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Comprehensive review of genetic factors contributing to head and neck squamous cell carcinoma development in low-risk, nontraditional patients

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Abstract

Background: The past 2 decades have seen an increased incidence of head and neck squamous cell carcinoma (HNSCC) in a nontraditional, low-risk patient population (ie, ≤45 years of age, no substance use history), owing to a combination of human papillomavirus (HPV) infection and individual genetic variation.

Methods: Articles positing genetic variants as contributing factors in HNSCC incidence in low-risk, nontraditional patients were identified using a PubMed search, reviewed in detail, and concisely summarized herein.

Results: Recent data suggest that common polymorphisms in DNA repair enzymes, cell-cycle control proteins, apoptotic pathway members, and Fanconi anemia-associated genes likely modulate susceptibility to HNSCC development in low-risk, nontraditional patients.

Conclusion: At present, there is a lack of robust, comprehensive data on genetic drivers of oncogenesis in low-risk patients and a clear need for further research on genetic alterations underlying the rising incidence of HNSCC in low-risk, nontraditional patients.

KEYWORDS
genetics, germline, head and neck squamous cell carcinoma (HNSCC), hereditary, personalized medicine

1 | INTRODUCTION

Each year, approximately 600 000 incident cases of head and neck squamous cell carcinoma (HNSCC) are diagnosed worldwide, leading to >350 000 deaths.[1] Cases of HNSCC encompass malignancies of the upper aerodigestive tract (ie, oral cavity, oropharynx, hypopharynx, and larynx) and classically afflicts older, white men with a heavy tobacco and/or alcohol use history.[2–4] In this population, chronic exposure to carcinogens results in accumulation of somatic alterations in an assortment of oncogenes (eg, *PIK3CA*), tumor suppressors (eg, *TP53*, *CDKN2A*, and *NOTCH1*), and cell-cycle regulators (eg, *CCND1*) that increase the risk of HNSCC over time.[5] Despite the high prevalence of tobacco and alcohol use in the general population, only a small proportion of individuals will

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ultimately develop HNSCC, indicating that genetic variants may modulate susceptibility in individual patients.

Over the last 2 decades, there has been an increasing prevalence of HNSCC in a nontraditional, low-risk patient population (ie, ≤45 years of age, no substance use history). A rapid rise in high-risk human papillomavirus (HPV)-associated HNSCC accounts for the majority of these cases,[6,7] although there remains a high-risk HPV-independent subset of low-risk patients who lack identifiable etiological factors.[8,9] The latter population is seen with particular frequency in cancers of the oral cavity.[10] Although differences in genetic drivers of oncogenesis between these younger, low-risk patients and their older, traditional counterparts require further elucidation, current evidence suggests that clinical outcomes (ie, disease-specific survival) are similar and comparably poor in these 2 patient groups.[8,11]

Recently, the International Head and Neck Cancer Epidemiology (INHANCE) consortium aimed to compare the role of family history of HNSCC, tobacco, and alcohol as oncologic risk factors among a large cohort of young (≤45 years) and older (≥45 years) patients.[12] Importantly, a higher proportion of oral tongue and oral cavity cancers was seen in young patients with HNSCC. Compared with older patients, these cancers were found to be less attributable to tobacco and alcohol use and had a higher association with a family history of early onset HNSCC. As such, differences in HNSCC etiology among low-risk, nontraditional patients and their older counterparts are evident. The association of HNSCC with family history in the setting of a weaker (or absent) contribution from tobacco and alcohol suggests a hereditary, germline component present in young patients that is absent in the traditional HNSCC cohort.

Herein, we present an overview of the current landscape of genetic determinants of HNSCC in low-risk patients, a population defined by young patient age and status as nonsmokers/drinkers. We discuss genetic polymorphisms in DNA repair enzymes and apoptotic pathway members, as well as known heritable forms of HNSCC, specifically familial HNSCC, and Fanconi anemia-associated HNSCC. Additionally, we address genetic susceptibility to HNSCC in relation to high-risk HPV serology, relevant to the soaring national prevalence of viral infection and epidemiologic rise in high-risk HPV-associated HNSCC. Finally, we conclude with an acknowledgment of current barriers to the understanding of genetic determinants of HNSCC in low-risk patients and implications for future research in this area.

1.1 | Genetic variants in DNA repair enzymes in patients with low-risk head and neck squamous cell carcinoma

Pathways for DNA damage repair fall into 3 broad categories determined by the nature and extent of genomic damage: (1) base excision repair; (2) nucleotide excision repair; and (3) double-strand break repair. Perturbations in cellular DNA repair pathways lead to genomic instability, aberrant cell function, and unchecked replication, all of which can promote tumor development.[13] Inherited diseases compromising DNA repair pathways, such as Fanconi anemia (double-strand break repair), xeroderma pigmentosum (nucleotide excision repair), and *BRCA1/2*-associated cancer syndromes (double-strand break repair) drastically increase an individual's lifetime risk of developing multiple cancers of different sites.[14] Functioning DNA repair machinery is essential to combat the accumulation of tumor-promoting mutations induced by chronic exposure to carcinogens in tobacco smoke, as in the case of HNSCC.[15] However, even in nontobacco users, polymorphisms in DNA repair genes may underlie oncogenesis by compromising genomic integrity, as clearly illustrated in the aforementioned hereditary syndromes. Current evidence on the association of polymorphisms in genes of DNA repair pathways with risk of HNSCC in low-risk patients is conflicting.

A systematic review and meta-analysis by Flores-Obando et al[16] examined associations in polymorphisms in DNA damage response genes with the risk of HNSCC development in 30 case-control studies comprising roughly 8000 patients with oral, pharyngeal, or laryngeal cancers and 12 000 matched controls. Focusing their analysis on DNA repair enzymes in the nucleotide excision repair (*XPA*, *XPD*, *XPC*, *XPF*, and *ERCC1*), base excision repair (*XRCC1*), and double-strand break repair (*XRCC3*) pathways, the authors reported an increased risk of HNSCC associated with mutations in *XPD codon 312* and *XRCC1 codon 399* in white and Asian populations and mutations in *XRCC1 codon 194* solely in Asian patients, although notably each of these associations was only marginally statistically significant. An updated meta-analysis with extensive subgroup stratification by smoking status, HNSCC subsite, and ethnicity by Lou et al[17] differed from Flores-Obando et al[16] in that the *XRCC1 codon 399* polymorphism had no significant impact on HNSCC susceptibility among 7000 patients with HNSCC (compared against 10 000 controls), alone or in an interactive fashion with smoking. However, the authors did note that *XRCC1 R194W* homozygosity and tobacco use interacted to produce a statistically significant increase in HNSCC risk in the patients studied. Although these results failed to support a clear susceptibility to HNSCC attributable to polymorphisms in genes of DNA repair machinery, they do suggest that subgroup stratification by smoking status may uncover correlations within low-risk populations. A second response to Flores-Obando et al[16] found no link between *XPD codon 312* polymorphism and HNSCC susceptibility in a meta-analysis of 9 case-control studies totaling 2700 HNSCC patients and 4500 controls.[18] However, stratification by age, smoking, or drinking status was not shown in this report,

thus limiting the ability to interpret the data in the context of these critical variables.

A recent genomewide association study of European patients with HNSCC within the INHANCE consortium identified a novel polymorphism at 4q21 (rs1494961) significantly associated with susceptibility to HNSCC in the roughly 2000 patients analyzed.[19] This site is positioned within the *HEL308* gene, a DNA-dependent ATPase and helicase important for DNA intra-strand cross-linking repair.[20] Sequencing of rs1494961 in an additional independent cohort of 5600 lung cancer cases and 9300 controls showed that this polymorphism was associated with the risk of lung cancer, suggesting that this variant may play an important role in tobacco-associated cancers in general. On subgroup stratification, the association of the rs1494961 variant with HNSCC risk was maintained in patients <50 years of age, although was lost in never-users of tobacco or alcohol, precluding any definitive conclusions regarding the role of rs1494961 in young patients with low-risk HNSCC. A second genomewide association study by Liang et al[21] identified a positive, multiplicative interaction between *HEL308* polymorphisms and cigarette use of >70 pack-years in 600 HNSCC cases, signifying that this genetic variant is less relevant to a low-risk, nontraditional patient population. Finally, a follow-up genomewide association study of a relatively small Chinese population with HNSCC revealed no association between rs1494961 polymorphism and HNSCC susceptibility (Table 1).[16–19,21,22]

Clearly, future confirmative studies and mechanistic investigations are needed to clarify inconsistencies in these posited associations between DNA repair polymorphisms (eg, *HEL308*) and HNSCC in low-risk, nontraditional patients. Such studies should stratify included HNSCC cases and controls by relevant clinical factors, such as age, tobacco use, and HNSCC subsite that may help uncover complicated gene-by-gene or gene-by-environment effects and generate robust, generalizable associations between polymorphisms in DNA repair machinery and HNSCC in low-risk patients.

1.2 | Genetic variants in apoptotic pathway members in patients with low-risk head and neck squamous cell carcinoma

Programmed cell death by apoptosis serves as a critical barrier to cancer development and progression.[23] Apoptotic signals are cued by various physiological stressors (eg, DNA damage and growth-factor deprivation) and trigger cellular suicide by caspase cascade activation. Dysfunction in this native defense mechanism promotes tumorigenesis and confers metastatic potential and treatment resistance in cancer cells.[24,25] One of the key regulators of apoptosis is p53 (encoded by the *TP53* gene), a tumor suppressor that induces cell-cycle arrest and apoptosis in response to DNA damage, oncogene activation, hypoxia, and numerous other stimuli.[26] Loss-of-function mutation in *TP53* is one of the most common acquired genetic events across all human cancers, including HNSCC, in which whole-exome sequencing has identified somatic silencing mutations in *TP53* in over 70% of tumors.[5,27]

In addition to acquired, tumor-promoting mutations in *TP53*, there are 2 germline polymorphisms at codon 72 of *TP53* generating an arginine (p53Arg) or proline (p53Pro) residue at this position that are common in the human population.[28] The p53Arg and p53Pro variants harbor minimal conformational differences in protein structure, yet several reports have posited that these polymorphisms may be important determinants of response to chemotherapies and differential oncologic outcomes.[29–31] Intriguingly, HNSCC cases have shown bias toward p53Arg homozygosity[30] and patients with this alteration respond less favorably to platinum-based chemoradiotherapy, perhaps due to negative influence of p53Arg on p73-dependent initiation of apoptosis.[31] At present, however, potential influences of p53 polymorphisms on HNSCC susceptibility alone, particularly in low-risk patients, have yet to be investigated.

The p53 upregulated modular of apoptosis (*PUMA*) plays a similar crucial role in promoting apoptosis through interaction with the antiapoptotic *Bcl-2*. *PUMA* expression is upregulated by p53, although likely has both p53-dependent and p53-independent roles in HNSCC as the expression of *PUMA* is sufficient to prevent HNSCC growth in vitro regardless of p53 mutational status.[32] At present, no somatic mutations within coding regions of *PUMA* have been identified in HNSCC, although 2 polymorphisms (rs3810294 and rs2032809) in the gene's promoter region have been proposed as potential risk factors for incident HNSCC in white populations.[33] These polymorphisms may result in differential binding affinities of transcription factors to the *PUMA* promoter, although the consequential effect on *PUMA* expression is still unclear. In a cohort study of 380 HNSCC cases and 335 controls, increased HNSCC risk was seen in patients with HPV16 seropositivity and a variant *PUMA* gene, an effect modification that was particularly pronounced in never-smokers, never-drinkers, young patients (<58 years), and in oropharyngeal subsites.[33] Mechanistically, the HPV16 E6 oncoprotein subverts apoptosis in infected cells by interfering with the p53/PUMA/Bax cascade, although functional evidence of any direct interaction between *PUMA* and E6 has yet to be elucidated.[34]

Last, polymorphisms in the promoter of baculoviral inhibitor of apoptosis repeat-containing 5 (*BIRC5*; also known as survivin) gene, have been implicated as risk factors for the development of HNSCC in Asian populations.[35] In HNSCC in vitro models and primary tumors, somatic mutations and overexpression of *BIRC5* correlates with resistance to conventional chemoradiotherapy,[36,37] although how the

TABLE 1 Summary of DNA repair pathway gene polymorphisms investigated in low-risk, non-traditional patients with head and neck squamous cell carcinoma

Gene investigated	Study design	Case/control size	Strength of HNSCC association OR (95% CI)	Subgroup stratification
XRCC1 R194W (homozygous)	Meta-analysis of 30 studies (Flores-Obando et al[16])	7291/12 052	**1.78** (1.13-2.82)	Asians
	Meta-analysis of 29 studies (Lou et al[17])	6719/9627	0.91 (0.77-1.08)	N/A (total population)
			2.53 (1.16-5.53)	Smokers
XRCC1 R399Q (homozygous and heterozygous)	Meta-analysis of 30 studies (Flores-Obando et al[16])	7291/12 052	**1.14** (1.01-1.27)	Whites
	Meta-analysis of 29 studies (Lou et al[17])	6719/9627	0.99 (0.90-1.09)	N/A (total population)
			0.7 (0.43-1.15)	Smokers
XPD D312N (heterozygous)	Meta-analysis of 30 studies (Flores-Obando et al[16])	7291/12 052	**1.14** (1.01-1.29)	N/A (total population)
	Meta-analysis of 9 studies (Hu et al[18])	2670/4452	1.11 (0.99–1.24)	N/A (total population)
HEL308 (homozygous and heterozygous)	Genomewide association study of European Studies in INHANCE Consortium (McKay et al[19])	8605/16 226	**1.13** (1.08-1.17)	N/A (total population)
			1.19 (1.08-1.31)	Age <50 y
			1.03 (0.93-1.14)	Never smokers
			1.04 (0.93-1.18)	Never drinkers
HEL308 (homozygous)	Case control study of patients in the Boston, MA area (Liang et al[21])	575/676	0.78 (0.57-1.06)	N/A (total population)
	Case control study of Chinese Nationals (Yuan et al[22])	397/900	0.98 (0.82-1.18)	N/A (total population)

Abbreviations: CI, confidence interval; HNSCC, head and neck squamous cell carcinoma; INHANCE, International Head and Neck Cancer Epidemiology; N/A, not applicable; OR, odds ratio.
Figure text in boldface indicates statistical significant.

promotor polymorphisms (at present, 5 identified in the oral cavity[38] and 1 in nasopharyngeal carcinoma[39]) relate to *BIRC5* expression has not yet been evaluated. Importantly, of currently identified *BIRC5* promoter polymorphisms, there is conflicting evidence on their association with HNSCC in low-risk patients. One investigation found that these polymorphisms confer elevated HNSCC risk in nonsmoking, nondrinking populations,[39] whereas another found that *BIRC5* variants confer susceptibility to HNSCC only in an interactive fashion with betel quid chewing or tobacco use.[38] These opposing conclusions highlight the need for further studies examining the association of genetic variants in apoptotic pathways with HNSCC in low-risk patients from multiple independent cohorts.

1.3 | Familial/hereditary head and neck squamous cell carcinoma

The vast majority of HNSCC are sporadic cancers, attributable to known etiologic factors, such as tobacco, alcohol, and HPV. At present, there is a paucity of data on the possible role of family history and importance of inheritance

patterns in HNSCC risk, particularly in low-risk patients. Previously mentioned herein, a 2015 INHANCE consortium study analyzed risk factors for incident HNSCC among 25 case-control studies and identified a convincing association between family history of early onset cancer and incident HNSCC in ever-smokers <45 years of age (odds ratio [OR] 2.27; 95% confidence interval [CI] 1.26-4.10).[12] This association was not present among older (≥45 years) patients with HNSCC (OR 1.10; 95% CI 0.91-1.31). Interestingly, an earlier INHANCE case-control study examining 9000 patients with HNSCC and 14 000 healthy controls similarly found that a family history of HNSCC in a first-degree relative conferred an increased risk of incident HNSCC in the patients examined (OR 2.2; 95% CI 1.6-3.1 for siblings and OR 1.5; 95% CI 1.1-1.8 for parents).[40] Importantly, this relationship was markedly stronger among patients with HNSCC with positive family history and tobacco and alcohol use (OR 7.2; 95% CI 5.5-9.5). These studies suggest that a familial/hereditary component to HNSCC may be attributable to inherited sensitivity toward tobacco-related and alcohol-related carcinogens, thus, this relationship is less relevant to a low-risk, never-smoker/never-drinker HNSCC population.

Inherited mutations in the *CDKN2A* gene, encoding the important tumor suppressor and cell-cycle regulating protein p16/INK4A, have been implicated in familial syndromes conferring a significantly increased lifetime risk of melanoma, pancreatic, lung, and breast cancer as well as of HNSCC.[41,42] Although the literature is limited, 2 recent case reports identified germline *CDKN2A* mutations in probands with HNSCC and melanoma among an extensive family history.[43,44] In the first, a novel single-nucleotide deletion (c.106delG) resulted in a premature stop codon (p.Ala36ArgfsX17).[43] In the second, a missense mutation (G302T) at exon 2 of *CDKN2A* with loss of heterozygosity was implicated.[44] A third *CDKN2A* germline variant termed p16-*Leiden* results in a 19-nucleotide deletion (c.225_243del19) and significantly heightens the lifetime risk of HNSCC development (relative risk 18.8; 95% CI 6.05-58.2).[45] Inherited *CDKN2A* loss-of-function mutations clearly elevate HNSCC risk in low-risk, never-smokers. Again, however, the risk of HNSCC in *CDKN2A* mutation carriers is amplified even more in ever-smokers, suggesting that such mutations may impair carcinogen metabolism and is, thus, less relevant to a low-risk HNSCC population.[46]

Nasopharyngeal carcinoma (NPC) is a poorly differentiated squamous cell carcinoma (SCC) of the posterior nasopharynx with a well-documented propensity for familial clustering.[47] A review of candidate-gene approaches and genomewide association studies to identify genomic loci conferring increased NPC risk by Bei et al[48] identified *ITGA*9 at chromosome 3p, *HLA-B/C* and *MICA* at chromosome 6p, and *CKDN2A/B* at chromosome 9q. A second study by Xiong et al[49] supported a link between familial variants in tumor suppressor gene clusters at chromosome 3p21 and NPC risk. Although further studies validating these findings have yet to be performed, it stands to reason that such germline variants likely contribute to NPC development in younger patients who lack identifiable risk factors.

1.4 | Fanconi anemia association in patients with low-risk head and neck squamous cell carcinoma

Fanconi anemia is an inherited bone marrow failure syndrome caused by biallelic inactivation of 1 of 17 genes (*FANCA* to *FANCQ*) inherited in an autosomal recessive or x-linked recessive pattern.[50] The *FANC* genes normally encode proteins that maintain genomic stability by repairing interstrand crosslinks (ICLs) in DNA (see Figure 1[1,50–52]). With an incidence of roughly 1 in 100 000 to 250 000 births, Fanconi anemia leads to a spectrum of phenotypic abnormalities that include congenital malformations (eg, short stature and microcephaly) and cytopenias.[53,54] Patients with Fanconi anemia are also particularly susceptible to development of malignancies, including leukemia, esophageal cancer, and HNSCC. The risk of HNSCC in Fanconi anemia, particularly of the oral cavity, is elevated roughly 500 to 700 times over the general population.[55] The median age of onset of HNSCC in patients with Fanconi anemia is a young 33 years of age and the vast majority (roughly 84%) of these patients are never-smokers/never-drinkers, suggesting minimal environmental contribution to HNSCC risk.[56]

Investigation into the association between Fanconi anemia and HNSCC begets questioning of whether inherited or sporadic *FANC* mutations may drive HNSCC development in low-risk, nontraditional patients without Fanconi anemia.[57] Tremblay et al[58] confirmed lower expression of *FANCA* and *FANCG* in oral cavity xenografts from young patients (<40 years), patients with HNSCC without Fanconi anemia as compared to an older patient (>60 years) cohort and healthy controls. The authors posited that attenuated expression of *FANCA* and *FANCG* may explain mechanistic differences in tumorigenesis between young, low-risk patients and their traditional HNSCC counterparts through defective carcinogen metabolism or DNA repair capabilities. Recently, Chandrasekharappa et al screened 417 patients for germline Fanconi anemia mutations in patients with head and neck cancer under the age of 50 years. They identified that 44% of the patients had the Fanconi anemia gene variants with 26% having variants predicted to be damaging. Additionally, they identified an increased mutational burden among 3 Fanconi anemia genes (*FANCD2*, *FANCE*, and *FANCL*) relative to the normal population; all of which are associated with other malignancies [PMID: 286978401]. Additionally, a recent investigation[59] utilizing 17 HNSCC cell lines found that *FANC* inactivation promotes genomic instability and

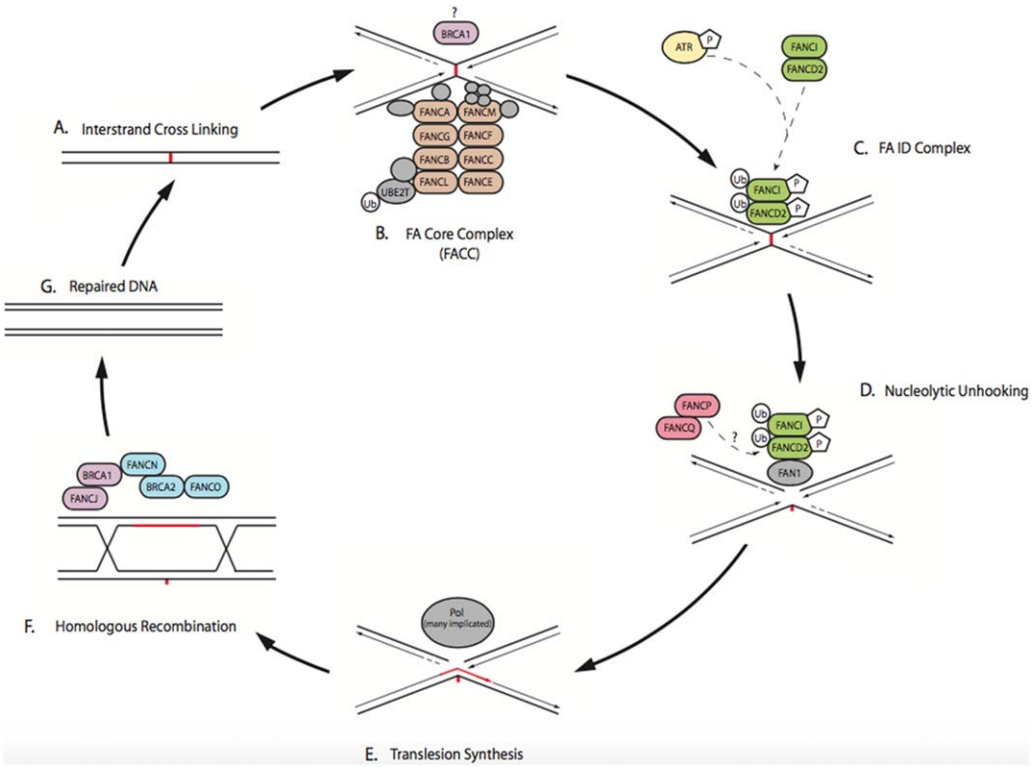

FIGURE 1 Interstrand crosslinking (ICL) repair pathway defective in patients with Fanconi anemia (the Figure is based on models put forth by Wang et al,[50] Ceccaldi et al,[51] and Garner et al[52]). A, ICL occurs. B, Fanconi anemia nuclear core complex (FACC) is a large, multi-subunit, E3 ubiquitin ligase (enzymatic activity provided by FANCL). The FACC is recruited to the site of ICL detected by stalled replication fork. The FANCM activates ATR serine/threonine kinase (ATR) via phosphorylation. The ATR functions downstream to phosphorylate FANCI in the ID complex. Many models propose BRCA1 associates with the initial FACC to assist with CMG helicase removal, allowing the replication fork to approach the ICL site more closely. C, The FACC recruits the phosphorylated Fanconi anemia ID complex (FANCI and FANCD2), and the FACC mono-ubiquitinates the Fanconi anemia ID complex. D, Nucleolytic "unhooking" of the ICL occurs through recruitment of downstream nucleases FAN1 (no mutations are currently identified in patients with Fanconi anemia), and multi-subunit FANCP(SLX4)/FANCQ(XPF). The FAN1 binds mono-ubiquitinated FANCD2. The FNACP(SLX4) contains ubiquitin binding sites and potentially interacts with FANCD2 as well. The role behind association of multiple nucleases, and their individual significance in nucleolytic processing, is unclear.[51] E, Translesion synthesis (TLS) is performed by TLS-specific polymerases that are recruited to the nuclear foci. Multiple TLS polymerases have been implicated, including REV1 and POLζ.[51] No mutations in TLS-specific polymerases have been identified in patients with Fanconi anemia to our knowledge.[1] F, Homologous recombination repair occurs through the recruitment of many repair factors. These include the FANCN(PALB2)/FANCD1(BRCA2)/FANCO(RAD51C) complex and FANCS(BRCA1)/FANCJ(BRIP1), which functions as a helicase and inhibitor of nonhomologous end joining. G, DNA has been successfully repaired by components in the Fanconi anemia pathway [Color figure can be viewed at wileyonlinelibrary.com]

tumorigenesis in only a small minority of non-Fanconi anemia HNSCC, prompting other authors to posit epigenetic modifications of *FANC* genes as potential drivers of sporadic HNSCC in patients with non-Fanconi anemia.[60,61]

Transcription of *FANC* genes seems to be regulated in synchrony with the cell cycle, as E2F, Rb, and ATR modulate expression of Fanconi anemia pathway components in human cancer cell lines.[62] Additionally, *FANC*-encoded proteins form complexes with the products of *BRCA1/2* genes (implicated in hereditary breast and ovarian cancer) to exert their ICL-repair functions.[63,64] In future studies, mutational status of upstream and downstream components of the Fanconi anemia pathway must be considered when clarifying associations between *FANC* aberrations and HNSCC risk in nontraditional patients.

Analysis of a 528-patient cohort of HNSCC cases from The Cancer Genome Atlas (TCGA) dataset using cBioPortal failed to demonstrate a consistent correlation between alterations in *FANC* genes and HNSCC (Figure 1[1,50–52] and Table 2[65,66]). However, only 42 of these 528 patients (8.0%) with HNSCC were <45 years of age, so drawing definitive conclusions regarding genetic profiling in low-risk, nontraditional patients with HNSCC is challenging. Despite the rising prevalence of a young, low-risk HNSCC population, the lack of genomic data from these patients precludes identification of causative genetic associations, prediction of clinical outcomes, and trials of personalized therapeutics.

The association between patients with HNSCC and Fanconi anemia, as well as the association between altered *FANC* genes and non-Fanconi anemia, low-risk patients with

TABLE 2 Alteration frequency of Fanconi anemia pathway and Fanconi anemia pathway-associated genes within the 528-patient The Cancer Genome Atlas head and neck squamous cell carcinoma provisional dataset retrieved using cBioPortal[65,66] [Color table can be viewed at wileyonlinelibrary.com]

Gene (alias)	No. of cases altered (504 total)	Percent of cases altered
Fanconi anemia pathway		
FANCA	11	2.20
FANCB	18	4
FANCC	13	2.60
FANCD1 (BRCA2)	27	5
FANCD2	13	2.60
FANCE	3	0.60
FANCF	5	1
FANCG	26	5
FANCI	9	1.80
FANCJ (BRIP1)	15	3
FANCL	6	1.20
FANCM	14	2.80
FANCN (PALB2)	8	1.60
FANCO (RAD51C)	3	0.60
FANCP (SLX4)	10	2
FANCQ (ERCC4)	5	1
FANCS (BRCA1)	13	2.60
Fanconi anemia pathway-associated		
BRCA2	27	5
BRCA1	13	2.60
RB	28	6
ATR	76	15

Color-coding of the genes in the table corresponds to the color-coding of gene products in Figure 1 depicting the Fanconi anemia-pathway.

HNSCC is in need of further characterization. To our knowledge, there has been limited investigation into the association of *FANC* genes with HNSCC in non-Fanconi anemia patients, with even fewer of these analyses considering low-risk patients specifically. In future mechanistic investigations of the Fanconi anemia pathway in HNSCC, it will be important to differentiate between somatic and germline mutations in *FANC* within non-Fanconi anemia patients with HNSCC.

1.5 | E2F Polymorphisms in patients with low-risk head and neck squamous cell carcinoma

The E2F family of transcription factors play a central role in modulation of cell-cycle progression and DNA synthesis and repair.[67] In quiescent cellular states, the Rb tumor suppressor protein (Rb) binds to E2F, preventing it from activating transcription machinery.[68] In the absence of Rb, or when Rb is sequestered by the HPV oncogenic protein E7, E2F becomes free to facilitate transcription of target genes that modulate the G1/S transition.[69] Dysregulation of the Rb-E2F pathway is seen almost universally across all human cancer types.

A recent investigation into the association of common polymorphisms in *E2F1* and *E2F2*, respectively, in 1100 HNSCC cases and 1090 healthy controls sought to correlate these genotypes with susceptibility to incident HNSCC.[70] The authors identified a statistically significant dose-response relationship conferring increased risk of incident HNSCC in patients harboring a greater number of these unique genetic variants (OR 1.62; 95% CI 1.14-2.30 for individuals with 9 to 10 polymorphisms versus individuals with 0 to 4; $P = .045$). Importantly, the joint effect of multiple *E2F* polymorphisms was particularly pronounced among adults <57 years of ages (OR 1.74; 95% CI 1.07-2.85), never-smokers (OR 1.85; 95% CI 1.07-3.17), never-drinkers (OR 2.19; 95% CI 1.22-3.95), and those with a family history of cancer in a first-degree relative (OR 1.64; 95% CI 1.05-2.57). These results suggest a convincing, additive effect of genetic variation within *E2F* genes on HNSCC risk, with enhancement of susceptibility in low-risk patients.

The authors of a recent investigation hypothesized that a common polymorphism (rs3213180) in the 3′UTR microRNAs (miRNAs) binding site of *E2F1* would be associated with the risk of oral cavity and oropharyngeal SCC and HPV status of oropharyngeal SCC, given the aforementioned role of HPV E7 in the Rb-E2F pathway.[71] Patients with *E2F1* rs3213180 polymorphism had an increased risk of developing oral cavity and oropharyngeal SCC (OR 3.3; 95% CI 2.4-4.6) regardless of HPV status. In individuals with HPV seropositivity and this *E2F1* polymorphism, never-smokers/drinkers had a particularly pronounced risk of oropharyngeal SCC compared to ever-smokers/drinkers (7.5-fold increased risk in never-smokers vs ever-smokers, and 4.9-fold increased risk in never-smokers vs ever-drinkers). In contrast, HPV seronegative individuals with *E2F1* polymorphisms had a similar risk of oral cavity and oropharyngeal SCC when comparing subsets of never-smokers/drinkers to ever-smokers/drinkers (0.97-fold decreased risk in never-smokers vs ever-smokers and 0.8-fold decreased risk in never-drinkers vs ever-drinkers). These complex interactions suggest that HNSCC susceptibility in HPV-positive, never-smokers/drinkers is due, at least in part, to specific interactions between variant *E2F1*-encoded proteins and the HPV E7 oncogene.

1.6 | Patients with high-risk human papillomavirus and low-risk head and neck squamous cell carcinoma

The incidence of high-risk HPV-associated HNSCC, particularly of oropharyngeal subsites, has soared in recent years and is most often seen in patients who lack traditional risk factors, namely tobacco and alcohol use.[6,72,73] Progression

TABLE 3 Alteration frequency of genes examined within this review, as reported in The Cancer Genome Atlas cohort and in Morris et al,[95] a precision oncology sequencing study of recurrent and metastatic head and neck squamous cell carcinoma from Memorial Sloan Kettering Cancer Center

	TCGA HNSCC cohort[5] (504 cases)		Morris et al[95] (132 cases)	
Gene (alias)	No. of cases altered	Percent of cases altered	No. of cases altered	Percent of cases altered
DNA repair genes				
XRCC1	5	1	0	0
XPD (ERCC2)	7	1.4	2	1.5
HEL308 (HELQ)	10	2	0	0
Apoptosis related				
TP53	363	72	62	47
PUMA (BCC3)	1	0.2	0	0
BIRC5	0	0	0	0
Familial HNSCC related				
CDKN2A	270	54	32	24
CDKN2B	143	28	10	8
ITGA9	6	1.2	0	0
HLA-B	24	5	0	0
HLA-C	5	1	0	0
MICA	6	1.2	0	0
Cell-cycle related				
E2F1	17	3	0	0
E2F2	4	0.8	0	0
HPV associated				
MDM2	24	5	3	2.3
MDM4	5	1	0	0
MCL1	13	2.6	9	7
NOXA (PAIMP1)	22	4	1	0.8
PUMA (BBC3)	1	0.2	0	0
TFGB1	4	0.8	0	0
TP53	363	72	62	47
TP73	8	1.6	0	0

Abbreviations: HNSCC, head and neck squamous cell carcinoma; HPV, human papillomavirus; TCGA, The Cancer Genome Atlas.
Many of these genes had high alteration rates (eg, *TP53, CDKN2A*), although those postulated to enhance HNSCC susceptibility in low-risk patients were less commonly altered in the overall TCGA cohort. The HNSCC TCGA provisional cohort had very few younger, low-risk patients (42 individuals were ≤45 years of age at diagnoses.

from oropharyngeal HPV infection to malignancy takes in excess of a decade and occurs in a small but significant minority of patients, indicating that genetic variation among individuals may play a pivotal role in differential risk of malignant transformation.[74,75]

Transforming growth factor-beta (TGF-β) is an anti-inflammatory cytokine with important roles in inflammation and immune responses that ultimately lead to HPV clearance or immune evasion and viral persistence.[76] Three distinct polymorphisms in the *TGF-β* gene (C509T, T869C, and G915C) have been shown to increase plasma levels of circulating TGF-β in human subjects.[77] As TGF-β suppresses proinflammatory responses mediated by Th1 and Th2 lymphocytes, elevated TGF-β levels may compromise immune surveillance and control of HPV-infected cells.[78] This immunosuppressive effect may be augmented by HPV-induced transcription of immunosuppressive cytokines, including TGF-β, as has been shown in cervical cancer models.[79] As such, Guan et al[80] hypothesized that TGF-β polymorphisms may contribute to differential genetic susceptibility to high-risk HPV-associated HNSCC. The authors genotyped TGF-β polymorphisms and confirmed HPV16 status in 200 primary tumors of the oropharynx. Patients with variant *TGF-β* genotypes were more than twice as likely to have an

HPV16-positive tumor (OR 2.28; 95% CI 1.16-4.50) compared with patients with wild-type *TGFβ*. Furthermore, a stratified analysis showed enhancement of this association among patients <54 years of age (OR 4.07; 95% CI 1.52-10.9), never-smokers (OR 3.76; 95% CI 1.15-12.3), and never-drinkers (OR 5.01; 95% CI 1.03-24.3). Similarly, 4 common polymorphisms (rs1800629, rs1799724, rs1800630, and rs1799964) in the gene promoter for tumor necrosis factor-alpha, a versatile proinflammatory cytokine involved in viral defense, have been proposed as susceptibility biomarkers for HPV16-associated oropharyngeal cancers in young, never-smokers/drinkers.[81] Finally, intriguing preliminary reports suggest that functional genetic polymorphisms of miRNAs likely contribute to variations in miRNA-mediated immune function and inflammation crucial for antiviral defense.[82,83] Taken together, the aforementioned studies provide evidence that genetic variation in immune-related genes augments the risk of high-risk HPV-mediated malignant transformation, particularly in young, low-risk HNSCC populations.

The critically important tumor suppressor p53 has been called the "guardian of the genome" for its role in activation of DNA repair pathways and initiation of growth arrest and apoptosis in response to genomic instability.[84] Inactivating *TP53* gene aberrations are oncogenic drivers in the vast majority of HNSCC and portend worse oncologic outcomes.[5,27] The HPV E6 oncoprotein complexes with human E6-associated protein to ubiquitinate p53, leading to its proteasomal degradation, a necessary cellular process for HPV-mediated carcinogenesis. As discussed above, a common polymorphism is observed at codon 72 of *p53*, replacing an arginine residue with proline; this amino acid change may influence the protein's susceptibility to E6-mediated degradation.[85] Variant genotypes at this *p53* locus enhance susceptibility to oropharyngeal cancers in an interactive and multiplicative fashion with HPV16 seropositivity and never-smoking status (OR 22.5; 95% CI 4.8-106.2).[85] Two linked polymorphisms (G4C14-to-A4T14) in exon 2 of *p73*, a close family member of *p53* that activates the promoter of several p53-responsive genes, confers similarly enhanced susceptibility to oropharyngeal cancers in HPV16-positive, never-smokers/drinkers.[86] Young (ie, <50 years), HPV16-positive individuals who harbor combined variants in both *p53* and *p73* and are never-smokers/drinkers have an even greater risk of incident oropharyngeal cancers.[87–89] Finally, *MDM2* and *MDM4* promoter polymorphisms synergize with HPV seropositivity to increase oropharyngeal cancer risk in young, never-smokers/drinkers.[90,91] MDM2 and MDM4 are p53-pathway members that inhibit p53-mediated transcriptional activity and target p53 for proteasomal degradation, thus inhibiting DNA damage repair, cellular growth arrest, and apoptosis.[91]

Components of the intrinsic apoptotic pathway may also be important contributors to differential risk of oropharyngeal cancer in young, HPV16-positive, never-smokers/drinkers. Overexpression of *Mcl-1* and deficiency of *NOXA,* antiapoptotic and proapoptotic members of the *Bcl-2* family, respectively, offer p53-dependent protection from apoptosis in multiple myeloma models, although confirmatory studies of this relationship in HNSCC are lacking.[92,93] Genetic variants in *Mcl1* and *NOXA* promoter regions may render cells more susceptible to HPV E6-mediated interference with the p53-NOXA-Mcl1 axis. To test this hypothesis, Zhou et al[94] analyzed 4 functional polymorphisms in the *NOXA* (rs9957673 and rs45589496) and *Mcl-1* (rs9803935 and rs3738485) promoters in 372 cases of oropharyngeal cancer and 315 healthy controls. Their results suggested a joint effect on oropharyngeal cancer risk in young, never-smoking/drinking patients with multiple *NOXA/Mcl-1* polymorphisms and HPV16 seropositivity.

2 | CONCLUSIONS

The rise in the number of low-risk, nontraditional patients with HNSCC in recent years should be met with a spirited effort to characterize novel germline and somatic genomic events that drive oncogenesis in this population. The current literature provides preliminary evidence of risk variants that may contribute to HNSCC development in low-risk patients, although conclusions are limited by conflicting findings, lack of consistent subgroup stratification, and small sample sizes.

Moving forward, we postulate that germline alterations in genes, such as *BRCA1*, *p53*, and *E2F*, which are implicated as oncologic drivers after somatic inactivation across human cancers, are likely to be ubiquitous and strongly conserved among young, low-risk HNSCC cases (Table 3[35]). Dedicated investigation of genetic aberrations in large populations of low-risk, nontraditional patients with HNSCC is imperative to advance our understanding of mechanisms of carcinogenesis, risk-stratify patients, and improve oncologic outcomes in this population. Alternatively, HNSCC development in low-risk patients may reflect exposure to unknown environmental factors, unclassified carcinogens, or a more complex interplay between multiple environmental influences and genetic variation.

Identifying pathogenic alterations in the low-risk HNSCC population will improve efforts to combat cancer progression. These studies may allow identification of at-risk patients from an early age, leading to subsequent modification of environmental and lifestyle factors, and may precede the development of novel, targeted therapies. Further research on low-risk, nontraditional patients with HNSCC is necessary and will ideally reveal a combination of actionable genetic drivers and modifiable lifestyle or environmental risk factors, thereby providing a means to reduce the prevalence, morbidity, and mortality of this disease.

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