Cutaneous Human Papillomaviruses in Head and Neck Cancers: Risk Factors or Innocent Bystanders?

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July 2022

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree

of Doctor of Philosophy (Ph.D.) in Epidemiology

﴿وَلا يُحيطونَ بِشَيءٍ مِن عِلمِهِ إِلّا بِما شاءَ﴾ [البقرة: ٢٥٥]

[And they encompass not a thing of His knowledge except for what He wills] (Quran 2:255)

DEDICATION

A large part of this work was carried out during the COVID-19 pandemic. I dedicate my work to the scientists and healthcare workers who lost their lives while fighting this disease.

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ABSTRACT

In high-income countries, human papillomavirus (HPV) is a major cause of head and neck cancers (HNC). While high-risk types from the α-genus like HPV16 have been studied extensively in the HNC literature, the role of other genera (β and γ), also called cutaneous HPV, is still poorly understood. However, recent studies have shown that β- and γ-HPV could be related to cancers in the skin, esophagus, and head and neck. There are few studies investigating their role in HNC, and none in the Canadian population. This dissertation research aims to address limitations in previous work and advance the research on the relation between cutaneous HPV and HNC.

The data for this project come from the Head and Neck Cancer (HeNCe) Life study. HeNCe investigators recruited incident HNC cases (460) and controls (458), frequency-matched by age and sex, from four main referral hospitals in Montreal, Canada. HeNCe collected information on sociodemographic and behavior characteristics using in-person interviews, and tested rinse and brush specimens for HPV genotyping. Tumor samples were retrieved from hospital archives for a subsample of cases (n=121) to investigate HPV in tumor tissues. Samples were tested for all three genera of HPV using several molecular techniques.

First, we described the prevalence of HPV genera and genotypes in oral and tumor samples, and examine the distribution according to age, sex, sexual behavior, smoking, alcohol consumption, and oral health indicators. Similar to the α-genus, γ-HPV distribution varied by smoking and sexual behavior. However, β-HPV did not show a difference in distribution by any of the typical cancer risk factors except for age.

Second, we estimated confounding-adjusted odds ratios (aOR) and 95% confidence intervals (CI) for the effect of HPV on HNC using logistic regression. α-HPV genus had a strong effect on HNC, particularly HPV16 (aOR=22.6; 95% CI: 10.8, 47.2). We found weaker evidence for γ-HPV (aOR= 1.29; 95% CI: 0.80, 2.08) and β-HPV was more common among controls than cases (aOR=0.80; 95% 0.57, 1.11). We conducted a quantitative bias analysis for the relation between HPV16 and HNC and found the effect would be underestimated when not accounting for the three epidemiologic biases: unmeasured confounding, selection bias, and measurement error. Multiple bias analyses for HPV16 increased the strength of the point estimate but also increased uncertainty (aOR=54.2, 95%CI 10.7, 385.9).

Finally, we estimated the interaction between HPV genera in HNC, particularly the interaction between HPV16 and infection with any β- or γ-HPV. Infection with HPV16 alone had a strong effect on HNC. The effect of coinfection between HPV16 and any cutaneous HPV was stronger than the effect of either one alone, but we did not find strong evidence for an additive interaction as the study was underpowered. However, the point estimate for interaction between HPV16 and any cutaneous HPV infection was positive with relative excess risk due to interaction (RERI) = 2.44 (95% CI -23.27, 28.15). Likewise, we did not find strong evidence for the interaction between HPV16 and β-HPV or γ-HPV, but the point estimate was in a negative direction with any β-HPV and a positive direction for any γ-HPV infection. Because of the limited sample size, results were imprecise and definite conclusions cannot be made.

Overall, we find little evidence for the effect of β- and γ-HPV on HNC. The absence of cutaneous HPV from tumor samples supports the hypothesis that these genera, while being cofactors to α-HPV, may not play a direct role in HNC development. Further improvement in methods used to study HPV is warranted to account for biases in HPV epidemiological studies. The transitory nature of HPV infection and the complex interaction between different genera and genotypes would be an area of potential research to help our understanding of HPV role in HNC.

RÉSUMÉ

Dans les pays à revenu élevé, le papillomavirus humain (HPV) est une cause majeure de cancers de la tête et du cou (HNC). Alors que les types à haut risque du genre α comme le HPV16 ont été largement étudiés dans la littérature sur les HNC, le rôle des autres genres (β et γ), également appelés HPV cutanés, est encore mal compris. Peu d'études ont été menées sur leur rôle dans les HNC, et aucune dans la population canadienne. Cette thèse de doctorat vise à aborder les limites des travaux antérieurs et à faire progresser la recherche sur la relation entre les HPV cutanés et les HNC.

Les données de ce projet proviennent de l'étude *Head and Neck Cancer Life Study* (HeNCe). HeNCe a recruté 460 cas incidents d'HNC et 458 témoins, appariés en fréquence pour l'âge et le sexe, dans quatre hôpitaux de référence de Montréal, au Canada. HeNCe a recueilli des informations sur les caractéristiques sociodémographiques et comportementales au moyen d'entretiens en personne, et a réalisé le génotypage du HPV. Des échantillons de tumeurs ont été récupérés dans les archives de l'hôpital pour un sous-échantillon de cas (n=121) afin d'étudier les HPV dans les tissus tumoraux. Les échantillons ont été testés pour les trois genres de HPV à l'aide de techniques moléculaires.

Tout d'abord, nous avons décrit la prévalence des genres et des génotypes de HPV dans les échantillons buccaux et tumoraux et examinons la distribution en fonction de l'âge, du sexe, du comportement sexuel, du tabagisme, de la consommation d'alcool et des indicateurs de santé buccale. Comme pour le genre α, la distribution du γ-HPV variait en fonction du tabagisme et du

comportement sexuel. Cependant, la distribution du β-HPV ne variait pas en fonction des facteurs de risque de cancer typiques, à l'exception de l'âge.

Deuxièmement, nous avons estimé les rapport de cotes (aOR) ajustés aux facteurs de confusion et les intervalles de confiance (IC) à 95 % pour l'effet du HPV sur les HNC en utilisant la régression logistique. Le genre α-HPV avait un effet important sur le HNC, en particulier le HPV16 (aOR=22,6 ; IC à 95% : 10,8, 47,2). Nous avons trouvé des preuves plus faibles pour le γ-HPV (aOR= 1,29 ; IC 95% : 0,80, 2,08); le β-HPV était plus fréquent chez les témoins que chez les cas (aOR=0,80 ; 95% 0,57, 1,11). Nous avons effectué une analyse quantitative de biais pour la relation entre HPV16 et HNC et avons constaté une sous-estimation de l'effet lorsqu'on ne prend pas en compte les biais épidémiologiques de confusion non mesurée, de sélection et erreur de mesure. Les résultats de l'analyse de biais pour le VPH16 suggèrent une relation plus forte, mais avec une plus grande incertitude (aOR=54,2, 95%CI 10,7, 385,9).

Enfin, nous avons estimé l'interaction entre les genres de HPV dans l'HNC. L'effet de la co-infection entre HPV16 et tout autre HPV cutané était plus fort que l'effet de l'un ou l'autre seul, avec une estimation ponctuelle de l'excès de risque relatif dû à l'interaction (RERI) de 2,44 (IC à 95 % -23,27, 28,15). Nous n'avons pas trouvé de preuves solides d'une interaction additive, car on manquait de puissance en raison de la taille limitée de l'échantillon. De même, nous n'avons pas trouvé de preuves solides pour l'interaction entre HPV16 et β-HPV ou γ-HPV pris séparément. Toutefois, les résultats étaient imprécis, et aucune conclusion ne peut être tirée.

Dans l'ensemble, nous trouvons peu de preuves de l'effet des β- et γ-HPV sur les HNC. L'absence de HPV cutané dans les échantillons de tumeurs soutient l'hypothèse selon laquelle ces genres pourraient ne pas jouer un rôle direct dans le développement des HNC. Il est nécessaire d'améliorer les méthodes utilisées pour l'étude du HPV afin de tenir compte des biais dans les études épidémiologiques. La nature transitoire de l'infection par le HPV et l'interaction complexe entre les différents genres et génotypes représente un domaine de recherche potentiel pour améliorer notre compréhension de l'étiologie des HNC.

LIST OF ABBREVIATIONS AND ACRONYMS

95% CI	95% Confidence Interval
DAG	Directed Acyclic Graph
EMM	Effect Measure Modification
HeNCe	The Head and Neck Cancer Life Course Study
HPV	Human Papillomavirus
HR-HPV	High Risk Human Papilloma Virus
IARC	International Association for Research on Cancer
ICD	International Classification of Diseases
LR-HPV	Low Risk Human Papilloma Virus
MAR	Missing at Random
OR	Odds Ratio
PCR	Polymerase Chain Reaction
QBA	Quantitative Bias Analysis
RERI	Relative Risk due to Interaction
RR	Risk Ratio
SCC	Squamous Cell Carcinoma
α-HPV	Alpha Human Papillomavirus
β-HPV	Beta Human Papillomavirus
γ-HPV	Gamma Human Papillomavirus

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ACKNOWLEDGEMENT

There is a long list of people who helped me get to this point and to whom I will always be grateful. My path to obtaining a Ph.D. degree was not straightforward. I ended up being a doctoral student in epidemiology in not one but two world-class universities— McGill University and the University of North Carolina at Chapel Hill (UNC). In these two institutions, I am thankful for the many professors who taught me over the past six years and the many colleagues and friends I met along the way.

I am particularly grateful for the support and guidance I received from my supervisors: Prof. Sam Harper and Prof. Belinda Nicolau. I appreciate their patience, guidance, and help whenever needed. It was not an easy path, but your help and motivation made success possible. Thank you for your advice and mentorship; I have learned many lessons from you that I will never forget.

I am also grateful to Prof. Jay Kaufman for introducing me to my supervisors and helping me move to McGill. I am thankful for the several discussions on methods, epidemiology, and many other topics over the years. I also thank Prof. Charles Poole of UNC, a wonderful scientist and human being. I owe a great deal of what I know about epidemiology to his classes. Both Jay and Charlie have an encyclopedic knowledge of history, people, and methods of epidemiology that is unparalleled.

Above all, I am grateful to my parents, Ms. Kawkab Baidar and Mr. Ahmed Al-Soneidar. Mom, thank you for your patience, prayers, and belief in me even when I doubted my own decisions. You will always be my best friend, and I hope that I have made you proud by completing this thesis. Dad, thank you for never doubting my ability to achieve success and for pushing me to be better. I have always looked up to you for your strength, courage, and resilience. A big thank you to my lovely sister, Sondos, whose love has been a great motivation. Thank you for taking the burden off my shoulders as I traveled the world on this learning journey.

I am also thankful for my dearly loved wife, Ahlam Al-Edlah. I could not have done it without you; your love and support over the years helped me reach this far. To my little two angels: my daughter, Jana, and son, Kenan, I am sorry for missing so many weekends, parties, and holidays. Your love has been a great source of strength; you all deserve credit for this work as much as I do.

I want to thank Prof. Gilles Paradis and Prof. Robert Platt, the two previous chairs of our department, for their generous support. I would also like to acknowledge the administrative help and assistance of Mr. André Yves Gagnon and Ms. Katherine Hayden. Thank you for answering my questions and putting up with my so-many emails.

Last, a list of friends and colleagues who have shared their wisdom and experience and whose advice has been fundamental to my success: Drs. Bob Scarfo, Sadeq Al-Maweri, Aderonke Akingukbe, and Veeral Sarayia. Thank you all.

STATEMENT OF FINANCIAL SUPPORT

Throughout my studies at McGill, I have been fortunate to receive funding from various sources that allowed me to conduct this dissertation research. Funding came in the form of studentships and fellowships and included: a doctoral fellowship from the government of Quebec Fonds de recherche du Québec- Santé (FRQS); Grad Excellence Awards from the Department of Epidemiology, Biostatistics and Occupational Health (EBOH); George G Harris Fellowship in Cancer Research from the Faculty of Medicine, McGill University; Wah Leung Fellowship from the Faculty of Dentistry, McGill University; a doctoral research award from Réseau de recherche en Santé Buccodentaire et Osseuse (RSBO); as well as additional funding from my supervisors Drs. Sam Harper and Belinda Nicolau.

I also received awards as a Hatton competitor from the Canadian and International Associations of Dental Research (IADR and CADR) to present at the IADR conference in Boston (July 2021); funding from the Behavioral Sciences, Epidemiology, and Health Services Research Group (BEHSR) of the American Association of Dental and Craniofacial Research (AADCOR) to present at the Oral Epidemiology Forum of in AADOCR 2022 conference in Atlanta, Georgia (March 2022); and a Graduate Research Enhancement and Travel Award (GREAT) from EBOH to present at the International Association for Dental Research (IADR) conference 2022 (June 2022).

CONTRIBUTION OF AUTHORS

This dissertation is composed of three manuscripts as listed below. I was responsible for conducting this work under the supervision of Prof. Sam Harper and Prof. Belinda Nicolau. I was solely responsible for conceptualizing the objectives, developing the research question, conducting data analysis, drafting manuscripts, editing final versions, and ultimately writing the chapters presented in this dissertation.

Prof. Sam Harper, my supervisor, is an Associate Professor of Epidemiology in the Department of Epidemiology, Biostatistics, and Occupational Health. He provided guidance and advice on study objectives, research questions, methodology and data analysis. He supervised the process from protocol development onwards. Prof. Harper reviewed all manuscripts and chapters and provided feedback and suggestions for improvement.

Prof. Belinda Nicolau, my co-supervisor, is a Professor at the Faculty of Dental Medicine and Oral Health sciences. She is the principal investigator of the Head and Neck Life Study (HeNCe). Dr. Nicolau supervised data collection and quality control, and provided guidance and advice on study objectives and protocol. Dr. Nicolau reviewed all manuscripts and chapters and provided feedback and suggestions for improvement.

In addition to my supervisors, other co-authors listed in the manuscripts below had the following contributions:

Manuscript I: *Prevalence of Alpha, Beta, and Gamma Human Papillomaviruses (HPV) and relation to behavioral factors*

- Dr. François Coutlée is a Professor of Microbiology in the Department of microbiology and immunology, Université de Montréal. He is the director of the microbiology laboratory at the CHUM Research Center. Samples from HeNCe were prepared and genotyped in the laboratory and under his supervision. He reviewed Manuscript I and provided valuable feedback.
- Drs. Tarik Gheit and Massimo Tomassino are microbiologists and virologists at the Infections and Cancer Biology Group, International Agency for Research on Cancer-World Health Organization, Lyon, France. They provided the in-house developed reagents used for genotyping cutaneous HPV in the laboratory. They also reviewed manuscript I and provided valuable feedback.

Manuscript II: *Do cutaneous human papillomavirus genotypes affect head and neck cancer? Evidence and bias-correction from a case-control study*

- Dr. Sreenath Madathil is an Assistant Professor at the Faculty of Dental Medicine and Oral Health Sciences, McGill University & Dr. Nicholas Schelcht is a Professor at the Department of Cancer Prevention and Control, Roswell Park Comprehensive Cancer Center, Buffalo, New York, USA: both co-authors have been collaborators on the HeNCe project, and both reviewed manuscript II and provided valuable feedback.

Manuscript III: *Interaction of HPV16 and Cutaneous HPV in Head and Neck Cancer*

- Dr. Babatunde Alli is a dentist and a Ph.D. student at McGill's Faculty of Dental Medicine and Oral Health Sciences. He assisted with the R code used in manuscript III and provided valuable comments on the manuscript.

CONTRIBUTION TO ORIGINAL KNOWLEDGE

Throughout my learning process and while working on this dissertation, I received feedback, guidance, and support from supervisors, co-authors, and colleagues, but the work presented here is my own. Work on this dissertation constitutes an original contribution to advancing our understanding of HPV-related head and neck cancer.

Manuscript I describes the prevalence of mucosal and cutaneous HPV in a sample of head and neck cancer patients and controls and explores variation by sociodemographic and behavioral variables. This is the first study to investigate cutaneous HPV in oral cell and tumor samples in the Canadian population.

Manuscript II estimates the conditional effect of HPV genera on HNC and conducts quantitative bias analysis (QBA) on the HPV16-HNC relation. While this research question has been answered in a single previous paper, we address some methodological limitations and is the first study to use QBA in the HNC literature.

Manuscript III investigates the interaction between HPV16 and cutaneous HPV on both additive and multiplicative scales. Interaction on the additive scale has been described as the most relevant for public health. To the best of my knowledge, this is the first study to explore the possibility of interaction between HPV genera as a risk factor for HNC.

CHAPTER 1: INTRODUCTION

1.1 Background

Head and Neck Cancer (HNC) is one of the most debilitating human diseases with a poor survival rate, high treatment cost, and increased morbidity following treatment (1,2). According to a recent report from GLOBOCAN, there were around 750 thousand incident cases and 370 thousand deaths of head and neck cancer (including cancers in the Lip, oral cavity, oropharynx, hypopharynx, and larynx) in 2020 globally (3). This makes HNC the sixth most incident cancer globally and the seventh in terms of mortality. The literature on this disease is vast, ranging from studying risk factors to treatment and survival. Over the past five decades, the incidence of HNC that is not related to human papillomavirus (HPV) has been declining due to extensive tobacco and alcohol regulations, while the incidence of HPV-related HNC has risen substantially (4–6). The rise in HPV-related lesions is more evident in developed countries such as Canada (7–9) and the United States (10). Such surge has been attributed to an increase in the incidence of a subsite of HNC, cancers in the oropharynx (11).

Research on the relation between HPV and HNC started in the 1990s and kept growing as the research on HPV grew. But it wasn't until 2007 that HPV16 was identified as a risk factor for HNC using epidemiologic evidence (12). We now recognize there are high-risk genotypes of HPV capable through different mechanisms of causing cancer, and some benign genotypes are either harmless or could cause benign lesions like warts (13).

It is important to note that over 200 genotypes of HPV have been identified to date (14), and only a minority have been linked to cancers in the cervix, vagina, vulva, penis, anus, and head and neck (15). HPV genotypes are divided into five genera (Alpha (α), Beta (β), Gamma (γ), Mu (μ), and Nu (ν)) (16), but only three genera have been detected in the oral cavity (α, β, and γ)(17). While most research has focused on studying α-HPV, mainly HPV16 and other high-risk genotypes, there is a growing literature on the role of other genera.

Some recent reports revealed that β- and γ-HPV genera, mostly detected in the skin and thus called *cutaneous* HPV, can be related to cancers in the skin and esophagus (18,19). More relevant to this dissertation work, cutaneous HPV can be detected in the oral cavity (17,20), and a recent article reported an association with HNC (21,22). This dissertation attempts to enhance our understanding of the role of these genotypes and aims to answer the research question presented in the title: are cutaneous HPV genera risk factors of HNC or just innocent bystanders?

1.2 Research Questions

Throughout my work on this doctoral dissertation, I focused on three aspects; each represents a publishable article that has either been published or will be submitted for peer review soon.

First, *what is the distribution and prevalence of cutaneous HPV among HNC patients and healthy people?* In the first manuscript (Chapter 4), I attempt to answer this question and investigate the determinants and characteristics of individuals infected with cutaneous HPV and mucosal HPV. I

also explore the distribution of the three genera of HPV (α-, β-, and γ) by important social and behavioral variables such as smoking, alcohol drinking, sexual behavior, and oral health measures. Also, for the first time, I investigate the presence of cutaneous HPV genotypes in HNC tumor lesions.

Second, *what is the effect of cutaneous HPV on the rate of HNC?* I attempt to answer this question in manuscript 2 (Chapter 5) by investigating the effect of the three genera on HNC while controlling for confounders. In this analysis, I look at the independent effect of cutaneous HPV and control for HPV16. I also conduct secondary analysis on the relation between HPV16, the most carcinogenic genotype, and HNC, including quantitative bias analysis to assess the potential role of unmeasured confounding, selection bias, and measurement error.

Third, *do mucosal and cutaneous HPV genotypes interact to cause cancer?* I aimed to answer this question while acknowledging the limitation of evidence on the physical dependence of exposure variables as assessed through an epidemiological study. It is crucial, however, to catch a glimpse at the possible direction of mechanistic interaction between these genotypes and how cutaneous HPV could play a synergistic or antagonistic role to that of α HPV.

At the end of this work, I discuss the findings of these three studies in view of what we know thus far from the literature and give some directions for future studies in this field. I hope this work will eventually drive our field forwards, enhance our understanding of how this terrible disease initiates and give hope for future research on etiology or prevention science.

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CHAPTER 2: LITERATURE REVIEW

2.1 Definition of HNC

Head and Neck Cancer (HNC) is a broad term used for all cancers in the head and neck region, including cancers of the oral cavity, anterior two-thirds of the tongue, the floor of the mouth, gingiva, hard palate, oropharynx, posterior third of the tongue, soft palate, and posterior pharyngeal wall and larynx (Figure 2.1). Virtually all of these cancers (92%) are squamous cell carcinomas (1), meaning they originate from the malignant transformation of cells in the epithelial layer of mucosa (squamous cells) (2,3). Due to histological similarities and relatively low incidence, these cancers have been historically studied together. However, anatomical differences may also result in different levels of exposure or susceptibility to a given risk factor (4). Global estimates indicate that 31% of all oropharyngeal cancers are attributed to HPV infection (5), with tobacco and alcohol causing the majority of remaining cases (6,7). In recent years, some research has focused on oropharyngeal cancers as a separate category within HNC, as they are closely related to human papillomavirus infections (HPV), have distinct molecular and epidemiological characteristics (8), respond better to treatment, and are characterized with better survival than other HNCs (9).

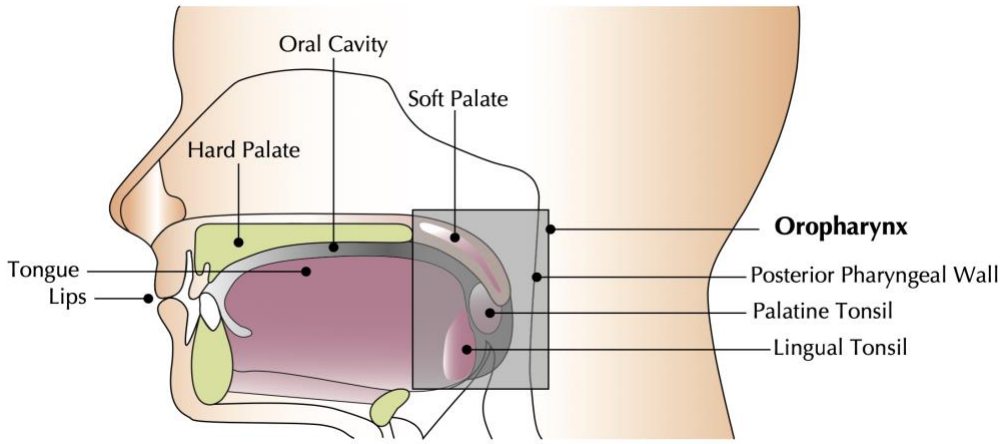


Figure 2.1 Anatomy of oral cavity and surrounding areas. Reprinted with permission from (10). Watters, A. L., Hansen, H. J., Patel, A. A., & Epstein, J. (2021). HNC. In Burket's Oral Medicine (pp. 211–257). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119597797.ch7

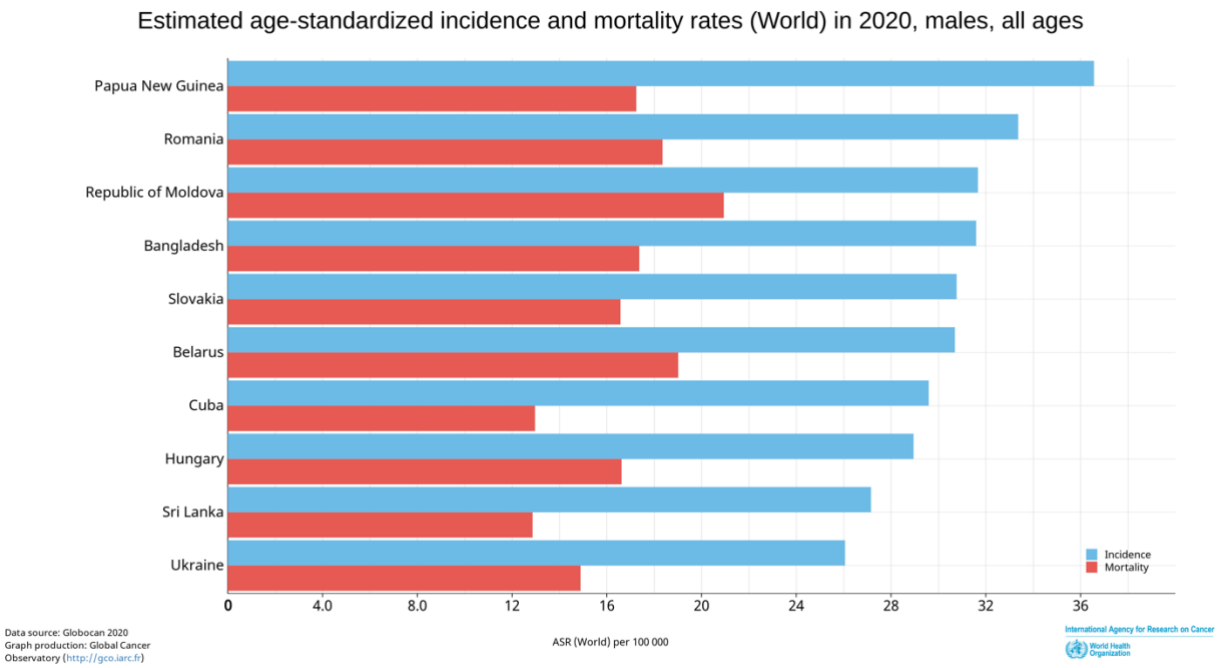
2.2 Epidemiology, Distribution and Global Burden

The ranking of HNC varies across countries, from the sixth (11) to the ninth (12) most commonly detected cancer annually. Such variation in ranking reflects the geographic variation in risk factors between different populations and demonstrates the heterogeneity of these cancers. Cancers of several organs in this region are sometimes reported separately or more often grouped as a single category.

HNC is a devastating disease with one of the highest morbidity and suicide rates of all cancers (13,14). Nearly two-thirds of these cancers are diagnosed at stage III and IV (15,16), which is reflected in poor survival rates. Because it is located in a complex anatomical region with many physiologic functions, HNC is also one of the most expensive solid tumors to treat (17), often requiring a multidisciplinary team of health professionals for treatment and rehabilitation. According to a recent report from GLOBOCAN (2020), there were almost 750 thousand incident

cases and 370 thousand deaths of HNC (including cancers in the lip, oral cavity, oropharynx, hypopharynx, and larynx) (18). This places HNC as sixth cancer in terms of incidence and seventh in terms of mortality globally (18).

Oral cavity cancer is common in developing countries such as Pakistan, India, and Sri Lanka, where tobacco, areca nut, and smokeless tobacco are widely used. Oropharyngeal cancer is mainly driven by HPV infection that is sexually transmitted and is more common in developed countries. For example, Denmark, France, and Romania have the world's highest age-standardized incidence rates (ASR), with 5.0, 4.3, and 4.3, respectively (19). Below are graphs from the Global Cancer Observatory of the International Association for Research on Cancer (IARC) that show estimated age-standardized incidence and mortality rates for 2020 for all groups of HNC (Figure 2.2). The disease predominantly affects men with a higher incidence and mortality (7). This could be because men are more likely to smoke, consume alcohol excessively, report several sexual partners and become infected with HPV. Figure 2.3 shows the highest ten countries with age-standardized incidence and mortality for oropharyngeal cancer.



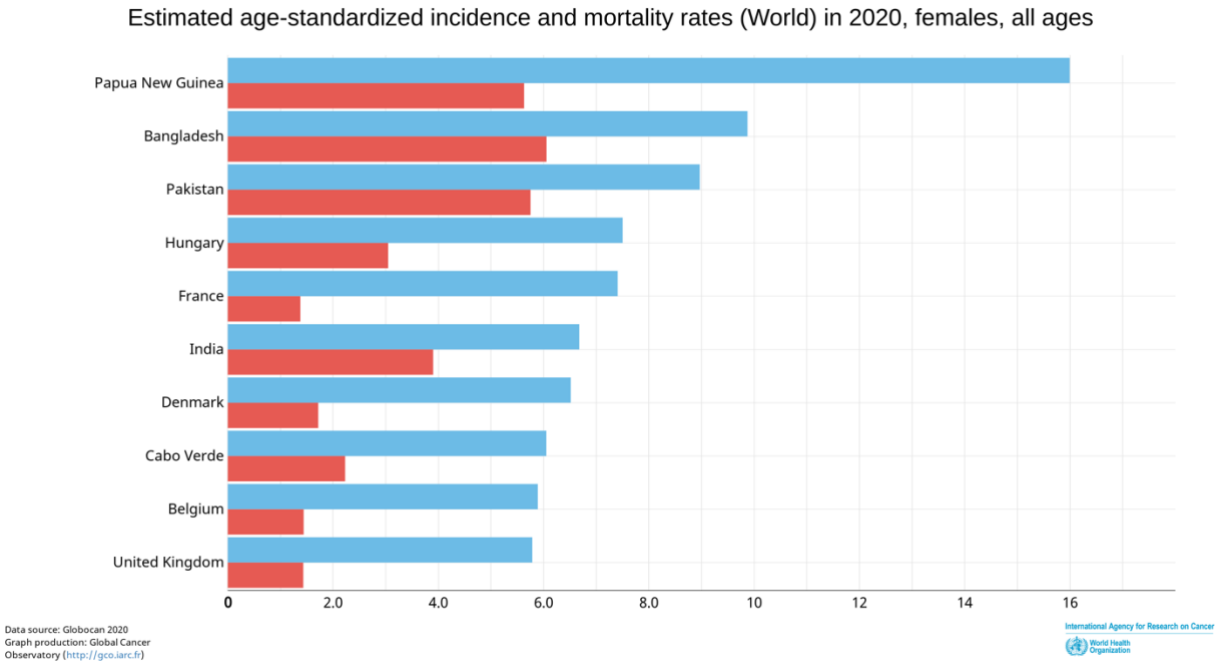


Figure 2.2 estimated age-standardized incidence and mortality for HNC. While incidence in developing countries is driven by smoking-related HNC, developed countries are mainly driven by HPV-related HNC. Data and graph are publicly available from the International Agency for Research on Cancer https://gco.iarc.fr/

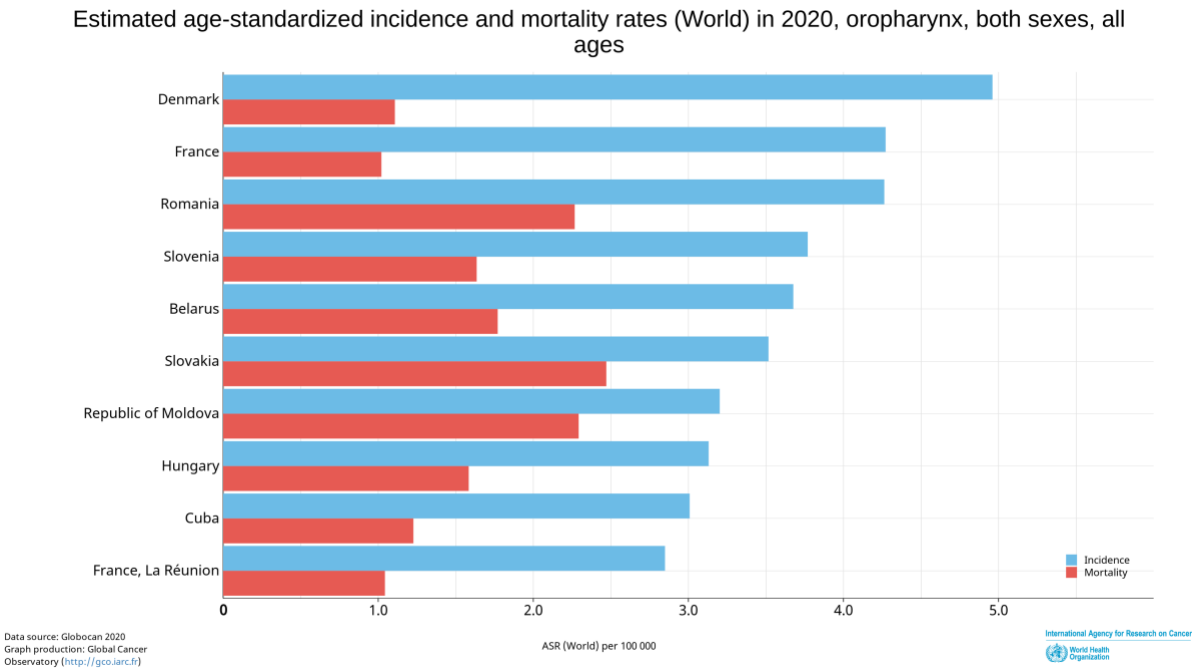


Figure 2.3 estimated age-standardized incidence and mortality Oropharynx only for both sexes. Oropharyngeal cancer is mainly affecting developed countries. Data and graphs are publicly available from the International Agency for Research on Cancer https://gco.iarc.fr/

2.3 The Epidemiology of HNC in Canada

One of every 89 Canadians is expected to develop cancer at some point in their lifetime (20). According to Canadian Cancer Statistics estimates for 2021 (21), HNC was the tenth most common cancer in Canada overall, sixth among males, and thirteenth among females. It is one of only four cancers with an increasing incidence trend and has a 65% five-year survival. The number of HNC cases projected for 2021 was five times that projected for cervical cancer (7,400 vs. 1,450). After many years of decline in HNC incidence, mainly due to anti-tobacco policies and regulations, there has been a recent surge of HNC in Canada (22,23). This is mainly caused by a subset of HNC in the oropharynx primarily driven by HPV infection. This recent increase in annual incidence change is more noticeable among men (1.5%) than women (0.8%) (24). One study from Ontario indicates that the overall annual percentage change for incidence of oropharyngeal

cancer can be as high as 4.6% (25). While the age at diagnosis for oral cavity cancer has been increasing yearly by 0.09 for women and 0.05 years for men, the age for diagnosis for oropharynx has decreased annually for both men and women (26).

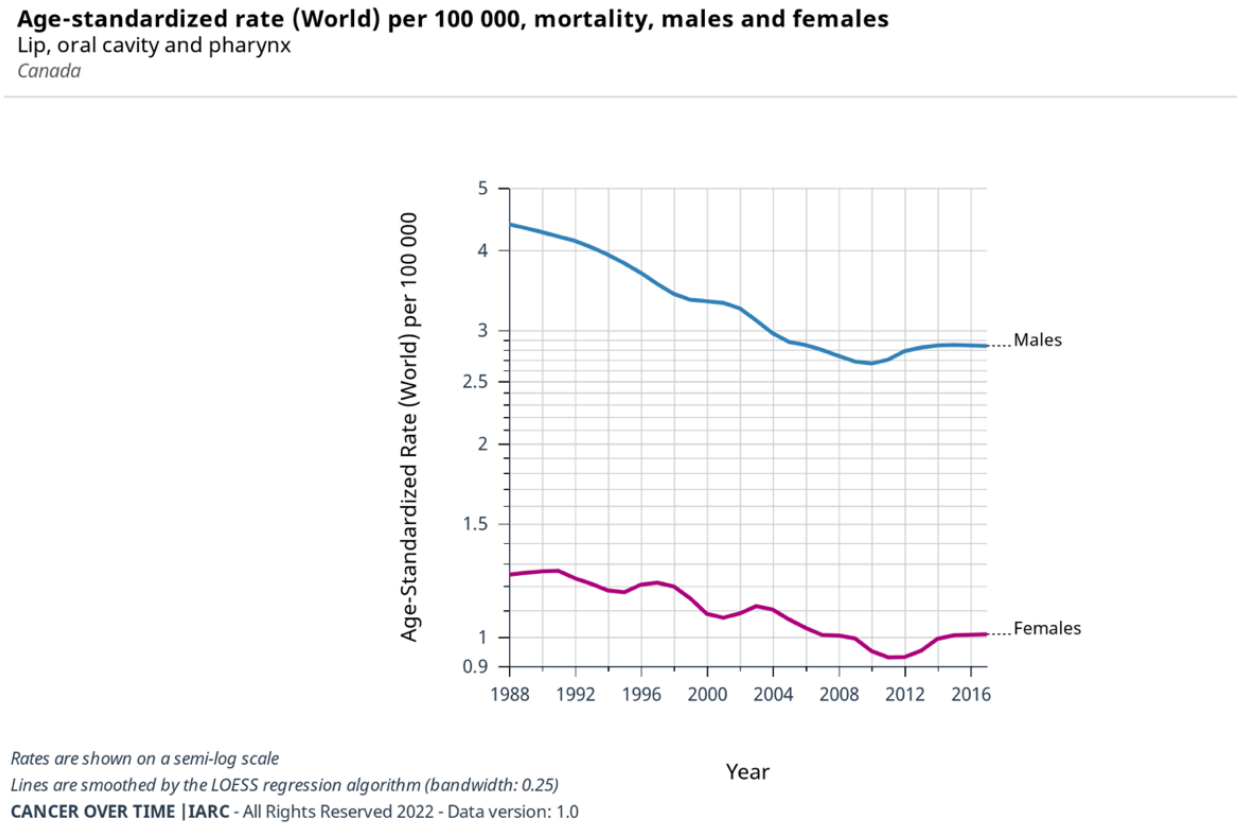


Figure 2.4 Trend of HNC Recent increase in cancer is driven by oropharyngeal cancer caused by HPV infection. Data and graph are publicly available from the International Agency for Research on Cancer https://gco.iarc.fr/overt

Canada and the United States have similar populations, disease distribution, and risk factors. Population-level incidence of HPV-related HNC in the US increased by 225% from 1988 to 2004 (6). Oropharyngeal cancer is now the fastest-rising incident cancer among young white men in the US (18), with around 70% of newly diagnosed linked to HPV (27). Further, in a recently

published article, Damgacioglu et al. (7) showed that the incidence of HPV-related HNC increased annually by 2.7% among men and 0.5% among women from 2001 to 2017, while mortality increased by 2.1% among men and decreased by 1.2% among women.

The estimated number of new cases of HNC in the United States in 2013 was 41,380, which represents 2.5% of all estimated new cancer cases (28). A report from the surveillance, epidemiology, and end results (SEER) program indicated that the 5-year survival (2010–2015) for HNC in the United States was 65.3% (29), which is slightly higher than that reported in Canada (64%) (30). The SEER report also estimates that 10,680 deaths, representing 1.8% of all cancer deaths, were projected for HNC in 2019. Comparing the age-standardized rate for HNC in Canada and the United States, 2020 GLOBOCAN estimates show that Canada has a lower incidence but slightly higher mortality than the US (Figure 2.4).

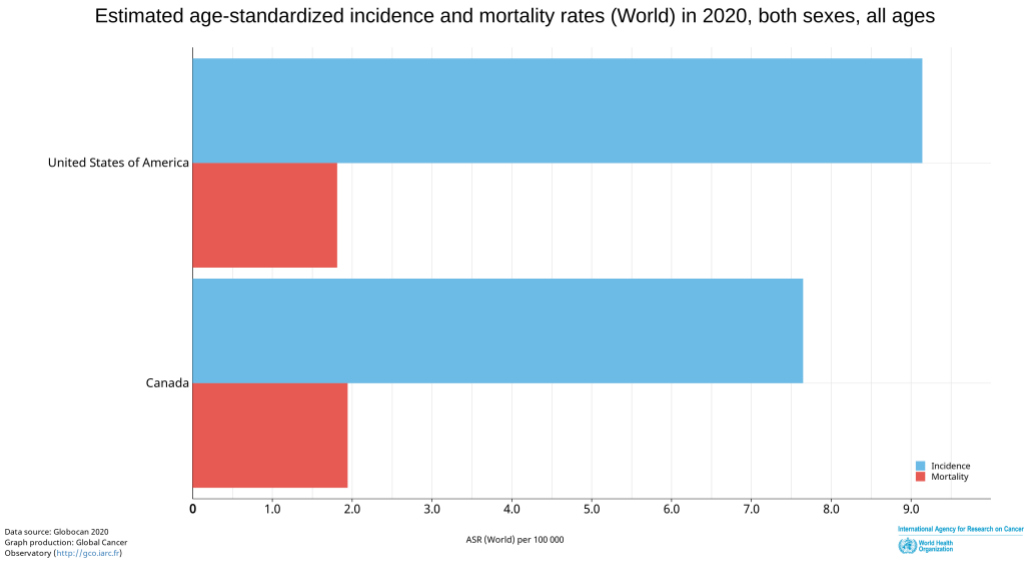


Figure 2.5 Comparing incidence and mortality between Canada and the United States. Data and graph are publicly available from the International Agency for Research on Cancer: https://gco.iarc.fr/overtime/en/dataviz/

2.4 Special Characteristics of Oropharyngeal Cancer

It is now recommended that HPV-positive HNC be evaluated separately from the rest cancers in the head and neck because 1) it has a unique strong association with the oropharynx, and 2) these cancers respond to chemotherapy and radiotherapy that de-intensification of treatment is now the standard of care (31), and 3) these patients are usually younger and healthier than those with tobacco-associated HNC (32). Indeed, patients with HPV-positive oropharyngeal cancers can have a five-year survival rate of up to 90% (31). For these reasons, recent changes in tumor classification and staging have been reflected in the eighth edition of the American Joint Committee on Cancer (AACC) Tumor, Node, and Metastasis (TNM) staging of head and neck tumors (33). The new system recognizes the difference in hazard of staging groups between the HPV-positive and HPV-negative tumors in terms of treatment intensity and prognosis. To make the new staging system applicable internationally, including in resource-scarce environments, HPV diagnosis was based on p16 expression. This is conducted using inexpensive immunohistochemistry that requires little technical expertise and can be obtained nearly everywhere. Based on the new classification system, there are now stages for lesions that are p16-positive and another system for those p-16 negative. Further details on implemented changes can be found in the 8th version of the AJCC Cancer Staging Manual (33,34).

2.5 Risk Factors and Predictors of HNC

HNC shares many risk factors with other cancer tumors. However, some factors are specific to this region of the human body. Below I highlight the main risk factors reported in the literature

and briefly explain the mechanism of action. Further information can be obtained from relevant references.

2.5.1 HPV Infection

HPV is a group of more than 200 types of viruses. HPV infection is primarily a sexually transmitted disease, and infection with certain types of HPV has been reported to cause some forms of cancer, including cancers of the penis, cervix, vulva, vagina, anus, and oropharynx (35). Previous work on the HeNCe project indicated that 40% of HNC and 60% of oropharyngeal cancers contained at least one genotype of HPV in oral samples (36). HPV genotype 16 (HPV16) is the type most often linked to cancer of the oropharynx, especially those in oral tonsils and the base of the tongue—section 2.7 details the classification, biology, and carcinogenic effects of HPV related to HNC.

2.5.2 Tobacco

Tobacco is the world's leading cause of preventable cancer. It has been studied extensively as a health determinant over the past few decades, and in Canada, almost 17% of annual deaths are due to tobacco-related diseases (37). It is now established that tobacco consumption, both through smoking and smokeless use, is a cause of many diseases, including cancer (38). Identifying tobacco as a health hazard took many decades of debate, and several attempts were halted by the tobacco industry's manipulation (39). It is now known that tobacco contains over 70 carcinogens that act on several pathways to cause cancer (40). The number of carcinogens varies by means of tobacco use as smoking contains many more carcinogens than smokeless use

(41). This is mainly because many carcinogens are formulated during burning (42). Some of these carcinogens are classified as strong, which means they have strong potency and a high risk of carcinogenic initiation, for example, polycyclic aromatic hydrocarbons (PAH), nitrosamines, and aromatic amines. Other carcinogens are less carcinogenic and are therefore described as "weak," such as acetaldehyde. In any case, the concentration and availability of these carcinogens vary greatly.

The effect of tobacco goes beyond direct exposure and extends to having systemic effects that cause cancer in organs other than those directly exposed to smoke. Carcinogens in tobacco require metabolic activation to react to the host's DNA. This reaction results in the formation of DNA adducts, which are believed to play a significant role in cancer induction. While there are innate mechanisms that work to repair damaged DNA and remove these adducts, smoking interferes with such natural pathways. Once these damages occur in specific locations of the human genome and are not repairable, the result would be the development of cancer (42,43). This is particularly the case if such DNA damage occurs in tumor suppressor genes such as TP53 or oncogenes such as RAS and MYC (42). Other unveiled mechanisms include gene promoter hypermethylation, decreased programmed cell death (apoptosis), and unregulated cell division. Further details can be found in references by the IARC (38) and others (42–44).

The relation between tobacco and HNC is well established and has been widely discussed in the literature (45–47). A meta and pooled analysis of several case-control studies of HNC showed that the relation between cigar smoking versus never smoking is strong OR =2.54 (95% CI 1.93,

3.34) (48). The association between smokeless use and HNC was slightly lower than that for smoking OR= 1.71 (95% CI 1.08, 2.70) (49). This relation seems to follow a non-linear dose-response pattern with increased frequency and duration leading to an increased risk of HNC (50,51). Madathil et al. investigated the relation between tobacco smoking latency and HNC (46) and found that exposure to smoking as far as 40 years before diagnosis increased the risk for HNC. Such strong relation was noticed among HPV-positive and HPV-negative individuals (46).

2.5.3 Alcohol

The causal relation between alcohol consumption and some types of cancer, such as oral, pharyngeal, laryngeal, and esophageal cancers, has been established in several observational and animal studies (52). It was previously thought that moderate alcohol drinking could have some health benefits, but recent studies have shown that there is no safe level of alcohol drinking (53). In addition to cancer, alcohol has been linked to liver disease (54) (stenosis, alcoholic hepatitis, fibrosis), cardiovascular disease (55) (stroke, hypertension, valve disease), and mental health problems (depression and suicidal thought) (56).

Alcohol contains ethanol and other additives that have been linked to cancer. Ethanol is oxidized in the liver to acetaldehyde and then to acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). In recent reports, IARC reviewed the evidence on the association between alcohol consumption and cancer and found that oral, lip, pharynx, larynx, and esophagus cancers are all strongly associated with daily alcohol drinking (57,58). There appears to be a monotonic relationship between alcohol and HNC, with higher consumption associated

with higher risk (59). This risk is even higher among populations and ethnicities with a deficiency of ADH or ALDH enzymes needed for the degradation of ethanol (60). Alcohol is an independent risk factor for cancer, but joint exposure to smoking and alcohol have a multiplicative effect on the risk of cancer development. Those jointly exposed to both are at a higher risk of developing oral cancer than those exposed to either factor independently (45). Further, previous results from the HeNCe project showed that those who are alcohol consumption with smoking and HPV infection elevated the risk of HNC substantially (61). Purdue et al. also showed that the effect on cancer seems comparable for beer and liquor, and all alcoholic beverages seem to increase the risk of HNC (62).

2.5.4 Age and Sex Differences

In all populations, HNC incidence is higher among men, with 4:1 to 10:1 odds. The median age of diagnosis is in the mid-sixties; for example, in Canada, the mediation age is 65 (20). The incidence rate, however, increases substantially among men older than 75 (29). Epidemiological studies have reported a higher incidence and mortality of HNCs among men than women, which holds for all age groups and geographic locations. This is mainly because men are more commonly exposed to risk factors such as smoking and alcohol (3,63). Even HPV-related cancer is more common among men, reflecting that oral HPV infection is more common among men who tend to report more sexual partners (64). It is worth noting that the relation between HPV16 and HNC follows a bimodal distribution, with the first peak taking place in the mid-20s and the other in the mid-50s (65). This relation with age does not seem to extend to oral infection with cutaneous HPV (66).

2.5.5 Socioeconomic Status

Socio-economic status (SES) is a construct that can be defined and measured in many several ways that is beyond the scope of this thesis (see Oakes and Andrade for further details (67)). People who are better socioeconomically have better health as SES is a strong risk factor of adverse health effects and has been studied extensively in the literature (68). Some authors argue that social factors are the primary health determinants that tend to define the particular elements people are exposed to in their environments (69). In a top-to-bottom approach, people living under unfavorable social conditions will be at a higher risk of smoking, improper housing, unhealthy diet, and other harmful exposures, making them more likely to develop cancer (70). In cancer studies, SES has been linked to a higher incidence of HNC and shorter survival after treatment (71). Again, the effect on incidence can be understood in that people with lower SES are usually exposed to unfavorable factors that can make them more likely to develop cancer. Further, lower SES affects the ability to receive diagnosis and treatment at the earliest stage possible, thus negatively affecting survival. Research using a life-course framework has demonstrated that SES advantages in childhood and early adulthood were associated with a lower risk of HNC (72). Blacks have a higher incidence of HNC and a 48% higher mortality of than whites (73). They also tend to have worse access to healthcare services and treatment (74), which explains worse outcomes among this minority group. For example, in a study by Ragin et al. that looked at the survival of HNC patients, non-Hispanic blacks in the United States had worse survival for laryngeal cancer but not for other subsites (74).

2.5.6 Diet and Nutrition

Diet quality has been linked to cancers, including HNC, although the evidence remains weak and inconsistent (75). There are many possible explanations for this association, one of which is that a healthy diet contains a good balance of nutrients needed for body growth and repair (76). Also, a good diet includes antioxidants such as carotenoids and vitamins C and E, which are necessary for breaking down toxins and for DNA synthesis (77). Research linking diet quality and HNC has shown that those eating large amounts of fresh vegetables and fruit are less likely to develop cancer, even after adjusting for other risk factors like HPV, smoking, and alcohol (77). On the contrary, the high intake of meat products, especially red meat and processed products, has been linked to an increased risk of HNC. In a recently published article by Saraiya et al. (78), the authors found a consistent relation between healthy diet scores and risk of HNC; the better the diet scores, the lower the risk of HNC. This relation did not seem to be site-specific or heterogeneous across modifiers like smoking, alcohol, or BMI. Nutritional and dietary factors vary considerably from one person to another and from time to time for the same individual. While these results are promising, there is a high chance of measurement error with nutritional exposures. The exact mechanism that links diet to HNC incidence is still largely unknown and should be a subject for future research.

2.5.7 Oral Health Indicators

Several studies in the literature have found an association between oral health indicators and HNC (79–81). Factors such as mouthwash use, gum bleeding, and the number of missing teeth increase the risk of HNC (79,82). Poor oral health status has also been associated with oral HPV infection (64,83). However, it is unclear if such a reported association exists because of

underlying biologic factors that trigger carcinogenesis and oral inflammation or because oral health directly affects oral cancer. Unfortunately, many studies in the literature suffer from limitations such as using a cross-sectional study design or unmeasured confounding that make establishing causality unattainable. Confounders such as SES can affect the number of missing teeth (exposure) and HNC (outcome), so missing such key factors increases the risk of bias. A recent study by Mazul et al. (84) used a population-based case-control study and found that poor oral health and low frequency of dental visits resulted in a higher risk of both HPV-positive and HPV-negative HNC.

2.5.8 Risk Factor Summary

As explained previously, tobacco and alcohol use remain HNC's most important risk factors, while HPV is the leading risk factor for oropharyngeal cancer, especially in developed countries. Relation between smoking and alcohol seems to follow a dose-response relation pattern, with no specific threshold for risk, and in which duration is just as important as frequency (51). HPV is now causing most cancers in the oropharynx, and the incidence of HPV-positive HNC now exceeds those of cervical cancer (21). Other etiologic factors reported but are less often discussed in the literature include betel quid, immunity, genetic predisposition and family history, and bacterial infection (51,85–87). Patients with HIV or those on immunosuppressant therapy are at higher risk of developing HNC cancers(86), and genetic mutations of p53, CYP, and GST have also been found among patients with rapidly progressing tumors (2). Several microbial risk factors have also been identified as causes of HNC, such as bacteria (*Fusobacterium nucleatum and Pseudomonas aeruginosa*) and other viruses (Epstein–Barr virus and Hepatitis C) (3). The role of

other exposures has been studied in the literature. Still, unlike smoking, alcohol, and HPV, the strength of association is weaker, and the relation may not translate into a direct causal effect.

2.6 Histopathology of HNC

Most HNCs are squamous cell carcinomas (90%), which means they arise from the epithelium in the oral mucosa. Histologically, they appear as islands or cords of transformed cells that have invaded the basement membrane layer. This is an essential distinction from premalignant lesions, like leukoplakia or carcinoma in situ, in which dysplastic cells have not invaded the basal layer of the mucosa. The invading cells might spread into nearby tissues, including nerves and blood vessels. Tissues under the microscope show eosinophilic cytoplasm, and well-differentiated lesions show keratin pearls. However, lesions that are caused by HPV are usually poorly differentiated, meaning they do not resemble the original tissue (epithelium) they originated from (3,88).

Further histologic characteristics can be found in relevant resources (3,89). HPV-positive oropharyngeal carcinomas are often poorly differentiated, non-keratinizing, with basaloid cytologic features. But although most of these lesions have such unfavorable characteristics and are diagnosed in late stages, they still have a better prognosis than HPV-negative lesions (3).

2.7 Human Papillomaviruses Biology

HPV is classified within a family of viruses known as papillomaviridae that can infect humans and animals. Only some infect humans and are thus called *human* papillomaviruses. HPV is responsible for more than 90% of anal and cervical cancers, 70% of vaginal and vulvar cancers, and more than 60% of penile cancers (27). These viruses are small, non-enveloped, icosahedral with DNA double strands and range from 5700 bp to almost 8600 bp (90,91). HPVs are organized into five major genera: alpha (α), beta (β), gamma (γ), mu (μ), and nu (ν). An up-to-date list of these genotypes and the respective classification date and relevant publications can be found in the international HPV reference center hosted at Karolinska Institutet in Stockholm, Sweden (https://www.hpvcenter.se/human_reference_clones/).

Of the five genera infecting humans, three genera, alpha (α), beta (β), and gamma (γ), have been detected in the oral cavity with oral samples (66). There are more genotypes in the β- genus than the other two in the oral cavity, but alpha has been more likely to be 'looked for' in the oral cavity (92). Based on accumulating evidence linking oral HPV infection to lesions in the oropharynx, the International Association for Research on Cancer (IARC) has classified a few genotypes as high-risk. Alpha papillomaviruses HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 that are labelled as "carcinogenic", while genotypes HPV26, 53, 66, 67, 68, 70, 73, and 82 are labelled as "probably carcinogenic."(93)

Attempts to study the relationship between HPV and cancer started in the 1960s after reports of malignant transformation of genital warts, and the first lab experiments began in 1972 (35). After

establishing HPV as a causal factor in cervical cancer, the role of HPV infection in oral papillomas and its relation to HNC's transformation was first proposed by Löning et al. (94), who discovered the presence of viral DNA in HNC tissues. The first epidemiologic evidence linking HPV to oropharyngeal cancer came from a hospital-based case-control study by D'Souza et al. (95). They have shown that participants with a high lifetime number of sexual partners had a higher risk of HPV infection, which increased cancer risk in the oropharynx. D'Souza et al. reported that oral HPV16 infection increases cancer risk by 14-fold (OR= 14.6; 95% CI, 6.3- 36.6) (95). Since then, several studies have found similar results in different populations across the globe. Laprise et al. (96) analyzed data from the HeNCe project and found that the prevalence of α-HPV was almost three times in cases than in controls (41% vs. 14 %). This makes those with HPV16 have 18 times the risk of HNC among those infected than those without infection (OR= 18.1; 95% CI, 9.1–35.8). Farsi et al. (36,61) found that those who tested positive for HPV alpha high-risk group and were smokers and drinkers had 50 times the odds of HNC compared to those with no HPV, smoking, or drinking (61). Such results indicate a likely interaction between HPV, smoking, and alcohol, making those double or triple exposed at a higher risk.

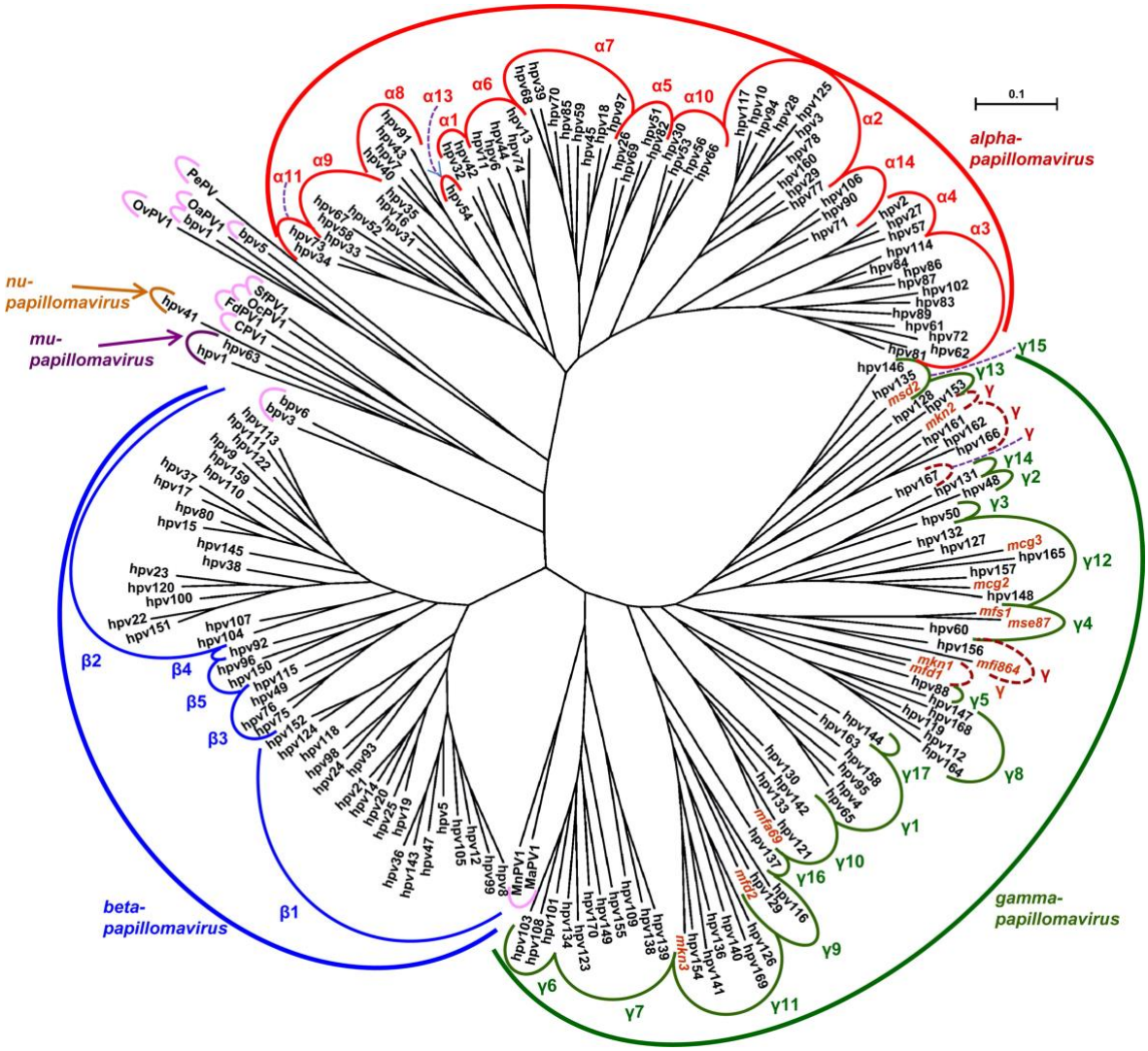


Figure 2.6. Phylogenetic tree based on the L1 ORF sequences of 170 HPV types, single animal papillomaviruses and newly identified human papillomaviruses using metagenomic sequencing. Reproduced with permission from (91) de Villiers, E.-M. (2013). Cross-roads in the classification of papillomaviruses. Virology, 445(1–2), 2–10, Available from https://doi.org/10.1016/j.virol.2013.04.023

2.8 Methods for HPV Detection

Overall, there are three general methodologies for detecting HPV among suspected cases: PCR-based techniques, In Situ Hybridization (ISH), and P16 Immunohistochemistry. PCR-based techniques have improved in recent decades and are now capable of detecting several genotypes using techniques such as bead-based multiplex genotyping (97). Recent developments also have

improved the sensitivity and specificity of PCR assays by combining multiplex PCR with DNA microarray primer extension that has a high capability to detect mucosal (α-HPV) (98) and cutaneous HPV (β-HPV) (99). However, a major limitation of PCR methods is that they depend on detecting viral DNA and do not distinguish between active and latent infections (100). In Situ Hybridization (ISH) uses type-specific probes to detect E6/E7 regions of the HPV messenger DNA. This means it can detect active infections. However, this technique is insensitive, expensive, non-automatic, and technically demanding. The most commonly used technique is p16 immunohistochemistry. In HNC treatment planning, HPV diagnosis is based on detecting a biomarker known as p16 rather than HPV genotyping. This marker is not perfect in 10% percent of the cases and could yield a positive result among patients with non-oncogenic variants of the virus (101). Knowing the HPV profile of patients would therefore be valuable for clinicians to understand the actual status of patients. Detecting multiple HPV infection, that is being infected with more than a single genotype, can only be done with PCR-essay or next-generation sequencing, which is time-consuming and costly.

2.9 Molecular Signature of HPV-Positive HNC

HPV-positive HNC is characterized by molecular characteristics that are distinct from that of HPV-negative tumors. This shows that the underpinning of carcinogenesis involves more than just HPV infection. Mirghani et al. (8) conducted a whole genome sequencing for HNC tumors and found a signature comprised of 224 genes that differ between HPV-positive and HPV-negative tumors. When they used a prediction model with information on these genes, they could correctly classify HPV infection in 93% of tumors. Lawrence et al. and the Cancer Genome

Atlas Network showed in their analysis of genomic profiles of 279 tumor lesions that those considered to be HPV-associated had unique mutations in *PIK3CA* oncogene as well as novel alterations that lead to loss of function in TRAF3 and amplification of the cell cycle gene *E2F1* (102). On the other hand, tumors caused by smoking that were also HPV-negative had a loss of function in *TP53,* the gene most frequently mutated in human cancer (103), and inactivation of *CDKN2A,* a gene that codes for several tumor suppressor proteins.

2.10 Course of HPV Infection

HPV infection is sexually transmitted; people with more sexual partners and those practicing oral sex are at higher risk for oral HPV infection (104,105). Following infection with HPV, the virus has three trajectories: 1) it gets cleared by the immune system of the host; 2) it becomes inactive latent infection; 3) the immune system fails to clear the infection, which remains active and becomes persistent. The infection can change status at any point in time with changes to the local environment (e.g., reduced immunity due to another systemic disease). Only persistent and active infections are capable of carcinogenesis. Figure 2.5 shows the three conditions for HPV infection.

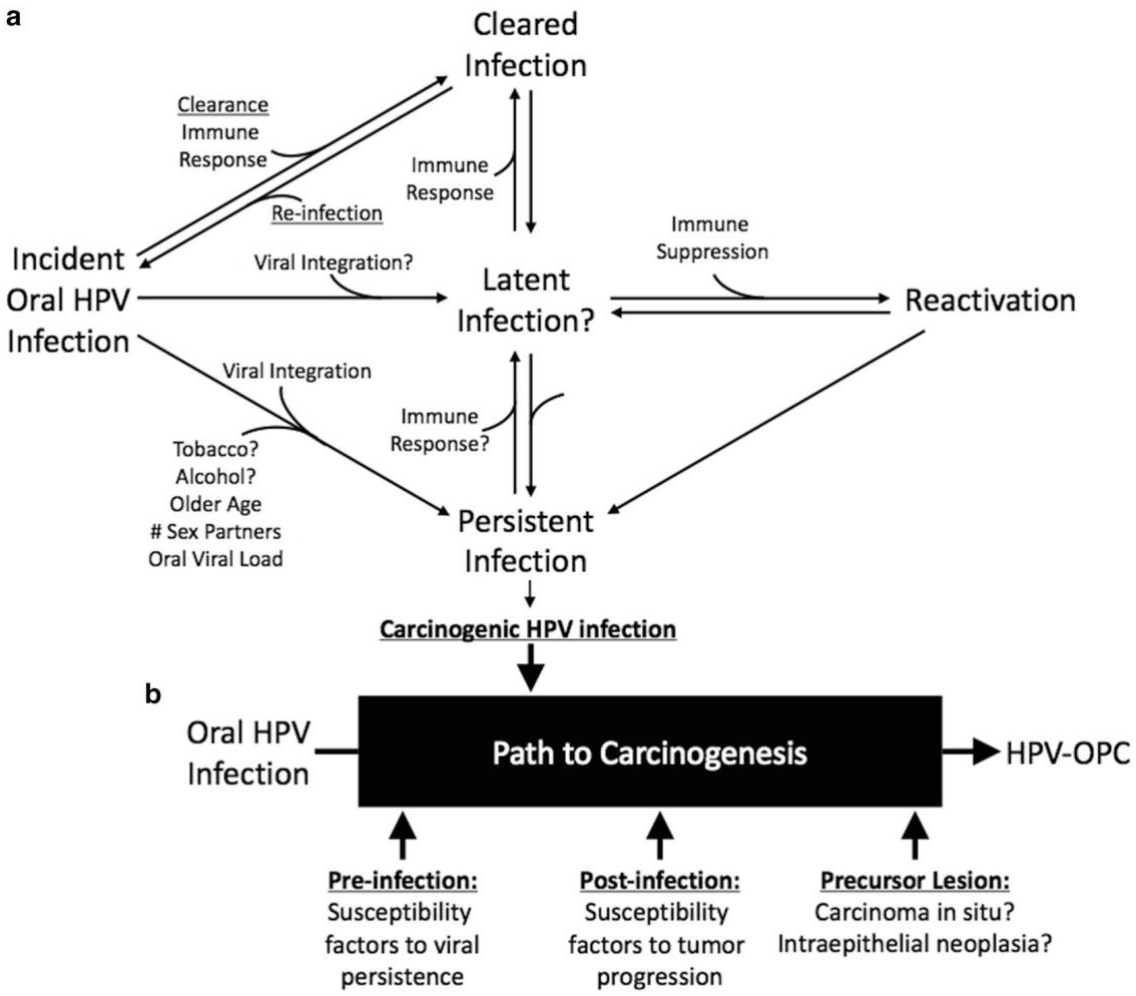


Figure 2.7 showing the possible trajectories for HPV infection: a) following infection with HPV the virus can get cleared, become latent, or persist as an active infection. Reproduced with permission from Faraji, F., & Fakhry, C. (2018). Human Papillomavirus and HNC. In M. L. Durand & D. G. Deschler (Eds.), Infections of the Ears, Nose, Throat, and Sinuses (pp. 349–364). Springer International Publishing. Available from https://doi.org/10.1007/978-3-319-74835-1_28

2.11 Biology and Detection of HPV

HPV genome has three major regions (Figure 2.6): the long control region (LCR), the early region (E6, E7, E5, etc.), and the late region (L1, L2). The LCR region is responsible for upstream regulation and is involved in DNA replication and transcription. The early region plays a role in virus replication, transcription, and even carcinogenic activity of the virus (92). It is responsible

for cell cycle deregulation, apoptosis inhibition, and cell polarity and attachment. The late region is responsible for encoding the capsid protein. To be classified as a novel genotype, L1 must share less than 90% of its sequence with other genotypes (90). Further articles on the biology of these viruses, protein structure, and classification can be found in references by Gheit (2019) (92) as well as in papers by the pioneers in HPV research zur Hauzen (2009) (35), and de Villiers (2013) (91).

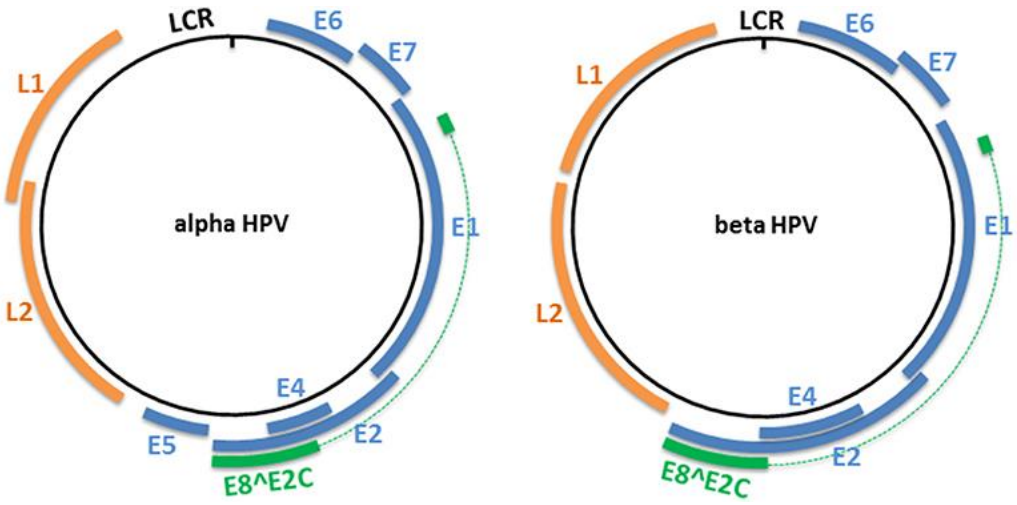


Figure 2.8 Genome organization of alpha and beta HPV. Obtained from Gheit, T. (2019). Mucosal and Cutaneous Human Papillomavirus Infections and Cancer Biology. Frontiers in Oncology, 9, 355. https://doi.org/10.3389/fonc.2019.00355

2.12 Multiple HPV Infection

Concurrent or multiple infection with more than one HPV type is found in 20% to 50% of cervical HPV-positive individuals (106). Some studies have reported that the tendency of multiple HPV types to cluster does not influence the course of HPV clearance (107). In contrast, others reported that having multiple HPV is a strong predictor of infection persistence (108,109). Rousseau et al.

found that multiple infection depended on age and cytologic abnormalities in cervical tissues (108). There is now consensus that women with multi cervical HPV are at a higher risk of developing cervical cancer. While the association between coinfection and cervical cancer has been widely described in the literature, only a handful of studies have investigated coinfection and HNC. However, all previous studies investigated the presence of several types from the α-HPV genus, and only one published study investigated multiple HPV infection across genera (110).

2.13 Role of β- and γ HPV in HNC

The role of cutaneous HPV (β and γ HPV genotypes) is still poorly understood, and we just started seeing publications on their possible role in HNCs. Unlike α-HPV, which have been isolated from mucosal tissues of the cervix and oral mucosa, historically, β and γ-HPV genera have been isolated from skin lesions (111,112). Recent studies have demonstrated a high prevalence of β- and γ-HPV in the oral cavity (110) and esophagus (113), showing a potential role in carcinogenesis. Hampras et al. (114) found that 36% of oral samples contained at least one genotype of β-HPV. This emerging evidence of increased HPV-related risk suggests a broader effect of HPV than initially thought, which may not be limited to one HPV genus or a specific location in the head and neck region. Indeed, Koppikar et al. (115) investigated the presence of HPV genotypes in 83 oral cancers and 19 lesions in other areas in the head and neck and found that β-HPV was indeed detectable in tumor lesions. However, whether these were in the oral cavity or the oropharynx is unclear. Lang Kuhs and colleagues analyzed oral samples of 500 women involved in an HPV16/18 vaccine trial in Costa Rica for all genera of HPV, both mucosal and cutaneous (116). They found the prevalence of β-HPV to be around 19%. However, it is unclear whether β or γ-

HPV can cause cancer on their own or whether a co-infection with α- HPV is needed for tumor development.

In a study by Paolini et al. (117), researchers collected oral samples (rinse and brush samples) from healthy individuals, patients with premalignant lesions, and HNC patients. They found the highest prevalence of cutaneous HPV to be among people with premalignant lesions (55%), followed by healthy individuals (42%), then by cancer cases (21%). However, cancer cases were more likely to be infected with a carcinogenic genotype from the α genus, especially HPV16. The first epidemiologic study to assess the relation between cutaneous HPV and HNC was conducted by Agalliu et al. (110), in which the authors reported an elevated risk of cancer among those who tested positive for β-HPV (OR= 1.74) and among those with any γ-HPV infection (OR=2.11). However, residual confounding by immunosuppression and tobacco smoking could explain these findings (118). Sabol et al. (119) also found an association between two genotypes of the β genus (HPV5 and HPV122) with HNC, but they did not report confidence intervals for their estimates. And Because their study had a small sample size, it probably suffered from imprecision.

2.14 Gaps in Knowledge and Rationale

While traditional risk factors of HPV such as smoking and alcohol consumption have been studied extensively in the literature, there are still many lingering questions about HPV genotypes and genera other than α-HPV. Some reports have reported the presence of β- and γ HPV genotypes in patients with HNC (115,120), while others found that only genotypes from α-genus are associated with cancer (117). Such conflicting reports open the door for speculation about the

role of cutaneous HPV in HNC. Are these genotypes potential suspects or simply bystanders in cancer development?

The prevalence and distribution of β- and γ-HPV types in HNC remain primarily unknown, with no data in Canada and only a small number of studies globally. Further, while some recent reports suggest mucosal and cutaneous HPV may not depend on each other (116), the interaction between these genera has not been investigated. Given the scarcity of information on the role of β- and γ HPV and the limitations of previous work, I intend to unveil the nature of their role in HNC development in this thesis. Specifically, I address three aims: 1) investigate the prevalence and distribution of all three genera of HPV in oral cell samples and tumor lesions, including variation in distribution by sociodemographic and oral health indicators. 2) Estimate the effect of cutaneous HPV on HNC while controlling for HPV16 infection, smoking, and other potential confounders. I will also be addressing potential methods limitations in previous studies using quantitative bias analysis to estimate the corrected effect of HPV on HNC. 3) To better understand the interplay between HPV genera, I investigate the interaction between HPV16 and high-risk genotypes from the α-genus with cutaneous HPV both on the additive and multiplicative scales. Chapters 4, 5, and 6 address each of these corresponding questions.

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CHAPTER 3: METHODS

3.1 Data Source and Overall Study Design

This dissertation uses data from the Head and Neck Cancer Life Study (HeNCe), an international multi-country hospital-based case-control study aiming to identify social and biological risk factors for head and neck cancer (HNC). HeNCe was conducted in three different countries (Canada, India, and Brazil) to identify etiologic factors of HNC that might vary by the geographic or social environment. As this project focuses on the relation between HPV and cancer, I only use data from the Canadian site. Samples in India tested all negative for HPV (1), and HPV was not measured in Brazil for technical and logistic reasons.

Case-control studies are often understood as a more efficient design to study a rare outcome that could be difficult to study using a cohort for either limited resources or time constraints (2). Older views on case-control studies promoted the idea of "cohorts in reverse" (3), a view that has been described as "trohoc fallacy" (4). More recently, some epidemiology textbooks now propose to think of case-control studies as sampled from cohorts, real or imaginary, in which we select all cases and then use some sampling strategy to obtain controls (2). Therefore, depending on the method used to sample controls, the calculated case-control odds ratio (*the estimator*), estimates the cohort's causal risk ratio, incidence rate ratio, or odds ratio (*the estimand* of interest) (5,6).

3.2 Defining the Source Population

HeNCe is a hospital-based case-control study. In this design, data should be thought of as coming from a dynamic population rather than one in which the cohort membership is fixed (7). Patients are assumed to be adults (men and women) residing in the Montreal metro area who would receive their tertiary care at the four referral hospitals in Montreal. Control subjects are sampled from the underlying dynamic population and represent the catchment population from which cases arise (7,8). To be included in the study, participants needed to: (a) be born in Canada; (b) speak either English or French (c) be at least 18 years old; (d) have no history of cancer, immunosuppressive condition or mental disorder; and (e) live within 50 km of the hospital. A total of 460 cases and 458 controls were recruited, representing participation rates of 47% and 54% for cases and controls, respectively (9).

3.3 Identification of Cases

Between September 2005 and November 2013, cases were recruited from the four major referral hospitals: Jewish General Hospital (n=89), Hôpital Notre-Dame (n=347), Montreal General Hospital (n=7), and Royal Victoria Hospital (n=17). Research assistants were hired in the four hospitals to collect data for the study. Cases were identified in tumor board meetings of each participant hospital based on the ICD-10 codes used to diagnose and refer patients. The following international classification of diseases codes (ICD-10) were used (i) oral cavity as ICD codes C02-C06 except for C02.4, C05.01, C05.2 (tongue, gum, floor of the mouth, palate and other unspecified parts of the mouth); (ii) oropharynx as ICD-10 codes C01, C02.4, C05.01, C05.2, C09, C10, C12 and C14 (base of the tongue, soft palate, tonsil, oropharynx, uvula); (iii) larynx and

hypopharynx as ICD-10 codes C13 and C32. It is worth noting that cancers of the nasopharynx and salivary glands were excluded because they have different etiology. The distribution of cancer cases in HeNCe is presented in Table 3.1. All cases were incident cases, meaning they were first cancer identified in patients. Also, the interview and sample selection took place within two weeks of cancer diagnosis and before treatment started. This ensures that treatment that might include radiotherapy or chemotherapy does not alter patients' biomarkers or biological samples needed for the study.

Table 3.1 Distribution of site and stage at diagnosis of cases in HeNCe

	Frequency	Percent
Cancer site		
Oropharynx	219	47.6%
Larynx	150	32.6%
Oral Cavity	91	19.8%
Cancer Stage*		
Stage 0	17	3.7%
Stage 1	86	18.7%
Stage 2	71	15.4%
Stage 3	62	13.5%
Stage 4	219	47.6%
Missing	5	1.1%

**Classification is based on the 7th version of The American Joint Committee on Cancer (AJCC)*

3.4 Identification and Selection of Controls

Controls in this study were selected to represent the *catchment area* or the secondary base from which the cases arise (2,8). Controls were selected from outpatient clinics in three of the four hospitals (Jewish General Hospital MUHC, Hôpital Notre-Dame CHUM, and Montreal General Hospital). Controls were selected from a list of non-chronic disease clinics unrelated to tobacco or alcohol consumption, the main risk factors for HNC. For example, we did not select patients

from clinics treating Chronic Obstructive Pulmonary Disease (CPOD) because of their association with smoking. Controls were selected from the following clinics: neurology (n=40, 8.7%), ENT (n=24, 5.2%), Endocrinology (n=3, 1.3%), Rheumatology (n=8, 1.7%), Orthopedics (n=24, 5.2%), Gastroenterology (n=53, 11.5%), Urology (n=18, 4%), Stomatology (n=80, 17,4%), Ophthalmology (n=104, 22.8%), Dentistry (n=2, 0.4%), Family medicine (n=46, 10.0%), nephrology (n=48, 9.9%), other or unknown (n=13, 1.9%). No single clinic contributed more than 30% of controls. Controls were frequency-matched to cases on sex and age (five-year categories). Figure 3.1 shows the distribution of control and cases across all hospitals.

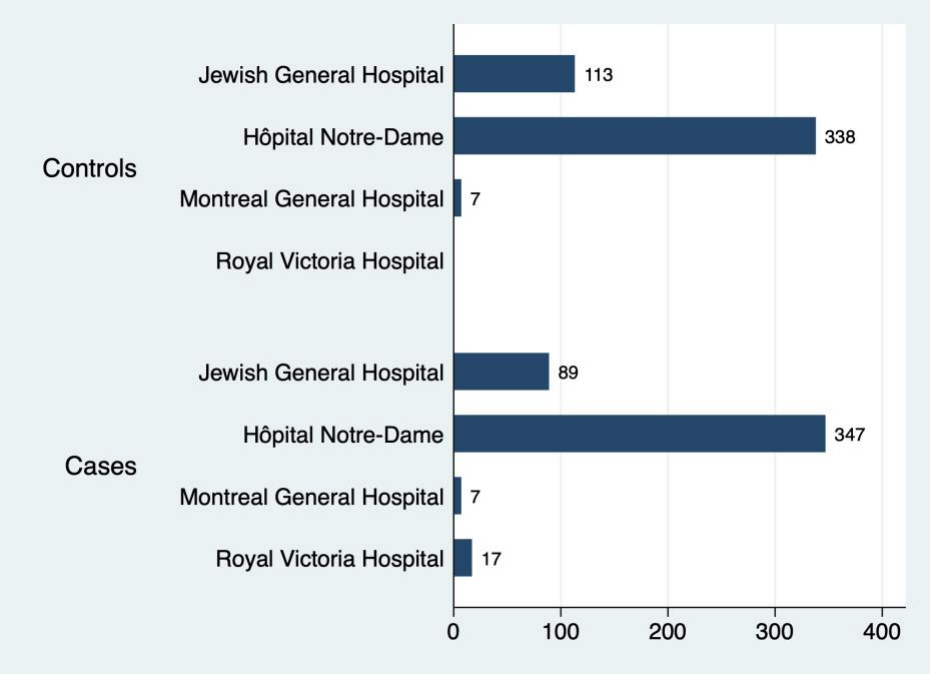

Figure 3.1 Distribution of cases and controls in HeNCe Canada

3.5 Ethics Approval

HeNCe was approved by the institutional review boards (IRB) of McGill University, Institut National de la Recherche Scientifique (INRS), and boards of participating hospitals from which cases and controls were selected. The purpose of the study and information on individual data for medical research was explained to the participants. All cancer patients and controls were given a consent form to sign. All data were later anonymized, and patients were given non-revealing identification numbers that concealed their identity.

3.6 Data Collection: Questionnaire and Medical Information

Information from participants was obtained in personal interviews rather than relying on self-report. This has been reported in the literature to reduce recall bias (10). Trained reviewers in HeNCe interviewed both cases and controls for approximately 2 hours employing a questionnaire and a life grid tool to aid in cross-referencing dates of personal and historical information (11). Interviews were structured, one-to-one, face-to-face, and conducted in either English or French. Questions covered 12 domains: general information, education, occupation and employment, housing and residential environment, smoking and chewing habits, drinking habits, dietary habits, oral health, family history of cancer, family environment in childhood, marriage and sex life, and social support. The questions in the interview were based on and derived from questions used in IARC studies on head and neck cancer as well as British cohort studies such as British Civil Servants, Whitehall II, and British Birth Cohort (BBC) 1946 (12–15). Information on cancer site,

stage, and clinical condition were obtained from patients' medical charts. The medical information on controls was used to confirm the stated reason for the visit in the interview.

Information on tobacco and alcohol consumption included current use as well as the history of use across the life course. For example, participants were asked whether they have ever smoked (yes vs. no), then if they gave a positive response, they were asked more detailed questions about their smoking habits. Age at smoking initiation and cessation, type of cigarettes (filter, non-filter, or hand-rolled), number of cigarettes, cigars, pipe smoked per day, the brand most frequently used (e.g., Camel, Marlboro, etc.), and frequency of smoking at each age period (average cigarettes per day). A similar approach was used for alcohol consumption, where individuals were also asked about the type and frequency of their consumed alcohol-containing beverage at each period. This approach is helpful in understanding not only the individual habit's frequency but also in estimating the cumulative lifetime exposure to such habits.

3.7 Data Collection: Biological Samples

Samples were collected in three forms: brushed exfoliated cells, mouthwash cells, and tissue samples. Brush and mouthwash samples were collected from both cases and controls. Participants were asked to take off any removable prostheses before sample collection. For mouth rinse, participants were asked to rinse their mouth for 15-30 seconds with an alcohol-based mouthwash solution and to spit the solution into a pre-labeled collection vial. For brush samples, participants were given an OralCDx brush (22) and asked to apply 20-30 gentle strokes in different mouth regions. However, cancer patients were instructed to apply strokes from and

around the tumor lesion, unless painful, while trying to avoid necrotic areas. After sample collection, the brushes were inserted into a PreservCyt® buffer bottle and transported to the microbiology and immunology laboratory at Notre-Dame Hospital, CHUM. Cell suspensions were centrifuged at 13,000 x g for 15 min at 22°C. Supernate was discarded, cell pellets were resuspended in 300 µL of 20 mmol/L Tris buffer (pH 8.3), and DNA was purified using the MasterPure™ Kit (Epicentre) (23). Extracted DNA was kept frozen until tested with PCR at -70°C. Paraffin-embedded biopsies were minced with a disposable scalpel blade, and slices were stored in xylene for one day and extracted with MasterPure™ (Epicentre, Madison, WI).

3.8 Sample Genotyping

More than 200 commercial HPV assays are currently in use for detecting HPV. Tests used for screening purposes mainly focus on maximizing clinical sensitivity. The use of partial HPV typing ensures high sensitivity for high-risk HPV (HPV16, HPV18, etc.). Tests with extended HPV typing require high analytical sensitivity to detect the largest number of HPV genotypes in any given sample. This is particularly needed in HPV vaccine research and for studies on HPV prevalence (24).

HPV testing depends on the detection and analysis of viral DNA. For this thesis, since we aimed to assess the prevalence of mucosal and cutaneous HPV in samples, our goal was to detect the largest number of genotypes in any given sample. We used several techniques for different samples: for biopsy samples, we used GP5/GP6 arrays with sequencing (25). For oral samples, we

used Linear Array to detect genital types and a special in-house array with Luminex to detect cutaneous types (26–28). Further details are given below.

3.8.1 Genotyping of Genital HPV (α) in Oral Samples

For genital genotypes (α-HPV), we used Linear Array genotyping assay from Roche Molecular Systems with PGMY09-PGMY11 primers (29). This technology uses consensus PCR that targets conserved regions of the HPV genome in which HPV amplicons generated from these primers can be detected by a non-isotopic reverse hybridization assay. Linear Array Samples were tested with β-globin primers GH20 and PC04 to ensure the quality of samples for genotyping. Linear array with PGMY primers is capable of identifying 37 genital HPV genotypes (29): HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82 (two variants), 83, 84, 89.

3.8.2 Genotyping of Cutaneous HPV (β and γ) in Oral Samples

We used HPV type-specific E7 PRC bead-based multiplex genotyping assay (TS-MPG) that was developed by the International Agency for Research on Cancer (IARC), Lyon, France, which combines multiplex OCR with bead-based Luminex technology (27). Technical details on the development of these methods and their ability to detect cutaneous HPV can be found in relevant publications (26). This technique is able to detect 43 β-HPV genotypes, namely, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93, 96, 98, 99, 104, 105, 107, 100, 110, 111, 113, 115, 118, 120, 122, 124, 143, 145, 150, 151 as well as 30 γ-HPV types, namely

4, 65, 95, 60, 48, 50, 88, 101, 103, 108, 109, 112, 116, 119, 121, 123, 126, 127, 128, 129, 130, 131, 132, 133, 134, 148, 149, 156, SD2.

3.9 Statistical Methods

Statistical methods for each manuscript are mentioned in each corresponding chapter. Manuscript I (chapter 4) is a descriptive paper that is among a handful of studies globally and the first in Canada to investigate the presence and distribution of cutaneous HPV in oral and tumor samples. I examine the distribution of HPV genera across several social, oral, and behavioral risk factors and calculate unadjusted case-control odds ratios.

In manuscript II (chapter 5), I estimate the effect of HPV on HNC. Potential confounders were identified using subject matter knowledge with the aid of Directed Acyclic Graphs (DAGs). To estimate the effects of the exposure on the outcome, I use unconditional logistic regression models. I also investigate the impact of epidemiologic biases on the relation between HPV16 and HNC.

In manuscript III, the goal is to measure interaction on the additive and multiplicative scales between alpha-HPV and infection with either beta or gamma HPV. Therefore, using logistic models with interaction terms between exposures, we obtain measures of multiplicative interaction and additive interaction as explained below.

3.10 The Logistic Model

Because this thesis uses data from a case-control design, in manuscripts II and II, the estimator of interest is the logistic model shown in the equation below. It is worth noting that because HeNCe used frequency matching, I use an unconditional rather than a conditional logistic model. Data that are frequency-matched on a few variables such as sex and age categories are "loose enough" that the unconditional model is considered the appropriate method (30,31). However, matching variables were also included in the models as they were also important confounders.

$$Pr(Case \mid X, C) = \frac{e^{(\beta_0 + \beta_1 X + \beta' C)}}{1 + e^{(\beta_0 + \beta_1 X + \beta' C)}}$$

Where X= exposure of interest (HPV), and C is a vector of covariates that constitute matching variables and the minimum sufficient set of confounders.

3.11 Directed Acyclic Graphs and Confounders Selection

I use logistic models that adjust for confounding in manuscripts II & III (Chapters 5 & 6). All confounders have been identified using substantive knowledge with the aid of DAGs. Since their first introduction to epidemiology by Greenland et al. (32), the use of DAGs has become standard in epidemiologic studies and are now taught in modern epidemiology textbooks (33,34). Recent papers have also introduced the use of DAGs to the oral health literature (35,36). DAGs are non-parametric methods, meaning they do not depend on knowledge of distribution parameters. The only require substantive knowledge usually obtained from researchers' expertise on the assumed

relation between variables as they would occur in nature. For example, in a study of the relation between HPV and HNC, we assume that HPV causes HNC (HPV → HNC), not the other way around. We, therefore, make the arrowhead point to HNC. We map out any other variable(s) related to the outcome or the exposure and could at least partially explain the observed results. For example, we assume that smoking affects both the exposure (HPV) and the outcome (HNC), thus acting as a confounder rather than a mediator.

In designing a DAG for this study, we used substantive knowledge and three general characteristics to identify confounders: 1) a confounder must be associated with the exposure in the source population; 2) a confounder must be a cause of the outcome either directly (being a direct cause), or indirectly (being a parent of a cause); 3) a confounder cannot be affected by the exposure of the outcome (not a mediator or a collider). Following identifying confounders, we choose the minimum sufficient set that closes the backdoor path from exposure to outcome (figure 3.2). By adjusting for confounding, we achieve conditional d-separation, meaning that exposure (X) is independent of outcome (Y) conditional on confounder (C). A more detailed DAG is given in manuscript II (Chapter 5).

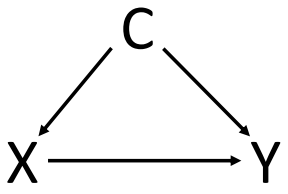

Figure 3.2 Exposure of interest X affect outcome Y. C is a confounder affecting both exposure A and outcome Y. Path X ← C → Y is a biasing backdoor path.

3.12 On modeling smoking and alcohol

Modeling smoking and alcohol use can be challenging. It is important to avoid assuming a linear relation between these covariates and the outcome and to avoid dichotomizing these variables, which can result in loss of power and residual confounding (16). We explored three techniques for modeling smoking and alcohol: a) using an indicator term and a centralized variable; b) using restricted cubic splines; c) using fractional polynomials. The first approach follows the recommendation by Leffondré et al. (17), which accounts for qualitative vs. quantitative differences between users and non-users. It is based on including a centered continuous variable for smoking pack-years by deducing the mean pack-years from each ever-smoker while keeping the never-smokers as zero. An indicator for ever vs. never smoker is added to the model. Using restricted cubic splines has been recommended by Harrel (18), while fractional polynomials have been recommended by Royston et al. and others (19–21). These two methods allow for flexible modeling of the relation between smoking and outcome. The final form for smoking and alcohol was decided based on the best model fit using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC).

3.13 Quantitative Bias Analysis

In manuscript II, I use quantitative bias analysis (QBA) to test the robustness of the estimated effect of HPV16 and HNC. QBA methods have been recently introduced to epidemiology. They have gained popularity since the publication of the book by Lash and colleagues (37), though the idea of quantifying bias dates back to at least the 1950s (38,39). These methods aim to quantify and minimize the effect of the three main biases in epidemiology (confounding, measurement

error, and selection bias). Using assumptions about the magnitude and distribution of bias parameters, these methods can produce bias-corrected point estimates and measures of uncertainty. Implementing QBA can be challenging because such analysis depends on assumptions about parameters for these biases. These assumptions can be obtained from information in the literature or guessed from expert judgment. For example, when the estimated relation between HPV and HNC is confounded by smoking, and if smoking was not measured during data collection, there is a high possibility for unmeasured confounding to bias obtained estimates. Such analysis, however, can be salvaged if we obtain information on the distribution of smoking in the general population among the exposed and unexposed to HPV, as well as information on the strength of association between smoking and outcome HNC.

Then using the equations for unmeasured confounding, we can obtain a bias-corrected estimate of the relation between HPV and cancer. The same approach can be extended to selection bias and information bias. For a combined analysis that includes multiple biases, we control for biases in reverse order of how they occur in nature: information bias, selection bias, then unmeasured confounding. The article by Lash et al. provides a brief overview and explanation (40). For more detailed and technical information, the reader can refer to the book by Lash et al. (37,41).

In manuscript II, we focused on biases of the relation between HPV16 and HNC. HPV16 is the most oncogenic genotype reported in the literature, and it is found in 90% of lesions in the oropharynx. More importantly, it was found in 83% of tumor samples in HeNCe—see manuscript I (Chapter 4). In each of the following sections, we explain how each of these biases was analyzed,

show correction equations and explain the theory and principles for choosing the parameters used in the analyses.

3.13.1 Exposure Misclassification (Information Bias)

HPV infection is transitory, which means that infection at one point in time may not be present at another. The majority of HPV infections clear within a year (47,48), and only persistent infections are problematic. This, in addition to the fact that HPV measurement accuracy may differ by sampling collection method or genotyping technique (49), causes a measurement error problem in HNC studies. To account for measurement error, we need to know sensitivity and specificity among cases and controls, but there is no gold standard for measuring HPV status. Measurement of HPV DNA or E6 or E7 messenger RNA (mRNA) is considered the most accurate method to confirm the presence of HPV in cancer lesions (49,50).

Because studies have shown that HPV is almost never found in non-malignant oropharyngeal tissue samples (51), we made the assumption that HPV status in tumors is the gold standard for true HPV exposure that is carcinogenic. We compared oral and tumor HPV among cases to obtain Se and Sp of HPV16 measurement. Then used these numbers for both cases and controls assuming non-differential misclassification, meaning that Se and Sp would not vary according to outcome status (Sp Controls = Sp Cases; Se Controls = Se Cases). Using these numbers in the equation below will allow us to obtain a bias-corrected odds ratio for the relation between HPV16 and HNC. See (Table 3.2) which shows how corrected data is obtained.

Table 3.2 Equations for obtaining corrected numbers of cases and controls from observed data				
	Observed		Corrected data	
	HPV =1	HPV =0	HPV =1	HPV =0
Cases	*a*	*b*	$\frac{a-Total\ Cases\ (1-SP_{cases})}{SE_{cases}-(1-SP_{cases})}$	*Total Cases – A*
Controls	*c*	*d*	$\frac{c-Total\ Controls\ (1-SP_{control})}{SE_{control}-(1-SP_{control})}$	*Total Controls – C*
Total	*a+c*	*b+d*	*A+C*	*B+D*
A+C represents corrected exposed and B+D represent corrected unexposed				

3.13.2 Selection Bias

Selection bias has also been called 'collider-stratification bias', although some authors argue that it can occur in the absence of collider stratification (44,45). One intuitive way to visualize selection bias is through DAGs. In case-control studies, disease status affects selection into the study by design, so there is an arrow from cases into study sample. If sampling into the study is also affected by exposure, for example, if controls were selected from conditions known to be affected by the exposure, then the estimates will be biased because the sample in hand mandates conditioning on a collider.

For example, in Figure 3.4, an arrow extends from cases (HNC) to selection into the study sample (S=1) by design. Suppose controls are selected from departments known to over or under-represent HPV exposure. In that case, there will be an arrow extending from HPV exposure to selection into the study, which is a collider on the HPV→S←HNC path. Such control sampling will create a biasing path that was once closed before. Berkson was the first to describe this

phenomenon in hospital-based case-control studies, which is now often called “Berkson’s bias” (46).

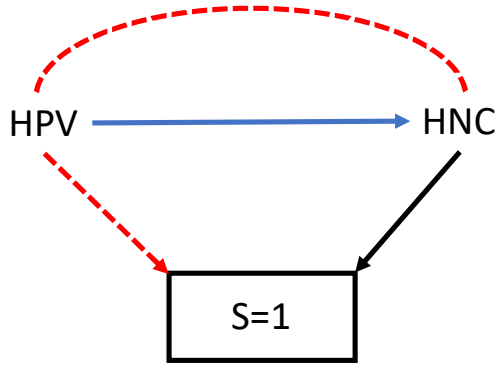

Figure 3.4. selection bias in case control studies can take place when the exposure affects selection into the study. This can happen when controls in hospital-based case-control studies are selected from departments in which these individuals are being treated for a disease that is affected by the same exposure.

In the HeNCe project, data collectors took some measures to minimize the possibility of selections bias: 1) controls were chosen from outpatient clinics in which people are treated for diseases unrelated to exposure; 2) HPV status was not known before selection into the study, so the selection was independent of HPV status; 3) No clinic contributed more than 20% of controls, which reduces the possibility of selection bias by chance; 4) the distribution of some confounders in controls like smoking and alcohol is similar to that of the general population in Quebec (9).

3.13.3 Unmeasured Confounding

For any unmeasured confounder (C) of the effect of HPV on HNC, we need to obtain three important parameters:

1) the strength of association between the confounder and outcome (C→ HNC) among the unexposed to HPV.

2) The strength of association between the confounder and the exposure in the source population, or the prevalence of confounder among the exposed.
3) And the prevalence of the confounder in the source population, or prevalence of the confounder among the unexposed.

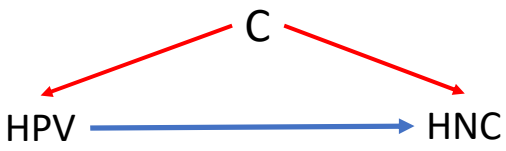

Figure 3.3. C is a vector of covariates that open a backdoor path from exposure HPV to HNC. Using substantive knowledge, we identify the minimum sufficient set of variables that if we control for which (for example in a logistic regression model), we obtain the conditional effect.

In manuscript II, we chose to perform the analysis considering poor oral health as a potential unmeasured confounder. Recent reports have indicated it is associated with both HPV status (42) and HNC (43). Thus, we referred to the literature to provide an educated guess about the association between poor oral health and HNC and the prevalence of poor oral health among those exposed and not exposed to HPV. We used the following equation to obtain bias-corrected odds ratio:

$$OR_{corr} = OR_{obs} \frac{OR_{LY}\, p_0 \; + \; (1 - p_0)}{OR_{LY}\, p_1 + (1 - p_1)}$$

Where,

OR_{corr} is odds ratios corrected for the unmeasured confounding.

OR_{obs} is observed odds ratios from the data.

OR_{CY} is the association between confounder C (poor oral health) and outcome Y (HNC).

p_0 is the prevalence of the confounder among the unexposed (HPV-negative).

p_1 is the prevalence of the confounder among the exposed (HPV-positive).

3.13.4 Combined Analysis

Combined analysis corrects for these three biases one at a time in the reverse order they occur during data collection. In nature, confounding happens in the source population, followed by selection bias that occurs when people get selected into the study sample, followed by misclassification or information bias that takes place when those selected are "measured" or classified as either exposed or non-exposed. Therefore, during combined analysis, we correct for the biases in this order: exposure misclassification, selection bias, then finally unmeasured confounding. In other words, we first correct for exposure misclassification using the relevant equations, then use misclassification-corrected estimates to correct for selection bias, and last, we use selection-bias-corrected estimates to correct for unmeasured confounding. The result would be artificial data that would have been obtained had none of the biases occurred. This data in turn is used to calculate the measure of effect (OR) between the exposure and the outcome.

3.13.5 Probabilistic Bias Analysis

All the above techniques assume a single correct value for the bias parameters, a deterministic value, but in reality, this is never the case (52). Therefore, in manuscript II, we use probabilistic bias analysis and assume that these parameters come from a known distribution. Then using sampling from such distributions, we run Monte Carlo simulations,

each time choosing bias parameters from these distributions and each time we conduct a combined analysis using the equations above. We then summarize the simulations by taking the median and 95% simulation intervals for corrected estimates of the relation between HPV and HNC. Our work followed the steps suggested by Fox et al. (41), which provide a framework for conducting probabilistic bias analysis:

1. Identify the likely sources of important bias in the data
2. Identify the bias parameters needed to adjust for the bias
3. Assign probability distributions to each bias parameter
4. Use simple bias analysis to incorporate uncertainty in the bias parameters and random error
    a. Incorporate bias parameter uncertainty by randomly sampling from each bias parameter distribution
    b. Genera bias-adjusted data using simple bias analysis methods and the sampled bias parameters
    c. Incorporate conventional random error by sampling summary statistics
5. Save the bias-adjusted estimate and repeat steps 4a-c
6. Summarize the bias-adjusted estimates with a frequency distribution that yields a central tendency and simulation interval

3.14 Measuring Interaction in Case-Control Studies

Interaction can mean two closely related but distinct concepts. Epidemiology and statistics have two different definitions, and this has been widely discussed and debated in the literature (53).

In this thesis, I will use the definition of interaction in epidemiology, which means the joint effect of two exposures on an outcome or the departure from additivity of independent effects (54). There is an important difference between interaction and effect measure modification (EMM). EMM represents when one exposure's effect on the outcome varies across the strata of a third variable (the modifier), but that third variable is not a causal exposure of the outcome. On the other hand, interaction occurs when the two exposure variables are both causal exposures of the outcome (55). A more detailed definition uses the counterfactual framework to distinguish between EMM and interaction and can be found in references by VanderWeele et al. (55,56) and Hernan and Robins(34).

Interaction as a departure from additivity is difficult to quantify directly in case-control studies, especially in hospital-based case-control studies where sampling fractions from the source population are unavailable. We, therefore, resort to proxies— on the multiplicative scale, interaction is assessed by including a product term between the two exposures in the logistic model. To measure EMM on the additive scale, Rothman suggested using a group of indices such as the Relative Excess Risk due to Interaction RERI, the Attributable proportion due to Interaction (AP), and Synergy Index (S). These measures require a rare disease assumption (<10%) for the odds ratio to approximate the risk ratio (57). The rare disease assumption holds in the case of HNC. Below are the equations for the three indices as estimated from odds ratios:

$$RERI = OR_{AB} - OR_A - OR_B + 1$$

$$AP = \frac{(OR_{AB} - OR_A - OR_B + 1)}{OR_{AB}}$$

$$SI = \frac{OR_{AB} - 1}{(OR_A - 1) + (OR_B - 1)}$$

There is a debate on the best proxy for measuring additive interaction in case-control studies. Skrondal showed that RERI and AP suffer from two problems he called the 'uniqueness' and the 'misspecification' problems and recommended using SI (58). VanderWeele (56) and Knol et al. (59) warn against using SI when one of the exposures is preventive. When one of the two exposures of interest is preventive, it is possible to have inconsistent additive interaction measures. SI can potentially show interaction in the opposite direction to that shown by RERI or AP. This inconsistency takes place because the denominator of SI, $(OR_A - 1) + (OR_B - 1)$, is negative. When this is the case, VanderWeele (2015) recommends not presenting SI (56), while Knol et al. (2011) suggest 'reverse-coding' the preventive exposure so that both variables increase risk (59). Kalilani and Atashili showed using simulations that using AP is more robust in case of common outcomes and closer to interaction on the risk scale (60).

In Chapter 6, we present all three measures of additive interaction. We calculate the interaction between several genotypes, for example, between HPV16 and infection with any other beta or gamma HPV as well as between high-risk alpha-9 and beta and gamma HPV. The additive

interaction was presented using all three indices, and confidence intervals were calculated using the delta method suggested by Hosmer and Lemeshow (61).

3.15 Sufficient Cause Model for Interaction of HPV

In manuscript III (chapter 6), we briefly explain interaction using sufficient cause models. Rothman introduced the sufficient cause model to epidemiology as a theory to explain how disease could develop under several mechanisms that may or may not include the exposure of interest. A detailed explanation can be found in Rothman, *Epidemiology: an introduction* (54), or Rothman et al. *Modern Epidemiology*(62)). In the context of interaction between two factors, say HPV16 and cutaneous HPV infection, Rothman explains that cases can happen under four different "classes". Each class represents several biological mechanisms. Class I is a combination of mechanisms that require the presence of HPV16 and another genotype from the beta and/or gamma genera. Class II is a combination of mechanisms that require only HPV16 infection, but not infection with beta or gamma genera. Class III represents mechanisms that require the presence of beta and/or gamma, but not HPV16. And last, Class IV is what Rothman describes as the background sufficient cause, which are the cases caused by some other exposures in the absence of both HPV16 and infection with beta/gamma HPV.

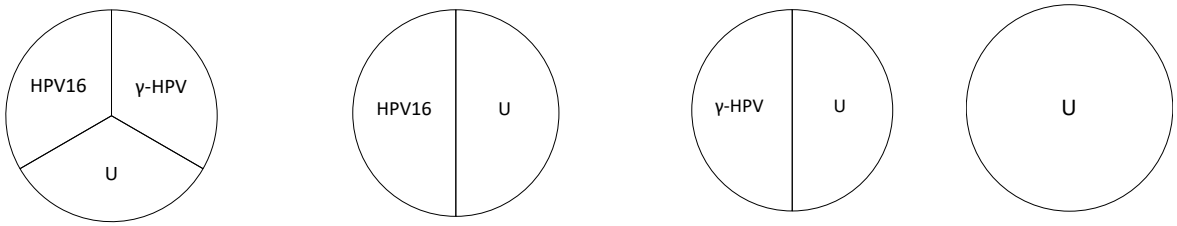

Figure 3.5 interaction can be seen as cases that are caused by mechanisms in which both HPV16 and Gamma (γ-HPV) played a role.

These four sufficient causes are essential to understand and illustrate Rothman's view of biologic interaction as a departure from additivity of effects. If indeed there is interaction at the biological level, then the excess number of cases will be decided by the pie on the left of figure 3.5. In the context of a case-control study, it is possible to approximate and estimate the proportion of sufficient causes from multiplicative models usually used for data analysis, i.e., from the logistic model. However, some assumptions need to be made for this equation to actually measure excess cases due to interaction, mainly that the model is correctly specified and there is no unmeasured confounding (63).

Since our study is frequency-matched, we used an unconditional multivariable logistic regression model to obtain the effect of the exposure on the outcome while controlling for confounders. The following equation shows the model in use:

$$\ln\left[\frac{Pr}{1-Pr}\right] = a_0 + a_1X_1 + a_2X_2 + a_3X_1X_2 + a_4C$$

Where X_1 represents the exposures of interest HPV16, X_2 represents β-HPV or γ-HPV, X_1X_2 represents a cross product of both exposures, and C represents a vector of confounders identified through analysis of a directed acyclic graph (DAG). The antilogarithm of the coefficient of the dichotomous exposure estimates the odds ratio of risk (the estimator), which in turn approximates the incidence rate ratio (the estimand). as this is a hospital-based case-control study in which the base represents a dynamic population from which controls are selected (7).

3.16 Missing Data

HeNCe has low numbers of missing data as interviewers ensured that participants answered all questions. Smoking was missing for one case, alcohol consumption for one case and one control, and education years variable was missing for one control. Missing education years was given mean value imputation— mean value of education years among controls. When calculating lifetime smoking pack-years and alcohol ethanol-liter consumption, missing observations were given mean value imputation.

There was also missingness in the variable of lifetime number of sexual partners. Among the analysis sample who were tested for α-HPV (controls n=429, cases n=389), 15 controls (3.5%) and 15 cases (3.85%) had missing values. As a sensitivity analysis, I explored using multiple imputation (MI) (64) with ordered logistic model, 30 imputation samples, and 300 between sample iterations to predict missingness in categories of lifetime sexual partners. The pooled estimate showed a minimal change in point estimate and standard errors compared to the complete-subject analysis. Because MI necessitates the assumption that missingness is conditional on observed data (i.e., missing at random), which may not be the case for sensitive personal questions like sexual behavior (57), I decided to proceed with a complete-case analysis.

3.17 Analysis Code

All analysis code used in this thesis can be found on the author's Open Science Framework profile at (https://osf.io/atwyj/?view_only=40809eeb842c46da8f64b7844bcf5b7e).

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CHAPTER 4: MANUSCRIPT I— ON THE PREVALENCE OF HPV GENERA

4.1 Preface Manuscript I

There are only a handful studies that investigate the presence of cutaneous HPV in oral cell samples or HNC tumor samples worldwide. However, these studies often had small sample size, used either oral cell samples or tumor samples but not both, and no study characterized the distribution of HPV distribution of all genera across key behavioral factors such as oral health behavior. Further, there are no studies for oral cutaneous HPV infection among the Canadian population. In this study, we fill this gap by using oral cell samples from HNC patients and non-cancer controls as well as tumor samples from HNC patients to investigate the prevalence of HPV genera. Our goal is to identify who is infected with HPV by age, sex, sexual behavior, smoking status, alcohol consumption, and oral health factors.

The sample is not representative of the general population of Canada or Quebec but results here have the potential to inform etiologic studies that aim to study the effect of cutaneous HPV on HNC. It will also be helpful for future studies aiming to estimate the national prevalence of oral HPV infection in Canada.

Title: Prevalence of Alpha, Beta, and Gamma Human Papillomaviruses (HPV) in head and neck cancer patients and non-cancer controls and relation to behavioral factors

Submitted for Peer Review

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4.2 Abstract

Objectives: Human papillomaviruses (HPV) are a major cause of a subset of head and neck cancer (HNC) that is increasing in incidence in developing countries. While oral alpha (α) HPV infection has been studied extensively in HNC, little is known about the role of other genera in these cancers. We investigate the prevalence of alpha (α-), beta (β)-, and gamma (γ) HPV among HNC cases and non-cancer controls, and their relationship with sociodemographic, behavioral and oral health factors.

Methods: We obtained oral rinse and brush samples from incident HNC cases (n=369) and hospital-based controls (n= 439), and tumor samples for a subsample of cases (n=121). We tested the samples using PCR with PGMY09-PGMY11 primers and Linear Array HPV genotyping for α-HPV, and type-specific multiplex genotyping (TS-MPG) for β-HPV and γ-HPV detection. We obtained sociodemographic and behavioral data from in-person interviews.

Results: The prevalence of α-, β-, and γ-HPV among controls was 14%, 56%, and 24%, respectively, whereas the prevalence among cases was 42%, 50%, and 33%, respectively. Compared to middle-aged participants (40 – 60 years), younger individuals (20-40 years) had a lower prevalence of all three HPV genera (α, β and γ-HPV) in both cases and controls. Prevalence of α- and γ-HPV, but not β-HPV, increased with the increase in sexual activity, smoking, and drinking habits. No HPV genus was associated with oral health status. Tumor samples included

HPV genotypes exclusively from the α-genus, mostly in the oropharynx and with HPV16 in over 80% of cases.

Conclusions: The distribution of α- and γ-HPV seems to vary based on sociodemographic and behavioral characteristics. β-HPV did not vary by behavioral factors. We did not observe the presence of cutaneous HPV in tumor tissues.

Keywords: Head and neck cancer, Cutaneous HPV, Alpha papillomaviruses, Beta papillomaviruses, Gamma papillomaviruses

4.1 Introduction

Human papillomavirus (HPV) is the most sexually transmitted infection in the world (1). For example, more than 70% of sexually active Canadian adults will be infected with HPV in their lifetime (2). HPV infection is now the leading cause of head and neck cancer (HNC) in developed countries (3), following successful public health efforts that reduced the risk from tobacco and alcohol (4). This increase in HNC incidence is mainly attributed to high-risk genotypes such as HPV16, the most studied genotype in the HNC literature (5).

More than 200 papillomaviruses genotypes have been discovered to date. It was once thought that HPV infection follows a certain "tropical distribution" with alpha (α) genus infecting genital and oral mucosa, while (β) and gamma (γ) genera infecting the skin. Thus, they were often called mucosal and cutaneous HPV, respectively (6). However, recent evidence suggests that genotypes from different genera can be found in several body parts. For example, genotypes from β and γ-HPV genera can be detected in the oral cavity mucosa (7,8) and could play a role in oropharyngeal cancer development (9).

While the HPV literature has focused on high-risk oncogenic viruses from the α-HPV genus, other genera might play some role in head and neck (9), esophagus(10), and skin (11) cancers. Cutaneous HPVs have only been recently studied in relation to the development of HNC, and co-infection with mucosal HPV has only been explored in a handful of articles (6,7). This long-time neglect of cutaneous HPV role in the oral cavity could be either due to technical limitations and non-availability of genotyping assays (7) or because they were primarily associated with benign

warts and thus were not considered important in HNC development. In this article, we aim to a) investigate the presence and distribution of the three main genera of HPV among HNC cases and healthy controls in oral and tumor samples; and b) describe the variation in the distribution of these genera according to sociodemographic factors, smoking and alcohol history, sexual behavior, and oral health status.

4.2 Methods

4.2.1 Study Population, Cases, and Controls Definition

This study uses data from the Head and Neck Cancer (HeNCe) Life Study that was conducted from September 2005 until November 2013. Extensive details about study populations have been discussed in previous publications (12,13). Briefly, this was a multi-center hospital-based case-control study of incident HNC patients using frequency-matched cancer-free controls. Incident HNC cases (n=460) and healthy controls (n=458) were recruited from the four main referral hospitals in Montreal, Québec. Case ascertainment was based on international codes of diseases (ICD-10) to include cancers of the oral cavity, oropharynx, larynx, and hypopharynx. Cancers of the lip, nasopharynx and salivary glands were excluded.

Non-cancer controls were selected from several outpatient clinics and were frequency matched on sex and age (within five years). None of the clinics contributed more than 20 % of the control population. To be eligible for the study, participants had to: be born in Canada, speak either English or French, be at least 18 years old, have no previous history of cancer, have no

immunosuppressive condition or mental disorder, and live within 50 km of the hospital they were recruited.

4.2.2 Data Collection

Trained interviewers administered a structured questionnaire using a life grid technique designed to improve recall by cross-referring personal and historical events with the information of interest (14,15). We collected data on an array of life course exposures, including socio-demographic (e.g., sex, age, education) and behavioral (e.g., tobacco smoking, alcohol consumption, sexual and oral health habits) factors, as well as self-reported oral health status.

4.2.3 Sample Collection

We collected two oral cell samples for HPV DNA detection (oral brush specimen and mouthwashes) from all participants and investigated the presence of HPV in tumor samples for a subset of cases from whom these samples were available. Total DNA was purified from exfoliated oral cavity epithelial cells obtained from a mouthwash rinse specimen, as described previously (13). Tissue block samples (n=121) were retrieved from hospital archives, and pathologists prepared them for HPV analysis.

4.2.4 Specimen Processing

Before detecting HPV DNA in both oral and tumor biopsies, β-globin testing was performed on 10 µL of extracted DNA by PCR using primers PC04 and GH20, followed by agarose gel electrophoresis to detect the presence of a 268 pb amplicon. Samples that were HPV-negative and β-globin negative were considered inadequate. This allowed quality control of extracted DNA

and ensured that enough human epithelial cells were available for PCR analysis. Detection and genotyping of oral α-HPV types have been previously described (13,16). Briefly, globin-positive DNA extracts were amplified using PGMY09-PGMY11 primers and the Linear Array HPV genotyping assay (LA-HPV, Roche Molecular Systems) for the 36 alphapapillomavirus, as previously described (17). Extracted DNA was also amplified with GP5+-GP6+ primers as described previously (18,19). In the presence of a 145 bp band on gel electrophoresis, genotyping was performed with Sanger PCR sequencing. Briefly, HPV amplicons of 145 bp were purified with the QIAquick gel extraction kit protocol (Quiagen Inc., Mississauga, Ontario) and sequenced with direct double-standard PCR-sequencing using a fluorescent cycle-sequencing method (BigDye terminator ready reaction kit, Perkin-Elmer Waltham, Massachusetts) on an ABI Prism 3100 Genetic Analyzer system.

Cutaneous HPV genotypes were detected in oral samples and biopsies using the type-specific multiplex genotyping (TS-MPG) assay, which combines multiplex PCR with a bead-based Luminex technology (20,21). Five microliters of the processed sample were amplified in a 9700 Thermal Cycler (Perkin-Elmer Cetus, Montreal, Canada) with the QIAGEN Multiplex PCR kit (Qiagen) according to the manufacturer's instruction, in a final reaction volume of 25µl. The PCR uses type-specific primers targeting the E7 region for the detection of 46 β-HPV types, namely 5, 8, 9, 12, 14, 15, 17,19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 75, 76, 80, 92, 93,96, 98, 99, 104, 105, 107, 100, 110, 111, 113, 115, 118, 120, 122,124, 143, 145, 150, 151, 152, 159, 174 as well as 52 γ-HPV types, namely 4, 48, 50, 60, 65, 88, 95, 101, 103, 108, 109, 112, 116, 119, 121, 123, 126, 127, 128,

129, 130, 131, 132, 133, 134, 148, 149, 156, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 175, 178, 179, 180, 184, 197, 199, 200, 201, 202 and SD2.

After PCR amplification, 10 µl of each reaction mixture was analyzed by MPG using Luminex technology (Luminex Corporation, Austin, TX) as described previously (22). The positivity of the assay was given by the intensity of the fluorescent signal detected by the Luminex apparatus and expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. The cut-off was calculated for each HPV-specific probe by considering the MFI values obtained with no respective PCR product. The cut-off was computed by adding 5 MFI to the median background value. All MFI values above the cut-off were considered positive.

4.2.5 Statistical Analysis

Because of logistic constraints and sample validity, we were not able to analyze all samples for all HPV genera. Overall, 834 individuals were tested for at least one genus (395 cases and 439 controls). We tested the presence of α-, β-, and γ-HPV in 818 (389 cases and 429 controls), 824 (391 cases and 433 controls), and 544 (246 cases and 298 controls) participants, respectively. We also investigated the presence of these viruses in a subsample of cases from whom the tumor samples were available (n=121).

To compare categorical and continuous variables between cases and controls, we calculated standardized difference scores as recommended by Yang and Dalton (29). Prevalence for any given HPV genotype infection was calculated as the percent of samples that tested positive for

the genotype of interest given the total sample for the respective genus. We also computed prevalence for subpopulations (sex, age groups, smoking status, etc.).

The appendix shows the findings obtained on tumor samples tested for all three genera. To measure both duration and frequency, smoking was calculated in pack-years and alcohol in ethanol-liters as explained previously (11), and oral sex intensity is measured in sex-years, as suggested by Drake et al. (22), which calculates sexual partners per 10 years since oral sex debut. All descriptive analyses were conducted with Stata/MP version 16.1 (StataCorp, College Station, Texas)(23). Code can be found in the Open Science Framework repository. (https://osf.io/atwyj/?view_only=40809eeb842c46da8f64b7844bcf5b7e)

4.3 Results

Table 4.1 shows the study characteristics of participants tested for the three genera (α, β, γ). Median years of education was slightly higher among controls than cases (14 vs. 12 years). Reporting ever smoking and alcohol were more common among cases with the life-time consumption for ever users almost double that of controls. When looking at oral health variables (regular visits to the dentist, tooth brushing, wearing dentures), it is noticeable that controls reported better oral health status than cases. Appendix Table 4.1 compares characteristics of the subsample tested for all three genera (α, β, γ) to the one tested for one or two genera.

Table 4.1. Sociodemographic and behavioral characteristics of HNC cases (242) and healthy controls (n=294)			
	Controls	**Cases**	**Standardized Difference**
	(n = 294)	**(n = 242)**	
Age (years)			
Median (Q1, Q3)	61.0 (54.0, 68.0)	60.0 (55.0, 68.0)	0.03
N (% Missing)	294 (0.0)	242 (0.0)	
Education years			
Median (Q1, Q3)	14.0 (11.0, 17.0)	12.0 (10.0, 14.0)	0.39
N (% Missing)	294 (0.0)	242 (0.0)	
Smoker			
Ever	214 (72.8)	207 (85.5)	0.33
Missing	0 (0.0)	1 (0.4)	
Smoking (pack-years) *			
Median (Q1, Q3)	14.1 (0.0, 32.5)	33.5 (10.5, 55.8)	0.33
N (% Missing)	294 (0.0)	242 (0.0)	
Drinker			
Ever	248 (84.4)	206 (85.1)	0.03
Missing	0 (0.0)	1 (0.4)	
Alcohol Ethanol-Liter*			
Median (Q1, Q3)	122.3 (11.7, 369.5)	220.3 (38.1, 626.5)	0.29
N (% Missing)	294 (0.0)	242 (0.0)	
Sex			
Male	201 (68.4)	182 (75.2)	0.15
Lifetime sexual partners			0.14
0-3	82 (27.9)	55 (22.7)	
3-7	92 (31.3)	74 (30.6)	
7-13	42 (14.3)	39 (16.1)	
>13	64 (21.8)	61 (25.2)	
Missing	14 (4.8)	13 (5.4)	
Ever practiced oral sex			
Yes	223 (75.9)	203 (83.9)	0.23
Missing	8 (2.7)	8 (3.3)	
Visited the dentist			0.46
At least once a year	201 (68.4)	112 (46.3)	
Once in 5 years	29 (9.9)	44 (18.2)	

Table 4.1. Sociodemographic and behavioral characteristics of HNC cases (242) and healthy controls (n=294)			
Only in pain or never	59 (20.1)	80 (33.1)	
Missing	5 (1.7)	6 (2.5)	
Toothbrushing frequency			
Once or more a day	232 (78.9)	160 (66.1)	0.12
Missing	52 (17.7)	70 (28.9)	
Partial/Complete Denture			
Yes	130 (44.2)	141 (58.3)	0.29
Missing	1 (0.3)	2 (0.8)	

Among non-cancer controls, the prevalence of α-, β-, and γ-HPV was 14%, 56%, and 24%, respectively. However, the prevalence among cases was 42%, 50%, and 33%, respectively. Younger individuals seem to have a lower prevalence of all three HPV genera, while middle-aged individuals (50-59 and 60-69) have the highest prevalence (Table 4.2). When we modeled the prevalence of HPV genera by age, "bimodal prevalence" was only detected for α-HPV among cases (Appendix Figure 4.1). All three genera are more common among males than females in both cases and controls, but this is more prominent for α- and γ-HPV than for β-HPV (Table 4.2). In both cases and controls, the prevalence of oral α-HPV and γ-HPV varied by the number of sexual partners and intensity of oral sex, showing what appears to be a dose-response relation. However, β-HPV showed less prominent differences (Table 4.2).

Table 4.2 Prevalence of HPV genera by sample, sociodemographic, and sexual behavior variables

	Controls (n= 458)						Cases (n= 460)					
	Alpha n tested (%) = 429 (93.7)		Beta n tested (%) = 433 (94.5)		Gamma n tested (%) = 298 (65.1)		Alpha n tested (%) = 389 (84.6)		Beta n tested (%) = 391 (85)		Gamma n tested (%) = 246 (53.5)	
	+ n (%)	Total	+ n (%)	Total	+ n (%)	Total	+ n (%)	Total	+ n (%)	Total	+ n (%)	Total
Overall HPV Positive	61 (14.2%)	429	242 (55.9%)	433	72 (24.2%)	298	163 (41.9%)	389	196 (50.1%)	391	82 (33.3%)	246
Age (years)												
20-39	0 (0.0%)	11	1 (9.1%)	11	0 (0%)	9	1 (14.3%)	7	2 (28.6%)	7	1 (20.0%)	5
40-49	7 (15.6%)	45	25 (55.6%)	45	9 (26.5%)	34	13 (37.1%)	35	15 (40.5%)	37	7 (28.0%)	25
50-59	23 (16.7%)	138	74 (52.5%)	141	22 (22.9%)	96	67 (50.0%)	134	70 (52.6%)	133	28 (31.1%)	90
60-69	18 (13.0%)	139	85 (61.2%)	139	23 (24.2%)	95	52 (40.6%)	128	64 (50.0%)	128	23 (31.5%)	73
70-79	12 (15.8%)	76	44 (58.7%)	75	11 (21.6%)	51	25 (36.8%)	68	38 (55.1%)	69	19 (42.2%)	45
80 ≤	1 (5.0%)	20	13 (59.1%)	22	7 (53. 9%)	13	5 (29.4%)	17	7 (41.2%)	17	4 (50.0%)	8
Sex												
Female	7 (5.3%)	132	72 (54.1%)	133	16 (17.0%)	94	26 (25.7%)	101	49 (49.5%)	99	17 (28.3%)	60
Male	54 (18.2%)	297	170 (56.7%)	300	56 (27.5%)	204	137 (47.6%)	288	147 (50.3%)	292	65 (35.0%)	186
Education												
Less than High School	11 (15.9%)	69	38 (52.8%)	72	9 (22.5%)	40	42 (44.7%)	94	47 (50.0%)	96	19 (38.0%)	50
High School	15 (16.9%)	89	54 (59.3%)	91	17 (24.6%)	69	61 (43.9%)	139	65 (45.8%)	142	30 (33.3%)	90
Technical or CEGEP	13 (11.30%)	115	62 (53.5%)	116	14 (17.1%)	82	39 (42.4%)	92	52 (58.4%)	89	19 (30.2%)	63
University Degree	22 (14.1%)	156	88 (57.1%)	154	32 (29.9%)	107	21 (32.8%)	64	32 (50.0%)	64	14 (32.6%)	43
Smoking												
Never	10 (9.0%)	111	63 (57.8%)	109	13 (16.1%)	81	31 (47.7%)	65	34 (52.3%)	65	9 (25.7%)	35
Ever	51 (16.0%)	318	179 (55.3%)	324	59 (27.2%)	217	132 (41%)	322	161 (49.7%)	324	73 (34.8%)	210
Missing		0		0		0		2		2		1
Drinking												
Never	7 (9.6%)	73	45 (59.2%)	76	10 (21.3%)	47	21 (33.3%)	63	28 (43.8%)	64	14 (40.0%)	35
Ever	54 (15.2%)	356	197 (55.2%)	357	62 (24.7%)	251	142 (43.8%)	324	167 (51.4%)	325	68 (32.4%)	210
Missing		0		0		0		2		2		1

Lifetime sexual partners												
0-3	10 (7.9%)	126	74 (59.2%)	125	14 (17.1%)	82	20 (21.5%)	93	50 (53.8%)	93	14 (25.5%)	55
3-7	15 (11.3%)	133	72 (52.9%)	136	22 (23.7%)	93	50 (41.3%)	121	63 (51.6%)	122	24 (32.4%)	74
7-13	8 (13.1%)	61	29 (48.3%)	60	9 (21.4%)	42	40 (58.0%)	69	32 (47.1%)	68	11 (28.2%)	39
>13	26 (27.7%)	94	55 (56.7%)	97	19 (28.4%)	67	50 (55.0%)	91	44 (47.3%)	93	30 (46.2%)	65
Missing		15		15		14		15		15		13
Ever practiced oral sex												
No	6 (6.3%)	95	59 (61. 5%)	96	15 (23.8%)	63	13 (25.0%)	52	23 (44.2%)	52	10 (32.3%)	31
Yes	55 (16.9%)	325	176 (53.8%)	327	55 (24.3%)	226	147 (45.2%)	325	167 (51.1%)	327	68 (32.9%)	207
Missing		9		10		9		12		12		8
Oral Sex Intensity*												
<1	7 (11.5%)	61	30 (50.0%)	60	4 (11.4%)	35	17 (27.9%)	61	37 (60.7%)	61	4 (13.3%)	30
1-5	21 (13.3%)	158	79 (49.7%)	159	21 (19.6%)	107	87 (51.8%)	168	82 (48.2%)	170	29 (27.4%)	106
>5	23 (28.4%)	81	43 (52.4%)	82	15 (25.4%)	59	33 (51.6%)	64	33 (52.4%)	63	23 (53.5%)	43
Missing		129		132		97		96		97		67

** Oral sex intensity measures sexual partners per 10 years since oral sex debut.*

Table 4.3 shows the distribution of HPV infection in oral samples. Almost 43% of all oral samples among cases tested positive for at least one genotype of α-HPV in comparison to only 15% of controls. Coinfection with more than a single genotype was more common among cases than in controls (71% vs. 77%). And of all the high-risk genotypes as classified by IARC, HPV16 was more common in cases than controls. Also, we found that infection with a beta genotype was more likely to occur with an alpha than with a gamma genotype (Table 4.3).

Table 4.3: Multiple infection distribution among cases and controls: HeNCe Life study (N=536)

HPV genera	**Controls N (%)**	**Cases N (%)**
Alpha HPV	44 (15%)	103 (42.6%)
Single Infection	32 (10.9%)	66 (27.3%)
Coinfection with other		
Alpha	12 (4.1%)	37 (15.3%)
Beta	28 (63.6%)	62 (60.2%)
Gamma	13 (29.6%)	40 (38.8%)
Beta and Gamma	29 (65.9%)	75 (72.8%)
Beta HPV	176 (59.9%)	144 (59.5%)
Single infection	53 (18.0%)	43 (17.8%)
Coinfection with other		
Beta	123 (41.8%)	101 (41.7%)
Gamma	63 (87.5%)	57 (69.5%)
Gamma HPV	72 (24.5%)	82 (33.9%)
Single infection	40 (13.6%)	39 (16.1%)
Coinfection with other		
Gamma	32 (10.9%)	43 (17.8%)

Figures 4.1 and 4.2 show the prevalence of β-HPV and γ-HPV genotypes in oral samples among cases and controls subsample tested for all genera. The prevalence of α-HPV is in the appendix

(Appendix Figure 4.2). The most common β-HPV genotypes were HPV38 (24%) and HPV76 (22%), with a slightly higher prevalence among healthy controls. The γ-genus were less prevalent than β-HPV. The most prevalent γ-HPV genotypes among controls were HPV128 (8%) and HPV50 (7%), while HPV156 was the most prevalent among HNC cases (6%). Appendix Figure 4.2 compares the distribution of HPV α-genus between cases and controls in oral samples. HPV16 was more likely to be detected among cases than controls (26% vs. 2%, respectively).

Figure 4.1 Frequency of Beta viruses detected in oral samples of HNC cases and controls

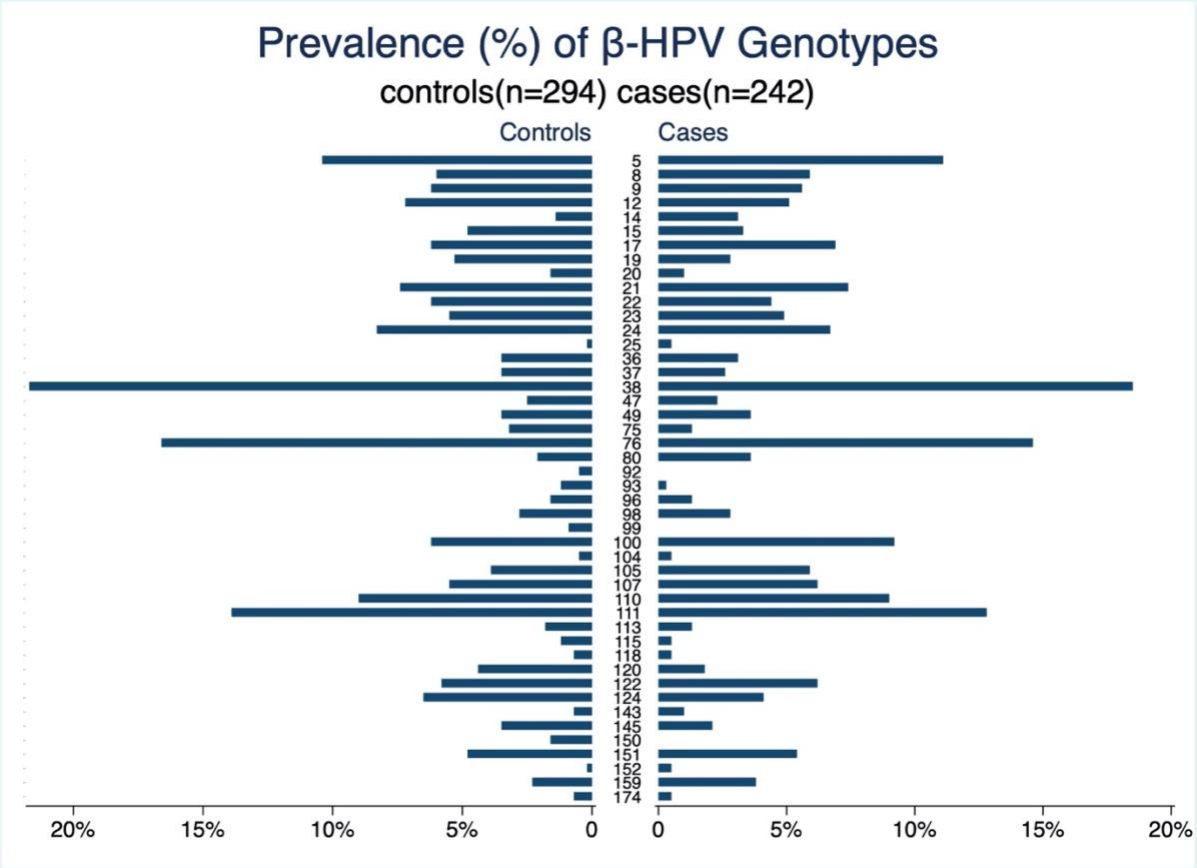
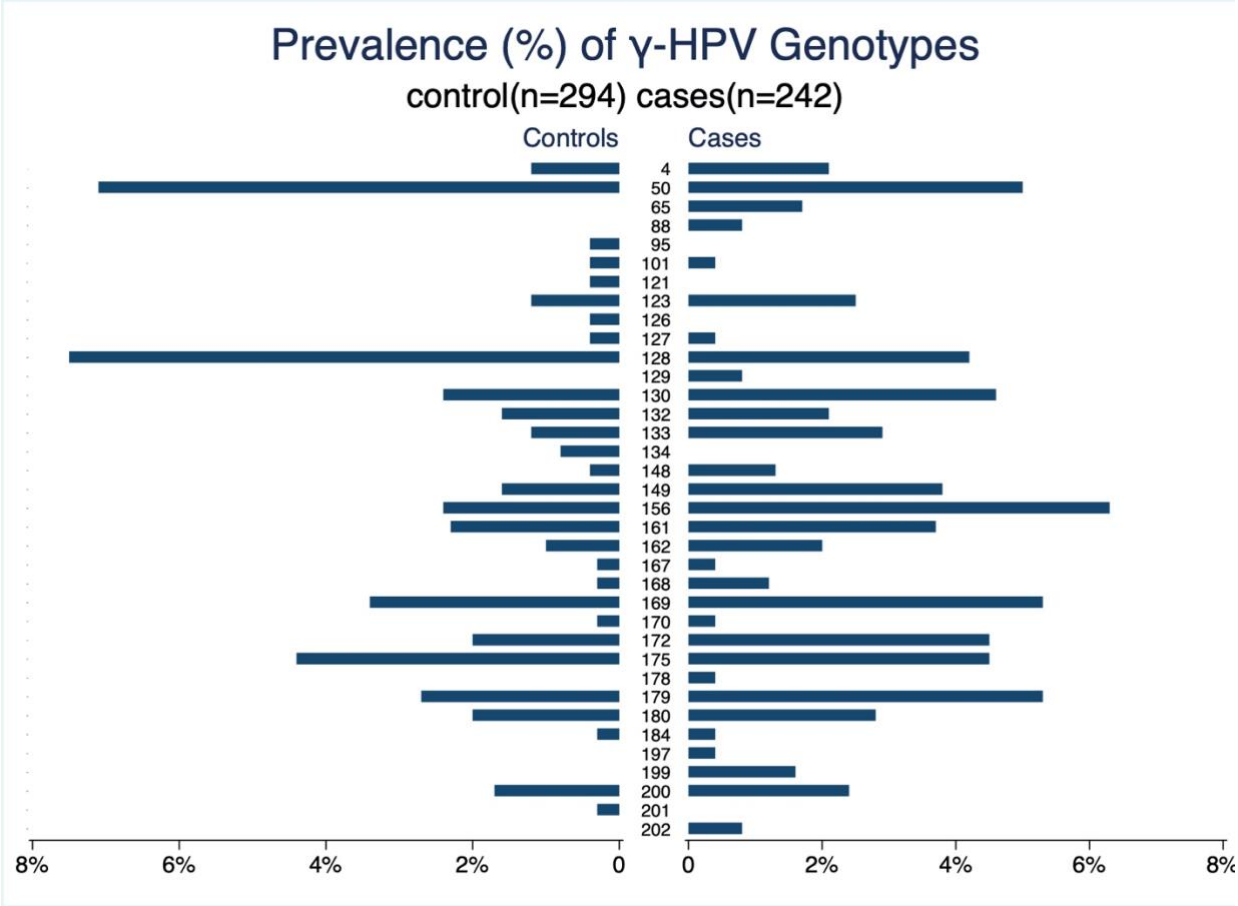


Figure 4.2 Prevalence of Gamma HPV in oral samples of HNC patients and non-cancer controls

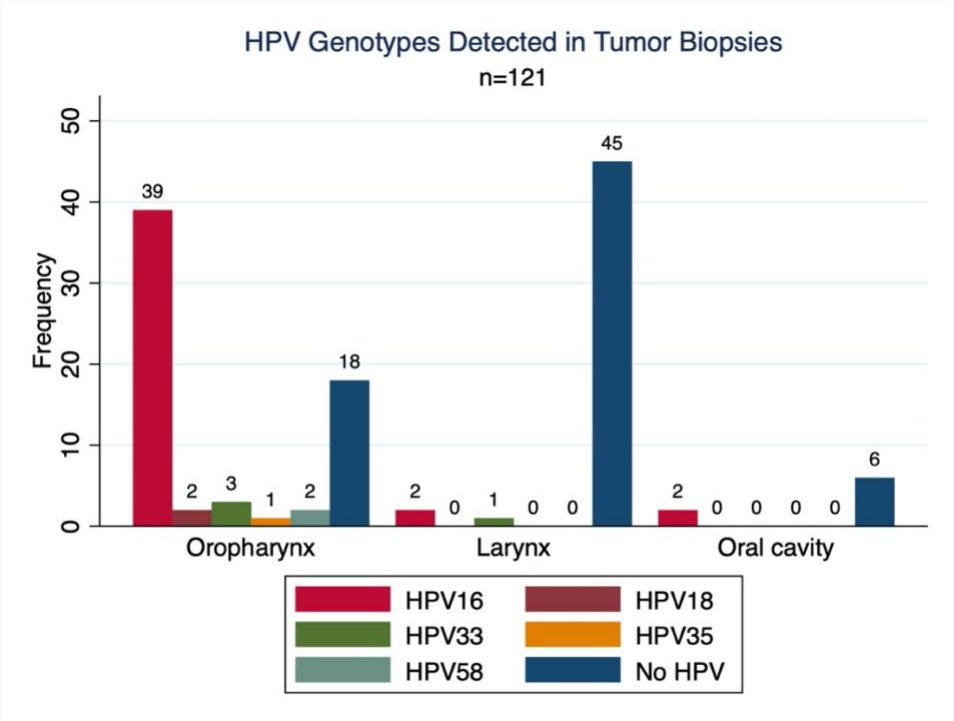


We found all common species of the β genus to be common among controls and cases without statistically significant differences in detection rates (Appendix Table 4.2). Despite the small sample size, the prevalence of γ-HPV was higher among cases when all types were considered, although the difference was smaller than that found with α-HPV genotypes. The difference in detection rates was significant for species 18 only. Species γ-11 and γ-12, often reported to be oncogenic, were only slightly more prevalent in cases than controls, and the unadjusted association was imprecise (Appendix Table 4.2). The relation between indicators of oral health

and detection of all genera among cases was not significant for any covariate (Appendix Table 4.3).

In the subsample of cases with tumor samples (n=121), HPV DNA was detected in 52 cases (43%), which were all from the α genus (Figure 4.3). Over 90% of these cancers (n=47) were in the oropharynx, which is the most affected site by HPV. HPV DNA was detected only in a few laryngeal (n=3) and oral cavity (n=2) cancers. We identified only five genotypes (Appendix Figure3): HPV16 (43 cases), HPV33(4 cases), HPV58 and HPV18 (2 cases each), and HPV35 (1 case). In all tumor samples, each sample contained a single genotype, and no tumor sample was infected by more than one genotype.

Figure 4.3. Frequency of HPV genotypes detected in tumor samples in a subsample of cases



4.4 Discussion

This is one of a handful of studies globally and the first in the Canadian population to use oral and tumor samples to measure the prevalence of several oral HPV genera. We also used innovative techniques of bead-based multiplex genotyping to identify the largest number of genotypes possible. This study is not without limitations, however. While not all HeNCe participants were tested and genotyped for HPV, we do not observe any differences in the distribution of characteristics between participants tested for all three genera and those tested for less than the three. Another limitation is that we are showing sample prevalence rather than population prevalence. To estimate the latter, we would need population controls and sampling fractions to obtain valid estimates. Last, while the study uses innovative methods to genotype and identify HPV, there is still the possibility of measurement error. Newer technologies, like next-generation sequencing (NGS) or deep-sequencing, although still prohibitively expensive, can detect rare genotypes and might better assess HPV in samples.

In our study, we found that 14% of controls and 42% of cases tested positive for at least one α-HPV genotype, which is higher than the sample prevalence reported by Agalliu et al. (13.9% of controls and 24.2% of cases) (9). While Winer et al. reported the prevalence of β-HPV and γ-HPV in oral samples to be 21% and 11%, respectively, the prevalence in HeNCe sample was much higher for both genera with β-HPV in controls = 56%, in cases = 50%, γ-HPV in controls= 24%, and in cases 33%. Our estimates, however, are slightly less than prevalence reported by Agalliu for oral samples of HNC cases and controls: any β-HPV (controls = 58.8%, cases=62.1%) and any γ-HPV infection (controls 34.5%, cases= 45.5%).

Cutaneous genotypes are highly prevalent in the anal canal of immunocompromised patients (20) and have been linked to skin cancer (11). However, it is unclear if they actually play a role biologically by initiating the carcinogenesis process or rather by helping more oncogenic genotypes get involved (25). One study reported an association between some genotypes or species of β- and γ-HPV types, but testing was done on mouthwash and not tissue biopsies (9).

Among both cases and control, the prevalence of α- HPV and γ- HPV infection was higher among individuals with more lifetime sexual partners and those who reported a high frequency of oral sex. Interestingly, similar to α-HPV infection, which is well known to be correlated with sexual behavior, we notice a similar pattern for γ but not β-HPV. This suggests that infection with β-HPV may not be related to sexual behavior. However, the prevalence of β-HPV infection was lower for people who reported ever practicing oral sex than those who did not (Table 4.2) among controls but not cases. The fact that β-HPV was more likely to occur with α-HPV than with γ-HPV could likely be due to the higher prevalence of β-HPV in the oral cavity as well as the rarity of γ-HPV. In a recently published article by Winer et al. (8), the authors found a strong correlation between β-HPV in oral and fingernail samples. This can mask the contribution of β-HPV infections in other anatomical sites if the infection is transmitted from fingers to the oral cavity.

HPV infection has been reported to be higher among men than women, which we observed for α- and γ-HPV, but not β-HPV. Unlike what has been reported in other studies(8), the prevalence

of β-HPV was not significantly higher among those with a greater lifetime number of sexual partners, which could be because β-HPV is not related to sexual behavior or sex (male vs. female).

The presence of mucosal HPV genotypes in tumor samples corroborates previous reports from the cervical and HNC literatures. However, the low prevalence of β and γ-HPV genotypes in oral cell samples and their absence in tumor tissues suggest they do not play a role individually in oral carcinogenesis. Our findings do not support a direct causal role for cutaneous genotypes in HNC carcinogenesis, but their absence from tumor samples does not rule them from intermediate stages that eventually lead to established cancer. A study by Donà et al. (2017) has found that both mucosal and cutaneous HPV genotypes were detected in papillomas in the head and neck region (26), and they have been detected in Actinic Keratosis, a precancerous lesion of the skin (27). HPV of this genus could play a role at the initial stages of cancer development as facilitators and then fade away once cancer has been clinically established. This hypothesis has been called “hit and run” (28,29). While we cannot confirm this hypothesis in our current study, it deserves to be investigated in further large prospective studies.

4.5 References

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4.6 Appendix — Manuscript I

Prevalence of Alpha, Beta, and Gamma Human Papillomaviruses (HPV) and relation to sexual, social, and oral health factors

Appendix Table 4.1 Comparison of sample tested for all HPV genera (α, β, γ) and those tested for either α, β, or both, but not γ-HPV.

	Not tested for all genera α and/or β (not γ tested) (N = 299)	Tested for all HPV genera α+ β+γ (N = 536)	p-value
Age (years)			0.071
Mean (SD)	62.25 (10.31)	60.86 (10.79)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Education years			0.056
Mean (SD)	12.65 (4.36)	13.23 (4.14)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Smoking (pack-years)			0.291
Mean (SD)	34.17 (43.59)	30.92 (42.01)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Alcohol Ethanol-Liter			0.457
Mean (SD)	474.60 (1015.72)	538.29 (1271.08)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Sex			0.809
Female	83 (27.8%)	153 (28.5%)	
Male	216 (72.2%)	383 (71.5%)	
Smoker			0.865
Never	65 (21.7%)	114 (21.3%)	
Ever	233 (77.9%)	421 (78.5%)	
Missing	1 (0.3%)	1 (0.2%)	
Drinker			0.050
Never	61 (20.4%)	81 (15.1%)	
Ever	237 (79.3%)	454 (84.7%)	
Missing	1 (0.3%)	1 (0.2%)	
Lifetime number of sexual partners			0.903
0-3	85 (28.4%)	137 (25.6%)	
3-7	95 (31.8%)	166 (31.0%)	
7-13	49 (16.4%)	81 (15.1%)	
>13	67 (22.4%)	125 (23.3%)	
Missing	3 (1.0%)	27 (5.0%)	
Ever practiced oral sex			0.806
No	55 (18.4%)	94 (17.5%)	

Yes	238 (79.6%)	426 (79.5%)
Missing	6 (2.0%)	16 (3.0%)

Note: Table S1 is intended to show that there are minimal differences between the sample tested for all genera and that who for logistical and cost reasons were tested for one or two genera. While sample to be tested for all genera was not selected completely at random, the table shows that covariate distribution was similar.

Appendix Table 4.2 Prevalence of and Association of Beta and Gamma HPV with cases and controls

α-HPV	Cases (N=389)	Controls (N=429)	Unadjusted OR (95 % CI)
Any alpha	163 (42)	61 (14)	4.4 (3.1, 6.2)
HPV16	106 (27)	10 (2)	13.4 (6.9, 29.1)
HPV a-9 ex. HPV16	29 (7)	9 (2)	3.8 (1.7, 9.1)

β-HPV	Cases (N=391)(%)	Controls (N=433)(%)	Unadjusted OR (95 % CI)
Any Beta	196 (50)	242 (56)	0.8 (0.6, 1.1)
Beta 1 Species	123 (31)	153 (35)	0.8 (0.6, 1.1)
Beta 2 Species	162 (41)	199 (46)	0.8 (0.6, 1.1)
Beta 3 Species	67 (1%)	91 (21)	0.8 (0.5, 1.1)

γ-HPV	Cases (N=246)	Controls (N=298)	Unadjusted OR (95 % CI)
Any Gamma	82 (33)	72 (24)	1.6 (1.1, 2.3)
Gamma 7 Species	14 (6)	10 (3)	1.7 (0.7, 4.5)
Gamma 8 Species	6 (2)	2 (<1)	3.7 (0.7, 37.7)
Gamma 9 Species	2 (<1)	0 (0)	NA
Gamma 10 Species	22 (9)	15 (5)	1.9 (0.9, 3.9)
Gamma 11 Species	14 (6)	11 (4)	1.6 (0.6, 3.9)
Gamma 12 Species	9 (4)	6 (2)	1.8 (0.6, 6.4)
Gamma 15 Species	13 (5)	8 (3)	2.0 (0.8, 5.7)
Gamma 18 Species	15 (6)	6 (2)	3.2 (1.1, 10.1)

Appendix Table 4.3 Distribution and relation (unadjusted odds ratios) between oral health behavior and HPV infection among HNC cases (n=389)

	Alpha infection			Beta infection			Gamma infection		
Teeth cleaning	*Negative*	*Positive*	*OR (95% CI)*	*Negative*	*Positive*	*OR (95% CI)*	*Negative*	*Positive*	*OR (95 % CI)*
One or more a day	150	108	Ref	130	129	Ref	112	50	Ref
Irregularly	9	8	1.2 (0.5, 3.3)	7	9	1.3 (0.5, 3.6)	8	4	1.1 (0.3, 3.4)
Missing	67	47		58	58		44	28	
Wearing Complete Denture									
No	120	87	Ref	101	107	Ref	85	41	Ref
Yes	102	76	1.0 (0.7, 1.5)	93	86	0.9 (0.6, 1.3)	78	40	1.1 (0.6, 1.8)
Missing	4	0		1	3		1	1	
Wearing Partial Denture									
No	115	90	Ref	96	109	Ref	96	34	Ref
Yes	44	26	0.8 (0.4, 1.3)	41	29	0.6 (0.4, 1.1)	24	20	2.4 (1.2, 4.8)
Missing	67	47		58	58		44	28	
Gum Bleeding									
No	118	84	Ref	105	98	Ref	81	39	Ref
Yes	41	32	1.1 (0.6, 1.9)	32	40	1.3 (0.8, 2.3)	39	15	0.8 (0.4, 1.6)
Missing	67	47		58	58		44	28	
Last visit to the Dentist									
Every 6 months	55	38	Ref	54	39	Ref	38	23	Ref
Once a year	53	45	1.2 (0.7, 2.2)	44	54	1.7 (1.0, 3.0)	36	17	0.8 (0.4, 1.7)
Once every 2-5 years	18	12	1.0 (0.4, 2.2)	10	20	2.8 (1.2, 6.6)	15	10	1.1 (0.4, 2.9)
Once every 5 years	17	12	1.0 (0.4, 2.4)	15	15	1.4 (0.7, 2.3)	13	7	0.9 (0.3, 2.6)
Only when in pain	34	29	1.2 (0.6, 2.4)	34	28	1.1 (0.6, 2.2)	30	11	0.7 (0.3, 1.4)
Never	43	26	0.9 (0.5, 1.7)	34	37	1.5 (0.9, 2.8)	26	14	0.9 (0.4, 2.0)
Missing	6	1		4	3		6	0	

Appendix Figure 4.1 Modeled HPV Prevalence Across Age for HNC cases and Controls

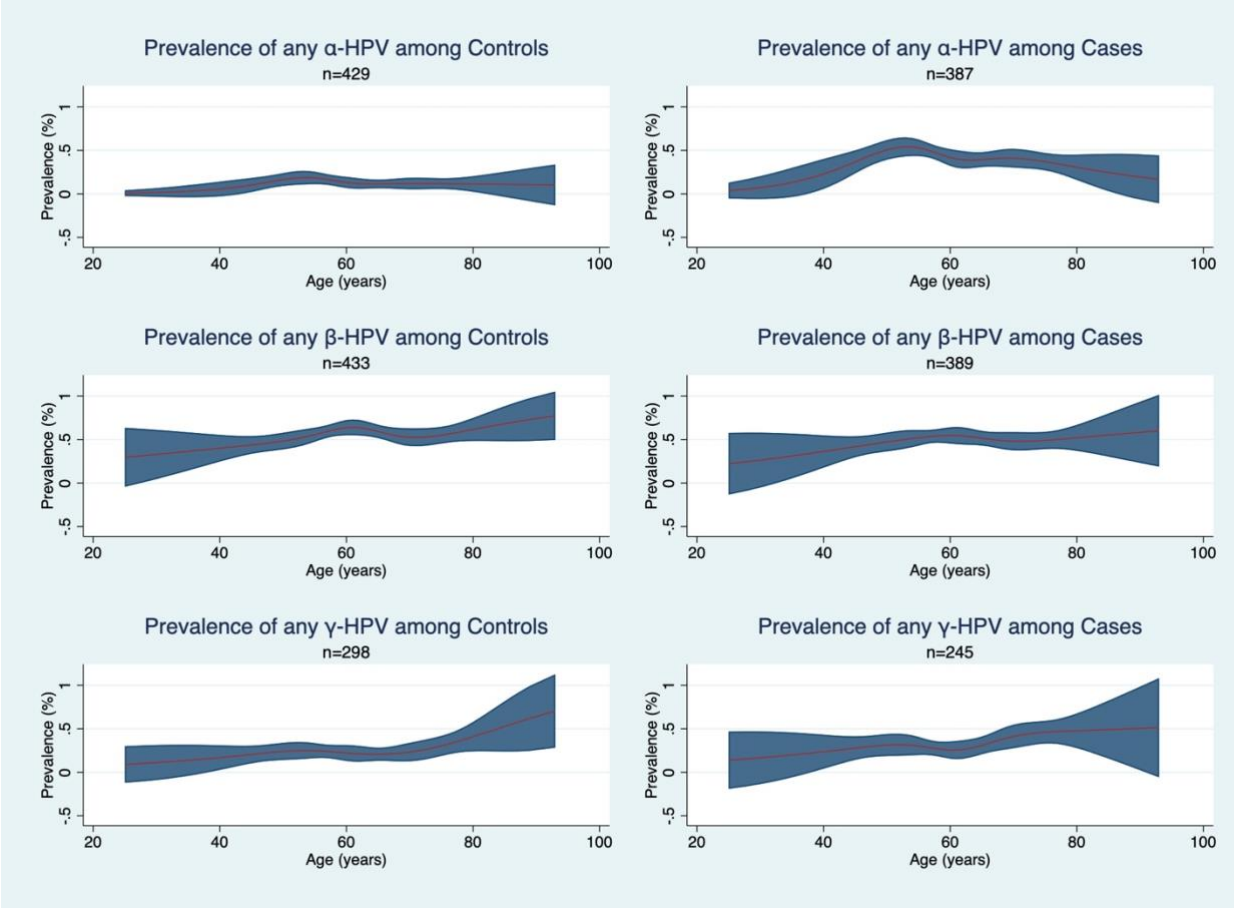
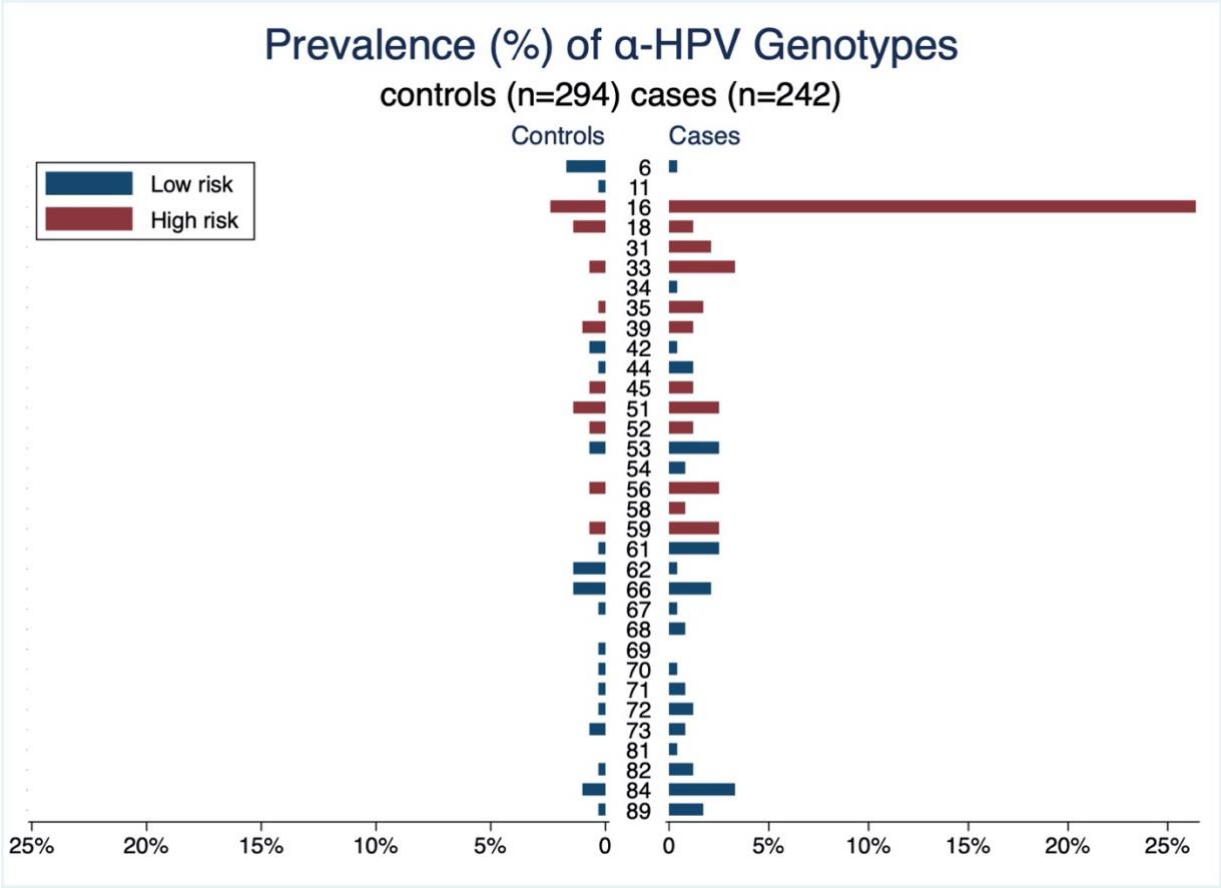


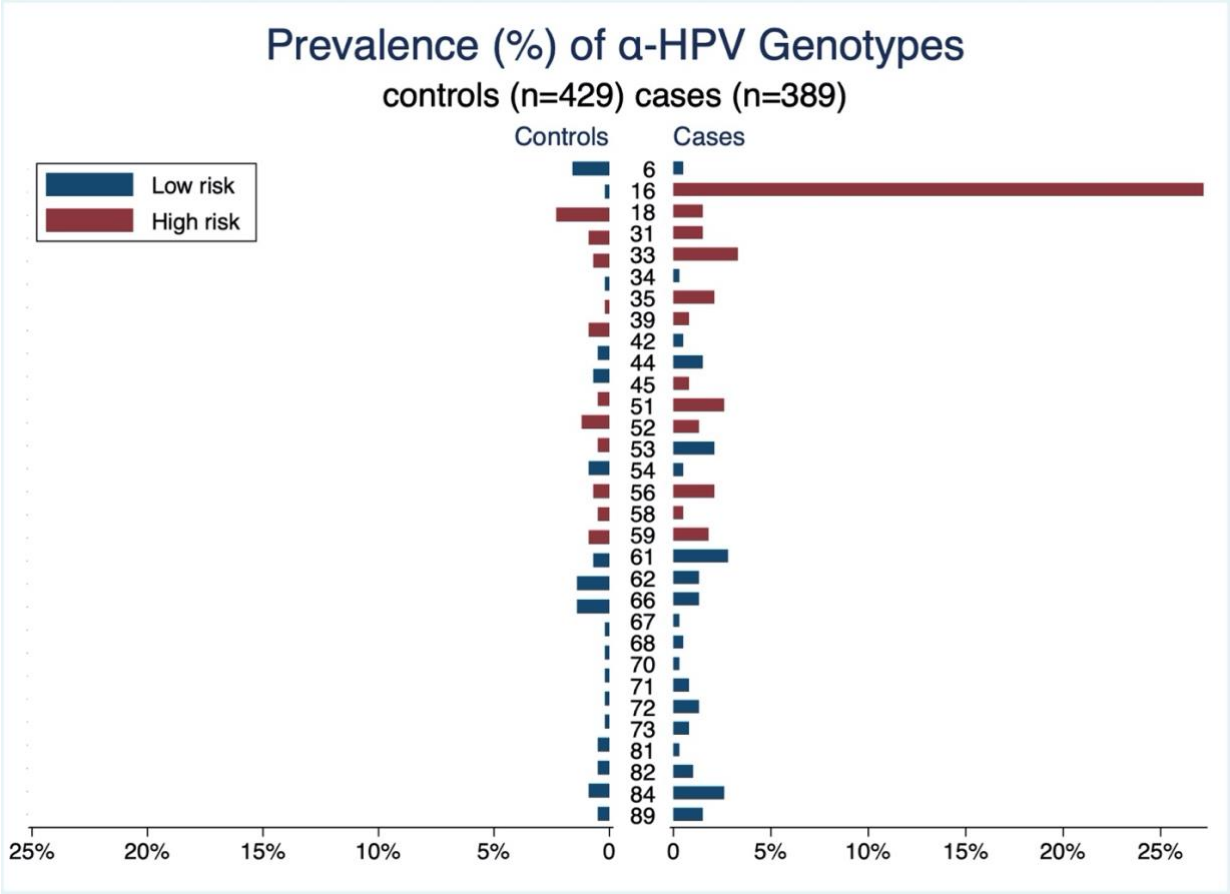
Figure 4.1 Modeled human papillomavirus (HPV) genera prevalence (%) and 95% CI by individual age in years. Age is modeled with restricted cubic splines with 5 knots and all models are adjusted for sex, smoking, and alcohol drinking.

Appendix Figure 4.2 Prevalence of α-HPV among cases and controls in sample tested for all genera

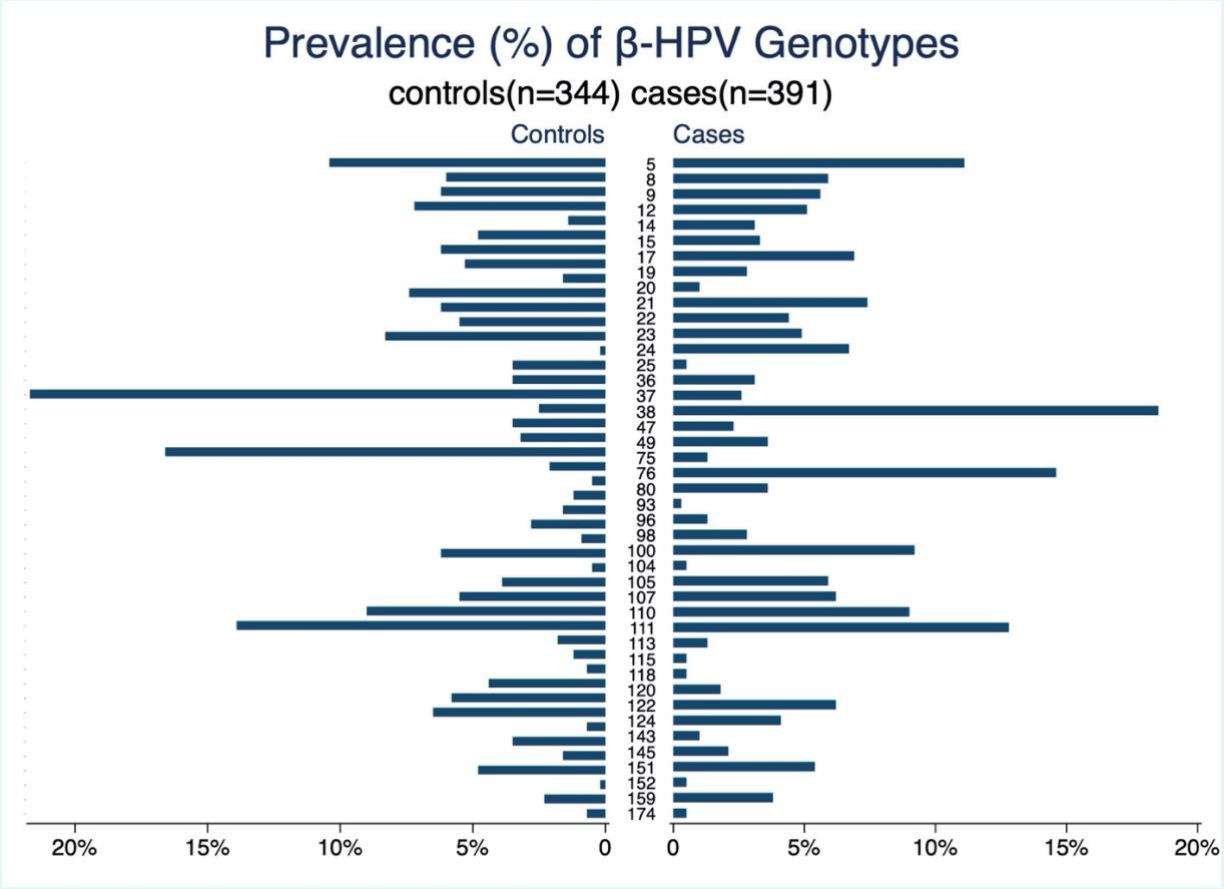


Note: High risk genotypes are identified by IARC include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59

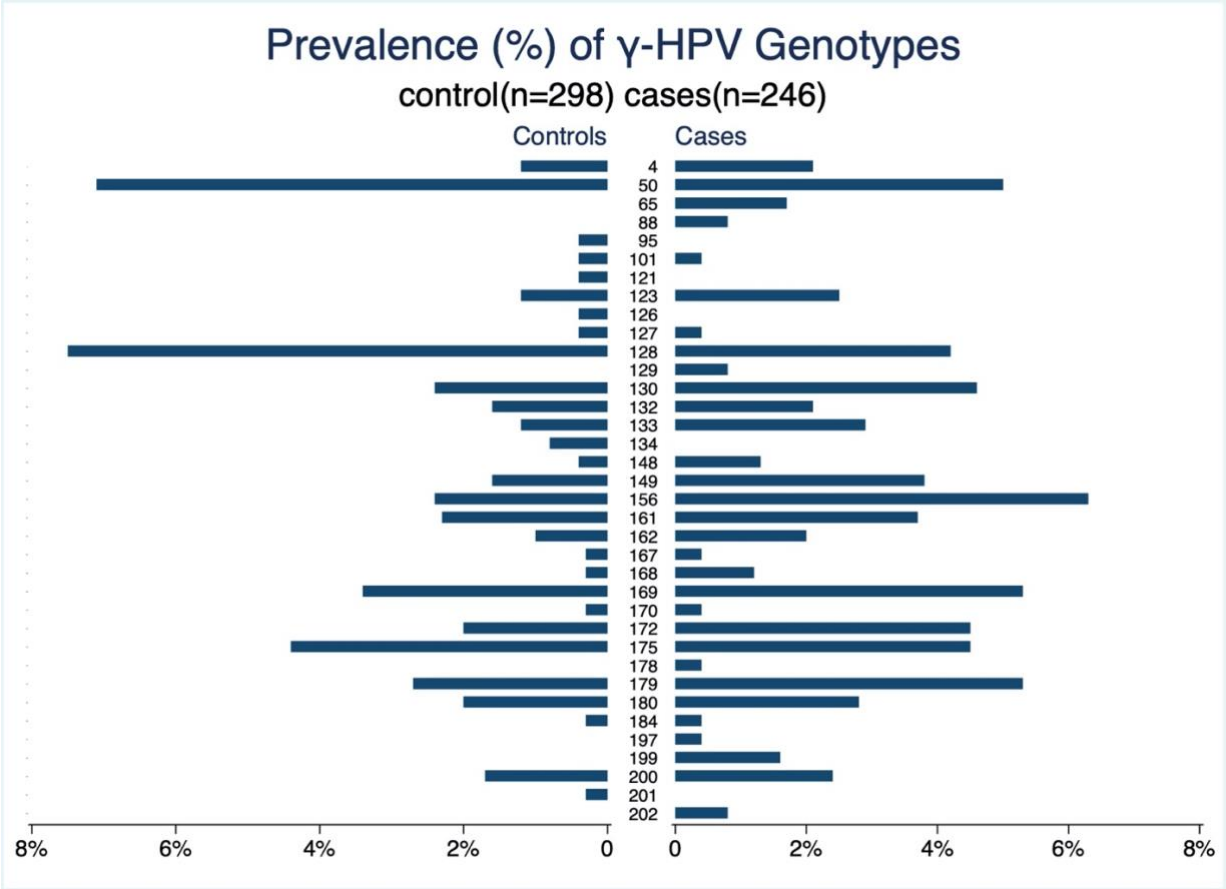
Figure S4 Prevalence of α-HPV among cases and controls in full sample tested for alpha



Appendix Figure 4.5 Prevalence of β-HPV among cases and controls in full sample tested for beta.



Appendix Figure 4.6 Prevalence of γ-HPV among cases and controls in full sample tested for Gamma.



CHAPTER 5: MANUSCRIPT II— ON THE EFFECT OF HPV GENERA ON HNC

5.1 Preface Manuscript II

The effect of α-HPV, especially that of HPV16, on HNC has been established through several animal and human studies. However, the information on the role of cutaneous HPV (β- and γ-HPV) is still scarce. Recently a single epidemiologic study showed that cutaneous HPV infection could be associated with HNC, but there are limitations regarding sample size and use of only mouthwash samples to measure HPV infection. In this study, we investigate the effect of HPV genera frequently detected in the oral cavity on HNC and address limitations of previous work. We use one of the largest case-control studies on HNC in Canada in which oral HPV was measured using mouthwash, brush, and tumor samples. Comparing HPV measurement between oral call samples and tumor samples is useful in understanding if these genera have a direct role in carcinogenesis.

In this article, we aim to estimate the conditional effect of cutaneous HPV, adjusted for HPV16 and confounding, on HNC. Further, we use quantitative bias analysis to show that the estimated relation between HPV16, the most frequently detected genotype in tumor samples, and HNC could be underestimated in the literature due to systemic biases (information bias, selection bias, and confounding).

Title: Do cutaneous human papillomavirus genotypes affect head and neck cancer? Evidence and bias-correction from a case-control study

Published in *Cancer Epidemiology*

https://doi.org/10.1016/j.canep.2022.102205

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5.2 Abstract

Background: Three genera of human papillomavirus (HPV) infect the oral cavity and oropharynx— alpha (α), beta (β) and gamma (γ). While α-HPV infection is an established risk factor for head and neck cancers (HNC), the role of other genera remains unclear. We aimed to estimate the effect of α-, β-, γ-HPV on HNC using a hospital-based case-control study.

Methods: We recruited incident HNC cases (396) and controls (439), frequency-matched by age and sex from four main referral hospitals in Montreal, Canada. We collected information on sociodemographic and behavior characteristics using in-person interviews, and tested rinse, brush and tumor specimens for HPV genotypes. We estimated adjusted odds ratios (aOR) and 95% confidence intervals (CI) for the effect of HPV on HNC using logistic regression, adjusting for confounding. We conducted probabilistic bias analysis to account for potential exposure misclassification, selection bias, and residual confounding.

Results: α-HPV genus had a strong effect on HNC, particularly HPV16 (aOR=22.6; 95% CI: 10.8, 47.2). β-HPV was more common among controls (aOR=0.80; 95% 0.57, 1.11). After adjustment for HPV16, we found weaker evidence for γ-HPV (aOR= 1.29; 95% CI: 0.80, 2.08). Combined bias analyses for HPV16 increased the strength of the point estimate, but added imprecision (aOR=54.2, 95%CI: 10.7, 385.9).

Conclusions: α-HPV, especially HPV16, appears to increase the risk for HNC, while there is little evidence for an effect of β- or γ-HPV. β-HPV may have a preventive effect, while γ-HPV may increase the risk of HNC, although to a lesser extent than that of α-HPV. Results for cutaneous HPV were imprecise and less conclusive. Due to possible epidemiologic biases, the true relation between HPV and HNC could be underestimated in the literature. Further improvement in current methods and more studies of the biologic mechanisms of the three genera in HNC development are warranted.

Keywords: Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Head and Neck Neoplasms, Epidemiologic Biases

5.3 Introduction

Over 90% of head and neck cancers (HNC) are squamous cell carcinomas that affect the oral cavity, lips, pharynx, or larynx (1). HNC is often diagnosed at late stages, has high morbidity and mortality (2), and one of the highest suicide rates of all cancers (3,4). Following many years of decline, the incidence of HNC has increased in recent decades in North America with an estimated annual percentage increase of 1.2% in Canada (5,6), and 0.8% in the United States (7). The rising incidence is mainly affecting the oropharynx and is driven by human papillomavirus (HPV) infection (8). Indeed, HPV-related HNC are now the most common cancers associated with these viruses in Canada (9).

Over 200 genotypes of HPVs have been discovered to date which are divided into five genera. Information on classification and biology of these viruses can be found elsewhere (10,11), and an up-to-date database of these viruses can be found in the International Human Papillomavirus Reference Center (12). Three of these HPV genera affect the oral cavity: alpha (α), beta (β), and gamma (γ) (13). It is worth noting that β- and γ-HPV genera have been often described as cutaneous HPV because they were first isolated from the skin, whereas α-HPV are known as mucosal for being isolated in cervical samples (11). Oral HPV infection by the α-genus, specifically HPV 16, has been responsible for the increase in HNC incidence (8,14). However, the frequent detection of β and γ HPV genera in oral samples and their presence in head and neck papillomas (13) raises questions about the potential etiological role in HNC. Yet, evidence on the effects of β and γ HPV on HNC is limited. In particular, the characteristics of the study population (e.g., age,

sex, ethnicity, behavior) and the methods used for oral sample collection, processing, and HPV detection (15) may affect the prevalence and effects of HPVs.

Agalliu et al., used a nested case-control design and found associations between oral infection by α-, β-, and γ-HPV and the development of HNC (16). However, this study sampled only 132 HNC cases and 396 controls (nested within 2 prospective cohorts) and used only mouthwash samples to detect oral HPV infection. Additional studies using a larger sample size, conducted in different populations and using multiple methods of sampling HPV are required to better characterize the role of β and γ HPVs in HNC.

Therefore, we aimed to estimate the effect of α-, β-, and γ- HPVs on HNC risk using oral samples from a large hospital case control study. We also investigated the presence of HPV infection according to α-, β-, and γ- HPVs in a subsample of participant cases (n=121) for whom tumors were available and evaluated concordance with oral HPV. Lastly, we conducted sensitivity analyses to test the robustness of our findings for possible sources of bias including HPV misclassification, selection bias, and residual confounding.

5.4 Methods

5.4.1 Data Source and Study Population

Data come from the Head and Neck Cancer Life study (HeNCe), which is a multi-country, multi-center, hospital-based case-control study, initiated to investigate the etiology of HNC. The study

was conducted in Canada, India and Brazil, but because no HPV viruses were detected in samples from India (17), and HPV was not measured for samples in Brazil, we used data from Canada only.

Data collection in HeNCe has been described previously (18,19). Briefly, data were collected from 2005 to 2013 at four main referral hospitals in Montreal, Quebec. To be included in the study, participants had to be: 1) born in Canada; 2) speak either English or French; 3) be at least 18 years old; 4) have no history of cancer, 5) have no immunosuppressive condition or mental disorder; and 6) live within 150 km of the hospital they were diagnosed and treated in.

5.4.2 Cases Assessment

Cases (n=396) were identified by the research team in hospital tumor board meetings and included lesions of anatomical sites in the oral cavity, tonsils, pharynx, hypopharynx, and larynx. Cases were included only if HNC was the first diagnosed cancer and patients had not been treated. Eligible cases were newly diagnosed and histologically confirmed (stage I to IV) squamous cell carcinomas of the upper aerodigestive tract. Case selection was based on relevant International Codes of Diseases (ICD-10), listed in the appendix.

5.4.3 Controls Selection

Hospital controls (n=439) were frequency matched to cases by sex and age within five-years. To mitigate the possibility of selection bias and Berkson's bias (20), controls were selected from outpatient clinics of diseases considered to be unrelated to traditional risk factors of cancer like tobacco or alcohol (e.g., neurology, urology, orthopedics, etc.). The participation of controls from

each outpatient clinic was restricted to fewer than 20% to limit overrepresentation of a single group. The overall sex- and age-specific distribution of smoking and alcohol—two strong predictors of HNC—among controls was not meaningfully different from the population of the province of Quebec (21).

5.4.4 HPV Measurement

Cases and controls provided two oral samples— mouthrinse and brushed exfoliated cells. Participants rinsed their mouth and gargled for 15-30 seconds with an alcohol-based solution and spit into a pre-labeled collection vial. To collect exfoliated cells, participants brushed their oral cavity using an OralCDx® brush which was inserted into a PreservCyt® buffer bottle and saved until laboratory processing . HPV testing has been explained in detail in previous publications (18,19). Briefly, DNA was extracted and purified using MasterPure™, and then tested for β-Globin. Those testing positive for β-Globin were considered adequate for polymerase chain reaction (PCR) with PGMY primers and the Linear array (22). FAP PCR with consensus primers have been used to analyze cutaneous HPV (beta- and gamma). Further details on specimen processing and HPV DNA amplification and typing are in the Appendix.

Among those with oral samples, we analyzed 818 participants (389 cases and 429 controls) for α-HPV, 824 participants (391 cases and 433 controls) for β-HPV, and 544 participants for γ-HPV (246 cases and 298 controls). Fewer samples were analyzed for γ-HPV due to a shortage of PCR reagents in laboratory. To avoid data sparsity, we did not create exposures at the species level

for γ-HPV. We were able to retrieve 121 cases with tumor samples preserved in Paraffin wax from hospital archives. HPV DNA was isolated and amplified using GP5+/GP6+ primer system.

5.4.5 HPV Positivity Definition

We considered individuals as HPV positive in the oral sample if they tested positive for an HPV genotype (for example HPV16) in either the brush or mouthrinse samples. If both rinse and brush biopsies were HPV negative for a specific genotype, then a participant was considered HPV negative for that genotype. We categorized as HPV-negative if no genotype was found in both rinse and brush biopsies. We created variables at the genus and species levels. For example, for any α-HPV, a participant takes the value of 1 (any α-HPV =1) if they tested positive for any genotype from the α-HPV genus in either oral sample. Otherwise, the variable takes a value of 0 (any α-HPV=0). The α-9 species contains several genotypes that are high-risk (16, 31, 33, 35, 52, 58, 67), in addition to estimating the effect on HNC for testing positive with HPV16, the most oncogenic genotype, we estimate the effect for testing positive for any other genotype in the α-9 species. Up to date classification of HPV can be found at the International Human Papilloma Reference Center(12).

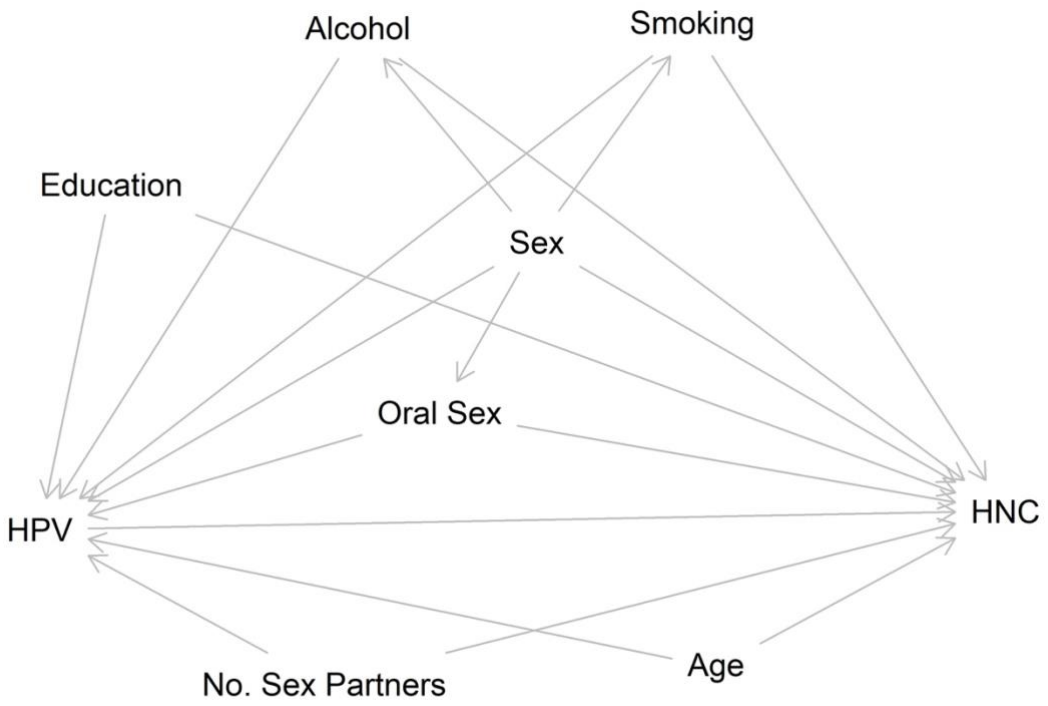
5.4.6 Confounding

Confounders to include in models were identified through a directed acyclic graph (DAG) (23,24) and are a priori known common risk factors of both exposure (HPV) and outcome (HNC) (19,21,25). We also included predictors of the outcome in regression models to improve precision (26) (figure 1). The final adjustment set included: sex, age, age when oral sex was first practiced,

life-time number of sexual partners, total education years, life-time smoking, life-time alcohol consumption. For models estimating the effect of β-HPV and γ-HPV, the adjustment set also included HPV16, the most common high-risk genotype among cases, to control for the possible effect of that genotype. All covariates were self-reported. Categorical variables included age of initial oral sex practice (never, >30 years, 17-30 years, ≤16 years) and number of sexual partners (0-3, 3-7, 7-13, >13). Age (years), education (years), smoking (pack-years), and alcohol drinking (ethanol-liters) were continuous variables.

As described in previous HeNCe publications (19), life-time exposure to smoking and alcohol are measured in pack-years and ethanol-liters, respectively. We modeled smoking (pack-years) and drinking (ethanol-liters) using restricted cubic splines to account for non-linearity. We considered 3-5 knots, as recommended by Harrel (27), then decided on using four knots as it provided the best fit.

Figure 5.1 Causal diagram of the relation between HPV and HNC and confounding variables



* Created with DAGitty package (version 0.3-1) in R (version 4.1.1).

5.4.7 Statistical Analysis

We used unconditional logistic regression to obtain adjusted odds ratios (OR) and 95% confidence intervals (CI) to estimate the effect of different genera and species of HPV and HNC. Stata/MP 16.1 was used for all analyses (StataCorp, College Station, TX), and figure 1 was created with DAGitty package (version 0.3-1) in R (version 4.1.1). Analysis code can be found on Open Science Framework (https://osf.io/dxc3b/?view_only=b4fd98a1c81d4d8084497715196792d3).

5.4.8 Quantitative Bias Analyses

Confounding, exposure misclassification, and selection bias threaten the validity of epidemiologic studies. To assess the sensitivity of our estimates to bias, we used probabilistic bias analyses (28,29). Because bias parameters (e.g., degree of misclassification) are not generally known with certainty, we used probability distributions for these parameters and Monte Carlo simulations to obtain bias-corrected estimates. All bias analyses were conducted for with HPV16 as the exposure variable since it is the most oncogenic genotype reported in the literature, and the most frequently found genotype in tumor samples of this study (n=43; 83%).

5.4.8.1 Measurement Error (HPV misclassification)

Measuring HPV using oral samples could have resulted in measurement error (15,30). We calculated a kappa statistic and 95% CI for agreement between oral and tumor measurement of HPV. Then, taking the measurement in tumor samples as the gold standard, we calculated sensitivity (Se) and specificity (Sp) for HPV measurement in oral samples. We applied probabilistic sensitivity analysis with prior triangular probability distributions for sensitivity and specificity (29) to get bias corrected odds ratios for the relation between HPV16 and HNC.

5.4.8.2 Selection Bias

Both disease status and exposure status must affect participation in a case-control study to generate selection bias(20). Cases affect selection by design, but exposure to HPV was not known to study participants when they enrolled in the study and controls were selected from clinics treating conditions unrelated to HPV. Both these factors make selection bias unlikely. However,

because controls in HeNCe were not randomly sampled from the source population, there is a possibility that controls could be under- or over-representative of HPV distribution in the source population. There are no studies on the prevalence of HPV16 in Canada, so we make the assumption that it is similar to that in the United States, which is 1.0% as reported by Gillison et al. (31). Because HPV16 prevalence among controls is 2.3% in our study, we conducted a sensitivity analysis under the assumption that HPV exposure is overrepresented in controls. And because the selection bias factor (28,29) should be less than 1.0 if exposure is overrepresented, we used a lognormal distribution with 95% prior probability of the bias factor falling between 1.0 and 0.45.

5.4.8.3 Uncontrolled Confounding

Residual confounding occurs if a confounder is missing from the adjustment set. We used poor oral health as an example of a plausible missing confounder. Poor oral health is known to be associated with the outcome (HNC) (32,33), and is also associated with the exposure oral HPV (34). To quantify the potential for residual confounding, we estimated the prevalence of poor oral health as 46% and 34% among HPV- exposed and unexposed, respectively, based on Bui et al.(34). Depending on how poor oral hygiene is measured (number of teeth, frequency of tooth brushing, etc.), the literature reports the odds ratio of the association between poor oral health and HNC in the range of 1.5 – 3.5 (32,33). Therefore, we used a probabilistic sensitivity analysis with triangular distribution for the prevalence of confounder by exposure levels and a lognormal distribution for the odds ratio of the association between confounder and the outcome.

Finally, we adjusted for all three biases in the following order: exposure misclassification, selection bias, confounding. Further details on the methods and bias parameters are provided in the appendix.

5.5 Results

Table 1 provides an overview of the HeNCe study population who were tested for HPV and included in the analysis. Controls generally had higher education and lower consumption of tobacco and alcohol relative to cases. The proportion of participants who reported ever practicing oral sex was nearly 10 percentage points higher among cases. Of all cases in HeNCe, over half (60%) were diagnosed in late stages (III and IV).

Table 1. Distribution of sociodemographic and behavior characteristics among cases and controls

	Controls (n=439)	Cases (n=396)
Age (years)		
Median (Q1, Q3)	61.0 (54.0, 69.0)	61.0 (55.0, 69.0)
Education years		
Median (Q1, Q3)	14.0 (11.0, 17.0)	12.0 (10.0, 14.0)
Smoking (pack-years) [a]		
Median (Q1, Q3)	25.0 (10.4, 45.3)	41.9 (22.3, 59.5)
Alcohol (ethanol-liter) [a]		
Median (Q1, Q3)	187.6 (58.4, 449.8)	301.7 (91.1, 831.5)
Sex		
Female	135 (30.8%)	101 (25.5%)
Male	304 (69.2%)	295 (74.5%)
Smoker		
Never	112 (25.5%)	67 (16.9%)
Ever	327 (74.5%)	327 (82.6%)
Drinker		
Never	78 (17.8%)	64 (16.2%)
Ever	361 (82.2%)	330 (83.3%)
Lifetime number of sexual partners		
0-3	128 (29.2%)	94 (23.7%)
3-7	138 (31.4%)	123 (31.1%)

7-13	61 (13.9%)	69 (17.4%)
>13	97 (22.1%)	95 (24.0%)
Missing	15 (3.4%)	15 (3.8%)
Ever practiced oral sex		
No	97 (22.1%)	52 (13.1%)
Yes	332 (75.6%)	332 (83.8%)
Missing	10 (2.3%)	12 (3.0%)
Tumor site ICD-10 code		
Oropharynx		190 (48.0%)
Larynx		131 (33.1%)
Oral cavity		75 (18.9%)
TNM Stage		
Stage 0		17 (4.3%)
Stage I		74 (18.7%)
Stage II		64 (16.2%)
Stage III		54 (13.6%)
Stage IV		182 (46.0%)
Unknown		5 (1.3%)
[a]Among ever users		

Table 2 shows numbers and percentages of participants testing positive for HPV as well as crude and confounder-adjusted odds ratios between HPV and HNC. The crude odds of testing positive for HPV16 were 16 times greater among HNC cases relative to controls, which increased to nearly 23 times greater after adjustment (aOR=22.6, 95% CI= 10.8, 47.2). The odds of any alpha-HPV were also 4.6 times higher among cases (95% CI= 3.1, 6.8). The odds of infection with α-9 genotypes other than HPV16 (i.e., HPV 31,33, 35, 52,58, 67) were roughly 3 times greater among cases relative to controls (aOR=2.8, 95% CI= 1.2, 6.2). However, the odds of infection with any β genotype (consistent across species) were roughly 20% lower among cases than controls. HNC cases had greater odds of infection with any γ-HPV, but the crude estimate was attenuated after adjustment for confounding (1.57 vs 1.29).

Table 2. Unadjusted and adjusted Relation between HPV and HNC

Exposure variable	Controls		Cases		Crude model	Adjusted model[a]
	N	%	N	%	OR (95% CI)	OR (95% CI)
HPV16	10	2.3%	106	27.3%	15.70 (8.07, 30.54)	22.59 (10.83, 47.17)
Any α-9 other than HPV16	9	2.1%	29	7.5%	3.76 (1.76, 8.05)	2.76 (1.23, 6.17)
Any α HPV	61	14.2%	163	41.9%	4.35 (3.10, 6.10)	4.62 (3.14, 6.79)
Any β-1	153	35.3%	123	31.5%	0.84 (0.63, 1.12)	0.82 (0.57, 1.17)
Any β-2	199	46.0%	162	41.4%	0.83 (0.63, 1.10)	0.78 (0.56, 1.09)
Any β-3	91	21.0%	67	17.1%	0.78 (0.55, 1.10)	0.59 (0.38, 0.92)
Any β HPV	242	55.9%	196	50.1%	0.79 (0.60, 1.04)	0.80 (0.57, 1.11)
Any γ-HPV	72	24.2%	82	33.3%	1.57 (1.08, 2.28)	1.29 (0.80, 2.08)
Any β or γHPV	251	58.0%	221	56.5%	0.92(0.72, 1.24)	0.90 (0.65, 1.25)

[a]Adjusted models control for age, sex, education, oral sex, lifetime number of sexual partners, smoking, and alcohol drinking; Adjusted models for Beta and Gamma HPV also adjust for HPV16 infection

5.5.1 HPV Measurement Agreement and Sensitivity Analysis Results

We compared HPV measurement agreement between oral samples and tumors using a sample of 121 HNC cases. Only 52 cases (43%) contained HPV viruses in tumors, which were all from the α genus. The kappa statistic for HPV16 measurement using oral samples and tumor samples was 0.53 (95% CI 0.36, 0.70), which is considered to be fair agreement beyond chance(35). HPV16 measurement in oral samples, compared to gold standard of tumor measurement, had 62% sensitivity (95% CI: 45%- 77%) and 89% specificity (95% CI: 80%- 95%).

Results for probabilistic bias analysis are summarized in table 3 and graphically in the Appendix. For exposure misclassification, the median bias-corrected OR for systemic and random error of the relationship between HPV16 and HNC was 42.7 with 95% simulation limits between 8.5 and 341. Selection bias correction inflated the observed odds ratio but to a lesser extent than exposure misclassification. However, correction for residual confounding moved the point estimate towards the null (table 3). When we adjusted for all systematic biases (misclassification of the exposure, then selection bias, then uncontrolled confounders) as well as for random error, the OR was 54.2 with 95% of simulations between 10.7 and 385.9.

Table 3. Correction for OR of HPV16-HNC association

Error corrected	Corrected for systemic error OR (95% simulation interval)	Corrected for systemic and random error OR (95% simulation interval)
Exposure misclassification	40.46 (10.91, 333.37)	42.68 (8.51, 340.97)
Selection bias	23.21 (15.60, 34.37)	23.08 (10.72, 49.85)
Residual confounding	13.56 (10.38, 17.35)	13.52 (6.56, 27.20)
Combined correction	53.37 (13.07, 319.58)	54.20 (10.70, 385.89)

5.6 Discussion

Our findings show that HNC cases were more likely than controls to be exposed to HPV16, any α-HPV, and any α-9 species other than HPV16. Consistent with what has been reported in the literature, the strength of effect was highest for HPV16, which remained strong after adjustment for confounding. Unlike the findings of Agalliu et al. who reported an increased risk of HNC with β-HPV infection, we found evidence consistent with lower odds of β-HPV infection among HNC cases, though a positive effect cannot be ruled out. The effect of infection with any β-3 species demonstrated the strongest evidence of a protective effect. The adjusted odds of γ-HPV were higher among HNC cases but estimated with imprecision. Given the small sample size and the sensitivity of these parameters to possible biases, we refrain from making definitive conclusions about the effect γ-HPV.

Our bias correction findings show that the relation between HPV16 on HNC may be underestimated in the literature if systematic biases are neglected or not accounted for by design

or in analysis. Selection bias, measurement error, and unmeasured confounding could theoretically happen in HNC studies. It is, therefore, recommended that better measurement methods be created(15,36,37), and more attention be given to study design to capture life-trajectory of HPV infection. Given that virtually all cervical cancers are caused by HPV infection, it should be no surprise that HPV infection could play a bigger role than initially thought in HNC development.

Our study has some limitations. Because we are using a hospital-based case-control design in which outcome and exposure are measured at the same time we cannot make conclusions about time individuals have been infected, or whether long-term infection raises the probability of HNC. Also, the smaller size of subgroups precluded providing conditional estimates for the effect of specific β- and γ-HPV genotypes without running into sparse data problems. Another limitation is that the bias correction analysis depends on bias parameters used, which we obtained from published articles and could be inaccurate. The small sample size also resulted in imprecise bias-corrected estimates and prevented us from obtaining estimates for subsites of HNC.

Our study also has strengths, however. This is one of the largest case-control studies in HNC in Canada, and the first to measure multiple HPV genera in oral and tumor samples. Also, the fact that life-time exposure of smoking and alcohol was measured using a life-grid technique decreased the possibility of recall bias (38). We also showed how much possible sources of bias might alter our estimates.

Little prior work has compared HPV measurement for all three major HPV genera in oral and tumor samples. The fact that we detected no β- or γ-HPV in tumors raises questions about the biologic mechanism for infection and the way different genera interact to initiate carcinogenesis. β- and γ-HPV genera have the potential for association without integration of virus in cancerous cells (as is observed with α-HPV) (39,40). Most HPV infections spontaneously clear within 1-2 years or can go into latency and become indetectable, whereas persistent and active infections can promote HNC carcinogenesis (41). A possible hypothesis that warrants further investigation is that non α-HPV, while not directly detected in tumor samples, could play a role in promoting HPV co-infection with high-risk genotypes like HPV-16 to become persistent. An alternative hypothesis is that β- or γ-HPV proteins in infected epithelial cells inhibit DNA damage repair mechanisms and apoptosis, which results in genomic instability and ultimately cancer (40).

Although previous studies (42,43) have linked beta viruses with skin cancer, tumors samples did not reveal viral DNA (42). This suggests that interaction between these viruses and UV light may play a role in carcinogenesis by inhibiting repair of UV-induced cell damages(44). The absence of cutaneous HPV from established tumor lesions could indicate that beta-HPV genera play a carcinogenic role during early stages of cancer, after which viral DNA becomes lost as the phenotype becomes evident(42). Such hypothesis, although not confirmed, could explain why tumor samples in our study contained no beta or gamma genotypes.

Results from this study are in alignment with finding reported by Agalliu et al. for α- and γ- HPV—both these genera were found to increase the risk of HNC. However, whereas Agalliu et al.

showed increase in the risk of HNC for β-HPV, our results were more consistent with a protective effect. This discrepancy could be explained by difference in populations, timing of HPV measurement (before vs after cancer development), sample size differences, or even HPV genotyping methods.

Our results also show that agreement between measurement of HPV using oral samples and tumor samples is only fair to good with high probability of measurement error. There is no gold standard for HPV16 measurement, and this opens the door for future research on improved methods to correct for misclassification of HPV in HNC studies. We also find that effects of HPV16 could be markedly underestimated in epidemiologic studies due to non-sampling biases.

To conclude, while we confirm the already known carcinogenic effects of α-HPV infection, especially that of HPV16, we find weak evidence of a protective effect for β-HPV and slightly harmful effect of γ-HPV. However, results were imprecise and warrant further investigation to reach conclusive evidence on the role of cutaneous HPV in HNC. Further, investigators need to pay close attention to systemic sources of bias which can greatly influence the estimates of the relation between HPV and HNC.

5.7 Acknowledgements

The authors would like to acknowledge and thank all the investigators, colleagues, and participants in HeNCe life study. We are especially grateful to Dr. Claudie Laprise for thoughtful discussions on this study, Dr. François Coutlée for assistance with HPV genotyping in his lab at

Centre hospitalier de l'Université de Montréal (CHUM), and Dr. Eduardo Franco for comments on an earlier draft of this paper.

Funding:

This work was supported by grants from the Canadian Institutes of Health Research (CIHR) [MOP 81172, MOP111207]; Fonds de recherche du Québec – Santé (FRQ-S), Ministère du Développement économique, de l'Innovation et de l'Exportationdu Québec: Programme de soutien à la recherche (PSR), volet: Soutien à des initiatives internationales de recherche et d'innovation (SIIRI).

Data availability:

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study and institutional restrictions.

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5.9 Appendix — Manuscript II

Do cutaneous human papillomavirus genotypes affect head and neck cancer? Evidence and bias-correction from a case-control study

1- ICD-10 codes for cancer lesions used in HeNCe:

Cases in HeNCe were identified using these codes:

Oral cavity	C02-C06 except C02.4, C05.01, C05.2
Oropharynx	C01, C02.4, C05.01, C05.2, C09, C10, C12 and C14
Larynx and hypopharynx	C13 and C32

Note: nasopharynx and esophagus cancers were not included due to their different etiologies.

2- Comparison of sample tested for all HPV genera (α, β, γ) and those tested for either α, β, or both, but not γ-HPV.

	Not tested for all genera α and/or β (not γ tested) (N = 299)	Tested for all HPV genera α+ β+γ (N = 536)	p-value
Age (years)			0.071
Mean (SD)	62.25 (10.31)	60.86 (10.79)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Education years			0.056
Mean (SD)	12.65 (4.36)	13.23 (4.14)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Smoking (pack-years)			0.291
Mean (SD)	34.17 (43.59)	30.92 (42.01)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Alcohol Ethanol-Liter			0.457
Mean (SD)	474.60 (1015.72)	538.29 (1271.08)	
N (% Missing)	299 (0.0%)	536 (0.0%)	
Sex			0.809
Female	83 (27.8%)	153 (28.5%)	

Male	216 (72.2%)	383 (71.5%)	
Smoker			0.865
Never	65 (21.7%)	114 (21.3%)	
Ever	233 (77.9%)	421 (78.5%)	
Missing	1 (0.3%)	1 (0.2%)	
Drinker			0.050
Never	61 (20.4%)	81 (15.1%)	
Ever	237 (79.3%)	454 (84.7%)	
Missing	1 (0.3%)	1 (0.2%)	
Lifetime number of sexual partners			0.903
0-3	85 (28.4%)	137 (25.6%)	
3-7	95 (31.8%)	166 (31.0%)	
7-13	49 (16.4%)	81 (15.1%)	
>13	67 (22.4%)	125 (23.3%)	
Missing	3 (1.0%)	27 (5.0%)	
Ever practiced oral sex			0.806
No	55 (18.4%)	94 (17.5%)	
Yes	238 (79.6%)	426 (79.5%)	
Missing	6 (2.0%)	16 (3.0%)	

Note: Table is intended to show that there are minimal differences between the sample tested for all genera and that who for logistical and cost reasons were tested for one or two genera. While sample to be tested for all genera was not selected completely at random, the table shows that covariate distribution was similar.

3- Biological samples collection:

HPV occupies the basal layer of the epithelium, so we aimed to obtain a sample from that layer. As described in the manuscript, samples were collected in mouthrinse and brush biopsies from cases and controls, and tumor samples for a subsample of cases. Collection of oral specimens was done by participants under the supervision and instruction of the interviewer. First, participants rinsed with PreservCyt solution their mouths for 15-30 seconds, then spit in a 60 ml wide mouth vial. Second, to collect brush biopsies, participants used 2 x OralCDx® brushes (OralScan Laboratories, NJ). Participants were instructed to apply 5-10 gentle strokes in the left and right buccal mucosa, lateral edges of the tongue, and left and right tonsils. Unless very painful

or uncomfortable, cancer patients were also asked to brush the lesion site while trying to avoid necrotic areas. For cases tumor samples, we retrieved formalin-fixed and paraffin-embedded tissue block samples from hospital archives for 121 cases. Hospital pathologists prepared samples and histologically evaluated to ensure they contained a section of the tumor of interest. All samples were sent to and processed at the Department of Microbiology-Infectiology, Notre-Dame Hospital, University of Montreal Hospital Centre (CHUM). Extracted DNS was kept frozen until tested with PCR.

4- HPV DNA genotyping

In order to check DNA integrity, we performed beta-globin testing on 10 µl of extracted DNA using primers PC04 and GH20 and gel electrophoresis. PCR using PGMY09-PGMY11 primers and typing was done with the Linear array for the alpha HPV genotypes: HPV6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, 89(1). Beta and gamma HPV genotypes were detected in oral samples and biopsies using the type-specific multiplex genotyping (TS-MPG) assay developed by the IARC, Lyon, France, which combines multiplex PCR with a bead-based Luminex technology(2,3). Consensus primers amplify a broad spectrum of skin HPV and has been used in previous studies by the lab group at CHUM(4). Technical details and microbiology lab procedures of FAP PCR can be found in other publications.

5- Bias Analysis:

This is a brief explanation of the bias analysis methods used in the paper, but more technical details can be found in specialized resources(5,6). A more accessible introduction to the topic can be found in the article by Lash et al.(7) which includes references to applications in many popular software packages.

Biases can threaten the internal and external validity of a given study leading to incorrect estimates. Systematic biases cannot be solved by using larger sample size, and unless we account for these biases, conclusions based on findings could be incorrect. Repeating studies with larger samples while maintaining systemic biases will increase precision but not accuracy of estimates. We make the distinction between confounding bias, selection bias, and misclassification bias (also called measurement error of a binary exposure). Definitions can be found in Porta's *A Dictionary of Epidemiology*(8).

To adjust for an unmeasured confounder, we need to make assumptions about two parameters: the distribution of the confounder in the population (p_0, p_1) and the confounder-outcome association in the absence of the exposure RR_{CD}. Using the equation below, we get a deterministic value for the confounding-adjusted relative risk:

$$RR_{adj} = RR_{obs}\frac{RR_{CD}p_0 + (1 - p_0)}{RR_{CD}p_1 + (1 - p_1)}$$

In our analysis, we used a probabilistic analysis in which we assumed the parameters are coming from a known distribution with and assigned:

- Pr(C=1| E=0) triangular distribution (min=0.20, mode=0.35, max=0.50).
- Pr(C=1| E=1) triangular distribution (min=0.30, mode=0.45, max=0.60).
- RR_{CD} log normal distribution (mean= 1.522, SD= 0.216)

To adjust for selection bias, we divide the observed odds ratios by the selection bias factor (SBF), which as shown in Modern Epidemiology, 4th edition, Page 730:

$$SBF = \frac{Sa_1 Sb_0}{Sa_0 Sb_1}$$

Where:

$Sa_0 =$ proportion of unexposed cases selected

$Sa_1 =$ proportion of exposed cases selected

$Sb_0 =$ proportion of unexposed controls selected

$Sb_1 =$ proportion of exposed controls selected

Assuming that selection probabilities for exposed and unexposed cases are equal, we looked at possible selection bias that could result when $Sb_0 > Sb_1$. i.e., when exposed controls are overrepresented. In our probabilistic analysis, we used a lognormal distribution for the selection bias (mean=-.392, SD=0.20).

To adjust for exposure misclassification, the sensitivity (Se) and specificity (SP) among cases and controls are required. We used the following parameters and a probability distribution:

Se among cases: triangular distribution (min 0.40, mode=0.6, max=0.8)

Sp among cases: triangular distribution (min 0.70, mode=0.90, max=1.0)

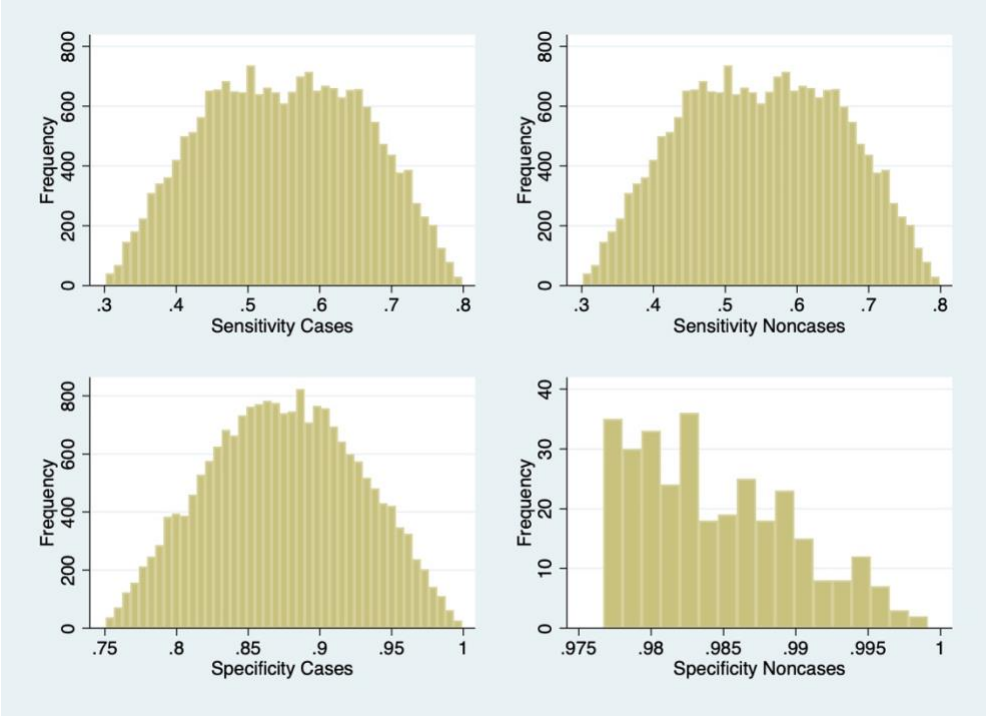
Se among noncases: triangular distribution (min 0.40, mode=0.6, max=0.8)

Sp among noncases: triangular distribution (min 0.70, mode=0.90, max=1.0)

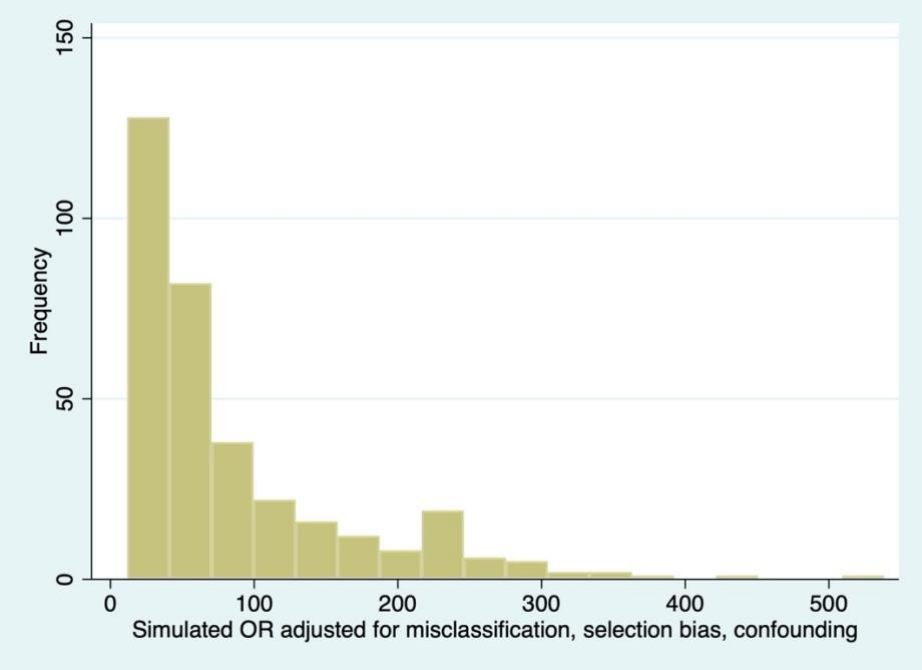
We also assumed the correlation between case and noncases sensitivities to be 0.8, and correlation between case and noncases specificities to be 0.8.

For combined bias analysis, we corrected for all biases in the opposite order they occur in nature: misclassification, selection bias, then confounding as explained above.

6- Simulation Graphs:



Appendix Figure 5.1 This diagram shows values of sensitivity and specificity that are compatible with the data. Only high values of specificity among non-cases are compatible, which indicates differential misclassification is not impossible in studies with HPV measurement using mouthrinse or brush samples.



Appendix Figure 5.2 This figure shows frequency of simulated OR after adjustment for all three sources of bias. We ran code with 20,000 Monte Carlo simulations.

Appendix References

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CHAPTER 6: MANUSCRIPT III— ON THE INTERACTION BETWEEN HPV GENERA

6.1 Preface Manuscript III

Results from animal studies in skin cancer literature suggest a possible interplay between cutaneous HPV from the β genus and other factors like ultra-violet exposure are needed to induce carcinogenesis process. It is not clear if cutaneous HPV (β and γ genera) are capable of causing HNC on their own or they need the presence of oncogenic genotypes from the α-HPV genus. We hypothesized that there could be an interaction between HPV of different genera and that these cutaneous HPV genotypes previously thought be benign could facilitate the role of high-risk genotypes of α-HPV like HPV16.

To address this gap in the literature, we investigated the possible interaction between infection with any genotype from β-HPV a γ-HPV, and coinfection with HPV16. Our goal was to find out if coinfection increases the risk of HNC risk compared to being infected with HPV16 alone or cutaneous HPV alone. Interactions were evaluated on the additive and multiplicative scales as interaction can be scale dependent.

Title: Interaction of HPV16 and Cutaneous HPV in Head and Neck Cancer

Submitted for Peer Review

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6.2 Abstract

Objectives: Human papillomavirus 16 (HPV16) is an established risk factor for Head and Neck Cancer (HNC). Recent reports have shown that genotypes from the beta (β) and gamma (γ) genera, also known as cutaneous HPV, can be found in the oral cavity, but their role is largely unidentified. We investigated the interaction between oral HPV16 and cutaneous HPV in HNC.

Methods: We use data on incident HNC cases (n=384) and frequency-matched hospital-based controls (n=423) from the HeNCe Life study in Montreal, Canada. Participants were tested for alpha HPV and cutaneous genera using oral mouth rinse and brush samples. We used logistic regression to obtain adjusted odds ratios (aOR) and 95% confidence interval (CI) as a measure of effect between HPV and HNC and assessed interaction between HPV genotypes on the multiplicative and additive scales.

Results: Prevalence of HPV infection was higher among cases (73%) than controls (63.4%), with cases more likely to be coinfected with more than a single genotype, 52.9% vs. 43.5%, respectively. Infection with HPV16 alone had a strong effect on HNC risk aOR=18.15 [6.19, 53.24], while infection with any cutaneous HPV, but not HPV16, appeared to have the opposite effect aOR=0.82 [0.59, 1.14]. The observed effect of joint exposure to HPV16 and any cutaneous HPV (aOR= 20.42 [8.32, 50.11]) was stronger than the expected effect based on an assumption of independent exposures but was measured with considerable imprecision. While the point

estimate suggests a positive interaction between HPV16 and cutaneous HPV, results were imprecise with relative excess risk due to interaction (RERI) = 2.44 [-23.27, 28.15].

Conclusion: There could be biologic interaction between HPV16 and genotypes from cutaneous genera, which warrants further investigation. Although cutaneous HPVs are not usually found in tumor tissues, they are cofactors that could interact with HPV16 in the oral cavity and thus strengthen the latter's carcinogenic effect.

Keywords: HPV, head and neck cancer, human papillomavirus, case-control studies

One sentence summary: there is preliminary evidence that HPV16 interacts with cutaneous HPV, which has a stronger carcinogenic effect than being infected with HPV16 alone.

6.3 Introduction

Human papillomavirus 16 (HPV16) as well as other genotypes of HPV known as high-risk genotypes are sexually transmitted viruses (1) and established causes of head and neck cancer (HNC), mainly in the oropharynx(2). A recent report in HPV-related literature by Agalliu et.al suggested that genotypes from β- and γ-HPV genera, also known as *cutaneous HPV* (as a result of being first discovered in skin warts), could also increase the risk of HNC (3). However, it is not clear if these viruses are carcinogenic *per se* or have rather a more limited role to assisting or enhancing the carcinogenic effect of known high-risk genotypes from the α-genus.

Agalliu et al. showed an increase in risk of HNC among those infected with cutaneous HPV even after adjusting for HPV16 status, suggesting an independent carcinogenic effect (3). We, however, found weak evidence for independent role of for cutaneous HPV. In another work that has been recently published (4), we found that γ-HPV could slightly increase the risk of HNC, but the magnitude of effect was small and was measured with imprecision for rare detection rate in oral samples. β-genus, on the other hand, did not show a harmful effect and was more common among controls than cases (4). Further, among genera isolated from tumor lesions we found only genotypes from the high-risk α-genus but no genotypes from the β and γ genera (5).

A possible hypothesis, often referred to as “hit and run”, which originated from animal studies of papillomaviruses and skin cancer, had been suggested for the role of cutaneous HPV (6,7). According to this hypothesis, cutaneous HPV could play a role in early stages of skin cancer by strengthening the role for high-risk genotypes, and then disappear once carcinogenesis has been

established. This explanation would be consistent with the observation that copies of β-HPV are more available in premalignant HPV than in established squamous cell carcinoma of the skin (8).

In this paper we aimed to investigate the role and possible interaction between genotypes from different HPV genera in HNC development. We measured coinfection and the potential combined effect of HPV16, the most implicated HPV genotype in HNC, and β- and γ-HPV genera. More generally, we also investigated the interaction between high-risk α-HPV genotypes, as defined by the international association for research on cancer (IARC) (2), and infection with the β- and γ-genera.

6.4 Methods

6.4.1 Study Population

We used a hospital-based case-control design from the Canada site of the multi-country head and neck cancer life study (HeNCe). Details on eligibility and study population can be found in previous publications (9–11). Briefly, incident HNC cases and hospital-based controls frequency-matched by sex and age (within five years) were recruited from four major referral hospitals in Montreal, Canada. Data collection took place from September 2005 to November 2013. The study was designed to represent the catchment area of the Montreal metro area in Quebec — i.e., people who would be treated in either of the four hospitals should they develop HNC. Controls were selected from patients with non-chronic diseases attending outpatient clinics from which the cases were recruited. Ethical approval for HeNCe was obtained from McGill University and all participating hospitals.

A total of 460 HNC cases and 458 controls were recruited into the HeNCe Canada study. Among these participants, 818 individuals (389 cases and 429 controls) were tested for the presence of α-HPV. Because of resource limitations that prevented β-HPV and γ-HPV testing of all samples, we prioritized β-HPV testing. β-HPV testing was performed on oral cell samples of 824 participants (391 cases and 433 controls), while 544 were tested for γ-HPV (246 cases and 298 controls). To test for the interaction between HPV16 or α-HPV with β-HPV, we used the data for those tested for both α- and β-HPV (384 cases and 424 controls). Likewise, to test for interaction between HPV16 or α-HPV with γ-HPV, we used data of participants tested for both genera (242 cases and 294 controls). We also investigated interaction in the sample tested for all HPV genera.

6.4.2 Data Collection

Detailed information on socio-demographic characteristics and potential confounders including lifetime history of tobacco smoking and alcohol drinking were collected using face-to-face interviews with a questionnaire employing the life-grid technique. Using an OralCDx® brush and mouthwash, epithelial cells were collected from various sites in the mouth, including the tumor site for cases, for HPV testing.

6.4.3 Oral HPV Testing

The HPV testing methodology for the HeNCe Life Study has been previously described (5). Cell suspensions were centrifuged at 13,000xg for 15 min at 22°C. Pellets were resuspended in 300 µL of 20 mmol/L Tris buffer (pH 8.3) and DNA was purified using the MasterPure™ Kit (Epicenter). Extracted DNA was kept frozen until tested with PCR at -70°C. We used several molecular techniques for testing and genotyping HPV genera. All HPV DNA samples were tested for β-globin, with positive samples considered for further HPV-genotyping and negative samples considered

inadequate for PCR. A polymerase chain reaction (PCR) assay with PGMY09-PGMY11-primers and linear array was used to test for α-HPV genotypes(12). This is capable of detecting 36 mucosal genotypes. To detect cutaneous genotypes, β- and γ-HPV, we used multiplex PCR with bead-based Luminex technology. This technique is capable of detecting 43 β-HPV genotypes and 52 γ-HPV genotypes (13).

6.4.4 Outcome Variable

The outcome of interest is incident Head and Neck Cancer (HNC). All cases were incident cancer cases, which represent first cancer a patient develops and before they receive any treatment. This precaution was taken to ensure that chemotherapy or any other treatment could not interfere with the patients' biomedical status. Cases ranged from carcinoma in situ to stage IV cancer and were all defined according to the International Classification of Diseases version 10 as follows: oral cavity cancers (OCC) (C02-C06), oropharyngeal cancer (OPC) (C01, C02.4, C05.01, C05.2, C09, C10, C13) and laryngeal cancers (LC) (C32). Cancers of the lip, nasopharyngeal cancers, and salivary glands were excluded due to their different etiologies.

6.4.5 Exposure Variables

We investigated interactions between the following combination of exposures: HPV16 vs any γ-HPV types; HPV16 vs any β-HPV types; HPV16 vs any β- or γ-HPV types; and any high-risk HPV vs any β- or γ-HPV types. In all models, we controlled for confounding for the main exposure with outcome and used the same sufficient set of confounders for both exposures which include age, sex, tobacco smoking, and alcohol use.

6.4.6 Statistical analysis

We used unconditional logistic regression analysis adjusting for confounding (age, sex, smoking, and alcohol consumption), and assumed an identical set of confounders for all genotypes, meaning that the minimum sufficient set needed to block the back door path for the first exposure is identical to the minimum sufficient set needed for the second exposure (14). We evaluated interaction on the multiplicative scale by including a cross-product (interaction term) between the two exposures of interest, which when exponentiated represents the ratio of the odds ratios (OR_11/OR_10 OR1_0). We also measured interaction on the additive scale (biologic interaction) using three indices — Relative Excess Risk due to Interaction (RERI), Attribution Proportion (AP), and Synergy Index (S) as suggested by Rothman (14,15). Each of these measures has advantages and limitations beyond the scope of this paper, and we present them all here as recommended in the literature (14,16,17).

RERI represents the difference between the observed joint effect of multiple exposures and the joint effect expected based on the individual independent effects of any two exposures under consideration, calculated as OR11 − OR01 − OR10 + 1; where a RERI of 0 indicates perfect additivity (i.e., no interaction), and a value of greater or less than 0 indicates positive or negative additive interaction, respectively (18). In addition, we estimated the AP as RERI / OR11. An AP of zero means no interaction or perfect additivity, greater than zero means positive interaction or more than additivity while less than zero means negative interaction or less than additivity. AP ranges from -1 to +1. The SI is the ratio of the combined effects and the individual effects. An SI of one means no interaction or perfect additivity, greater than one means positive interaction or more than additivity while less than one means negative interaction or less than additivity. SI

ranges from zero to infinity. Wald-type 95% confidence intervals (CI) were estimated for all individual and joint estimates with 95% CI estimated for the measures of additive interaction using the delta method (19). All interaction analyses, measures of interaction, and effect estimates were reported in two-by-two tables as recommended by Knol and VanderWeele (16). Tables were created with InteractionR package (20) in R version 4.1.1, while graphs showing adjusted predicted probability of HNC for each level of the interaction between α-HPV and each of β and γ HPV were created with Stata/MP version 16.1 (StataCorp, College Station, Texas).

6.5 Results

Table 6.1 describes the relevant characteristics of the participants tested for all genera and stratified by case-control status. By design, most of the cases and controls were men, with no difference in age in both groups – approximately 61 years of age. However, a relatively higher proportion of cases were tobacco smokers compared to controls, and the smokers among cases were heavier smokers throughout life compared to the smokers in the control group (47 pack-years vs 32 pack-years) (Table 6.1). A much higher proportion of the cases were HPV16 positive compared to controls (26.6% vs 2.4%). For cutaneous HPV, infection with any γ-HPV was more common in cases (34% vs 35%), while any β-HPV had a similar prevalence between cases and controls (59% vs 60%). Prevalence of HPV infection overall was higher among cases than controls, with cases more likely to be coinfected with more than a single genotype, respectively.

Table 6.1. Distribution of sociodemographic and behavior characteristics among cases and controls

	Controls	Cases
	N = 294	N = 241
Age (mean (SD))	60.73 (11.06)	60.91 (10.34)
20-39 years	9 (3.1)	5 (2.1)
40 – 49 years	34 (11.6)	24 (10.0)
50 -59 years	94 (32.0)	89 (36.9)
60 – 69 years	93 (31.6)	72 (29.9)
70 -79 years	51 (17.3)	44 (18.3)
80 years and above	13 (4.4)	7 (2.9)
Sex (%)		
Female	93 (31.6)	60 (24.9)
Male	201 (68.4)	181 (75.1)
Years of education (mean (SD))	13.95 (4.24)	12.37 (3.85)
Ever smoker (%)		
Ever	214 (72.8)	207 (85.9)
Never	80 (27.2)	34 (14.1)
Smoking pack-years (mean (SD))	32.32 (44.79)	46.50 (41.64)
Ever drinker		
Ever	248 (84.4)	206 (85.5)
Never	46 (15.6)	35 (14.5)
Ethanol liter-years (mean (SD))	450.32 (972.15)	855.93 (1688.93)
HPV16 (%)		
Negative	287 (97.6)	177 (73.4)
Positive	7 (2.4)	64 (26.6)
Any beta HPV (%)		
Negative	118 (40.1)	98 (40.7)
Positive	176 (59.9)	143 (59.3)

Table 6.1. Distribution of sociodemographic and behavior characteristics among cases and controls		
Any gamma HPV (%)		
Negative	222 (75.5)	159 (66.0)
Positive	72 (24.5)	82 (34.0)

As expected, infection with HPV16 in the absence of β-HPV had a strong effect on HNC OR=16.9 [6.9, 41.5], while infection with any β-HPV but no HPV16 appeared protective OR=0.73 [0.53, 1.01] (Table 6.2). The risk of HNC among those infected with both HPV16 and any β-HPV genotype is lower than it is among those exposed to HPV16 alone OR=16.9 [6.9, 41.5]. All measures of additive interaction are consistent and show a negative interaction (RERI<0, AP <0; SI<1). Figure 6.1 shows the marginal predicted probability of HNC as estimated from the logistic model with interaction between α-HPV and β-HPV. There is an increase in the probability of HNC when infected with low-risk α-HPV and an even higher probability of HNC when infected with high-risk α-HPV. Co-infection with β-HPV shows a slight reduction of risk when coinfection with high-risk genotypes (Figure 6.1), although with an overlap in confidence limits.

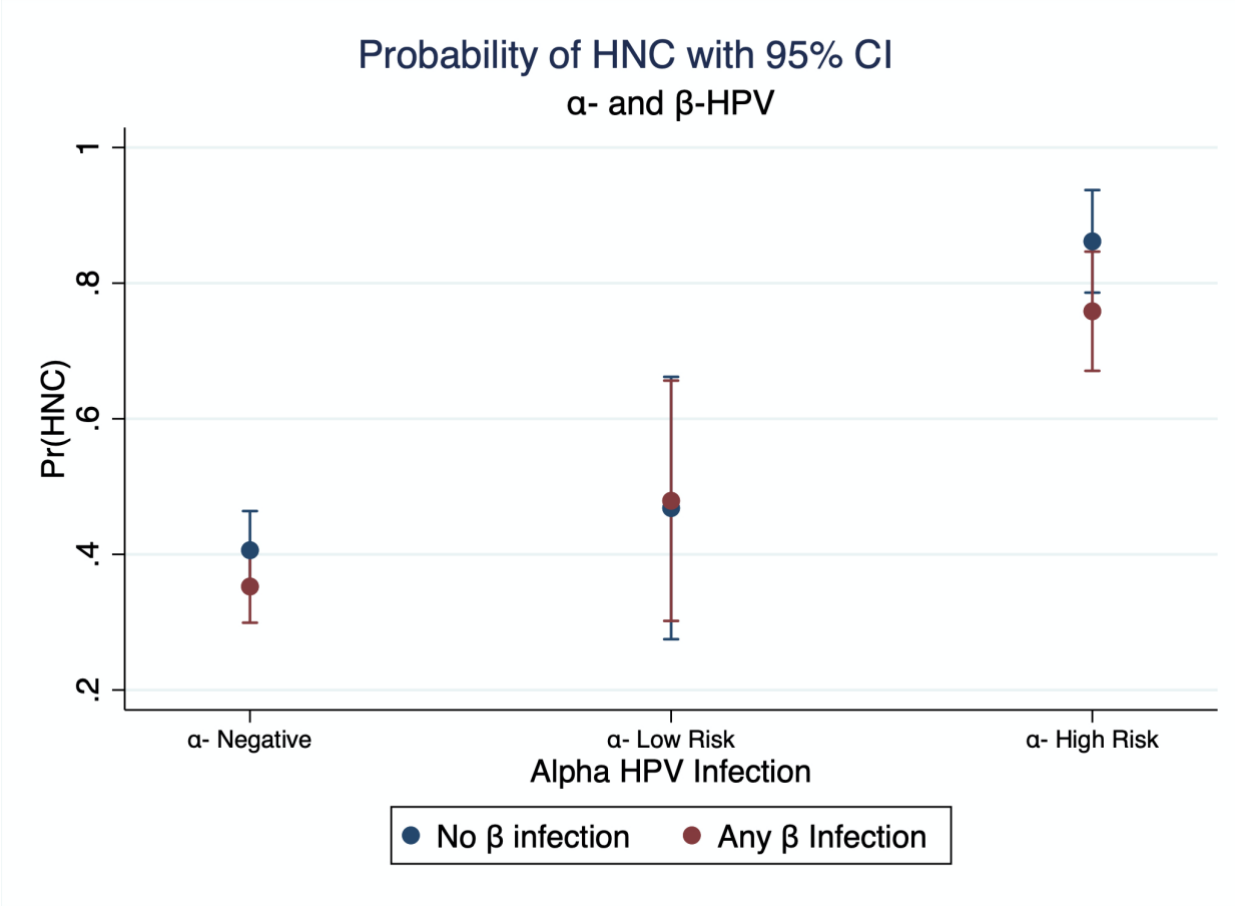

Figure 6.1 Interaction between α-HPV and β-HPV Marginal estimation of probability of HNC. from a logistic model with interaction between α-HPV and β-HPV. High Risk genotypes as identified by IARC: HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59. Model is adjusted for age, sex, smoking, and alcohol.

Table 6.2. Interaction between Infection with HPV16 and infection with any β-HPV genotype

Interaction of HPV16 and Beta HPV

	Beta HPV absent		Beta HPV present		Effect of Beta HPV within the strata of HPV16
	N cases/controls	OR [95% CI]	N cases/controls	OR [95% CI]	OR [95% CI]
HPV16 absent	140/183	1 [Reference]	137/230	0.73 [0.53, 1.01]	0.73 [0.53, 1.01]
HPV16 present	51/4	20.86 [7.19, 60.56]	54/6	16.87 [6.85, 41.54]	0.81 [0.21, 3.1]
Effect of HPV16 within the strata of Beta HPV		20.86 [7.19, 60.56]		23.05 [9.35, 56.82]	
Multiplicative scale		1.1 [0.28, 4.4]			
RERI		-3.72 [-29.7, 22.26]			
AP		-0.22 [-1.87, 1.43]			
SI		0.81 [0.19, 3.38]			

ORs are adjusted for age, sex, smoking, and alcohol consumption. Values in parentheses are 95% confidence intervals. Abbreviations: RERI, relative excess risk due to interaction; AP, attributable proportion; SI, synergy index. Multiplicative interaction (OR11 / OR01 * OR10) is measured from a logistic model as and exponent of the coefficient of the interaction term of the two variables e^(β(A*B))

Table 6.3 shows that infection of HPV16 in the absence of γ-HPV had a strong effect on HNC OR= 18.5 [6.77, 50.57]. There was a weak relation between infection with any genotype from the γ-genus without HPV16 and HNC OR=1.05 [0.67, 1.66]. Those coinfected with both HPV16 and any γ-HPV had a stronger effect on HNC than being infected with one but not both genera OR = 35.98 [8.05, 160.82], but measured with considerable imprecision. Marginal predicted probability of HNC, shows that coinfection with any γ-HPV may increase the risk of HNC among those infected with α-HPV (Figure 6.2). Additive interaction between HPV16 and γ-HPV indicates that the excess risk on the odds ratio scale that is attributed due to interaction (RERI) is 17.44 [-38.34, 73.21], and that 48% of the effect among those jointly exposed to HPV16 and any γ-HPV is due to interaction (AP= 0.48 [-0.41, 1.38]). The direction of measures of additive interaction may suggest a positive mechanistic interaction between HPV16 and γ-HPV, although results are imprecise due to small sample size.

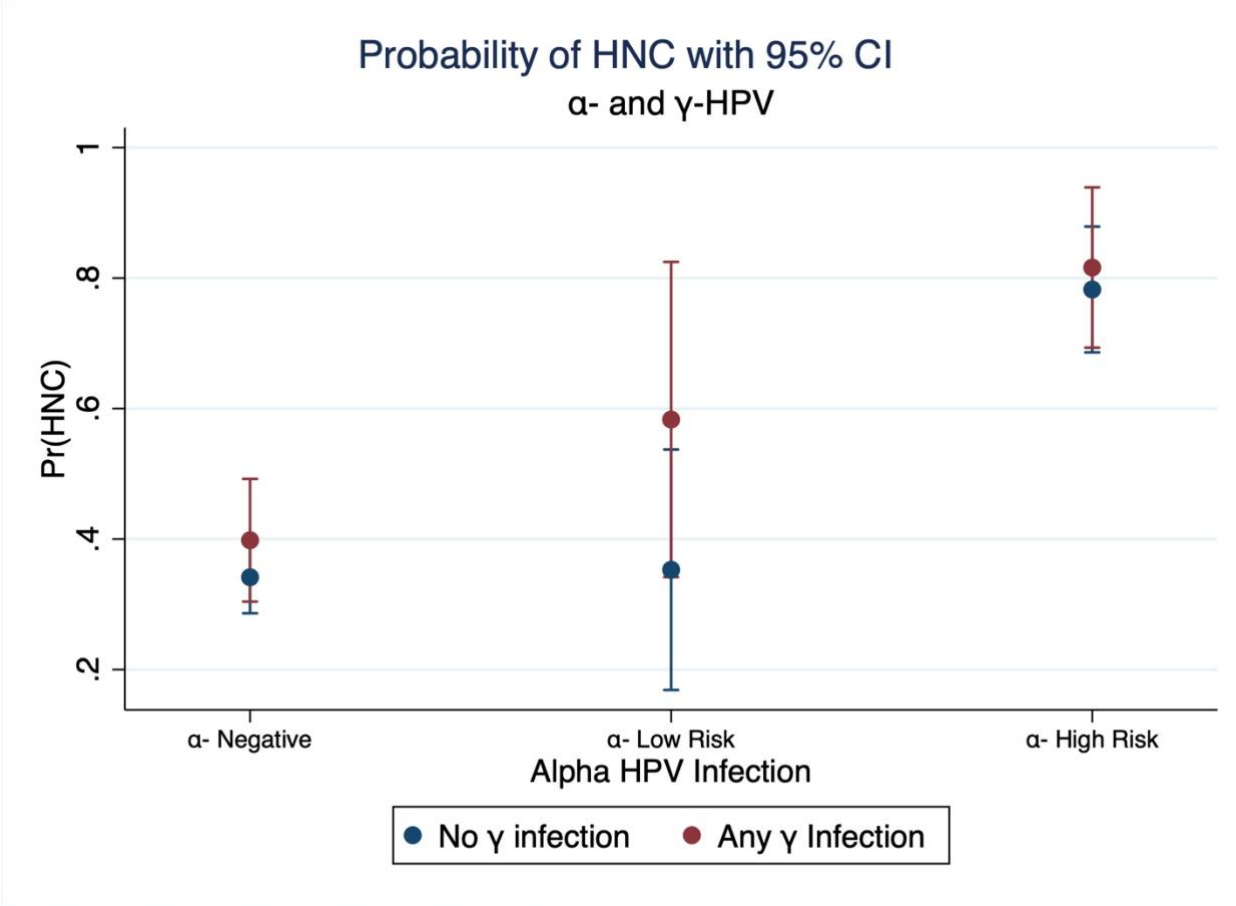

Figure 6.2 Interaction between α-HPV and γ-HPV . Marginal estimation of probability of HNC from a logistic model with interaction term between α-HPV and γ-HPV. Model is adjusted for age, sex, smoking, and alcohol.

Table 6.3. Interaction between Infection with HPV16 and infection with any γ-HPV genotype

Interaction of HPV16 and Gamma HPV

	Gamma HPV absent		Gamma HPV present		Effect of Gamma HPV within the strata of HPV16
	N cases/controls	OR [95% CI]	N cases/controls	OR [95% CI]	OR [95% CI]
HPV16 absent	122/217	1 [Reference]	55/70	1.05 [0.67, 1.66]	1.05 [0.67, 1.66]
HPV16 present	37/5	18.5 [6.77, 50.57]	27/2	35.98 [8.05, 160.82]	1.95 [0.34, 11.15]
Effect of HPV16 within the strata of Gamma HPV		18.5 [6.77, 50.57]		34.25 [7.38, 158.85]	
Multiplicative scale		1.85 [0.3, 11.24]			
RERI		17.44 [-38.34, 73.21]			
AP		0.48 [-0.41, 1.38]			
SI		1.99 [0.33, 12.17]			

ORs are adjusted for age, sex, smoking, and alcohol consumption. Values in parentheses are 95% confidence intervals. Abbreviations: RERI, relative excess risk due to interaction; AP, attributable proportion; SI, synergy index. Multiplicative interaction (OR11 / OR01 * OR10) is measured from a logistic model as and exponent of the coefficient of the interaction term of the two variables e^(β(A*B))

Appendix Table 6.1 shows that infection with HPV16 in the absence of cutaneous genera (β and γ-HPV) had a strong effect on HNC risk adjusted OR=18.15 [6.19, 53.24], while the effect of infection with any cutaneous HPV alone on HNC risk is OR=0.82 [0.59, 1.14]. The joint effect of being infected with HPV16 and any cutaneous HPV was stronger than the effect of either one alone 20.42 [8.32, 50.11]. As expected, when the analysis was restricted to oropharyngeal cancer (Appendix Table 6.2), the effect of HPV16 alone on the risk of the OPC was more pronounced OR=40.13 [13.31, 120.99], and while being infected with any cutaneous HPV alone on the risk of OPC appears to be protective OR= 0.71 [0.45, 1.13], coinfection with HPV16 and any cutaneous HPV leads to a higher risk for OPC than being infected with either exposure alone OR= 53.9 [19.56, 148.51]. All measures of additive interaction suggest a positive interaction between HPV16 and cutaneous HPV.

6.6 Discussion

Interaction happens when one variable's effect on an outcome varies or interacts depending on the value of another variable (21). Using a sufficient causal framework, interaction takes place when two exposures are both components of the same sufficient cause, meaning that for some individuals in the population the two exposures need to be present for the outcome to develop (14,22), and will not experience the outcome in the absence of both exposures. In this study, we evaluated, the role of cutaneous HPV as a potentially important cofactor for the development of HNC of viral etiology. To the best of our knowledge, this is the first epidemiolocal evidence studying the interaction of HPV genera in the HNC literature. While our results were imprecise,

we report a preliminary view on possible a positive interaction between cutaneous HPV and HPV16, and this interaction is more profound in the oropharynx.

Having positive interaction on the additive scale means that for some individuals in the sample, the presence of two of these risk factors, for example, α-HPV and HPV16, needs to happen for cancer to develop. However, in case-control studies, we can only infer the direction of interaction, but not the magnitude as baseline risks are unknown (17). VanderWeele argues that if RERI>1 we can make the assumption that there is a sufficient cause interaction between the two exposures, when RERI >2 as was the case for HPV16 and γ-HPV and HPV and any cutaneous HPV, exposures can have epistatic interaction (23). This means there are individuals in the population who will have the outcome (HNC) if and only if both exposures are present. While the point estimate of RERI for the interaction between HPV16 and γ-HPV is indeed >2, and the RERI, the wide confidence interval makes reaching a conclusion impossible.

The results above support the 'hit and run' hypothesis stemming from animal studies of HPV genotypes and carcinogenesis (6,7). According to this hypothesis, cutaneous HPV genotypes promote and assist the carcinogenic effect of mucosal HPV (α-HPV) but are not necessarily carcinogenic in themselves in the oral mucosa. Combining the evidence from this study and what has been reported in animal models, strengthens the hypothesis that the carcinogenicity of HPV16 and high-risk genotypes from the α-genus is increased in case of coinfection with cutaneous HPV. However, reaching a conclusion on the dynamics and interplay between these viruses is difficult from studies looking at HPV infection in a snapshot rather than over time.

A major limitation of this study is the small sample size that leads to large imprecision in our interaction estimates. Although our study is one of the largest case-control studies of HNC in Canada, HeNCe was not designed for interaction analysis, and we were limited by the logistic and cost of genotyping cutaneous HPV. Future studies should consider planning combining data from several studies, for example in a consortium, as large sample sizes are warranted to quantify interaction (24). We conclude that while α-HPV is still the main player in HNC development, the role of cutaneous HPV as a helping factor should be considered.

Acknowledgment

W.A.A and B.Y.A are recipients of a doctoral fellowship from the Fonds de recherche du Québec – Santé (FRQS). BN is a Canada Research Chair (tier 2) in Life Course Epidemiology. The authors are grateful for HeNCe patients and participants and colleagues who helped collect data for this study.

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Appendix Table 6.1 Interaction between HPV16 and infection with any Cutaneous HPV (β or γ genotype)

Interaction of HPV16 and Any Beta or Gamma

	Any Beta or Gamma absent	Any Beta or Gamma present	Effect of Any Beta or Gamma within the strata of HPV16
	OR [95% CI]	OR [95% CI]	OR [95% CI]
HPV16 absent	1 [Reference]	0.82 [0.59, 1.14]	0.82 [0.59, 1.14]
HPV16 present	18.15 [6.19, 53.24]	20.42 [8.32, 50.11]	1.12 [0.29, 4.32]
Effect of HPV16 within the strata of Any Beta or Gamma	18.15 [6.19, 53.24]	24.78 [10.14, 60.58]	
Multiplicative scale	1.37 [0.34, 5.46]		
RERI	2.44 [-23.27, 28.15]		
AP	0.12 [-1.07, 1.31]		
SI	1.14 [0.27, 4.78]		

ORs are adjusted for age, sex, smoking, and alcohol consumption. Values in parentheses are 95% confidence intervals.
Abbreviations: RERI, relative excess risk due to interaction; AP, attributable proportion; SI, synergy index. Multiplicative interaction (OR_{11} / OR_{01} * OR_{10}) is measured from a logistic model as and exponent of the coefficient of the interaction term of the two variables $e^{\beta(A*B)}$

Appendix Table 6.2 Interaction between HPV16 and infection with any Cutaneous HPV In Oropharynx

Interaction of HPV16 and Any Cutaneous HPV (Oropharyngeal Cancers Only)

	Any Cutaneous HPV absent	Any Cutaneous HPV present	Effect of Any Cutaneous HPV within the strata of HPV16
	OR [95% CI]	OR [95% CI]	OR [95% CI]
HPV16 absent	1 [Reference]	0.71 [0.45, 1.13]	0.71 [0.45, 1.13]
HPV16 present	40.13 [13.31, 120.99]	53.9 [19.56, 148.51]	1.34 [0.33, 5.44]
Effect of HPV16 within the strata of Any Cutaneous HPV	40.13 [13.31, 120.99]	75.96 [27.68, 208.43]	
Multiplicative scale	1.89 [0.43, 8.27]		
RERI	14.07 [-51.65, 79.78]		
AP	0.26 [-0.78, 1.3]		
SI	1.36 [0.32, 5.73]		

ORs are adjusted for age, sex, smoking, and alcohol consumption. Values in parentheses are 95% confidence intervals. Abbreviations: RERI, relative excess risk due to interaction; AP, attributable proportion; SI, synergy index. Multiplicative interaction (OR_{11} / OR_{01} * OR_{10}) is measured from a logistic model as and exponent of the coefficient of the interaction term of the two variables $e^{\beta(A*B)}$

CHAPTER 7: DISCUSSION

7.1 Summary of Findings

The overall goal of this dissertation has been to elucidate the role of cutaneous HPV, which are represented mainly by the beta and gamma genera, in HNC and to provide insight into their potential role in carcinogenesis. While recent reports from the HNC literature have shown an elevated risk of HNC due to infection with cutaneous genotypes (1), results shown here for γ-HPV agree with prior research, while those for β-HPV are in conflict with prior research. We used several molecular techniques to genotype HPV in oral and tumor samples (Chapter 4), examined the distribution of genera and genotypes in cases and controls (Chapter 4), estimated the conditional effect of cutaneous HPV on HNC (Chapter 5), and finally investigated interaction with the high-risk genotype HPV16 (chapter 6). The collective result of this dissertation shows that there is limited evidence to support an independent effect of cutaneous HPV on HNC, and that their role could either be harmless or just limited to aiding mucosal genotypes from the α-genus. Inference from epidemiological studies on the physical nature of these viruses is limited and could only be studied in animal models due to ethical reasons.

Unlike previous studies that discovered an elevated risk of HNC with β-HPV (1,2), we found an apparent preventive role, which I believe could be an artifact because viruses in this genus have been reported to disappear from mature and established cancer (3,4). The fact that β-HPV were more commonly detected in controls relative to cases does not necessarily mean that infection with these genotypes actually protects against HNC. Differences from previous reports could also

be related to the genotyping technology used; while we used bead-based multiplex genotyping, Agalliu et al. used next-generation sequencing (1). Unfortunately, there are no studies that compare the sensitivity and specificity of these two methods in oral HPV infection.

We also found that in comparison to controls, a substantially higher proportion of oral cell samples of cases were infected with at least one genotype of HPV, meaning they are more likely to display 'multiple infection'. Interestingly, while γ-HPV infection mimics the distribution and behavior of mucosal HPV, β-HPV genotypes are less likely to be correlated with sexual behavior. Such findings may indicate the need for distinguishing β from γ-HPV when studying their role as potential risk factors of HNC.

Findings in chapter 5 show that the effect of HPV16 on HNC could be underestimated in the literature if systematic biases are neglected or not accounted for by design or during data analysis. Selection bias, measurement error, and unmeasured confounding could theoretically happen in HNC studies. Therefore, rather than simply acknowledging the existence of these biases as a limitation in any given study, it is important to attempt to use quantitative bias analysis methods to reduce or eliminate bias whenever possible. It is also essential to recommended better measurement methods be created (5–7) and more attention be given to capture life-trajectory of HPV infection. Given that virtually all cervical cancer is caused by HPV infection, it should be no surprise that HPV infection could play a bigger role than initially thought in HNC development.

The interplay between different HPV genera and genotypes over time is still largely an uncharted terrain. While current IARC classification makes a distinction between high-risk and low-risk HPV genotypes, it is possible that such distinction is artificial, and that interaction and coexistence between these types are essential to trigger the carcinogenic process. While HeNCe study was not designed for interaction analysis, the preliminary findings of interaction analysis results (Chapter 6) show that there could be 'exposure-based antagonism' between HPV16 and infection with any β-HPV (8). On other hand, the interaction with γ-HPV could be synergistic as that those individuals coinfected with HPV16 and any genotype from the γ-genus were at a higher risk of HNC. However, the rarity of γ-HPV in oral samples, and the small sample tested for gamma, made results inconclusive with wide confidence intervals for both additive and multiplicative interactions. Such limitations need to be addressed in future studies that are designed with enough power to study interactions.

7.2 Strengths and Limitations

Research in this dissertation has a number of strengths. These three papers are among the earliest studies that investigate the presence of cutaneous HPV in oral samples, first in the Canadian population and first to investigate the presence of all three genera in both oral and tumor samples. Using several innovative molecular techniques allowed us to analyze and identify several genotypes for the largest number of participants. For the detection of cutaneous HPV, we used sensitive, type-specific, bead-based, multiplex genotyping assay (TS-MPG) that can detect more genotypes of low copy numbers than older techniques (9).

Also, the third manuscript (chapter 6) is the first study to investigate possible mechanistic interaction between mucosal and cutaneous HPV in the head and neck region, which opens the door for more studies in the future to investigate etiologic role for multiple HPV infection. If indeed interaction is the cause for a large number of HPV-related cancers in the head and neck region, then future preventive measures should be aimed at preventing coinfection from taking place. Current HPV vaccines approved in Canada target 2 , 4, 9 genotypes (10)— all from the α-genus. If cutaneous HPV does indeed play a role in carcinogenesis by interacting with α-HPV, then it might be worth investigating if future vaccines should have a wider valency against most common genotypes from other genera.

A limitation of our study, as with other HPV-related investigations, is the possibility of information bias (measurement error) of HPV. There is no gold standard to measure HPV status and no FDA-approved tests to detect HPV DNA or mRNA in saliva. HPV studies often depend on mouthrinse or brush samples to collect samples, then use some molecular technique for genotyping HPV DNA or E6/E7 RNA. These methods can vary in performance. In a systematic review that investigated the sensitivity and specificity of oral HPV detection for HPV-positive HNC, Gipson et al. found that studies reported sensitivity as low as 12% and as high as 93% (5). Specificity also varied between studies from 88% to 100%. Such variation in methodology highlights the importance of continuous improvement for methods to measure both mucosal and cutaneous HPV.

The use of hospital-based rather than population-based controls could have limitations on external validity. If controls are not selected from the secondary base that produced the cases,

we could have selection bias that jeopardizes external validity (11). However, as explained in methods chapter (Chapter 3), investigators in HeNCe selected controls from a variety of departments and none of which represents more than 20% of controls. This minimizes the possibility of selection bias and external validity violation.

Also, restricting the base to people living within 50 km of the hospital means that cases and controls are more likely coming from the same catchment area, which covers people who would seek medical treatment in these hospitals if they ever develop the disease. It is important to highlight that since citizens in Montreal are all covered by public insurance, there is no concern about people seeking care in different hospitals based on coverage of their health insurance.

Since HPV status was not known before selection into the study and patients were selected from clinics that are not related to HPV-related diseases, the possibility that the distribution of exposures (HPV genotypes) differs from the study base is minimal. In other words, selecting people into the study could not have been differential to factors related to HPV exposure. As for generalization, it can be argued that these sampled are generalizable to the overall population of Quebec. Cases and controls are coming from the four main referral hospital in Montreal, which is not only the largest metro area in Quebec, but also where almost half of Quebec's population lives.

Another limitation is related to logistical constraints that forced us to direct resources to identifying and genotyping β-HPV, which is the most detected genus in the oral cavity (12). As

discussed above, the different relation between β and γ HPV with sexual behavior may require further investigation.

7.3 Future Direction of Research

Given the findings of this study, there are some directions for future research on the role of cutaneous HPV in HNC. First, animal models have shown that β-HPV, although expressed their oncoproteins E6 and E7 in epithelial layers of the skin, are still not able to result in cancer and rather required interaction with environmental factors (UV light) (3,13). The tissues in the head and neck area are not directly exposed UV light, and I therefore hypothesize that there could be another trigger mechanisms that could play a role in place of UV light to cause carcinogenesis in the head and neck. That additional trigger could be genetic or some other environmental factor (smoking, alcohol, etc.), so HPV interaction with other factors need to be examined further.

Second, cutaneous HPV particularly form the β-genus have been shown to be associated with premalignant lesions in the skin (13,14). Weissenborn and colleagues (14) found that these genotypes were more common in Actinic Keratosis, a skin premalignant lesion, than in established squamous cell carcinomas, which corroborates our finding of no cutaneous HPV in tumor tissues (chapter 4). It is still to be determined whether these genotypes have any role in oral premalignant lesions such as leukoplakia, erythroplakia, or lichen planus.

Third, a major limitation in HPV studies is that HPV is measured at one point in time. This allows taking a “snapshot” of current HPV status but limits the ability to evaluate the course of infection

over time. We do not understand the natural history of HPV infection and how different genotypes of HPV interact with the natural immune system over time. This minimizes what we can infer about the biology of HPV infection and the dynamics of it from epidemiologic data alone. It is therefore important to have longitudinal studies in the future that measure HPV at different times and follow the clinical condition of patients in order to understand how these genera interact over time to cause in relation to clinical phenotypes.

Fourth, in this dissertation I have shown that cutaneous HPV, unlike mucosal HPV, are not strongly related to sexual behavior. All studies on β- and γ HPV and HNC have been from developed countries where tobacco use is no longer the main driving factor of HNC. In previous study of HeNCe that investigated the role of HPV in oral samples from India (15), a more conservative country than Canada, investigators did not detect any mucosal HPV genotype in oral samples. As a quality control check, investigators sent forty randomly selected samples from India to be analyzed and genotyped for mucosal HPV in Canada to ensure that absence of HPV from Indian samples was not due to laboratory quality-control problem. Given that cutaneous HPV can be found in oral and skin samples and not related to sexual practice, it is worth investigating in future studies whether these genotypes are present in oral samples in population in south-east Asia, Arab countries, and Africa. While genotyping for cutaneous HPV is still too costly, with the advancement of genotyping technology the cost likely to go down in the future.

Fifth, the role of immunity system is very important in clearing infections including that with HPV. It is important to understand the role of immune status in clearing β- and γ-HPV, and whether

the risk of head and neck cancer is modified in immunocompromised people. Such studies could reveal answers for how and when do people get infection with cutaneous HPV.

7.4 Overall Conclusion

Cutaneous HPV (β- and γ) are present in the oral cavity but absent in head and neck cancer tumor tissues. I found little evidence that they play a direct role in cancer development, but they could play some assisting role in early subclinical stages then fade away in the background. Further studies are warranted to understand their mechanism of action.

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Appendix – HeNCe Life Study Questionnaire

CONFIDENTIAL

MULTI CENTER STUDY OF ORAL CANCER: A LIFE COURSE APPROACH

UNIT OF EPIDEMIOLOGY & BIOSTATISTICS
INRS-INSTITUT ARMAND-FRAPPIER – LAVAL – CANADA

FACULTY OF DENTISTRY & DEPARTMENT OF EPIDEMIOLOGY
MCGILL UNIVERSITY – MONTREAL – CANADA

DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY
CENTRE DE RECHERCHE DU CHUM – MONTREAL – CANADA

DEPARTMENT OF EPIDEMIOLOGY AND POPULATION HEALTH
ALBERT EINSTEIN COLLEGE OF MEDICINE – NEW YORK – USA

HOSPITAL DO CÂNCER-DEPARTAMENTO DE CIRURGIA DE CABEÇA E PESCOÇO
SÃO PAULO – BRASIL

GOVERNMENT DENTAL COLLEGE –MEDICAL COLLEGE CAMPUS
KOZHIKODE – SOUTH INDIA

DEPARTMENT OF CLINICAL VIROLOGY – CHRISTIAN MEDICAL COLLEGE
VELLORE – SOUTH INDIA

2011

TABLE OF CONTENTS

0 2 - ___ ___ ___ - ___
Country ID N°

A. MEDICAL INFORMATION

Interviewer Reminder: Prior to interview, obtain information below from research file or medical records.

Identification Number..... 0 2 - ___ ___ ___ - ___
Country: (01) Brazil (04) United Kingdom **Country** **Participant**
(02) Canada (05) India
(03) South Africa

Medical file N°..... ___ ___ ___ ___ ___ ___ ___ ___ ___ ___

A1 Status.....
(01) Case (02) Control

A2 Subject's Initials (Surname, Name).....

A3 Hospital / recruitment site.....
(01) Jewish General Hospital MUHC (02) Hôpital Notre-Dame CHUM
(03) Montreal General Hospital (04) Royal Victoria Hospital

A4 Control Department *(Code 88 for cases)*.....
(01) Neurology (04) Rheumatology (07) Urology
(02) Ear, Nose, Throat (05) Orthopaedics (08) Other, specify:
(03) Endocrinology (06) Gastroenterology _____

For controls only:

A5 Main Diagnosis for being seen at this department (LC)..... ___ ___ ___ - ___
Condition description: _____ (I.C.D.10)

For cases only:

A6 Cancer site: _____
(01) Pharynx (C146,148,149) (02) Larynx (C161) (03) Oral cavity (C141,143,144,145)

A7 Global TNM stage T_____ N_____ M____ ➔ Global Staging **(LC)** _____

A8 Date of Diagnosis..... ___ ___ - ___ ___ - ___ ___ ___ ___
(99-99-9999) Don't know Day Month Year

A9 Time since Diagnosis (months)..... ___ ___ ___

Initial treatment modality(ies)

A10 Surgery..... **8 8**
(01) No (02) Yes

A11 Date of surgery..... **8 8** - **8 8** - **8 8**
Day Month Year

A12 Radiotherapy..... **8 8**
(01) No (02) Yes

A13 Date of radiotherapy..... **8 8** - **8 8** - **8 8**
Day Month Year

A14 Chemotherapy..... **8 8**
(01) No (02) Yes

A15 Date of chemotherapy..... **8 8** - **8 8** - **8 8**
Day Month Year

For all subjects:

A16 Initials of the person who collected the medical data (Surname, Name)..... | | |

A17 Date medical data collected..... | | - | | - | | | | |
(99-99-9999) Don't know Day Month Year

B. GENERAL INFORMATION

B1 Date of Interview..... [ ][ ] - [ ][ ] - [ ][ ][ ][ ]
Day Month Year

B2 Time of beginning of Interview..... [ ][ ] - [ ][ ]
Hour **Minute**

B3 Interview..... [ ][ ]
(01) Original (02) Duplicate *(6-12 weeks later)* (03) Duplicate *(+12 weeks later)*

B4 Sex..... [ ][ ]
(01) Female (02) Male

Interviewer Reminder: **Present life grid here**. See instructions in guidebook.

B5 What is your date of birth?..... [ ][ ] - [ ][ ] - [ ][ ][ ][ ]
(99-99-9999) Don't know Day Month Year

B6 How old are you?..... [ ][ ][ ]

B7 Do you consider yourself living in a rural (farm) or an urban (city) area?... [ ][ ]
(01) Urban (02) Rural (GO TO B9)

B8 What city do you live in? (LC)..... [ ][ ]
Name of City: _____ Postal Code: _____ - _____

Interviewer Reminder: Confirm name of city from list of codes. Rural area is in the farm

B9 How many years have you been living there? (Last consecutive years)..... [ ][ ]
(00) Less than one year

B10 In which city / place did you live in just before?(LC)..... [ ][ ]
Name of city: _____ Postal Code: _____ - _____
(00) Rural area

B11 Were you born in a rural (farm) or an urban (city) area?..... [ ][ ]
(01) Urban (02) Rural (GO TO B13)

B12 In what city were you born in? (LC)..... [ ][ ]
Name of city: _____ Postal Code: _____ - _____
(00) Other country

B13 How many years did you live there?..... [ ][ ]
(00) Less than one year

B14 In this list, which group best describe you? ☐☐

(01) White (Caucasian)
(02) Black
(03) Asian Indian
(04) Asian Pakistani
(05) Asian Bangladeshi
(06) Chinese
(07) Mixed ethnic group
(08) Aboriginal
(09) Other, specify: _____ ☐☐

B15 To which of these religions do you identify with? ☐☐

(00) None (GO TO B18)
(01) Muslim
(02) Christian
(03) Jewish
(04) Buddhist
(05) Hindu
(06) Other, specify: _____ ☐☐

B16 Do you practice this religion?..... ☐☐

(00) No (GO TO B18) (01) Yes

B17 How old were you when you started practicing this religion?..... ☐☐

(00) My whole life

B18 *For the interviewer:* **(Language used in this questionnaire)?**..... ☐☐

(01) English
(02) French

C. EDUCATION

This section is about your education. Firstly,

C1 Did you ever attend school?..... ☐☐
(00) No (GO TO SECTION D)
(01) No, but I can read and write (GO TO SECTION D)
(02) Yes

Let's start by looking at when you started school, when you stopped and interruptions in between. We will use this grid to help us out. I will ask you more specific questions about your education afterwards.

Interviewer Reminder: Collect general information using the **life grid**, referring to it later when asking questions C2 through C9.
- Situate years of **formal** education i.e. that were successfully completed at school.
- Do **NOT** consider regular interruptions (ex.: summer time) or kindergarten. But DO consider interruptions for medical reasons, evacuations, etc...

C2 How many years of formal education do you have? (Subtract years failed)..... ☐☐

C3 What was the highest degree or qualification that you obtained?..... ☐☐
(00) None (GO TO C5)
(01) Elementary / primary school
(02) High school
(03) Technical qualification
(04) CEGEP (non-technical)
(05) University
(06) Post-graduate

C4 How old were you when you obtained this degree?..... ☐☐
(99) Don't know

C5 Have you ever failed a school year?..... ☐☐
(00) No
(01) Yes, once
(02) Yes, twice
(03) Yes, 3 or more times

C6 Have you ever interrupted your full time education?..... ☐☐
(00) No (GO TO SECTION D)
(01) Yes

C7 How many years of formal education did you have when you FIRST interrupted your full time education?..... ☐☐

C8 How old were you when you FIRST interrupted you full time education?..... ☐☐

D. OCCUPATIONS & EMPLOYMENT

In this section I would like to ask you a few questions about jobs you may have had.

Interviewer Reminder: A job is a **continuous period of time of ONE YEAR OR MORE working and paid by the same employer** even though the participant may have had different positions during that period. If the participant was self-employed, a job is considered to be a period of time doing the same type of self-employed work.

D1 Have you ever had a job in your life?..... [ ][ ]
(00) No (GO TO SECTION E) (01) Yes

D2 Which of the options below best describes your work situation in the past 7 days?..... [ ][ ]
(01) Full time work (30+ hours / week)
(02) Part time work (< 30 hours / week)
(03) Unemployed
(04) Fully retired from work
(05) Permanently sick or disabled
(06) On sick leave
(07) Other, specify: _____ [ ][ ]

Let's look at the different jobs you've had, the different positions you may have held and the times you may have been unemployed. Again, we will use this grid to help us out and refer to it for the specific questions I will have afterwards.

Interviewer Reminder: Collect general information using the **life grid**, referring to it later when asking questions D2 through D112.

- Going back to an old employer, even if for more than one year, is considered to be another, separate job and should be counted as such.
- Seasonal work (6 months full time) done for more than 2 years in a row counts as 1 job.
- Include army service IF paid or compensated for.
- Include "informal work", i.e. direct selling, itinerant seller, undeclared work. Count different contracts, odd jobs, etc… as one job IF done continuously over at least one year. Subject should consider all different work related activities in this period as a whole whilst describing this job through the related questions.
- Mark periods of unemployment on life grid (refer to description in box below).

Do **NOT** include:

- Summer or holiday time jobs while at school or full-time education.
- Part-time jobs done at the same time as full-time education.
- Part time jobs done at the same time as a full-time job.

D3 Since you started working how many jobs have you had?..... [ ][ ]
(01) (02) (03) (04) (05) (06) (07) (08) (09 or more)

Interviewer Reminder: Unemployment means being out of a work for **at least 3 MONTHS**. You do not have to be registered as unemployed BUT you must be enabled to work and actively or passively looking for work.

Do **NOT** include:
- Holidays or vacations while attending full-time education
- Interruptions due to seasonal work
- Maternity leaves, Sabbatical leaves
- Deliberate choice to exclude oneself from the workforce, i.e. living on inheritance, housewife...

D4 Since you started working how many times have you been unemployed?.... ☐☐
(00) None (GO TO D6)
(01) (02) (03) (04) (05) (06) (07) (08) (09 or more)

D5 Please describe the longest periods of your life in which you were unemployed.

From age?	**To age?**	**# Months**
☐☐	☐☐	☐☐
☐☐	☐☐	☐☐
☐☐	☐☐	☐☐
☐☐	☐☐	☐☐
☐☐	☐☐	☐☐

FIRST JOB

Interviewer Reminder: Confirm which job is 1st job with life grid.

I would like to ask you a few questions about your **first job**. So,

D6 You were doing that job...

From age? [ ][ ] **To age?** [ ][ ] **i.e. # Years** [ ][ ]

D7 Did you occupy different positions at that job?..... [ ][ ]
(00) No (Fill in FIRST column only) (01) Yes

FIRST **LAST**

D8 Please describe your job / different positions (LC)..... [ ][ ][ ] [ ][ ][ ]

FIRST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

LAST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

D9 What did the company you worked for specialise in? (LC)..... [ ][ ][ ]
_____

Interviewer Reminder: Confirm job / position code with list of codes for Q D8 and D9.

D10 Were you an employee or self-employed?..... [ ][ ] [ ][ ]
(01) Employee (02) Self-employed (GO TO D12)

D11 Were you an employee? (GO TO D13)..... [ ][ ] [ ][ ]
(01) Not supervising others
(02) Foreman, supervisor, team leader
(03) Manager: Firm of <25 employees
(04) Manager: Firm of 25+ employees
(05) Professional

D12 Were you self employed?.....

(01) Without incorporated business
(02) With incorporated business but without employees other than family members
(03) With <25 employees
(04) With 25+ employees
(05) Professional

D13 Did you work?.....

(01) Full time (30 hours +) (02) Part time (<30 hours)

D14 How many hours a week?.....

D15 How much were you paid PER YEAR at that time?.....

FIRST: $

LAST: $

Describe: _____

- Calculate average amount in Canadian dollars
- Average: hourly rate x 35 hours x 50 weeks OR Min + Max / # yrs, prorated
- Self-employed: average earnings per year as per income tax declarations if submitted

Now I would like to ask you a few questions about work environmental hazards. Consider your job in general, regardless of the different positions you may have occupied.

D16 Did your work often involve exposure to chemical hazards such as dust, oils, solvents or thinners, smoke, gas, etc…?.....

(00) No (GO TO D24) (01) Yes (99) Don't know (GO TO D24)

Did it involve exposure to...?

D17 Dust (Silica dust, saw dust, sanding dust, epoxy-resins, welding...).....

(00) No (01) Yes

D18 Oils (Mineral oil, lubricants...).....

(00) No (01) Yes

D19 Solvents or thinners (acetone, paint thinners, chlorinated solvent (trichloroethylene), solvent of cellulose...).....

(00) No (01) Yes

D20 Smoke (Gas from motors, coal, wood, rubber...).....

(00) No (01) Yes

D21 Gas (Oxygen, ammonia...).....

(00) No (01) Yes

D22 Did your work involve working with substances such as: asphalt, alcohol, gasoline, glue, mercury, kerosene, etc?..... [ ][ ]
(00) No (01) Yes

D23 Did your work <u>often</u> involve exposure to other chemicals?..... [ ][ ]
(00) No (01) Yes, specify (ex.: cigarette smoke): _____ [ ][ ]
_____

D24 Did your work <u>often</u> involve exposure to physical hazards such as humidity, high temperatures, pressure (physiological), electro-magnetic radiations, etc…?..... [ ][ ]
(00) No (GO TO **<u>Interviewer Reminder</u>** preceding D30) (01) Yes
(99) Don't know (GO TO **<u>Interviewer Reminder</u>** preceding D30)

Did it involve exposure to...

D25 Humidity?..... [ ][ ]
(00) No (01) Yes

D26 High temperatures?..... [ ][ ]
(00) No (01) Yes

D27 Pressure (physiological; ex.: loud noise, underwater work, gravity changes)?..... [ ][ ]
(00) No (01) Yes

D28 Electromagnetic radiations (x-rays, microwaves, radioactive substances)? [ ][ ]
(00) No (01) Yes

D29 Did your work <u>often</u> involve exposure to other physical hazards?..... [ ][ ]
(00) No (01) Yes, specify: _____ [ ][ ]
_____

<u>Interviewer Reminder</u>: If D16 **<u>OR</u>** D24 are (01) Yes, then ask D30. If not, go to D31.

D30 Did you use any kind of protection for chemical / physical hazards?..... [ ][ ]
(00) No (02) Yes, sometimes
(01) Yes, most of the time (03) Yes, rarely

D31 Was your first job the same one as your longest job?..... [ ][ ]
(00) No (01) Yes, the same one as my longest job (GO TO D58)
(02) Yes, the same one my whole life (GO TO SECTION E)

0 2 - [ ][ ][ ] - [ ]
Country ID N°

LONGEST JOB

Now I would like to ask you some questions about your **longest job**. I will be using the same set of questions I used in the previous section. So,

Interviewer Reminder: Confirm which job is longest job with life grid.

D32 You were doing that job...

From age? [ ][ ] **To age?** [ ][ ] **i.e. # Years** [ ][ ]

D33 Did you occupy different positions at that job?..... [ ][ ]
(00) No (Fill in FIRST column only) (01) Yes

FIRST **LAST**

D34 Please describe your job / different positions (LC)..... [ ][ ][ ] [ ][ ][ ]

FIRST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

LAST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

D35 What did the company you worked for specialise in? (LC)..... [ ][ ][ ]
_____

Interviewer Reminder: Confirm job / position code with list of codes for Q D34 and D35.

D36 Were you an employee or self-employed?..... [ ][ ] [ ][ ]
(01) Employee (02) Self-employed (GO TO D39)

D37 Were you an employee...?..... [ ][ ] [ ][ ]
(01) Not supervising others
(02) Foreman, supervisor, team leader
(03) Manager: Firm of <25 employees
(04) Manager: Firm of 25+ employees
(05) Professional

D38 Were you self employed?..... ___ ___

(01) Without incorporated business
(02) With incorporated business but without employees other than family members
(03) With <25 employees
(04) With 25+ employees
(05) Professional

D39 Did you work...?..... ___ ___

(01) Full time (30 hours + / week) (02) Part time (<30 hours / week)

D40 How many hours a week?..... ___ ___

D41 How much were you paid PER YEAR at that time?..... **FIRST: $** ___ ___ ___ ___ ___ ___

LAST: $ ___ ___ ___ ___ ___ ___

Describe: _____

- Calculate average amount in Canadian dollars
- Average: hourly rate x 35 hours x 50 weeks OR Min + Max / # yrs, prorated
- Self-employed: average earnings per year as per income tax declarations if submitted

Now I would like to ask you a few questions about work environmental hazards. Consider your job <u>in general</u>, regardless of the different positions you may have occupied.

D42 Did your work <u>often</u> involve exposure to chemical hazards such as dust, oils, solvents or thinners, smoke, gas, etc…?..... ___ ___

(00) No (GO TO D50) (01) Yes (99) Don't know (GO TO D50)

Did it involve exposure to...?

D43 Dust (Silica dust, saw dust, sanding dust, epoxy-resins, welding...)..... ___ ___

(00) No (01) Yes

D44 Oils (Mineral oil, lubricants...)..... ___ ___

(00) No (01) Yes

D45 Solvents or thinners (acetone, paint thinners, chlorinated solvent (trichloroethylene), solvent of cellulose...)..... ___ ___

(00) No (01) Yes

D46 Smoke (Gas from motors, coal, wood, rubber...)..... ___ ___

(00) No (01) Yes

D47 Gas (Oxygen, ammonia...)..... ___ ___

(00) No (01) Yes

D48 Did your work involve working with substances such as: asphalt, alcohol, gasoline, glue, mercury, kerosene, etc?.....

(00) No (01) Yes

D49 Did your work <u>often</u> involve exposure to other chemicals?.....

(00) No (01) Yes, specify (ex.: cigarette smoke): _____

_____

D50 Did your work <u>often</u> involve exposure to physical hazards such as humidity, high temperatures, pressure (physiological), electro-magnetic radiations, etc…?.....

(00) No (GO TO **<u>Interviewer Reminder</u>** preceding D56) (01) Yes

(99) Don't know (GO TO **<u>Interviewer Reminder</u>** preceding D56)

Did it involve exposure to...

D51 Humidity?.....

(00) No (01) Yes

D52 High temperatures?.....

(00) No (01) Yes

D53 Pressure (physiological; ex.: loud noise, underwater work, gravity changes)?.....

(00) No (01) Yes

D54 Electromagnetic radiations (x-rays, microwaves, radioactive substances)?

(00) No (01) Yes

D55 Did your work <u>often</u> involve exposure to other physical hazards?.....

(00) No (01) Yes, specify: _____

_____

<u>Interviewer Reminder</u>: If D42 **<u>OR</u>** D50 are (01) Yes, then ask D56. If not, go to D57.

D56 Did you use any kind of protection for chemical / physical hazards?.....

(00) No (02) Yes, sometimes

(01) Yes, most of the time (03) Yes, rarely

D57 Was your longest job the same one as your latest or current job?.....

(00) No

(01) Yes, the same one as my latest / current job (GO TO SECTION E)

LAST / LATEST JOB

Finally about your last / latest job...

Interviewer Reminder: Confirm which job is last/latest job with life grid.

D58 You were doing that job...

From age? [ ][ ] **To age?** [ ][ ] **i.e. # Years** [ ][ ]

D59 Did you occupy different positions at that job?..... [ ][ ]
(00) No (Fill in FIRST column only) (01) Yes

FIRST **LAST**

D60 Please describe your job / different positions (LC)..... [ ][ ][ ] [ ][ ][ ]

FIRST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

LAST POSITION

Job Title: _____
Work environment: _____
Most frequent tasks: _____
_____
_____

D61 What did the company you worked for specialise in? (LC)..... [ ][ ][ ]
_____

Interviewer Reminder: Confirm job / position code with list of codes for Q D60 and D61.

D62 Were you an employee or self-employed?..... [ ][ ] [ ][ ]
(01) Employee (02) Self-employed (GO TO D66)

D63 Were you an employee...?..... [ ][ ] [ ][ ]
(01) Not supervising others
(02) Foreman, supervisor, team leader
(03) Manager: Firm of <25 employees
(04) Manager: Firm of 25+ employees
(05) Professional

D64 Were you self employed...?..... ☐☐ ☐☐

(01) Without incorporated business
(02) With incorporated business but without employees other than family members
(03) With <25 employees
(04) With 25+ employees
(05) Professional

D65 Did you work...?..... ☐☐ ☐☐

(01) Full time (30 hours + / week) (02) Part time (<30 hours / week)

D66 How many hours a week?..... ☐☐ ☐☐

D67 How much were you paid PER YEAR at that time?..... **FIRST: $** ☐☐☐☐☐☐

LAST: $ ☐☐☐☐☐☐

Describe: _____

- Calculate average amount in Canadian dollars
- Average: hourly rate x 35 hours x 50 weeks OR Min + Max / # yrs, prorated
- Self-employed: average earnings per year as per income tax declarations if submitted

Now I would like to ask you a few questions about work environmental hazards. Consider your job <u>in general</u>, regardless of the different positions you may have occupied.

D68 Did your work <u>often</u> involve exposure to chemical hazards such as dust, oils, solvents or thinners, smoke, gas, etc…?..... ☐☐

(00) No (GO TO D76) (01) Yes (99) Don't know (GO TO D76)

Did it involve exposure to...?

D69 Dust (Silica dust, saw dust, sanding dust, epoxy-resins, welding...)..... ☐☐

(00) No (01) Yes

D70 Oils (Mineral oil, lubricants...)..... ☐☐

(00) No (01) Yes

D71 Solvents or thinners (acetone, paint thinners, chlorinated solvent (trichloroethylene), solvent of cellulose...)..... ☐☐

(00) No (01) Yes

D72 Smoke (Gas from motors, coal, wood, rubber...)..... ☐☐

(00) No (01) Yes

D73 Gas (Oxygen, ammonia...)..... ☐☐

(00) No (01) Yes

D74 Did your work involve working with substances such as: asphalt, alcohol, gasoline, glue, mercury, kerosene, etc?..... ☐☐

(00) No (01) Yes

D75 Did your work <u>often</u> involve exposure to other chemicals?..... ☐☐

(00) No (01) Yes, specify (ex.: cigarette smoke): _____ ☐☐

_____

D76 Did your work <u>often</u> involve exposure to physical hazards such as humidity, high temperatures, pressure (physiological), electro-magnetic radiations, etc…?..... ☐☐

(00) No (GO TO **<u>Interviewer Reminder</u>** preceding D85) (01) Yes

(99) Don't know (GO TO **<u>Interviewer Reminder</u>** preceding D85)

Did it involve exposure to...

D77 Humidity?..... ☐☐

(00) No (01) Yes

D78 High temperatures?..... ☐☐

(00) No (01) Yes

D79 Pressure (physiological; ex.: loud noise, underwater work, gravity changes)?..... ☐☐

(00) No (01) Yes

D80 Electromagnetic radiations (x-rays, microwaves, radioactive substances)?..... ☐☐

(00) No (01) Yes

D81 Did your work <u>often</u> involve exposure to other physical hazards?..... ☐☐

(00) No (01) Yes, specify: _____ ☐☐

_____

<u>Interviewer Reminder</u>: If D68 **<u>OR</u>** D76 are (01) Yes, then ask D82. If not, GO TO SECTION E.

D82 Did you use any kind of protection for chemical / physical hazards?..... ☐☐

(00) No (02) Yes, sometimes

(01) Yes, most of the time (03) Yes, rarely

E. HOUSING CONDITIONS & RESIDENTIAL ENVIRONMENT

In this section I would like to ask you a few questions about your housing conditions and residential environment at different times in your life. We will use the grid first to look at the different addresses you lived at, noting the times you moved from one place to another.

Interviewer Reminder: Collect general information using the **life grid**, referring to it later when asking questions E1 through E181.

- **An address is a place where the participant lived for at least 1 YEAR.**
- Moving back to an old address *within the same time period* is considered to be a separate place of residence and should be counted as such as long as it is for at least one more year.
- Moving back to an old address in *another time period* is always considered a separate place of residence as long as it is for a longer period of time than previously.
- If an address overlaps two time periods, consider it the main residence in a period only if the participant lived there for the longest time.
- If "boarding school" (E9), answers should pertain to the residence when child was back home.
- If person changed living place many times within the same year or over many years (ex.: gypsies, travelers, musicians touring, homeless) do not count any addresses. Rather, record the number of years spent with this housing pattern in E2, E4 and E6. If this pattern is present for the longest time in one period of life, note age span for that period and answer (06) to E9.

E1 Up until you were 16 years old (incl.) at how many *different* addresses did you live?
(01) (GO TO E3)... (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]

E2 Up until you were 16 years old (incl.) how many times (total) did you spend changing living places more than once in the same year?
(00) (01) (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]

E3 Between the ages of 17 and 30 (incl.) at how many *different* addresses did you live?
(01) (GO TO E5)… (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]

E4 Between the ages of 17 and 30 (incl.) how many times (total) did you spend changing living places more than once in the same year?
(00) (01) (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]

E5 From the age of 30 (excl.) until today at how many *different* addresses did you live?
(01) (GO TO E7)… (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]
If the respondent is less than 30 years old, mark (88) and GO TO E7

E6 From the age of 30 (excl.) until today how many times (total) did you spend changing living places more than once in the same year?
(00) (01) (02) (03) (04) (05) (06) (07) (08) (09 or more)..... [ ][ ]

CHILDHOOD RESIDENCE

I would like to ask you a few questions about the residence / home in which you lived **for the longest time during your childhood**. By childhood I mean up to age 16 (incl.).

Interviewer Reminder: Identify and confirm longest residence in childhood using the life grid.

E7 You lived at that place...?

From age? ☐☐ **To age?** ☐☐ **i.e. # Years** ☐☐

E8 Do you remember what the POSTAL CODE is for this residence? _____ - _____

For all the following questions, refer to the situation that was present "MOST OF THE TIME" while living in that residence.

Interviewer Reminder: Immediate family means: husband / wife & children and extended family means mother, father & own family.

E9 What type of setting were you living in at that place?..... ☐☐

(01) With immediate family
(02) With extended family
(03) Foster home (GO TO E43)
(99) Don't know
(04) Boarding school, monastery (GO TO E43)
(05) Institution (ex.: psychiatric hospital, rehabilitation centre) (GO TO E43)
(06) Pattern of many different living places (GO TO E43)
(07) Other, specify: _____ ☐☐

E10 Who was the owner of the place?..... ☐☐

(01) My family or a member of my family
(02) State or municipality
(03) Private owners / company (renting)
(04) Other, specify: _____ ☐☐
(99) Don't know

E11 How many people lived in the household? (At once, for the longest period of time).. ☐☐
(Include borders, live-in maids, roommates...) (99) Don't know

Interviewer Reminder:
QE11: Include people who were permanent residents and those who were living in the house for the longest period of time.
QE12: Rooms include: kitchen, living room, dining room, bedroom, furnished basement. Do **NOT** include: toilet, bathroom, laundry room, hallway, garage, patio.

E12 How many rooms did your place have? (If renovated, count # rooms during longest period living there)..... ☐☐
(99) Don't know

E13 Were some or all of these rooms damp / humid / wet? (For example: wallpaper peels of wall, mould grows on internal walls, clothes stem when aired after storage)..... ☐☐
(00) No (01) Yes, all (02) Yes, some (99) Don't know

Now, I will read a list of facilities you may have had in the place where you lived. We would like to know **which of these facilities were present inside your childhood residence.**

E14 Did your home have a bathroom (indoor toilet, bath and/or shower)?..... ☐☐
(00) No (GO TO E16) (01) Yes (99) Don't know (GO TO E16)

E15 How many?..... ☐☐

E16 Did your home have a sewage system?..... ☐☐
(00) No (02) Yes, a septic tank
(01) Yes, a central public system (99) Don't know

E17 Did your home have running cold water?..... ☐☐
(00) No (02) Yes, an independent one (rural) i.e. outside the house
(01) Yes, a central public system (urban) i.e. inside the house (99) Don't know

E18 Did your home have electricity?..... ☐☐
(00) No (02) Yes, by a generator / battery only
(01) Yes, by a central system (99) Don't know

E19 Did your home have running hot water?..... ☐☐
(00) No (01) Yes (99) Don't know

E20 Did your house have a wood (or coal) stove?..... ☐☐
(00) No (GO TO E26) (01) Yes (99) Don't know (GO TO E26)

E21 Was the stove located inside the house?..... ☐☐
(00) No (GO TO E26) (01) Yes (99) Don't know (GO TO E26)

E22 Was the stove located in an area with any ventilation / windows?..... ☐☐
(00) No (01) Yes (99) Don't know

E23 Did the stove have a chimney?..... ☐☐
(00) No (01) Yes (99) Don't know

E24 How often did you use the stove to <u>cook</u>?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week (05) Only during the winter

E25 How often did you use the stove to <u>heat</u> your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

E26 Did you use any other kind of method to <u>heat</u> your home?.....

(00) No (GO TO E30) (01) Yes

E27 What kind of material did you use?.....

(01) Electricity (03) Gas (05) Wood (06) Other, specify: _____
(02) Petrol (04) Coal (99) Don't know

E28 In what kind of appliance was this material used?.....

(01) Furnace with chimney (05) Fireplace with chimney
(02) Furnace without chimney (06) Baseboards
(03) Open fire (07) Radiators
(04) Fireplace without chimney (08) Other, specify: _____
(99) Don't know

E29 How often did you use this method to heat your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

I will now read a **list of household goods** you may have had in your childhood residence or not. You may find that some of these appliances were not applicable to the epoch you were a child. Chose the answer that best represents your situation, regardless.

E30 Did your place have a refrigerator?.....

(00) No, it had no appliance to cool food (02) Yes
(01) No, it had an ice box (99) Don't know

E31 Did your place have a radio?.....

(00) No (01) Yes (99) Don't know

E32 Did your place have a TV?.....

(00) No (02) Yes, color
(01) Yes, black and white (99) Don't know

E33 Did your place have a machine to wash clothes (inside own dwelling)**?**.....

(00) No, it had no appliance to wash clothes (02) Yes
(01) Yes, it had a clothes ringer (99) Don't know

E34 Did your place have a system to play recorded music?.....

(00) No, it had nothing to play recorded music
(01) Yes, it had a gramophone
(02) Yes, a record player
(03) Yes, a cassette player
(04) Yes, a CD player
(99) Don't know

E35 Did your place have a vacuum cleaner?.....

(00) No, it had no appliance to vacuum
(01) No, it had a non-electric device to vacuum
(02) Yes
(99) Don't know

E36 Did your place have a VCR?.....

(00) No, it had no appliance to watch recorded images
(01) No, it had a less sophisticated image viewing machine
(02) Yes (VCR or DVD)
(99) Don't know

E37 Did your place have a computer?.....

(00) No, that did not exist at the time (01) No (02) Yes (99) Don't know

Also, I would like to ask you...

E38 Did your household have a car?.....

(00) No (GO TO E40) (01) Yes (99) Don't know (GO TO E40)

E39 How many?.....

Finally, I would like to ask you a few questions about the **residential area** where you lived during your childhood. Could you tell me **how common** each of these situations were in your neighbourhood... (Use Answer Sheet)

(00) Not common (01) Common (02) Very common (99) Don't know

E40 Noise from neighbouring apartments, streets, trains, airplanes, industry, etc….

E41 Smoke, dust or smell from industry, traffic, sewage or from other sources.....

E42 Cigarette, cigar and/or pipe smoke from residents in this household.....

LONGEST RESIDENCE IN EARLY ADULT LIFE (17-30 yrs)

Now I would like to ask you a few questions about the residence / home in which you lived **for the longest time during your early adult life, that is between the ages of 17 (incl.) and 30 (incl.)**. I will use the same set of question I used in the previous sections.

Interviewer Reminder: Identify / confirm longest residence in early adulthood using life grid.

E43 Is this residence the same one as your childhood residence?..... ☐☐
(00) No (01) Yes (GO TO E80)

E44 You lived at that place...?

From age?	To age?	i.e. # Years
☐☐	☐☐	☐☐

E45 Do you remember what the POSTAL CODE is for this residence? _____ - _____

For all the following questions, refer to the situation that was present "MOST OF THE TIME" while living in that residence.

E46 What type of setting were you living in at that place?..... ☐☐
(01) With immediate family / alone
(02) With extended family
(03) Foster home (GO TO E80)
(04) Boarding school, monastery (GO TO E80)
(05) Institution (ex.: psychiatric hospital, rehabilitation centre) (GO TO E80)
(06) Pattern of many different living places (GO TO E80)
(07) Other, specify: _____ ☐☐
(99) Don't know

E47 Who was the owner of the place?..... ☐☐
(00) Myself (even if bought after renting)
(01) My family or a member of my family
(02) State or municipality
(03) Private owners / company (renting)
(04) Other, specify: _____ ☐☐
(99) Don't know

E48 How many people lived in the household? (At once, for the longest period of time).. ☐☐
(Include borders, live-in maids, roommates...) (99) Don't know

Interviewer Reminder:
QE48: Include people who were permanent residents and those who were living in the house for the longest period of time.
QE49: Rooms include: kitchen, living room, dining room, bedroom, furnished basement. Do **NOT** include: toilet, bathroom, laundry room, hallway, garage, patio.

E49 How many rooms did your place have? (If renovated, count # rooms during longest period living there)..... ☐☐
(99) Don't know

E50 Were some or all of these rooms damp / humid / wet? (For example: wallpaper peels of wall, mould grows on internal walls, clothes stem when aired after storage)..... ☐☐
(00) No (01) Yes, all (02) Yes, some (99) Don't know

Now, I will read a list of facilities you may have had in the place where you lived. We would like to know **which of these facilities were present inside your early adulthood residence.**

E51 Did your home have a bathroom (indoor toilet, bath and/or shower)?..... ☐☐
(00) No (GO TO E53) (01) Yes (99) Don't know (GO TO E53)

E52 How many?..... ☐☐

E53 Did your home have a sewage system?..... ☐☐
(00) No (02) Yes, a septic tank
(01) Yes, a central public system (99) Don't know

E54 Did your home have running cold water?..... ☐☐
(00) No (02) Yes, an independent one (rural) i.e. outside the house
(01) Yes, a central public system (urban) i.e. inside the house (99) Don't know

E55 Did your home have electricity?..... ☐☐
(00) No (02) Yes, by a generator / battery only
(01) Yes, by a central system (99) Don't know

E56 Did your home have running hot water?..... ☐☐
(00) No (01) Yes (99) Don't know

Could you please tell me…

E57 Did your house have a wood (or coal) stove?..... ☐☐
(00) No (GO TO E63) (01) Yes (99) Don't know (GO TO E63)

E58 Was the stove located inside the house?..... ☐☐
(00) No (GO TO E63) (01) Yes (99) Don't know (GO TO E63)

E59 Was the stove located in an area with any ventilation / windows?..... ☐☐
(00) No (01) Yes (99) Don't know

E60 Did the stove have a chimney?..... ☐☐
(00) No (01) Yes (99) Don't know

E61 How often did you use the stove to <u>cook</u>?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week (05) Only during the winter

E62 How often did you use the stove to <u>heat</u> your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

E63 Did you use any other kind of method to <u>heat</u> your home?.....

(00) No (GO TO E67) (01) Yes

E64 What kind of material did you use?.....

(01) Electricity (03) Gas (05) Wood (06) Other, specify: _____
(02) Petrol (04) Coal (99) Don't know

E65 In what kind of appliance was this material used?.....

(01) Furnace with chimney (05) Fireplace with chimney
(02) Furnace without chimney (06) Baseboards
(03) Open fire (07) Radiators
(04) Fireplace without chimney (08) Other, specify: _____
(99) Don't know

E66 How often did you use this method to heat your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

I will now read a **list of household goods** you may have had in your early adulthood residence or not. You may find that some of these appliances were not applicable to the epoch you were 17 to 30 years old. Chose the answer that best represents your situation, regardless.

E67 Did your place have a refrigerator?.....

(00) No, it had no appliance to cool food (02) Yes
(01) No, it had an ice box (99) Don't know

E68 Did your place have a radio?.....

(00) No (01) Yes (99) Don't know

E69 Did your place have a TV?.....

(00) No (02) Yes, color
(01) Yes, black and white (99) Don't know

E70 Did your place have a machine to wash clothes (inside own dwelling)**?**.....

(00) No, it had no appliance to wash clothes (02) Yes
(01) Yes, it had a clothes ringer (99) Don't know

E71 Did your place have a system to play recorded music?.....

(00) No, it had nothing to play recorded music
(01) Yes, it had a gramophone
(02) Yes, a record player
(03) Yes, a cassette player
(04) Yes, a CD player
(99) Don't know

E72 Did your place have a vacuum cleaner?.....

(00) No, it had no appliance to vacuum
(01) No, it had a non-electric device to vacuum
(02) Yes
(99) Don't know

E73 Did your place have a VCR?.....

(00) No, it had no appliance to watch recorded images
(01) No, it had a less sophisticated image viewing machine
(02) Yes (VCR or DVD)
(99) Don't know

E74 Did your place have a computer?.....

(00) No, that did not exist at the time (01) No (02) Yes (99) Don't know

Also, I would like to ask you...

E75 Did your household have a car?.....

(00) No (GO TO E77) (01) Yes (99) Don't know (GO TO E77)

E76 How many?.....

Finally, here are a few questions about the **residential area** where you lived during your early adulthood. **How common** was it in your neighbourhood to have... (Use Answer Sheet)

(00) Not common (01) Common (02) Very common (99) Don't know

E77 Noise from neighbouring apartments, streets, trains, airplanes, industry, etc….

E78 Smoke, dust or smell from industry, traffic, sewage or from other sources.....

E79 Cigarette, cigar and/or pipe smoke from residents in this household.....

LONGEST RESIDENCE IN LATER ADULTHOOD (30 yrs +)

Now let's talk about your **longest residence in later adulthood**, that is after age 30 (excl.).

Interviewer Reminder: Identify / confirm longest residence in later adulthood using life grid.

E80 Is this residence the same one as the residence you lived in for the longest time between the ages of 17 and 30 or your childhood residence?..... ☐☐

(00) No (01) Yes, same as longest residence between ages of 17-30 (GO TO SECTION F)
(02) Yes, same as childhood residence (GO TO SECTION F)
(03) Yes, same one in the three periods of my life (GO TO SECTION F)
(88) None of the above, ex.: subject is less than 30 yrs old (GO TO SECTION F)

E81 You lived at that place…?

From age? ☐☐ **To age?** ☐☐ **i.e. # Years** ☐☐

E82 Do you remember what the POSTAL CODE is for this residence? _____ - _____

For all the following questions, refer to the situation that was present "MOST OF THE TIME" while living in that residence.

E83What type of setting were you living in at that place?..... ☐☐

(01) With immediate family / alone
(02) With extended family
(03) Foster home (GO TO SECTION F)
(04) Boarding school, monastery (GO TO SECTION F)
(05) Institution (ex.: psychiatric hospital, rehabilitation centre) (GO TO SECTION F)
(06) Pattern of many different living places (GO TO SECTION F)
(99) Don't know
(07) Other, specify: _____ ☐☐

E84 Who was the owner of the place?..... ☐☐

(00) Myself (even if bought after renting)
(01) My family or a member of my family
(02) State or municipality
(03) Private owners / company (renting)
(04) Other, specify: _____ ☐☐
(99) Don't know

E85 How many people lived in the household? (At once, for the longest period of time).. ☐☐
(Include borders, live-in maids, roommates…) (99) Don't know

Interviewer Reminder:
QE85: Include people who were permanent residents and those who were living in the house for the longest period of time.
QE86: Rooms include: kitchen, living room, dining room, bedroom, furnished basement. Do **NOT** include: toilet, bathroom, laundry room, hallway, garage, patio. If renovated, count # rooms during longest period living there.

E86 How many rooms did your place have? (If renovated, count # rooms during longest period living there)..... ☐☐
(99) Don't know

E87 Were some or all of these rooms damp / humid / wet? (For example: wallpaper peels of wall, mould grows on internal walls, clothes stem when aired after storage)..... ☐☐
(00) No (01) Yes, all (02) Yes, some (99) Don't know

Now, I will read a list of facilities you may have had in the place where you lived. We would like to know **which of these facilities were present inside your later adulthood residence.**

E88 Did your home have a bathroom (indoor toilet, bath and/or shower)?..... ☐☐
(00) No (GO TO E90) (01) Yes (99) Don't know (GO TO E90)

E89 How many?..... ☐☐

E90 Did your home have a sewage system?..... ☐☐
(00) No (02) Yes, a septic tank
(01) Yes, a central public system (99) Don't know

E91 Did your home have running cold water?..... ☐☐
(00) No (02) Yes, an independent one (rural) i.e. outside the house
(01) Yes, a central public system (urban) i.e. inside the house (99) Don't know

E92 Did your home have electricity?..... ☐☐
(00) No (02) Yes, by a generator / battery only
(01) Yes, by a central system (99) Don't know

E93 Did your home have running hot water?..... ☐☐
(00) No (01) Yes (99) Don't know

Could you please tell me…

E94 Did your house have a wood (or coal) stove?..... ☐☐
(00) No (GO TO E100) (01) Yes (99) Don't know (GO TO E100)

E95 Was the stove located inside the house?..... ☐☐
(00) No (GO TO E100) (01) Yes (99) Don't know (GO TO E100)

E96 Was the stove located in an area with any ventilation / windows?..... ☐☐
(00) No (01) Yes (99) Don't know

E97 Did the stove have a chimney?..... ☐☐
(00) No (01) Yes (99) Don't know

E98 How often did you use the stove to <u>cook</u>?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week (05) Only during the winter

E99 How often did you use the stove to <u>heat</u> your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

E100 Did you use any other kind of method to <u>heat</u> your home?.....

(00) No (GO TO E104) (01) Yes

E101 What kind of material did you use?.....

(01) Electricity (03) Gas (05) Wood (06) Other, specify: _____
(02) Petrol (04) Coal (99) Don't know

E102 In what kind of appliance was this material used?.....

(01) Furnace with chimney (05) Fireplace with chimney
(02) Furnace without chimney (06) Baseboards
(03) Open fire (07) Radiators
(04) Fireplace without chimney (08) Other, specify: _____
(99) Don't know

E103 How often did you use this method to heat your home?.....

(00) Never (02) 5-6 times a week (04) 1-2 times a week
(01) Everyday (03) 3-4 times a week

I will now read a **list of household goods** you may have had in your later adulthood residence or not. You may find that some of these appliances were not applicable to the epoch you were in later adulthood. Chose the answer that best represents your situation, regardless.

E104 Did your place have a refrigerator?.....

(00) No, it had no appliance to cool food (02) Yes
(01) No, it had an ice box (99) Don't know

E105 Did your place have a radio?.....

(00) No (01) Yes (99) Don't know

E106 Did your place have a TV?.....

(00) No (02) Yes, color
(01) Yes, black and white (99) Don't know

E107 Did your place have a machine to wash clothes (inside own dwelling)**?**.....

(00) No, it had no appliance to wash clothes (02) Yes
(01) Yes, it had a clothes ringer (99) Don't know

E108 Did your place have a system to play recorded music?.....

(00) No, it had nothing to play recorded music
(01) Yes, it had a gramophone
(02) Yes, a record player
(03) Yes, a cassette player
(04) Yes, a CD player
(99) Don't know

E109 Did your place have a vacuum cleaner?.....

(00) No, it had no appliance to vacuum
(01) No, it had a non-electric device to vacuum
(02) Yes
(99) Don't know

E110 Did your place have a VCR?.....

(00) No, it had no appliance to watch recorded images
(01) No, it had a less sophisticated image viewing machine
(02) Yes (VCR or DVD)
(99) Don't know

E111 Did your place have a computer?.....

(00) No, that did not exist at the time (01) No (02) Yes (99) Don't know

Also, I would like to ask you...

E112 Did your household have a car?.....

(00) No (GO TO E114) (01) Yes (99) Don't know (GO TO E114)

E113 How many?.....

Finally, here are some questions about the **residential area** where you lived during your later adulthood. **How common** was it in your neighbourhood to have... (Use Answer Sheet)

(00) Not common (01) Common (02) Very common (99) Don't know

E114 Noise from neighbouring apartments, streets, trains, airplanes, industry, etc...

E115 Smoke, dust or smell from industry, traffic, sewage or from other sources.....

E116 Cigarette, cigar and/or pipe smoke from residents in this household.....

Country: 0 2 - ID N°: _ _ _ - _

F. SMOKING AND CHEWING HABITS

Now I would like to ask you some questions about your smoking and/or chewing habits.

F1 Have you ever smoked in your life? (or chewed, any product, any amount)..... _ _
(00) Never (GO TO F6) (01) Yes (I still do) (02) Yes, but only in the past

Think of the periods in your life during which you smoked cigarettes, cigars, pipe, chewed tobacco products and/or took drugs, the amount you smoked / chewed / took and other details about the products. Please try to summarise the most important changes in the amount and type of product.

Interviewer Reminder: Use **life grid** if necessary to help answer Q F2 to F8.
- Avoid overlapping years for the same product, type of cigarette or amount smoked, i.e. record 30-40, 41-45 rather than 30-40, 40-45.
- Only note changes occurring for **one year or more**.
- Exclude quitting during pregnancy(ies) if for less than one year.

F2 Do / did you smoke cigarettes?..... _ _
(00) No (GO TO F3) (01) Yes (02) Yes, only in the past

From age	To age (A)	Type (B)	Brand	#cigarettes/Day (D)
_ _	_ _	_ _	_____	_ _
_ _	_ _	_ _	_____	_ _
_ _	_ _	_ _	_____	_ _
_ _	_ _	_ _	_____	_ _
_ _	_ _	_ _	_____	_ _
_ _	_ _	_ _	_____	_ _

To Age (A)	Type (B)	No/Day (D)
If still smoking, write age at time of interview	(01) Filter (02) Non-filter (03) Hand rolled	(00) If less than daily Make average if not constant frequency

Section F – Smoking and Chewing Habits

0	2	-				-	
Country			ID N°				

F3 Do / did you smoke cigar?..... ☐☐

(00) No (GO TO F4) (01) Yes (02) Yes, only in the past

From age	To age (A)	Brand	#cigars/Day (D)
☐☐	☐☐	_____	☐☐
☐☐	☐☐	_____	☐☐
☐☐	☐☐	_____	☐☐

To Age (A)	No/Day (D)
If still smoking, write age at time of interview	(00) If less than daily Make average if not constant frequency

F4 Do / did you smoke pipe?..... ☐☐

(00) No (GO TO F5) (01) Yes (02) Yes, only in the past

From age	To age (A)	Brand	Unit (C)	#/Day(D)
☐☐	☐☐	_____	☐☐	☐☐
☐☐	☐☐	_____	☐☐	☐☐
☐☐	☐☐	_____	☐☐	☐☐

To Age (A)	Unit (C)	No/Day (D)
If still smoking, write age at time of interview	(01) Grams (02) Pipes	(00) If less than daily Make average if not constant frequency

F5 Do / did you <u>smoke or inhale</u> drugs (marijuana, grass, dope, joints...) at least once a week for at least 6 months in your lifetime?..... ☐☐

(00) No (GO TO F6) (01) Yes (02) Yes, only in the past

From age	To age (A)	Type (B)	Unit (C)	#/Day(D)
☐☐	☐☐	☐☐	☐☐	☐☐
☐☐	☐☐	☐☐	☐☐	☐☐
☐☐	☐☐	☐☐	☐☐	☐☐

To Age (A)	Type (B)	Unit (C)	No/Day (D)
If still smoking, write age at time of interview If less than one year, write same age From and To	(01) Marijuana (02) Grass (03) Crack (04) Hashish	(01) Grams (02) Joints	(00) If less than daily Make average if not constant frequency

F6 Do / did you <u>use any other drugs</u> (cocaine, heroin, LSD...) at least once a week for at least 6 months in your lifetime?.....

(00) No (GO TO SECTION G) (01) Yes (02) Yes, only in the past

From age	To age (A)	Type (B)	Unit (C)	#/Day(D)

To Age (A)	Type (B)	Unit (C)	No/Day (D)
If still using, write age at time of interview If less than one year, write same age From and To	(01) Cocaine (02) Acid / LSD (03) Speed (04) Heroin	(01) Grams (02) Joints (03) Injections (04) Pills	(00) If less than daily Make average if not constant frequency

Country 0 2 - ID N° _ _ _ - _

G. DRINKING HABITS

Now I would like to ask you some questions about your drinking habits.

G1 Have you ever drunk alcoholic beverages <u>at least once a month</u>?..... _ _

(00) No (GO TO SECTION H) (01) Yes, I do (02) Yes, only in the past

We can use the grid to help us describe the periods in your life during which you consumed alcoholic beverages. Please try to summarise the most important changes in your life regarding the amount and type of beverage.

<u>Interviewer Reminder</u>: Use **life grid** if necessary to help answer Q G3.

- Avoid overlapping years for the same beverage i.e. record 30-40, 41-45 rather that 30-40, 40-45. Ask about each beverage separately.
- Note only changes occurring for **<u>one year or more</u>.**
- Exclude quitting during pregnancy(ies) if for less than one year.

G2 When do / did you usually drink alcoholic beverages?..... _ _

(01) With meals (03) Both

(02) Between meals (04) Only at social events

G3 Beverage (A)	If (A) = (05), Then specify other beverage	From age	To age	Unit (B)	Consumption (how many)	Per (C)
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _
_ _	_ _	_ _	_ _	_ _	_ _	_ _

Beverage (A)
(01) Wine
(02) Beer / cider
(03) Hard liquor (>35) (whisky, cognac, vodka, brandy, grappa, marc, gin, rum)
(04) Aperitif (<35) (Martini, port, sherry, vermouth)
(05) Other, specify: _____

Unit (B)
(01) Small glass (50ml) (1-2oz)
(02) Medium glass (100ml) (2-3oz)
(03) Big glass (250ml) (7oz) (1/2 pint)
(04) ½ small bottle (330ml) (1beer)
(05) Bottle (700-750 ml) (21oz)

Per (C)
(01) Day
(02) Week
(03) Month

Country: 0 2 - ID N°: _ _ _ - _

H. DIETARY HABITS

Now, I have some questions about your dietary habits from your childhood (0-16 yrs).

H1 Please name 5 foods (any type) which you ate the most often during your childhood, starting with the most frequent.

1 _____
2 _____
3 _____
4 _____
5 _____

H2 If applicable, please name 5 foods (any type) which you did <u>not</u> eat during your childhood for any reason (religious beliefs, dislike, allergies, etc…).

1 _____
2 _____
3 _____
4 _____
5 _____

I would like to ask you a few questions about a list of foods that you ate during your childhood. Could you please tell me how often you ate the following foods during the ages of 0-16 yrs... (Use <u>Answer Sheet</u>)

(00) Sometimes	(01) Often	(02) Very Often	(99) I don't know

H3 Meat (all kinds)..... _ _

H4 Fish..... _ _

H5 Dairy products (milk, yogurt, cheese)..... _ _

H6 Vegetables..... _ _

H7 Fruits..... _ _

H8 Candies & Desserts..... _ _

H9 Chips & Fried Snacks..... _ _

H10 Did you eat spicy foods during your childhood?..... _ _

(00) No
(01) Yes, a little bit (mild)
(02) Yes, moderately spicy
(03) Yes, very spicy

Section H – Dietary Habits

0	2	-				-	
Country			ID N°				

Now, I have some questions about your dietary habits. As these habits may have changed somewhat according to your health status, **please tell me about your usual habits before diagnosis of the disease / being seen at this clinic**. How frequently did you consume the following foods and beverages?

Interviewer Reminder: Adapt portions to ones in table below.
- If less than once a week, **code (98)**.
- If not consumed at all, **code (00)**.
- **If don't not know code (99)**.

	Unit	Food item	Frequency (Per week)
H11	1 glass (200ml).....	Milk.....	
H12	1 pot (125g).....	Yoghurt.....	
H13	1 teaspoon.....	Butter.....	
H14	1 serving (50g) (2 slices).....	Bread.....	
H15	1 serving (4 full tablespoons).....	Pasta or rice.....	
H16	1 serving (100g) (1 side dish).....	Maize (Corn based dishes, polenta).....	
H17	1 serving (80g) (medium piece)..	Red meat (beef).....	
H18	1 serving (100g) (medium piece)	Pork.....	
H19	1 serving (160g) (medium piece)	Chicken.....	
H20	1 serving (80g) (medium piece)..	Lamb.....	
H21	1 serving (150g) (medium piece)	Fish.....	
H22	1 serving.....	Ham (2 slices), salami (4 slices), sausages (1)	
H23	1.....	Egg.....	
H24	1 serving (50g).....	Cheese.....	
H25	1 medium.....	Potatoes.....	
H26	1 serving (50g) (1 side dish).....	Raw green vegetables and salads.....	
H27	1 serving (50g) (1 side dish).....	Cruciferae (broccoli, cabbage, Brussels sprouts)	

H28	1 medium.....	Carrots.....	
H29	1 medium.....	Fresh tomatoes (in season).....	
H30	1 serving (4 full tablespoons).....	Pulses (chickpeas, beans, lentils, etc.).....	
H31	1 serving (50g) (1 side dish).....	*As a summary, how often would you say you eat any kind of vegetable (except potatoes)?*.....	
H32	1 glass (200ml).....	**Fresh** fruit juices.....	
H33	1 medium.....	Apples or pears.....	
H34	1 medium.....	Citrus fruit (oranges, grapefruit, lemons) (in season).....	
H35	1 medium.....	Bananas.....	
H36	1 medium.....	*As a summary, how often would you say you eat any kind of fresh fruit (including fruit salads)?*.....	
H37	1 slice or cup.....	Cake and desserts.....	
H38	1 portion.....	Chips and fried snacks.....	

H39 Which type of fat did you predominantly use to season vegetables?.....

(00) I don't use any fat	(04) Raisin oil	(08) Other vegetable oil	(12) I don't use animal fat
(01) Olive oil	(05) Corn oil	(09) Margarine	
(02) Dandelion oil	(06) Sunflower oil	(10) Butter	(13) Other fat
(03) Coconut oil	(07) Soy bean oil	(11) Pork fat	(99) Don't know

H40 Which type of fat did you predominantly use for cooking?.....

(00) I don't use any fat	(04) Raisin oil	(08) Other vegetable oil	(12) I don't use animal fat
(01) Olive oil	(05) Corn oil	(09) Margarine	
(02) Dandelion oil	(06) Sunflower oil	(10) Butter	(13) Other fat
(03) Coconut oil	(07) Soy bean oil	(11) Pork fat	(99) Don't know

H41 On average, how often did you eat barbecued food in the summer?.....

(00) I never eat BBQ
(01) Less than once a month
(02) Once a month
(03) Less than once a week
(04) Once or twice a week
(05) 3 to 5 times a week
(06) More than 5 times a week
(99) Don't know

H42 On average, how often did you eat barbecued food in the winter?.....

(00) I never eat BBQ
(01) Less than once a month
(02) Once a month
(03) Less than once a week
(04) Once or twice a week
(05) 3 to 5 times a week
(06) More than 5 times a week
(99) Don't know

H43 Did you drink coffee?.....

(00) No (GO TO H44) (01) Yes (02) Yes, only in the past

From age	**To age**	**# Cups**	**Per (C)** (01) Day, (02) Week, (03) Month

H44 How many cups of tea do you drink per day?.....

(00) I don't drink tea (98) Less than one a day

H45 How many cans of regular soda do you drink per day?.....

(00) I don't drink regular soda (98) Less than one a day

H46 How many cans of diet soda do you drink per day?.....

(00) I don't drink diet soda (98) Less than one a day

Section H – Dietary Habits

0	2	-				-	
Country			**ID N°**				

Interviewer Reminder: Note weight and height in measure used by participant. Later, use conversions to record weight in **kgs** and height in **cms**. See Interviewer's guide for conversions.

H47 If you remember, can you tell me what your weight was two years ago?..... ☐☐☐
(_____ lbs) , i.e. _____**kgs** (999) Don't know

H48 Can you tell me what your weight was at age 30?..... ☐☐☐
(_____ lbs) , i.e. _____**kgs** (999) Don't know

H49 Can you tell me what your weight was at age 20?..... ☐☐☐
(_____ lbs) , i.e. _____**kgs** (999) Don't know

H50 What is your height?..... ☐☐☐
(_____ feet _____ inches) , i.e. _____**cm** (999) Don't know

H51 Physical Activity and Hobbies
We would like to know which activities and hobbies you have during your adulthood. Please indicate if you have participated in the following activities regularly i.e. for at least 6 months.

							Frequency				
Activities	**Y**	**N**	**Don't know**	**Age at start**	**Age at end**	**# months**	**# times**	**per day**	**per week**	**per month**	**Total years**
Walking (for exercise)											
Jogging or running											
Aerobics											
Golf											
Racket sports (tennis, squash, etc…)											
Bowling or curling											
Swimming											
Skiing or skating											
Biking											
Dancing											
Gardening											
Outdoor physical work (mowing the lawn, shovelling, raking…)											
Household work											
Construction work (sawing, sanding, etc…)											
Car maintenance / work											
Other physical activities											
1)											
2)											
3)											
4)											

I. ORAL HEALTH

I am going to ask you some questions about your oral health **before your diagnosis / being seen at this clinic** and at a different time in your lifetime.

I1 Did you wear complete dentures?..... [ ][ ]
(00) No (GO TO I4) (02) Yes, top only
(01) Yes, bottom only (GO TO I3) (03) Yes, top AND bottom

I2 At what age did you start wearing complete top dentures? (Years)..... [ ][ ][ ]

I3 At what age did you start wearing complete bottom dentures? (Years)..... [ ][ ][ ]
Code (888) if QI1 = (02)

Interviewer Reminder: If both top AND bottom complete dentures, i.e. (03) to Q I1, skip to I10.

I4 Did you wear partial dentures?..... [ ][ ]
(00) No (02) Yes, bottom only
(01) Yes, top only (03) Yes , top AND bottom

Interviewer Reminder: Refer to life grid to separate each life period.

I5 How often did you clean your teeth?..... [ ][ ]
(00) Never (03) Every other day (3-4 times a week)
(01) Less than once a week (04) Once a day
(02) 1-2 time a week (05) Twice or more a day

I6 Did you use dental floss?..... [ ][ ]
(00) No (02) Yes, once a week
(01) Yes, daily (03) Rarely

I7 Did you use toothpicks / sticks?..... [ ][ ]
(00) No (02) Yes, once a week
(01) Yes, daily (03) Rarely

I8 Did you use any kind of substance to clean your teeth?..... [ ][ ]
(00) No (02) Other, specify: _____ [ ][ ]
(01) Toothpaste

I9 Did your gums bleed when you cleaned your teeth?..... [ ][ ]
(00) No (01) Sometimes (02) Always or almost always

I10 Did you use mouthwash?..... ☐☐
(00) No (GO TO I13) (01) Yes

I11 How often did you use mouthwash?..... ☐☐
(01) Less than once a week (03) Every other day (3-4 times a week) (04) Once a day
(02) 1-2 times a week (05) Twice or more a day

I12 What was the brand name of the mouthwash? (LC)..... ☐☐
Brand name: _____

Now, let's look at your oral health habits and oral health at different periods of your life.

I13 In the <u>last 20 years</u>, how often did you see a dentist?..... ☐☐
(00) Never (03) Every 2 –5 years
(01) Every 6 months (04) Once every 5 years
(02) Every year (05) Only when I had pain

I14 Have you ever had a tooth extracted?..... ☐☐
(00) No (GO TO I16) (01) Yes

I15 How many teeth extractions had you had?
Up until you were 16 of age..... ☐☐
Between 17-30 years of age..... ☐☐
After 30 years of age but before the diagnosis of the disease..... ☐☐

(00) None	(02) 6–15	(04) 21-30	(99) Don't know
(01) 1-5	(03) 16-20	(05) More than 30	

I16 Have you ever had a filling?..... ☐☐
(00) No (GO TO SECTION J) (01) Yes

I17 How many fillings had you had?
Up until you were 16 of age..... ☐☐
Between 17-30 years of age..... ☐☐
More than 30 years of age..... ☐☐

J. FAMILY HISTORY OF CANCER

Interviewer Reminder:
- Family includes these **biological** relatives: father, mother, brother, sister, son, daughter, aunt, uncle, grandmother, grandfather.
- Input one person per line in chart below.

J1 Has any member of your family ever had cancer?..... ___

(00) No (GO TO SECTION K) (01) Yes (99) Don't know

J2

Relationship (A)	Status (B)	Current / last Age (C)	Type of cancer	Type of tumour (LC)	Age at Diagnosis (D)
				-	
				-	
				-	
				-	
				-	
				-	

Relationship (A)	Status (B)	Current / last Age (C)	Age at diagnosis (D)
(01) Mother (02) Father (03) Sister (04) Brother (05) Daughter (06) Son (07) Grand-mother (08) Grand-father (09) Aunt / uncle	(00) Deceased (01) Alive	(999) Don't know If alive, give present age If deceased, give age at death	(999) Don't know

K. FAMILY ENVIRONMENT IN CHILDHOOD

I would like to ask you a few questions about your parents (mother and father), or the women or men who cared for you during your childhood, that is from your birth until you were 16 (incl.). If you were cared for by only one person, please respond only to the questions related to that person. We may refer to the grid to help us out at times.

This first set of questions is related to their level of education and their occupation.

K1 At your birth, how old was your <u>natural</u> father?..... ☐☐
(99) Don't know

K2 At your birth, how many years of education did your father / the man who cared for you most of your childhood have?..... ☐☐
(99) Don't know

K3 What was his longest occupation during your childhood? (LC)..... ☐☐☐
Describe: _____
(999) Don't know

K4 At your birth, how old was your <u>natural</u> mother?..... ☐☐
(99) Don't know

K5 At your birth, how many years of education did your mother / the woman who cared for you most of the time during your childhood have?..... ☐☐
(99) Don't know

K6 What was her longest occupation during your childhood? (LC)..... ☐☐☐
Describe: _____
(999) Don't know

<u>Interviewer Reminder</u>: Confirm occupation codes in K3 and K6 with list of codes.

Now I have a few questions on family environment during your childhood.

K7 In total, how many brothers and sisters do you have? (natural only)..... ☐☐

K8 What was your birth order in your family (at time you were 16 years old)?..... ☐☐
(00) Only child (02) Second child (04) Fourth child or more
(01) First child (03) Third child

K9 Did your family have continuous financial difficulties during your childhood? ☐☐
(00) No (01) Yes (99) Don't know

K10 Did your parents argue or fight during your childhood?.....
(00) Never (02) Often
(01) Sometimes (99) Don't know

K11 How often did your father use to drink alcohol during your childhood?.....
(00) Never (02) Once a week / weekends (04) Everyday
(01) Occasionally (03) 3-4 times a week (99) Don't know

K12 How often did your mother use to drink alcohol during your childhood?.....
(00) Never (02) Once a week / weekends (04) Everyday
(01) Occasionally (03) 3-4 times a week (99) Don't know

K13 Did your father smoke? (any product).....
(00) No (01) Yes (99) Don't know

K14 Did your mother smoke? (any product).....
(00) No (01) Yes (99) Don't know

The following six questions relate to your natural parents.

K15 Were you ever separated from your <u>biological mother</u> <u>for a year or more</u> during your childhood?.....
(00) No (GO TO K18) (01) Yes (99) Don't know

K16 How old were you?

From age?	**To age?** *(max = 16)*	**i.e. # Years**

K17 Why did the separation happen?.....
(00) Parents separated / divorced (03) Adoption
(01) Mother died (04) Other, specify: _____
(02) Mother ill

K18 Were you ever separated from your <u>biological father</u> <u>for a year or more</u> during your childhood.....
(00) No (GO TO K21) (01) Yes (99) Don't know

K19 How old were you?

From age?	**To age?** *(max = 16)*	**i.e. # Years**

K20 Why did the separation happen?.....
(00) Parents separated / divorced (03) Adoption
(01) Father died (04) Other, specify: _____
(02) Father ill

Now I would like to ask you a few questions about your mother / father figure during your childhood.

K21 Who was the woman who cared for you most of your life during your childhood?
(00) None (GO TO K29)
(01) Biological mother
(02) Step mother
(03) Adoptive mother
(04) Grand-mother
(05) Other, specify: _____

Here are some questions about how you remember your **MOTHER** (or the woman who cared for you) during the years you were growing up, that is, until you were age 16 – incl. (Use Answer Sheet)

(01) A great deal	(02) Quite a lot	(03) Little	(04) Not at all

K22 How much did she understand your problems and worries?.....

K23 How much could you confide in her about things that were bothering you?....

K24 How much love and affection did she give you?.....

K25 How much time and attention did she give you when you needed it?.....

K26 How strict was she with the rules for you?.....

K27 How harsh was she when she punished you?.....

K28 How much did she expect you to do your best in everything you did?.....

Now I would like to ask you how you remember your **FATHER** (or the man who cared for you) during the years you were growing up that is, until you were 16 years old. (Use Answer Sheet)

K29 Who was the man who cared for you most of your life during your childhood?.....
(00) None (GO TO K37)
(01) Biological father
(02) Step father
(03) Adoptive father
(04) Grand-father
(05) Other, specify: _____

(01) A great deal	(02) Quite a lot	(03) Little	(04) Not at all

K30 How much did he understand your problems and worries?.....

K31 How much could you confide in him about things that were bothering you?...

K32 How much love and affection did he give you?.....

K33 How much time and attention did he give you when you needed it?..... ☐☐

K34 How strict was he with the rules for you?..... ☐☐

K35 How harsh was he when he punished you?..... ☐☐

K36 How much did he expect you to do your best in everything you did?..... ☐☐

I have only a few more questions about your childhood. You do not have to answer if you do not feel comfortable doing so. **Did any of the following things happen during your childhood...? (0-16 yrs)**

K37 Were you physically abused?..... ☐☐
(00) No (01) Yes

K38 Were you sexually abused?..... ☐☐
(00) No (01) Yes

K39 Were your parents divorced?..... ☐☐
(00) No (01) Yes

Finally,

K40 Can you remember any life event(s) in your childhood that have either positively or negatively impacted upon you?..... ☐☐
(00) No (GO TO SECTION L) (01) Yes

K41 Can you tell me what? (Describe) (**LC**)..... ☐☐
1 _____ ☐☐
2 _____ ☐☐
3 _____ ☐☐
4 _____ ☐☐
5 _____ ☐☐

K42 Could you please tell me how much impact this (se) event (s) had on your life?
(Use Answer Sheet).....

-4 -3 -2 -1 0 1 2 3 4
Very negative no impact Very positive

Event 1 ……..score: _____…….. ☐☐
Event 2 ……..score: _____…….. ☐☐
Event 3 ……..score: _____…….. ☐☐
Event 4 ……..score: _____…….. ☐☐
Event 5 ……..score: _____…….. ☐☐

K43 For each of the following diseases, can you tell me if you ever had it and, if so, how often?

Presence (A)	Frequency (B)
(00) No	(01) Once
(01) Yes	(02) Sometimes
(99) Don't know	(03) Often

	Presence (A)	Frequency (B)
Measles	[ ][ ]	[ ][ ]
Mumps	[ ][ ]	[ ][ ]
Chicken pox	[ ][ ]	[ ][ ]
Whooping cough	[ ][ ]	[ ][ ]
Scarlet fever	[ ][ ]	[ ][ ]
Rheumatic fever	[ ][ ]	[ ][ ]
Infectious hepatitis	[ ][ ]	[ ][ ]
Tuberculosis	[ ][ ]	[ ][ ]
Asthma attack	[ ][ ]	[ ][ ]
Disease of the ear(s)	[ ][ ]	[ ][ ]
Disease of the nose	[ ][ ]	[ ][ ]
Disease of the throat	[ ][ ]	[ ][ ]

Other diseases:
Specify (ex.: chronic heartburn, bulimia):

_____	[ ][ ]	[ ][ ]
_____	[ ][ ]	[ ][ ]
_____	[ ][ ]	[ ][ ]
_____	[ ][ ]	[ ][ ]
_____	[ ][ ]	[ ][ ]

L. MARRIAGE, INTIMACY & LIFE AS A COUPLE

Now, I would like to ask you some questions about marriage and living as a couple.

L1 What is your marital status?..... ☐☐

(01) Single
(02) Living with a husband / wife (married)
(03) Living with a partner in common law
(04) Divorced
(05) Widowed
(06) Separated

Interviewer Reminder: Use **life grid** if necessary to help answer Q L2 to L26.

L2 How many times have you been married or lived in common law?..... ☐☐

(01) None (GO TO L8) (01) Once (Fill in first column only) (02) More than once

At the time you FIRST / LAST got married or FIRST / LAST lived in common law...

	FIRST	LAST
L3 How old were you?.....	☐☐	☐☐
L4 How many years did your partner go to school for? (until today)	☐☐	☐☐
L5 What was your partner's longest occupation? (until today) **(LC)**	☐☐☐	☐☐☐

FIRST partner: _____

LAST partner: _____

L6 How did the relationship end?..... ☐☐ ☐☐

(00) Still ongoing! (GO TO L8)
(01) Divorce
(02) Separation
(03) Partner deceased

L7 How old were you when the relationship ended?..... ☐☐ ☐☐

L8 In your whole life, how many (biological) children have you had?..... ☐☐

(00) None (GO TO L10) *(Do NOT include miscarriage or stillborn)*

L9 With how many <u>different</u> partners?..... ☐☐

(00) All with the same one

I will ask you some questions regarding your sexuality. The reason I am asking these questions is because medical science has found some links between viruses that are sexually transmitted and some types of cancers. <u>You have no obligation to answer these questions if you do not feel comfortable doing so.</u>

L10 Have you ever had sexual intercourse?..... ☐☐

(00) No (GO TO L14) (01) Yes
(99) Prefer not to say / Don't know

L11 How old were you when you had sexual intercourse for the first time?.....
(99) Prefer not to say / Don't know

L12 How many sexual partners have you had in total in your life? (regular and casual)
Up to 16 yrs old.....
Between 17-30 yrs old.....
After 30 yrs old.....

Answer's options L12 and L13		
(00) None	(03) 06-10	(06) 51-100
(01) One	(04) 11-20	(07) More than 100
(02) 2-5	(05) 21-50	(99) Prefer not to say / Don't know

L13 How many of these were prostitute? (99) Prefer not to say / Don't know
Up to 16 yrs old.....
Between 17-30 yrs old.....
More than 30 yrs old.....

L14 Have you ever had oral sex? (your mouth and a woman / man genitals).....
(00) No (GO TO L17) (99) Prefer not to say / Don't know (GO TO L17)
(01) Yes

L15 How old were you when you had oral sex for the first time?.....
(99) Prefer not to say / Don't know

Answer's options Q16	
(00) Occasionally	(02) Most of the time
(01) Frequently	(99) Prefer not to say / Don't know

L16 How often?
Up to 16 yrs old.....
Between 17-30 yrs old.....
After 30 years old.....

L17 Have you ever had non-consenting sex?.....
(00) No (GO TO L19) (99) Prefer not to say / Don't know (GO TO L19)
(01) Yes

L18 How old were you or from what age to what age? *(mark same age if one episode or if during less than one year)* (99) Prefer not to say / Don't know

From age? **To age?** **i.e. # Years**

L19 Have you ever had skin warts?.....
(00) No (GO TO L22) (99) Prefer not to say / Don't know (GO TO L22)
(01) Yes

L20 If yes, where? (01) Yes (00) No (99) Prefer not to say / Don't know

Hands.....
Feet.....
Head and Neck.....
Other, specify: _____.....

L21 At which age, were you? (99) Prefer not to say / Don't know

Hands.....
Feet.....
Head and Neck.....
Other, specify: _____.....

L22 Since you started your sexual life have you ever had Candida Albicans?.....

(00) No (GO TO L24) (99) Prefer not to say / Don't know (GO TO L24)
(01) Yes

L23 If yes, where? (01) Yes (00) No (99) Prefer not to say / Don't know

Genital.....
Mouth.....
Other, specify: _____.....

L24 Have you ever had a sexually transmitted disease?.....

(00) No (GO TO SECTION M) (99) Prefer not to say / Don't know (GO TO SECTION M)
(01) Yes

L25 If yes, which ones? (01) Yes (00) No (99) Prefer not to say / Don't know

Gonorrhea.....
Syphilis.....
Herpes.....
Chlamydia.....
AIDS.....

L26 At which age, were you? (99) Prefer not to say / Don't know

Gonorrhea.....
Syphilis.....
Herpes.....
Chlamydia.....
AIDS.....

(*Note other types of sexually transmitted diseases in the* ***Participant's comments*** *on page 53.*)

M. SOCIAL SUPPORT

Finally I would like to ask you some questions about your friends, relatives and the people you live with.

M1 Is there <u>someone in particular</u> in your life that you think would listen to you and give you emotional support if you needed it?..... ☐☐
(01) Yes (00) No (GO TO M9)

	1st PERSON	2nd PERSON
M2 What is your relationship with this person?.....	☐☐	☐☐
(01) Spouse / partner (living together) (05) Neighbour		
(02) Boyfriend / girlfriend (06) Colleague		
(03) Parent (07) Son / daughter		
(04) Brother / sister (08) Other family member (cousin, etc)		
(09) Friend		
(10) Other, specify: _____	☐☐	☐☐
M3 Does he/she live near enough to come around if something came up? (01) Yes (00) No	☐☐	☐☐
M4 On average how often have you <u>seen</u> him / her in the last year?.....	☐☐	☐☐
(01) Not in the last year (04) 1 or 2 times a week		
(02) Less than once a month (05) 3+ times a week		
(03) Less than once a week		
M5 Would you prefer to see him / her more / less often or is this about right for you?..... (01) Less often (02) About right (03) More often	☐☐	☐☐
M6 How long have you known him / her for? (Years).....	☐☐	☐☐
M7 Would you say that you could talk frankly and share your feelings with him / her?.....	☐☐	☐☐
(00) No (02) Yes, about most things		
(01) Yes, about some things (03) Yes, about anything		

M8 Apart from this person / these two people, is there anyone else in particular that you think would listen to you and be supportive if you needed it?..... ☐☐
(00) No (01) Yes

M9 In your life in general, do you think you have enough opportunities to talk openly and share your feelings about things?..... ☐☐
(00) No (01) Yes

M10 In general, do you prefer to keep your feelings to yourself?..... ☐☐
(00) No (01) Yes

M11 Can you remember any life event(s) in your adulthood that have either positively or negatively impacted upon you?..... | |

(00) No (GO TO M14) (01) Yes

M12 Can you tell me what? (Describe) **(LC)**.....

1_____ | |

2_____ | |

3_____ | |

4_____ | |

5_____ | |

M13 Could you please tell me how much impact did this (se) event (s) have in your life?
(Use Answer Sheet).....

-4	-3	-2	-1	0	1	2	3	4
Very negative				no impact				Very positive

Event 1 ……..score: _____……... | |

Event 2 ……..score: _____……... | |

Event 3 ……..score: _____……... | |

Event 4 ……..score: _____……... | |

Event 5 ……..score: _____……... | |

M14 Do you have any brothers or sisters of a similar age (±5 yrs) that would be interested in participating in this interview?..... **8 8**

(00) No (01) Yes

M15 10% of participants of this study will be re-interviewed. Do you agree to be re-contacted for you to participate a second time?..... | |

(00) No (01) Yes

M16 Incomplete questionnaire?..... | |

(00) No (01) Yes

If YES, reason:_____

M17 Time of end of interview..... | | - | |

Hour **Minute**

M18 Interviewer's initials?..... | |

M19 Initials of data enterer into FileMaker?..... | |

Participant's comments:

_____

_____

_____

N. BIOLOGICAL SAMPLING

N1 Was a mouthwash sample taken?..... ___
(00) No (01) Yes (02) Yes, but taken with water

N2 Was a sample for HPV analysis taken?
(this sample is taken from the lesion site for cases, from healthy buccal cells for controls).... ___
(00) No (01) Yes

N3 Was a sample for genetic analysis taken?
(this sample is taken from healthy buccal cells from both the cases and controls)..... ___
(00) No (01) Yes

N4 Please document below if there was any comments from the biological sampling (e.g., occurrence of untoward / adverse events such as patient discomfort, bleeding).

_____

_____

N5 Were all 3 above samples delivered to Dr Coutlée's laboratory?..... ___
(00) No (01) Yes

N6 Date of Sample Delivery..... ___ - ___ - ___
Day Month Year

N7 Please document below if there was any comments from the state of the sample (e.g., leaking of vials, etc…).

_____

_____

N8 HPV ANALYSIS

		Mouthwash		HPV		GEN	
	HPV type	**Present**	**Not-present**	**Present**	**Not-present**	**Present**	**Not-present**
N8a	6						
N8b	11						
N8c	16						
N8d	18						
N8e	26						
N8f	31						
N8g	33						
N8h	35						
N8i	39						
N8j	40						
N8k	42						
N8l	45						
N8m	51						
N8n	52/33/35/58						
N8o	52tm						

		Mouthwash		HPV		GEN	
	HPV type	**Present**	**Not-present**	**Present**	**Not-present**	**Present**	**Not-present**
N8p	53						
N8q	54						
N8r	55						
N8s	56						
N8t	58						
N8u	59						
N8v	61						
N8w	62						
N8x	64						
N8y	66						
N8z	67						
N8aa	68						
N8bb	69						
N8cc	70						
N8dd	71						
N8ee	72						
N8ff	73						
N8gg	81						
N8hh	82						
N8ii	83						
N8jj	84						
N8kk	IS39						
N8ll	CP6108						

N9 Mouthwash comments: _____

N10 HPV Sample comments: _____

N11 GEN Sample comments: _____

How many different types of HPV were found in…

N12 Mouthwash?..... ___

N13 HPV sample?..... ___

N14 GEN sample?..... ___

N15 GENETIC ANALYSIS

		Mouthwash	HPV	GEN
N15a	Concentration (ng/ul)			
N15b	PCR (+/-)			
N15c	Notes			