

Supplementary Information

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Supplementary Table S1. Baseline Population Characteristics and Smoking and Drinking Exposures in the subset of VOYAGER data used for GWAS of HPV-positive and HPV-negative HNSCC. The continuous variables are compared using analysis of variance and categorical variables are compared using chi-squared test. All tests are two-sided.

Variables		Control (n= 3469)	HPV-Negative Cases (n=2326)	HPV-Positive Cases (n=1105)	P value
Age	Median (IQR)	59.1 (52.0 to 67.0)	60.7 (53.0 to 68.8)	56.9 (51.0 to 62.6)	4E-21
Sex	Female	1220 (35.2)	763 (32.8)	177 (16.0)	1E-32
	Male	2249 (64.8)	1563 (67.2)	928 (84.0)	
Ethnicity (self-reported)	White/Caucasian	2283 (65.8)	1695 (72.9)	1007 (91.1)	1E-71
	Black/African/Caribbean	180 (5.2)	147 (6.3)	14 (1.3)	<0.001
	unknown	995 (28.7)	466 (20.0)	64 (5.8)	
	Other	11 (0.3)	18 (0.8)	20 (1.8)	
Cancer Site	Oral Cavity	-	1943 (83.5)	-	<1E-158
	Oropharynx	-	383 (16.5)	1105 (100.0)	
Stage	I		549 (24.5)	33 (3.0)	<1E-158
	II		451 (20.1)	54 (4.9)	
	III		301 (13.4)	141 (12.9)	
	IV		915 (40.9)	850 (77.7)	
Exposures					
Smoking Initiation	No	1495 (43.2)	459 (20.7)	334 (32.0)	4E-67
	Yes	1969 (56.8)	1758 (79.3)	711 (68.0)	
Age of Smoking initiation	Median (IQR)	18.0 (15.0 to 20.0)	16.0 (15.0 to 19.0)	16.0 (14.0 to 18.0)	8E-18
Cigarettes per day	Median (IQR)	8.0 (0.0 to 20.0)	20.0 (10.0 to 30.0)	20.0 (10.0 to 25.0)	3E-141
Smoking Cessation	Current	656 (33.5)	999 (57.2)	222 (31.4)	4E-56
	Former	1302 (66.5)	747 (42.8)	485 (68.6)	
Drinks per week	Median (IQR)	5.8 (1.5 to 12.9)	14.1 (4.5 to 35.3)	10.0 (3.0 to 22.5)	5E-91

Supplementary Table S2: Factorial MR with One-Sample Results for smoking and drinking phenotypes on HPV-negative and HPV-positive HNSCC. All tests are two-sided. The one-sample MR and factorial MR were analyzed based on the logistic model.

Model	<u>Exposures</u> Phenotype	<u>Outcomes</u>			
		HPV-negative HNSCC vs controls		HPV-positive HNSCC vs controls	
		Effect size (se)	<i>P</i> value	Effect size (se)	<i>P</i> value
One-Sample MR	DPW	0.015 (0.004)	5.14E-4	2.51E-4 (0.006)	0.969
	SI	0.550 (0.089)	5.76E-10	0.364 (0.085)	1.84E-05
	ASI	-0.063 (0.014)	5.49E-06	-0.087 (0.017)	6.96E-07
	CPD	0.019 (0.004)	5.74E-06	-0.003 (0.005)	0.529
	SC	-0.543 (0.251)	0.031	0.389 (0.284)	0.171
Factorial MR	Interaction effect of DPW with SI	-0.100 (0.589)	0.866	0.254 (1.726)	0.883
	Interaction effect of DPW with ASI	0.031 (0.827)	0.970	-0.003 (0.012)	0.818
	Interaction effect of DPW with CPD	-0.001 (0.001)	0.584	-2.62E-4 (1.00E-4)	0.133
	Interaction effect of DPW with SC	0.108 (0.111)	0.330	0.044 (0.036)	0.221

MR, Mendelian Randomization; DPW, Drinks Per Week; SI, Smoking Initiation; ASI, Age of Smoking Initiation; CPD, Cigarettes Per Day; SC, Smoking Cessation, HNSCC, Head and Neck Squamous Cell Carcinoma

Supplementary Table S3. Univariable Mendelian Randomisation of All Smoking and Alcohol Consumption Exposures on Oral and Oropharyngeal Cancer Stratified by HPV status. All tests are two-sided. The Odds ratios for age of smoking initiation, Cigarettes per day, CSI and drinks per week are for a standard deviation change.

HPV status	Method	Smoking Initiation		Age of Smoking Initiation		Cigarettes Per Day		CSI		Drinks Per Week	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
		SNPs, <i>N</i> = 57		SNPs, <i>N</i> = 5		SNPs, <i>N</i> = 15		SNPs, <i>N</i> = 90		SNPs, <i>N</i> = 25	
<i>HPV-negative HNSCC vs controls</i>	MR	29.5 (3.46, 251)	3.11E-03	0 (0, 9.9)	0.25	1.91 (1.10, 3.30)	0.04	2.46 (0.19, 31.5)	0.49	13.4 (3.36, 53.7)	1.26E-03
	Egger	1.82 (1.20, 2.76)	4.99E-03	4.05 (0.5, 32.8)	0.19	1.59 (1.17, 2.17)	3.07E-03	2.59 (1.37, 4.92)	3.45E-03	6.79 (2.68, 17.2)	5.21E-05
	IVW	2.20 (1.20, 4.04)	0.01	9.6 (0.77, 120)	0.08	1.54 (1.02, 2.31)	0.04	3.87 (1.6, 9.33)	2.61E-03	9.78 (3.24, 29.5)	5.25E-05
	Weighted median	3.28 (0.92, 11.6)	0.07	13.4 (0.42, 429)	0.22	1.58 (1.01, 2.48)	0.07	6.78 (0.89, 51.7)	0.07	10.4 (3.5, 31.1)	3.14E-04
<i>HPV-positive HNSCC vs controls</i>	MR	6.88 (0.35, 135)	0.21	0.37 (0, 64E06)	0.91	0.65 (0.31, 1.35)	0.27	0.42 (0.02, 9.06)	0.58	4.53 (0.93, 22.0)	0.07
	Egger	2.37 (1.33, 4.24)	3.46E-03	4.96 (0.15, 169)	0.37	0.80 (0.53, 1.21)	0.3	2.60 (1.20, 5.65)	0.02	3.58 (1.27, 10.1)	0.02
	IVW	2.41 (1.04, 5.58)	0.04	6.62 (0.21, 207)	0.28	0.68 (0.40, 1.14)	0.14	1.45 (0.47, 4.41)	0.52	3.17 (0.79, 12.7)	0.1
	Weighted median	2.63 (0.53, 13.19)	0.24	10.0 (0.05, 1972)	0.44	0.66 (0.39, 1.09)	0.13	1.03 (0.08, 13.9)	0.98	2.82 (0.73, 10.8)	0.14
	Weighted mode										

HPV, human papillomavirus; MR, Mendelian randomization; OR, odds ratio; CI, confidence interval; IVW, inverse variance-weighted

Supplementary Table S4: Univariable MR Results for the Association of Number of Sexual Partners and Risk Tolerance with HPV positive and HPV negative HNSCC. All tests are two-sided. The Odds ratios correspond to a standard deviation change in number of sexual partners and risk tolerance. The results presented are for the Inverse Variance Weighted Method

	HPV-positive HNSCC vs Controls		HPV-negative HNSCC vs Controls	
	OR (95% CI)	P value	OR (95% CI)	P value
Number of sexual Partners	2.11 (0.89, 4.98)	0.090	0.99 (0.5, 1.95)	0.976
Risk Tolerance	0.5 (0.09, 2.89)	0.436	0.25 (0.06, 1.12)	0.070

CSI, Comprehensive smoking index; HPV, Human papillomavirus; HNSCC, Head and Neck Squamous Cell Carcinoma

Supplementary Table S5: Heterogeneity of SNP effects in Inverse Variance Weighted and MR-Egger analysis. Inverse Variance Weighted and MR-Egger are two-sided tests.

Outcome	Exposure	MR Egger			Inverse variance weighted		
		Q	Q degrees of freedom	Q P value	Q	Q degrees of freedom	Q P value
HPV+ HNSCC vs Controls	Smoking Initiation	62.255	55	0.234	62.829	56	0.247
HPV- HNSCC vs Controls	Smoking Initiation	48.329	55	0.726	55.068	56	0.51
HPV+ HNSCC vs Controls	Age of Initiation	7.634	3	0.054	7.886	4	0.096
HPV- HNSCC vs Controls	Age of Initiation	1.6	3	0.659	4.822	4	0.306
HPV+ HNSCC vs Controls	Cigarettes Per Day	12.609	13	0.478	13.078	14	0.52
HPV- HNSCC vs Controls	Cigarettes Per Day	12.706	13	0.471	13.318	14	0.502
HPV+ HNSCC vs Controls	Smoking Cessation	6.49	3	0.09	6.927	4	0.14
HPV- HNSCC vs Controls	Smoking Cessation	11.199	3	0.011	11.235	4	0.024
HPV+ HNSCC vs Controls	CSI	75.102	88	0.835	76.549	89	0.824
HPV- HNSCC vs Controls	CSI	106.469	88	0.088	106.472	89	0.1
HPV+ HNSCC vs Controls	Drinks Per Week	22.996	23	0.461	23.146	24	0.511
HPV- HNSCC vs Controls	Drinks Per Week	31.167	23	0.119	33.406	24	0.096

CSI, Comprehensive smoking index; HPV, Human papillomavirus; MR, Mendelian Randomization

Supplementary Table S6: I-squared statistics for assessing the NOME (No Measurement Error) Assumption for MR-Egger Analysis. MR-Egger is a two-sided test.

Exposure	F-statistic	HPV-positive HNSCC versus Controls		HPV-negative HNSCC versus Controls	
		I^2 Weighted	I^2 Unweighted	I^2 Weighted	I^2 Unweighted
Smoking Initiation	47.912	0.397	0.464	0.42	0.464
Age of Smoking Initiation	42.3	0	0.647	0	0.647
Cigarettes Per Day	133.593	0.978	0.978	0.979	0.978
Smoking Cessation	72.44	0.754	0.873	0.721	0.873
CSI	48.502	0.507	0.686	0.518	0.686
Drinks Per Week	92.188	0.965	0.974	0.964	0.974

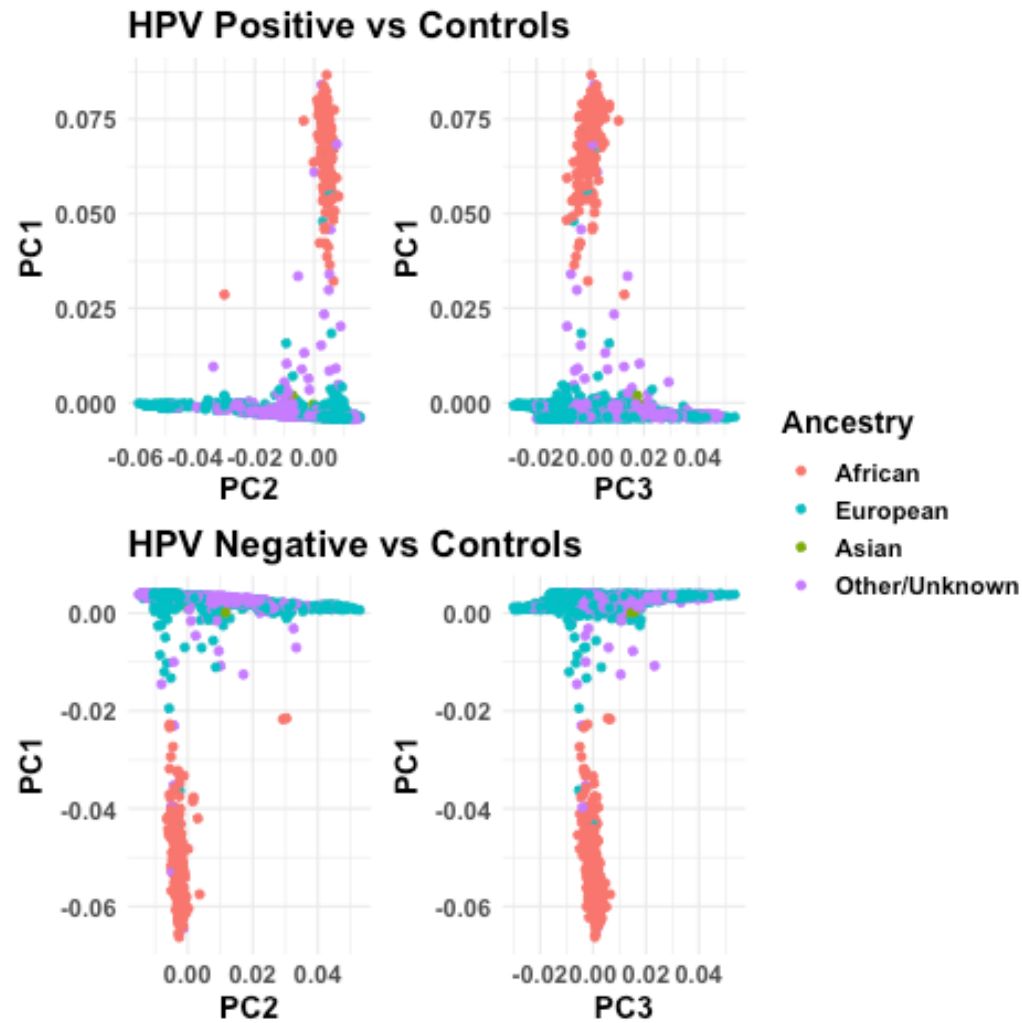
CSI, Comprehensive smoking index; HPV, Human papillomavirus; MR, Mendelian Randomization

Supplementary Table S7: Directional Pleiotropy using MR- Egger Intercept. MR-Egger is a two-sided test.

Outcome Exposure	HPV-positive HNSCC versus Controls			HPV-negative HNSCC versus Controls		
	Intercept	Standard Error	<i>P</i> value	Intercept	Standard Error	<i>P</i> value
Smoking Initiation	-0.03	0.042	0.479	-0.078	0.03	0.012
Age of Smoking Initiation	0.055	0.176	0.774	0.153	0.085	0.171
Cigarettes Per Day	0.019	0.027	0.505	-0.016	0.02	0.448
Smoking Cessation	-0.063	0.14	0.684	-0.013	0.136	0.928
CSI	0.029	0.024	0.232	0.001	0.02	0.966
Drinks Per Week	-0.007	0.018	0.702	-0.021	0.016	0.211

CSI, Comprehensive smoking index; HPV, Human papillomavirus; MR, Mendelian Randomization

Supplementary Figure S1: Population Stratification in the Study Population by Principal Component Analysis (PCA).



HPV, Human papillomavirus; PC, Principal Component