disease process, this review serves as a first step toward appreciating our existing knowledge base and identifying what remains to be elucidated.

Conclusions

The rise in OTSCC among young patients without traditional risk factors represents a unique and puzzling clinical entity. Though a discrete epidemiologic cohort, distinctive underlying etiologic and genomic factors have yet to be identified. Our review suggests that *TP53* mutations in YLERs with OTSCC likely occur at a rate similar to that in the ''traditional'' cohort of older smokers, and no additional genetic mutations were found to be specific to this cohort. Overall, few studies with relatively small sample sizes have aimed to characterize the genetic landscape of oral cavity tumors in this growing patient population. Factors such as the oral microbiome, sex, ethnicity, and various underlying genetic elements may play unique roles in driving tumor formation in these patients. Future studies are needed to explore unidentified tumor susceptibility genes or alternative mechanisms of carcinogenesis in this cohort.

Author Contributions

Rohini R. Bahethi, study selection, screening, analysis, manuscript drafting; **Katelyn O. Stepan**, study selection, screening, analysis, manuscript drafting; **Rachel Pinotti**, study selection and search, manuscript drafting; **Ryan Li**, supervision, analysis; **Nishant Agrawal**, supervision, analysis; **Sidharth V. Puram**, supervision, analysis; **Brett A. Miles**, supervision, manuscript editing; **Brittany Barber**, supervision, manuscript editing.

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