Oral Co-infection with Multiple Alpha-Human Papillomavirus and Head and Neck Cancer Risk.

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DEDICATION

I dedicate this work to God and my parents.

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This thesis stands as a testament not solely to my individual efforts but significantly to the collective support, guidance, and encouragement I have been fortunate enough to receive from many. First and foremost, I extend my deepest gratitude to my supervisor, Dr. Belinda Nicolau. I consider myself profoundly blessed to have been under her tutelage for the past two years. Her mentorship has been nothing short of transformative, providing me with the resources, opportunities, and support needed to shine in this academic endeavor. Her unwavering support and guidance have illuminated my path through the complexities of academic research.

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

HNC: Head and neck cancer

IARC: International Agency for Research on Cancer

HPVs: Human papillomaviruses

LR-HPVs: Low-risk HPVs

HR-HPVs: High-risk HPVs

ORFs: Open reading frames

MHC-class I: Major-Histocompatibility Complex class I

FDA: Food and Drug Administration

HeNCe: Head and Neck Cancer Life Study

ICD-10: International Classification of Diseases 10th Revision

PCR: Polymerase chain reaction

α-HPV: alpha Human papillomavirus

DAG: Directed acyclic graph

VIF: Variance inflation factor

IPTW: Inverse Probability of Treatment Weighting

CCW-TMLE: Case-control weighted targeted maximum likelihood estimation

ATU: Average treatment effect on the untreated

ATT: Average treatment effect on the treated

ATE: Average treatment effect

RERI: Relative excess risk due to interaction

LCA: Latent class analysis

CI: Confidence interval

OR: Odds ratios

RR: Relative risk

AIC: Akaike Information Criterion

BIC: Bayesian Information Criterion

AP: Attributable proportion

S: Synergy index

ABSTRACT

***Objectives**:* In Canada, the incidence of human papillomavirus (HPV)-related head and neck cancer (HNC) is increasing, and has recently surpassed that of cervical cancer, making it the most common HPV-associated cancer. While multiple oral HPV infections have been observed in several studies, the role of these infections in HNC aetiology remains unclear. Additionally, evidence of the effectiveness of HPV vaccination in reducing HNC incidence is limited. We therefore investigated HPV co-infection patterns, estimated the extent to which multiple HPV infections are associated with HNC risk, and estimated the effect of eliminating all vaccine-targeted HPV genotypes on HNC incidence in a sample of Canadians.

***Methods**:* We used data from a hospital-based case-control study. Incident HNC cases (n=460) and frequency-matched controls (n=458) by age and sex were recruited from four main referral hospitals in Montreal. In-person interviews collected information on an array of life course exposures, and exfoliated cells from the mouth and cancer site were analyzed by PCR to detect α-HPV genotypes. We assessed the independence of co-infecting α-HPV genotypes using a Poisson model and estimated the odds ratios (OR) and 95% confidence intervals (CI) for the association between multiple α-HPV infections and HNC using logistic regression. We also emulated a target trial and used targeted maximum likelihood estimation (TMLE) to evaluate the effect [average treatment effect (ATE), average treatment effect on the treated (ATT), average treatment effect on the untreated (ATU)] of HPV vaccination on HNC.

***Results**:* Of 225 HPV-positive individuals (164 cases, 61 controls), 34.76% of cases and 31.15% of controls had multiple α-HPV infections. The distribution of multiple α-HPV infections was considerably different than expected under a mutually independent model of infection. Participants infected with multiple α-HPV genotypes, including co-infection with HPV 16 [OR= 22.09; 95%CI: 4.31, 404.74] and excluding it [OR= 1.90; 95%CI: 0.86, 4.28], had increased HNC risk, compared to those with no α-HPV infection. In the entire population [ATE= -0.007, 95% CI; -0.008, -0.005] and among individuals with no vaccine-targeted HPV genotype [ATT= -0.04, 95% CI; -0.05, -0.03], there was a 0.7% and 4%-point reduction in HNC risk, respectively. In contrast, among individuals with at least one vaccine-targeted HPV genotype [ATU= 0.05, 95% CI; -0.03, 0.14], there was a 5%-point increase in HNC risk.

***Conclusion**:* Multiple oral α-HPV infections are common and increase HNC risk, with this risk greatly heightened when HPV 16 is one of the infecting genotypes. Conversely, HPV vaccination holds promise in reducing the incidence of HNC. Future studies can elucidate mechanisms underlying codependence of oral α-HPV genotypes and assess which α-HPV genotypes are more or less likely to be involved in oral co-infection.

RÉSUMÉ

Objectifs: Au Canada, l'incidence du cancer de la tête et du cou (HNC) lié au virus du papillome humain (VPH) augmente et a récemment dépassé celle du cancer du col de l'utérus, ce qui en fait le cancer associé au VPH le plus courant. Bien que de multiples infections orales au VPH aient été observées dans plusieurs études, le rôle de ces infections dans l'étiologie du HNC reste incertain. De plus, les preuves de l'efficacité de la vaccination contre le VPH pour réduire l'incidence du HNC sont limitées. Par conséquent, nous avons étudié les schémas de co-infection par le VPH, estimé dans quelle mesure plusieurs infections par le VPH sont associées au risque de HNC et estimé l'effet de l'élimination de tous les génotypes de VPH ciblés par la vaccination sur le risque de HNC dans un échantillon de Canadiens.

Méthodes: Nous avons utilisé les données d'une étude cas-témoins en milieu hospitalier. Les cas incidents de HNC (n = 460) et les témoins appariés en fréquence (n = 458) selon l'âge et le sexe, ont été recrutés dans quatre grands hôpitaux de référence à Montréal. Des entrevue en personne ont permis de recueillir des informations sur un éventail d'expositions au cours de la vie. Également, les cellules exfoliées de la bouche et du site cancéreux ont été analysées par PCR pour détecter les génotypes α-HPV. Nous avons évalué l'indépendance des génotypes co-infectants du VPH-α à l'aide d'un modèle de Poisson et estimé les rapports de cotes (OR) et les intervalles de confiance (IC) à 95 % pour l'association entre plusieurs infections au VPH-α et le HNC à l'aide d'une régression logistique. Nous avons également simulé un essai cible et utilisé l'estimation du maximum de vraisemblance ciblée (TMLE) pour évaluer l'effet [effet moyen du traitement (ATE), effet moyen du traitement sur les sujets traités (ATT), effet moyen du traitement sur les sujets non traités (ATU)] de la vaccination contre le VPH sur HNC.

Résultats: Sur 225 individus positifs au VPH (164 cas, 61 témoins), 34,76 % des cas et 31,15 % des témoins présentaient de multiples infections au VPH-α. La distribution de plusieurs infections par le VPH-α était considérablement différente de ce qui était attendu dans le cadre d'un modèle d'infection mutuellement indépendant. Les participants infectés par plusieurs génotypes α-HPV, dont [OR= 22,09 ; IC95% : 4,31, 404,74] et excluant la co-infection par HPV-16 [OR= 1,90 ; IC à 95 % : 0,86, 4,28], présentaient un risque accru de HNC, par rapport à ceux qui n'étaient pas infectés par le α-HPV. Une réduction de 0,7 % du risque de HNC [ATE= -0,007, IC à 95 % ; -

0,008, -0,005], une réduction de 4% du risque de HNC [ATT= -0,04, IC à 95 % ; -0,05, -0,03] et une augmentation de 5% points du risque de HNC [ATU= 0,05, IC à 95 % ; -0,03, 0,14] ont été observés dans l'ensemble de la population, chez les individus sans VPH ciblé par le vaccin et chez ceux présentant au moins un génotype de VPH ciblé par le vaccin, respectivement.

Conclusion: Les infections orales multiples à l'α-HPV sont courantes et augmentent le risque de HNC, ce risque étant considérablement accru lorsque le HPV 16 est l'un des génotypes infectieux. À l'inverse, la vaccination contre le VPH semble prometteuse pour réduire l'incidence du HNC. Des études futures pourront élucider les mécanismes sous-jacents de la co-dépendance des génotypes oraux du α-HPV et évaluer quels génotypes α-HPV sont plus ou moins susceptibles d'être impliqués dans la co-infection orale.

CONTRIBUTION OF AUTHORS

Mary Amure, MSc. Candidate, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: Conceptualized study, carried out statistical analysis, interpretated findings and wrote manuscript.

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Belinda Nicolau, Professor, Faculty of Dental Medicine and Oral Health Sciences, McGill University, Montreal, Quebec, Canada: HeNCe Life study principal investigator, supervised the candidate, contributed to conceptualization, statistical analysis, reviewed, and contributed to writing.

1 INTRODUCTION

Head and neck cancer (HNC) commonly refers to all carcinomas arising from the epithelial lining of the sinonasal tract, oral cavity, pharynx, and larynx and show microscopic evidence of squamous differentiation. (1) Globally, more than 660,000 new cases and 325,000 deaths were reported in 2020. (2) By 2030, the number of new cases is anticipated to rise by 30%. (3) Given the increasing trend of HNC, its highly aggressive nature, (4) recurrence-prone characteristics, (4, 5) cost of treatment, (6) high suicide rates, (7) understanding the aetiology of HNC plays a key role in reducing HNC burden.

While recent changes in behavioural exposure to traditional risk factors for HNC, such as tobacco and alcohol, (8) have led to a decrease in overall HNC incidence, (9, 10) a subgroup of these cancers has seen a substantial increase. This increase has been attributed to human papillomavirus (HPV), which has significantly altered the epidemiological landscape of HNC. (10, 11) Indeed, rates of HPV-related HNC have dramatically increased in developed countries since the 1980s (12) and have now surpassed the annual incidence and mortality of cervical cancer, the most well-known HPV-related malignancy in Canada. (13, 14) Important gaps, however, persist regarding pathways through which oral HPV infection drives carcinogenesis.

Several studies examining the role of HPV in cervical cancer have shown a positive association between multiple HPV infections and cervical neoplasia, (17, 18) suggesting a potential involvement of multiple oral HPV infections in the development and progression of HNC. Importantly, although multiple oral HPV genotypes have been identified in HNC, (13, 15, 16) their role in HNC aetiology has remained unexplored.

Evidence of the effectiveness of HPV vaccination in reducing HNC incidence is also limited. Currently, the only evidence of the utility of HPV vaccination in HNC prevention is the lower incidence of chronic oral HPV infection in vaccinated groups compared to unvaccinated groups (17) However, while this is promising, the limitations of using a surrogate marker of efficacy, such as chronic oral HPV infection in this case, emphasizes the need to reassess the effectiveness of HPV vaccination in preventing HNC by exploring alternative methods.

This thesis investigates the co-infection patterns of oral HPV genotypes and estimates the extent to which multiple oral HPV infections increase HNC risk among a sample of the Canadian population. We will also assess the impact of a hypothetical intervention eliminating all vaccine-targeted HPV genotypes on HNC incidence.

2 LITERATURE REVIEW

2.1 Head and Neck Cancer Definition

HNC refers to a broad category of different tumour types emerging from various anatomic structures including soft tissues, craniofacial bones, salivary glands, skin, and mucosal membranes. More than 90% are squamous cell carcinomas, such that the phrase "head and neck cancer" is frequently used to refer to all carcinomas arising from the epithelium lining the sinonasal tract, oral cavity, pharynx, and larynx and showing microscopic evidence of squamous differentiation. (1)

2.2 Epidemiology of HNC

The most recent global statistics on the incidence and mortality of HNC are available in the Globocan 2020 database from the International Agency for Research on Cancer (IARC). (2) Globally, the estimated burden of HNC is 5.3% of all cancers. (18) It is the seventh most common malignancy worldwide, accounting for more than 660,000 new cases and 325,000 deaths annually. (2) The global incidence and mortality trends show an increase in both developed and developing countries, generally higher among males than female, and among older individuals (after the fifth decade). (19) The number of new cases is anticipated to rise by 30% (i.e., 1.08 million new cases annually) by 2030. (3) This is consistent with Canada's projection; by 2030, the incidence of HNC is expected to increase by 40% in Canada. (20) Lip and oral cavity cancers contribute about half of the global incidence of HNC, whereas pharyngeal and laryngeal cancers account for approximately one-fourth. (2) The survival rates vary greatly depending on the primary site and aetiology. Over the past three decades, there has been a modest improvement in the survival rate for HNC, for example, the 5-year survival rate increased from 55% from 1992–1996 to 66% from 2002–2006. (21) In addition to deaths directly attributable to HNC, survivors have the second highest rate of suicide (63.4 cases per 100,000 individuals) after those with pancreatic cancer (86.4 cases per 100,000 individuals), compared with survivors of other cancers (23.6 cases per 100,000 individuals). (7)

2.3 Risk factors of HNC

Majority of HNC are due to acquired genotoxic exposure rather than inherited high penetrance oncogenic mutations. (22) The heterogeneity in the global incidence and mortality of HNC is mainly attributed to variations in exposure to the risk factors. All of which play a role, individually or in combination, in the development of HNC. HNC is strongly associated with environmental and lifestyle risk factors, particularly tobacco use (both smoked and smokeless), the chewing of areca nut (betel nut), regular alcohol consumption, diets poor in antioxidant vitamins and minerals, UV light from the sun, indoor and outdoor air pollution, chronic trauma, chronic inflammation, occupational exposures to radiation or chemical carcinogens and increasingly, to certain viruses, notably 'high-risk' genotypes of the HPV family. (23)

2.3.1 Tobacco use

Tobacco is a major independent risk factor for the development of HNC. It is consumed in various forms of smoking products, including cigarettes, cigars, beedi/bidi, pipes, (24) and smokeless products, including chewing tobacco, oral snuff, moist pouches, gutkha and betel quid. (25) Associations between smoking tobacco products (cigarettes, cigars, and pipes) and HNC have been previously described in the International Head and Neck Cancer Epidemiology Consortium, with all three products independently associated with increased risk of these cancers. (26)

More than 70 carcinogenic combustion products are present in tobacco smoke. (27) Intensity, duration, and total pack-years of tobacco smoking are all, in a dose-dependent manner, associated with an increased risk of HNC. (24) When compared to current smoking, cessation of smoking for 1–4 years reduces HNC risk by 30%, but it takes 20 years to reach the risk of a never smoker. (28) Among the different HNC subtypes, oral cavity cancer is the least associated with smoking in any form. (29-31) This could be attributed to the aerodynamics of respiratory flow in the upper airway, which changes to turbulent in the larynx from laminar in the oral cavity, thus making the larynx and pharynx more exposed to inhaled air, and consequently, smoke, than the oral cavity. (31)

Similar to smoking tobacco products, smokeless tobacco products contain numerous carcinogens, including several tobacco-specific *N*-nitrosamines, and polycyclic aromatic hydrocarbons (PAH). (32) They are also associated with an increased HNC risk. (33-35) At least 28 carcinogens are

locally exposed to the oral mucosa as a result of chewing tobacco. (36) Betel quid chewing, a smokeless tobacco habit, is a mixture of tobacco, areca nut, and other ingredients (lime, spices). Between 10 to 20% of the world's population (600–1200 million people) engage in this habit, making betel quid the fourth most frequently consumed psychoactive substance after nicotine, ethanol, and caffeine. (37) Its prevalence among adults in South-East Asia is notably high, with rates ranging from 25% to 50%, with peaks of 80–90% in some regions. (38). Chewing tobacco and betel quid chewing are both classified as Class 1 carcinogens by the International Agency for Research on Cancer (IARC). A recent study (39) reported a strong association between HNC and betel quid chewing (OR 8.23, 95% CI 5.31–12.75), even in never-tobacco smokers (OR 13.7, 95% CI 3.62–51.9) and mainly for oral cavity cancers. (OR 18.5, 95% CI 10.3–33.2) A nonlinear dose–response risk for oral cavity cancer was also observed; the risk increased steeply at low doses and plateaued at high exposures (> 425 chew-years). (40)

2.3.2 Alcohol consumption

According to the World Health Organization (WHO) estimates, about two billion people globally consume alcohol, and almost 80 million have diagnosable alcohol abuse disorders. IARC has classified alcoholic beverages and acetaldehyde, the main metabolite of ethanol, as a Class 1 carcinogen. (41) Epidemiological studies conducted in different populations have reported an association between alcohol consumption and HNC risk, with a dose–response relationship on intensity. (29, 31, 42) Differential risks among HNC-subtypes have also been identified, with laryngeal cancer being the least associated with alcohol consumption. (31, 43) This is most likely due to the larynx having the least direct exposure to alcohol compared to the oral cavity and pharynx. Further, similar HNC risks between intake of beer, wine, liquor, and HNC have been reported, implying that ethanol and its metabolites are the principal carcinogenic agents in these alcoholic beverages rather than other constituents. (31, 44, 45) Alcohol consumption of at least three drinks per day increases HNC risk (OR 2.04, 95% CI 1.29-3.21) in never-users of tobacco. (30)

Tobacco smoking and alcohol drinking behaviours account for 75% of HNC cases when used in combination. (46) Studies have confirmed a multiplicative interaction between alcohol consumption and smoking in relation to HNC. (29, 31) Since alcohol can act as a solvent for

carcinogens in cigarette smoke and increase the mucosa's permeability to these carcinogens, the interaction effect between alcohol consumption and smoking is biologically plausible. As a result, the carcinogenic effects of both factors are likely to be amplified when they are together.

2.3.3 Other risk factors of HNC

In addition to the risk factors mentioned above, several other factors are associated with HNC risk. Men have a two to five-fold greater risk of HNC than women. (9) This difference is most likely attributed to men having higher rates of substance abuse, particularly tobacco, than women. (9) Some dietary factors increase susceptibility to HNC, while some play a role in protecting individuals from HNC. While a higher frequency of fruit and vegetable intake is inversely associated with HNC risk (47-51), ingestion of red and processed meat is positively associated with increased risks of HNC. (48, 49, 51) Also, oral health factors such as poor oral hygiene, periodontal diseases, and wearing ill-fitting dentures have been associated with a higher risk of HNC. (52) There has been extensive discussion in the literature on the socioeconomic spectrum in the burden of HNC and the elevated risk linked with low socioeconomic status. (53-55) While there is a distinct social gradient in the incidence of HNC, with the disease primarily affecting individuals of lower socioeconomic status worldwide, this gradient is less evident among cases of HNC related to HPV. (56)

2.4 Human papillomaviruses (HPVs) infection

2.4.1 Definition and Biology

HPV infection has been identified as the cause of approximately 5% of all cancers worldwide. (57) The HPV family consists of circular, double-stranded DNA viruses of 8000 base pairs that encode accessory proteins (E5, E6, and E7) and proteins involved in virus replication (E1 and E2/E4) and assembly (L1 and L2).

This family of viruses, which includes more than 200 HPV types, is phylogenetically classified into genera, species, and types (Figure 1). Three main genera of the papillomaviridae infect humans: alpha papillomavirus (α), beta papillomavirus (β), gamma papillomavirus (γ), all of which contain virus types that infect specific regions of the cutaneous epithelium, but the alpha papillomavirus (α-HPV) genus also contains HPV types that infect the oral and genital mucosal

epithelium. (58) The mucosal α-HPV types are further categorized as low-risk HPVs (LR-HPVs) and high-risk HPVs (HR-HPVs) according to their oncogenic potential. (59) Based on epidemiological evidence, 18 α-HPV types are high-risk HR-HPVs; HPV 16,18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82. (60, 61). They cause squamous intraepithelial lesions that can progress to squamous cell carcinoma in the head and neck region and/or anogenital tract, in contrast to LR-HPVs, such as HPV 6 and HPV 11, which cause benign papilloma/condyloma.

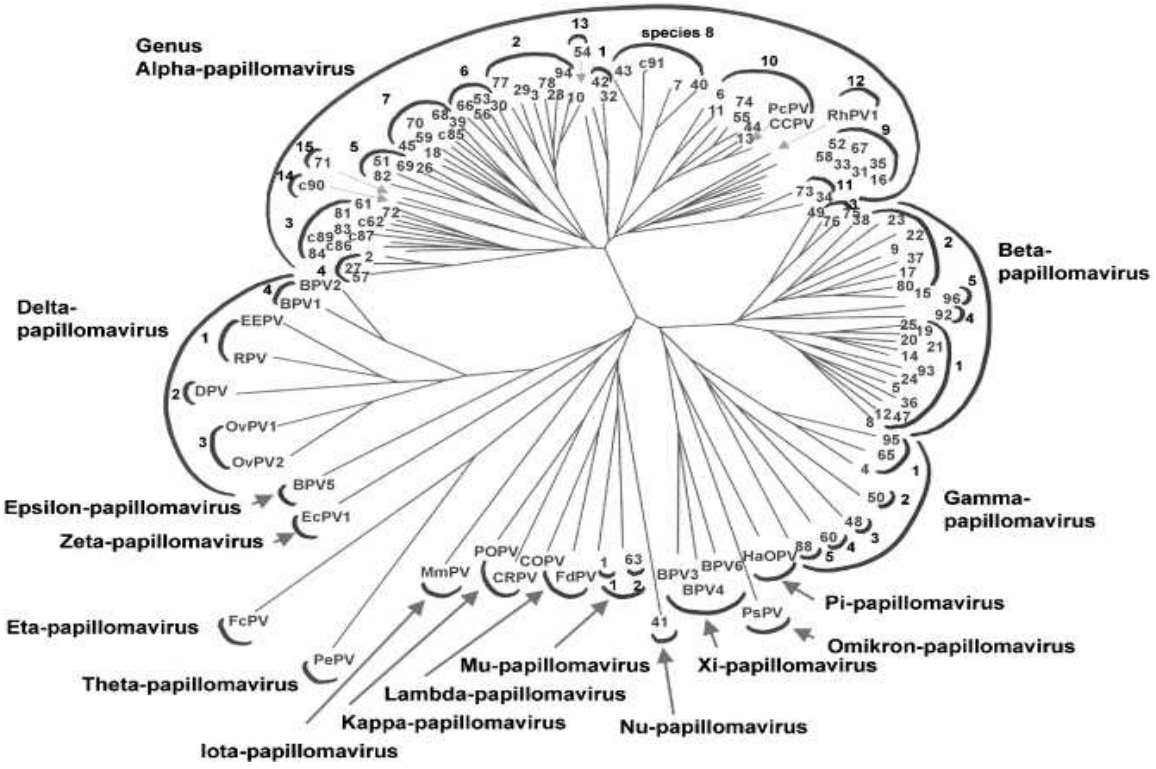

Figure 1: Phylogenetic classification of the Papillomaviridae. [Reused with permission from (58)].

2.4.2 Epidemiology of oral HPV infection

"HPV is the most common sexually transmitted infection." (62) Globally, men and women have a 50% chance of being infected with HPV at least once. (63) The lifetime probability of acquiring HPV among sexually active adults ranges from 53.6% to 95.0% for women and 69.5% to 97.7% for men. (64) The global prevalence of oral HPV infection in healthy individuals varies between 4.5%-5.5%, with a prevalence of 2.6%-3.9% and 1%-1.3% for high-risk types and HPV 16, respectively. (65-68) This prevalence increases in high-risk populations, e.g., HIV-positive individuals with overall oral HPV infection estimated at 34% and HPV 16 infection at 5.7%. (69)

HPV 16 is the most prevalent genotype everywhere, with the highest estimates observed in Europe and North America. (65) The age distribution of oral HPV infection in the general population is bimodal; the first peak is 30-34 years old, and the second peak is 60-64 years old. (12, 70) The first peak may represent an increase in sexual activities, while the second peak could be explained by an age-related impairment in immunologic responses to HPV infections, (71) or increased HPV persistence with age. (72) There is also evidence for sex differences in the prevalence of oral HPV infection. The prevalence of oral HPV infection is higher in men than women. Specifically, rates of oral HPV infection overall, high risk, and HPV 16 are approximately 3-fold higher (10.1% vs 3.6%), more than 5-fold higher (7.3% vs 1.4%), and more than 5-fold higher (1.6% vs 0.3%) in men compared to women, respectively. (70, 73) One explanation for this could be behavioural differences between men and women; men have higher lifetime sexual activities than women. (74) Also, there might be higher infection risk when performing oral sex on a woman than performing oral sex on a man. (75) A woman's higher seroconversion rate in response to genital HPV infection (76) could confer greater protection against subsequent oral infection. (77)

2.4.3 Risk factors for oral HPV

2.4.3.1 Sexual behaviour

Oral HPV infection is predominantly sexually transmitted. (70, 78) It is associated with several measures of sexual behaviour, with increased prevalence among individuals with a higher number of lifetime sex partners, (70) higher number of lifetime oral sexual partners, (79) who have ever received oral sex, (67, 79, 80) who first performed oral sex at 18 years or younger. (70) Nonsexual transmission of HPV to the oral cavity through autoinoculation or salivary transmission is also plausible. (79, 81, 82)

2.4.3.2 Smoking and Alcohol consumption

There is an association between oral HPV infection and smoking, as shown by multiple epidemiologic studies. (67, 70, 79, 83) Smoking increases the persistence of incident oral HPV infection in men after a follow-up period of seven years. (84) This can be explained biologically. Cigarette smoke extracts decrease epithelial barrier function, thus facilitating the entry of the virus. (85) Further, cigarette smoke exposure markedly impacts the immune system, thus compromising appropriate immune and inflammatory responses and increasing the likelihood of HPV infection

and persistence. (86-89) Alcohol consumption is also an independent risk factor for oral HPV. (79) The prevalence of oral HPV infection is higher among heavy alcohol drinkers and increased with intensity. (70) Alcohol could be associated with oral HPV infection due to sharing of cups, or to a local effect that enhances invasiveness of the virus, or to associations with risky sexual behaviour. (79)

2.4.3.3 Poor oral health

A history of oral disease increases the risk for oral HPV infection. (90) Bui et al. (91) demonstrated that self-reported poor oral health was an independent predictor of oral HPV infection. To infect the oral cavity, HPV penetrates epithelial wounds to reach the basal layer of epithelium. (92) Poor oral health, including ulcers, mucosal disruption, and chronic inflammation, may therefore increase susceptibility to HPV infection. Oral mucosal injuries from mechanical stimulation of dentures also contribute to oral HPV infection. (93)

2.4.3.4 Other risk factors for oral HPV

Bimodal peaks of HPV infection at the ages of 30-34 and 60-64 have been shown, suggesting that increased sexual activity and aging-related declines in oral immune capabilities may have contributed to an increase in infection rates. (70) Men are at a higher risk of oral HPV compared to women. (81, 94, 95) This is partly because men are more likely to have more lifetime sexual and oral sexual partners. (94) In addition, men are generally more susceptible to infections due to weaker immune responses to infection. (96) Oral HPV prevalence is also higher among whites than other races. (97)

2.4.4 Natural history of oral HPV infection

2.4.4.1 Molecular structure

As previously mentioned, HPVs are small, double-stranded, non-enveloped DNA viruses. The DNA of approximately 8000 base pairs contains eight open reading frames (ORFs) and core genes involved in replication (E1, E2) and packaging (L1, L2). The remaining genes, E6, E7, E5, and E4, drive cell cycle entry, immune evasion, and virus release. The DNA molecule also contains a non-coding region- long control region (LCR)- responsible for regulating viral expression. (59,

98-101) HPV influences key pathways in the hallmark of carcinogenesis through these molecular players. (99, 102)

2.4.4.2 Initial infection and progression

HPVs are highly epitheliotropic. (103) The oral mucosal epithelium comprises the stratified squamous epithelium (surface) and the lamina propria (deeper). The stratified squamous epithelium cells are arranged in layers according to their stage of cellular differentiation - stratum basale, stratum spinosum, stratum granulosum, and stratum corneum in keratinized areas; stratum basale, stratum filamentosum, and stratum distendum in nonkeratinized areas. The basal cells are the proliferating pool, while the cells at the surface are terminally differentiated. (104, 105) The life cycle of HPV, directly linked to epithelial cell differentiation, is initiated by the infection of basal epithelial cells at sites of injury. (Figure 2) (100, 103) The L1 capsid protein attaches to heparan sulphate proteoglycans on the epithelial cell surface, and the virus enters the cell by micropinocytosis. (106-108) Upon entry into the basal cells, HPV travels to the nucleus. The viral genome then enters the nucleus following membrane breakdown during mitosis. (109) Inside the nucleus, the virus genome is amplified to 50–100 copies by expressing E1 and E2 viral replication proteins. (110) During the division of infected cells, the viral genomes segregate equally into daughter epithelial cells. (111) Following basal cell division, infected daughter cells may stay in the basal layer or move into the suprabasal epithelial layers and begin to differentiate. (112) The viral genome's continued presence in actively dividing basal cells for several years results in persistent infection. (113) In a normal infection on the other hand, upon basal cell division, an infected daughter cell will become a transit-amplifying cell which will complete epithelial differentiation and travel up through the various epithelial layers. Notably, once detached from the basement membrane, uninfected keratinocytes exit the cell cycle and begin to synthesize keratin, but HPV-infected cells, triggered by the expression of the viral E6 & E7 proteins, enter into S-phase. (114, 115) Viral genome amplification to thousands of copies per cell occurs as a result of this S phase entry. (116) Eventually, virions are released into the environment as the upper layer of the epithelium is shed, allowing for infectivity and transmission.

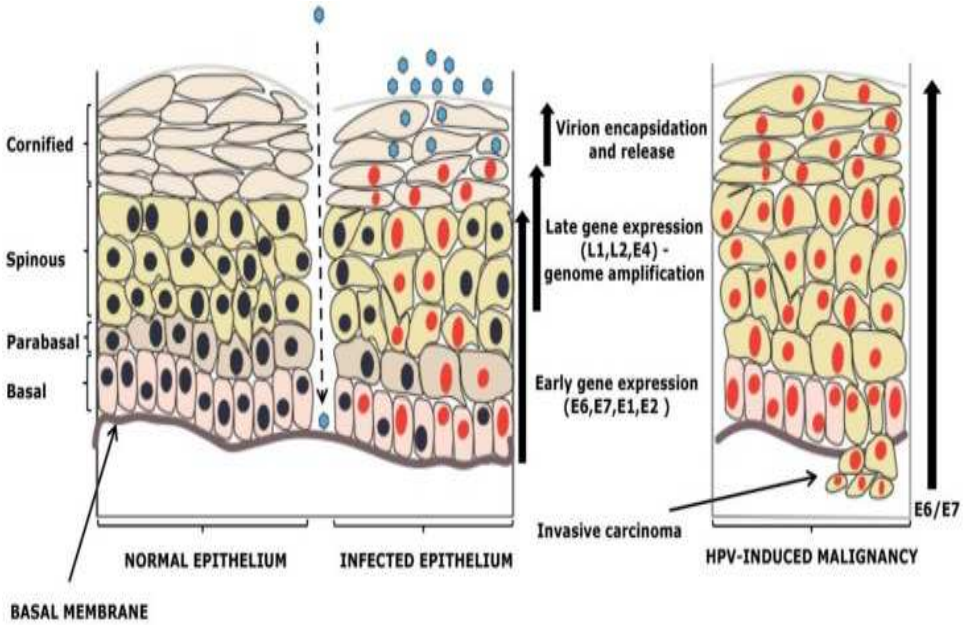

Figure 2: HPV lifecycle and cancer development. [Figure source: Tomaić 2016, (117) used under the Creative Commons Attribution (CC-BY) license].

2.4.4.3 Carcinogenesis

The majority of HPV infections are self-limiting and regress without treatment. A persistent infection with a HR-HPV is the main risk factor for carcinogenesis. (118) HR-HPV genome integration has been associated with persistent infection. (119) During the infectious process, the virus may exist episomally, integrate into the host cell genome, or coexist (episomal/integrated). The virus, in its integrated form, has the ability to produce changes in cell functions that favor both the replication of the viral particles and the malignant transformation of the cell. (120) Although HPV integration can happen across the human genome, it is more common in chromosomal regions such as *3q28*, *4q13.3*, *8q24.21*, *13q22.1*, and *17q21* or near clusters of microRNAs. (121) In the HPV genome, on the other hand, the *E2* ORF is usually the most affected location by the integration process. (122) A break in the E2 gene, the main repressor of *E6* and *E7* oncogeneses, allows for their unbalanced expression. (123) These oncogenes then impair multiple key regulatory pathways of the cell cycle and elicit all the known hallmarks associated with cancer. (99) Among other disruptive effects, E6 inhibits p53, a crucial tumor suppressor gene, thereby allowing unchecked proliferation of the basal cells, and E7 inhibits retinoblastoma protein (pRb), another tumor suppressor gene, thus allowing for cell cycle deregulation. (117, 124, 125) The disruption

of the Rb pathway results in an accumulation of $p16^{INK4a}$, (126) one very important indicator of malignancy due to HPV. (127) Additionally, E6 and E7 inhibit the human telomerase reverse transcriptase suppressors, an enzyme that prevents the telomeres from shortening, giving rise to cell immortality. (128) All of these activities, together with other genomic mechanisms, (120) act to promote dysregulated cell proliferation, immortalization, malignant transformation, cell invasion, and eventually metastasis. (99)

2.4.4.4 Immunobiology

The viral oncogenes further act to help the immortalized and uncontrollably dividing cells to evade the immune system. Through several mechanisms: altering host gene expression, dysregulating protein functions, altering cytoplasmic trafficking of host proteins, the viral oncogenes help HPV-infected cells evade host immune defenses. (129) E6 downregulates Interferon Regulatory Factor 3, a known transcription factor of Interferon β, thereby decreasing the immune response against HPV-transformed cells. (130) E6 and E7 downregulates the expression of proinflammatory cytokines and chemokines, IL-8, IL-18, CCL2, and CCL20. (131-133) By inhibiting the Major-Histocompatibility Complex class I (MHC-class I) from moving to the cell surface, E5 promotes its retention in the Golgi apparatus, therefore reducing the ability of the complex to present viral antigens to the T-cells, facilitating immune evasion. (134) In general, evasion of the immune system aids HPV persistence and ultimately tumorigenesis.

2.4.5 HPV-related HNC

The first report that tied HPV to HNC was published in 1983. (135) Since then, researchers have investigated the potential role of HPV in HNC pathogenesis, with results suggesting that HPV-positive HNC have different epidemiologic, clinical, and molecular features than HPV-negative HNC. (136) In 2007, IARC recognized HPV as a carcinogen for the head and neck region. (137)

Over the past few decades, there has been a significant increase in the incidence of HPV-related HNC, particularly oropharyngeal cancers. (138, 139) Indeed, HPV prevalence in oropharyngeal tumors increased substantially from 16.3% during the 1980s to 72.7% during the 2000s. (138) It is, however, important to note that the mere presence of HPV DNA in malignant tissues does not establish causality. Distinctively, a cell that has become malignant due to HPV oncogenesis will

typically overexpress $p16^{INK4a}$ (as explained in section 2.4.4.3). (127) The most oncogenic HPV genotype as it relates to HNC is HPV16, accounting for over 80% of HPV-related HNC. (140, 141) In Ndiaye's review (141) on global estimates of the attributable fraction of HPV in HNC, HPV 16 accounted for 82·2% of all HPV DNA positive cases, and HPV 18 was detected in 2·5% of all HNC. Goodman et al. (142) also reported the incidence of HPV genotypes in tumour tissue of 378 oropharyngeal cancer patients, identifying HPV 16 in 322 (61%) of the samples and other HR-HPV genotypes in 56 (11%) of the samples, including HPV 33, HPV 18, HPV 35, HPV 31, HPV 52, HPV 39, and HPV 45.

Geographically, the burden of HPV-related HNC is higher in developed than less developed countries. (143) Countries in which the age-standardized incidence rates of HPV-related HNC are relatively high (over 1.25 per 100,000) are located in Northern America and Europe. (143) Globally, HPV-related HNC most frequently arise in the oropharynx, followed by oral cavity and laryngeal regions. (141, 143)

Individuals with HPV-positive HNC typically present at a younger age. (144-147) Tobacco and alcohol have been responsible for a smaller proportion of HNC cases in younger individuals (<45 years) compared with the older age groups, (46) indicating that other factors, e.g., HPV infections, are more important risk factors in this group of individuals. Indeed, Gillison et al. (145) observed that HPV-positive HNC patients were younger by about five years on average when compared to HPV-negative HNC patients. Similarly, Ringstrom et al. (146) reported that patients with HPV 16-positive HNCs were 8.4 years younger than those with HPV 16-negative HNC. Individuals with HPV-positive HNC also have higher socioeconomic status, lower rate of alcohol consumption (136, 146), and are less likely to have a history of tobacco use when compared to individuals with HPV-negative HNC. (145) Gillison et al. (148) reported that HPV 16-positive HNC was independently associated with several measures of sexual behaviour, measures of tobacco smoking, and alcohol drinking. Associations increased in strength with increasing number of oral sex partners. By contrast, HPV 16-negative HNC was associated with measures of tobacco smoking, and alcohol drinking but not with any measure of sexual behaviour. Associations increased in strength with increasing intensity, duration, and cumulative pack-years of tobacco smoking, increasing years of heavy alcohol drinking. In another study, when compared with HPV-

negative oropharyngeal cancers, HPV-positive oropharyngeal cancers were less likely to occur among moderate to heavy drinkers and smokers. (136)

There is strong evidence that HPV-positive status is an important prognostic factor for HNC outcomes. It is associated with favourable treatment and survival outcomes. (136, 149-151) In a prospective clinical trial evaluating the association of tumor HPV status with response to treatment and survival in patients with HNC, it was demonstrated that in comparison to patients with HPV-negative HNC, those with HPV-positive HNC had better response rates following induction chemotherapy (55% vs. 82%) and chemoradiation treatment (57% vs. 84%). Furthermore, they showed that at a median follow-up of 39.1 months, patients with HPV-positive HNC had an improved overall survival of 33%, and reduced risk of progression and death from any cause compared to those with HPV-negative HNC. (152) Similarly, Gillison et al. (136) found that patients with HPV-positive HNC had a 59% reduction in risk of death from cancer when compared with HPV-negative HNC patients. There are plausible explanations for these findings. High doses of radiation therapy has been shown to increase MHC-class I, E6, and E7 expression, resulting in increased immune surveillance, which could, in turn, contribute to improved treatment outcomes and survival. (153) The absence of field cancerization (154) in HPV-positive cancers may be another factor leading to a better prognosis. Field cancerization refers to the presence of early genetic changes in the epithelium, from which multiple independent lesions can arise. It is typically seen in alcohol and tobacco-related tumours. (136, 152)

2.4.6 Multiple oral HPV infections

Multiple HPV infections refer to concurrent HPV infection with multiple genotypes (two or more) at an anatomical site, e.g., oral cavity, cervix. Globally, several studies in HNC literature have detected the presence of multiple oral HPV genotypes; (13, 15, 16) however, its prevalence in the general population and its role in HNC development have not been extensively studied. One of the few studies on this subject is a population-based study conducted in the U.S. Bui et al. (155) showed the prevalence of multiple oral HPV infection to be 1.5%, with a higher prevalence in men (2.5%) compared to women. (0.4%) These estimates might have been underestimated because of the method used to collect samples (oral swish only). A study on global estimates of HPV-attributable fractions in HNC found the proportion of HNC with multiple infections to be 2·1%,

1·0%, and 0·3% in the oral cavity, laryngeal, and oropharyngeal cancers, respectively. (141) Having a new sex partner in the past year, being male, and being a current cigarette smoker is associated with an increased risk of multiple oral HPV infections. (155)

Unlike oral co-infections, multiple cervical HPV infections and their relation to cervical cancer have been extensively studied. At the cervical site, infection with multiple HPV genotypes is relatively common worldwide, with prevalence ranging from 0.3% to 12.0%. (156) Globally, the proportion of HNC with multiple HPV infections (1·1%) is ten times lower than in cervical cancer (11·2%). (157) This difference could be explained by the increased exposure of the cervix to HPV. (141)

Natural seroconversion induced by infection by one HPV type is not associated with immune protection against reinfection with the homologous HPV type or its genetically related types. (158) In fact, prior infection with one HPV genotype increases the likelihood of acquiring another genotype. (159-162) Dickson et al. (156) found that different HPV types exhibit varying relationships with each other, with some types showing positive relationships and others showing negative relationships. In positive relationships, HPV genotypes are more likely to occur together in multiple infections than expected by chance alone. This suggests cooperative interactions where certain HPV genotypes support or facilitate each other's presence within the tissue, leading to a higher likelihood of them being detected together in multiple infections. On the other hand, negative relationships indicate that HPV genotypes are less likely to co-occur in multiple infections than expected by chance, suggesting competitive interactions in which certain HPV genotypes inhibit or compete with each other within the tissue, leading to a lower likelihood of them being present together in multiple infections. Notably, α-9 HPV genotypes exhibited a complex interplay of both cooperative and competitive interactions within the species; meanwhile, other species exhibited either cooperative or competitive relationships within their respective groups. (156)

From cervical cancer literature, multiple HPV infections have been shown to increase the risk of high-risk cervical lesions (161, 163-167). However, there is inconsistency regarding how multiple infections increase this risk. Some studies show that multiple HPV genotypes act synergistically (166), while others show that multiple HPV infections have no synergistic or additive effect on the

development of high-risk cervical lesions. (161, 167) The heterogeneity in these findings may be attributable in part to the fact that most studies have used prevalence analysis by cross-sectional detection of type-specific HPV infections, which may underestimate the cumulative effects due to exposure to different HPV genotypes over time. Longitudinal studies like Trottier et al. (166) have the benefit of allowing the assessment of lesion risk in the context of co-infections detected sequentially throughout periods relevant to the natural history of cervical carcinogenesis. Multiple HPV genotypes could also facilitate persistent HPV infection. (168) Persistent high risk HPV infection, in turn, is an important predictor for the development of cancer. (169) Interestingly, Salazar et al. observed a reduced risk of cervical disease in the presence of multiple infections, suggestive of possible intergenotypic competition or a more effective immune response triggered by multiple infections. (170)

3 RATIONALE

Despite the decrease in tobacco smoking, a major risk factor for HNC, (8, 10, 171) the incidence of a subgroup of these cancers has increased substantially in recent decades in Canada (172, 173) and other high income countries. (139, 174, 175) This rising incidence has been attributed to HPV, the main driver of a subset of HNC - oropharyngeal cancers. (9, 138, 176) In fact, HPV-related HNC have surpassed the annual incidence and mortality of cervical cancer, the most well-known HPV-related malignancy, in Canada and other high-income countries. (13, 14) Although this upward trend might be partly due to an increase in cervical cancer screening, which has aided the identification of pre-malignant lesions, the incidence of HPV-related HNC has increased substantially, leading some authors to refer to is as an epidemic. (10, 177) Notably, there are several aspects of the role of oral HPV infection in HNC aetiology that remain poorly understood. One of these is oral infection with multiple HPV genotypes.

Several studies have shown a positive association between multiple HPV infections and cervical neoplasia. (166, 178) Importantly, people who had multiple genital HPV infections had a low clearance frequency, (179) thus persistent infection, which is essential for carcinogenesis. (180-182) This evidence suggesting the role of multiple infections in cervical cancer supports that multiple oral HPV infections can potentially play a role in the development and progression of HNC. However, although, several studies have detected the presence of multiple oral HPV genotypes both in the healthy population and in individuals with HNC, (13, 15, 16) investigations examining the role of multiple infections in HNC are currently lacking.

Assessing multiple oral HPV infections is also important because of its implication in prevention. Removing certain genotypes by type-specific vaccination could either result in non-targeted genotypes occupying the niche vacated by the vaccine targets, thus increasing their prevalence, (183) or decrease the prevalence of non-targeted genotypes because of cross-type immunity. (184, 185) These concerns require a solid understanding of the equilibrium in the distribution of oral HPV genotypes, an equilibrium that the introduction of vaccination into a population may modify. Studying co-infection patterns with samples retrieved before the introduction of vaccination may

aid in better characterizing this equilibrium, upon which the impact of type-specific vaccination on non-targeted oral HPV genotypes can be assessed.

Apart from the potential contribution of multiple infections, the rising burden of HPV-related HNC also call for the evaluation of the efficacy of HPV vaccination, particularly given the challenges of screening. (12) There are currently three Food and Drug Administration (FDA)-approved HPV vaccines that protect against HPV 16 and 18 (Cervarix), HPV 16, 18, 6, and 11 (Gardasil), HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 (Gardasil 9). While these vaccines were licensed based on cervical clinical outcomes (Cervical Intraepithelial Neoplasia grade 2 or higher), they have not been tested for HNC prevention. This is primarily due to the difficulties in detecting HNC lesions, particularly oropharyngeal, the rarity of the disease, and the long interval between infection and the occurrence of cancer, all of which have precluded clinical efficacy trials. (12, 186-188) In 2014, however, the IARC HPV Working Group recommended using persistent oral HPV infection as the end-point for evaluating vaccine efficacy in clinical trials, (189) and studies have shown decreased prevalence of persistent oral infection in vaccinated groups. (188, 190, 191) Although these studies have shown promising results, evidence of effectiveness against HNC is still limited, especially considering the limitations of using a surrogate of efficacy and the limited follow-up time. Additional evidence is therefore needed to establish the efficacy of HPV vaccination on HNC.

4 HYPOTHESIS AND OBJECTIVES

I hypothesize that multiple oral α-HPV genotypes interact cooperatively to increase the risk of HNC more than single α-HPV infection; and eliminating all vaccine-targeted HPV genotypes considerably reduces HNC risk.

The objectives of this thesis are:

1. To investigate the co-infection patterns of oral α-HPV genotypes among a sample of the Canadian population
2. To estimate the extent to which multiple oral α-HPV infections increase the risk of HNC among a sample of the Canadian population
3. To evaluate the effect of eliminating vaccine-targeted HPV genotypes on HNC incidence among a sample of the Canadian population

Specifically, we aim to:

1. Describe the distribution of co-infecting α-HPV genotypes among cases and controls
2. Estimate the HNC risk conferred by co-infection of HPV 16 and other α-HPV genotypes
3. Estimate the HNC risk conferred by co-infection of targeted and non-targeted α-HPV genotypes
4. Evaluate the effect of eliminating vaccine-targeted HPV genotypes on HNC incidence

5 METHODOLOGY

5.1 Study Design

Data from the Head and Neck Cancer (HeNCe) Life Study was used for this project. The HeNCe Life Study is an international, hospital-based case-control study designed to investigate the life course aetiology of HNC. The study was conducted in three countries: (i) 2005 to 2013 in Montréal, Canada; (ii) 2008 to 2012 in Kozhikode, India; and (iii) 2003 to 2005 in São Paulo, Brazil. The protocols employed in all three countries were akin yet tailored to the specific context of each country. There were four recruitment centres in Canada and two in both India and Brazil:

- Montreal, Canada: Montreal General Hospital, Jewish General Hospital, Royal Victoria Hospital, and Notre Dame Hospital
- Kozhikode, South India: Government Medical and Dental College hospitals
- São Paulo, Brazil: AC Camargo Hospital, Hospital Beneficência Portuguesa

For the purpose of this thesis work, only data from the Canadian site were utilized.

5.2 Eligibility Criteria

The participants had to:

i. Be born in Canada and live within 50km from the study site. This geographical restriction was to ensure that the cases and controls originated from the same secondary study base. (192)

ii. Speak either English or French (the local language in Montreal, Canada). The interview process followed a life course perspective, so the language restriction was important to allow the interviewer to build a good rapport with participants and ensure the quality of the data collected.

iii. Be at least 18 years old; HNC typically manifests in adulthood, with the median age of diagnosis for most anatomic sites in the sixth to seventh decades of life. (193)

iv. Have no history of cancer. Including individuals with a cancer history would have resulted in a biased risk estimate.

v. Have no history of cognitive or mental disorders. This was to ensure the accuracy of retrospective data collected.

5.3 Case and Control Definition

Cases (n= 460) were individuals who had incident, untreated, primary squamous cell carcinomas of the head and neck region (oral cavity, pharynx, and larynx). They were identified in tumor board meetings of each participant hospital. Case-finding was done through histological examination, which is considered the diagnostic gold standard for HNC. (194) Lesions located on the tongue, gingiva, floor of the mouth, palate, retromolar area, vestibule, buccal mucosa, and tonsil were included in oral cavity cancers (ICD-10 codes C00.3 – C06.9, C09); those located in the oropharynx and hypopharynx were included in pharyngeal cancers (C10, C13, C14); and those in the supraglottic, glottic or subglottic regions were included in laryngeal cancers (C32). Malignant neoplasms of the external lip (C00.0-C00.2), major salivary glands (C07, C08), esophagus (C15), and nasopharynx (C11) were excluded due to their different histology and aetiology. (195)

Controls (n= 458) were recruited from several outpatient clinics at the same hospitals as cases and were frequency-matched to cases by sex and age (5-year brackets). To avoid biased estimates of the association between exposure and outcome, matching was only considered for risk factors whose confounding effects needed to be controlled for but which were not of scientific interest as independent risk factors in the study. (196) The outpatient clinics selected were clinics in which diseases considered to be unrelated to major HNC risk factors (e.g., tobacco and alcohol) were treated, e.g., Orthopedics, Dentistry, Ear, nose, and throat (ENT), and Gynecology. A total of 13 outpatient clinics were utilized, with six having a major contribution. To avoid overrepresenting any disease group, no outpatient clinic contributed more than 20% of all controls. Controls were recruited within months of recruiting a case in the corresponding group. Please refer to Supplementary Table 3 to see the distribution of the clinics where controls were selected.

5.4 Ethical Approval and Informed Consents

The HeNCe Life Study protocol was reviewed and approved by the ethics committees of all participating hospitals, and McGill University. All the study participants provided written informed consents.

5.5 Data Collection

5.5.1 Recruitment procedure

The recruitment process commenced with acquiring a list of potential participants from each recruitment clinic's appointment register. A research assistant then explained the study protocol to the potential participants, addressed their inquiries, and evaluated their eligibility for participation. Subsequently, individuals who expressed interest and met the eligibility criteria were invited to review and sign a consent form (available both in French and English). Route sheets were used to schedule interviews, which took place either at the participants' residence or at the recruitment hospital.

5.5.2 Study Measurements

5.5.2.1 Interview

The interview questionnaire (Appendix- Study Questionnaire) was designed using questions from different studies: British Birth Cohort (BBC) 1946, BBC 1958, British Civil Servants: Whitehall Study II, and IARC research on HNC. (197-199) Following focus group discussions with stakeholders, modifications were made to the questionnaire to suit the local context better, and then it was translated into French. As a quality assurance measure, it was back-translated into English and tested during the pilot study.

In the main study, each participant underwent a face-to-face interview that lasted approximately 1.5 to 2 hours. Information was collected on demographics, indicators of socioeconomic position (e.g., education, occupation, housing conditions, and other amenities), participants' and their parents' behavioural factors (e.g., smoking, paan/betel quid chewing, alcohol consumption), childhood and adulthood dietary habits (e.g., diet patterns and consumption of spices), medical history, general health and built, oral hygiene and oral health (e.g., decayed, missing and filled teeth), history of sexual practices and sexual-transmitted diseases, family history of cancer, marital life, and social support.

The interviews were conducted using the life grid technique, a validated technique used to collect life course data. (200) Evidence suggests that conducting interviews in a way compatible with

memory retrieval processes enhances recall. (201) The life grid technique involves cross-referencing the dates of any changes in the areas of interest, for example, housing, against dates in the individual's personal life, such as marriage, as well as against events in the outside world, like wars. (200)

5.5.2.2 Biological sample collection

For genetic analyses and HPV genotyping, two oral brush samples (using Oral CDx® brush) and one oral rinse sample (using alcohol-based mouthwash) were collected from each participant. The brushes were firmly pressed against the lesion (oral cavity cases) or normal buccal mucosa (controls and cases) and rotated until pinpoint bleeding appeared. The brushes were then swirled vigorously into a PreservCyt® buffer (Hologic, Bedford, Massachusetts) to transfer the epithelial cells. (16) With an alcohol-based mouthwash solution, each participant was requested to rinse their mouth and gargle vigorously for 15-30 seconds. The rinse was then collected into a pre-labeled container. These samples were kept at 4 °C until DNA extraction using the MasterPure™ DNA purification kit (Epicenter, USA). Extracted DNA was stored at -80 °C until genotyping. (16)

5.5.2.3 HPV DNA detection and genotyping

HPV DNA was detected and genotyped using the Linear Array (Roche Molecular Diagnostics, Pleasanton, California). DNA samples (10 µl) were analysed for the presence of the β-globin gene using polymerase chain reaction (PCR) and agarose gel electrophoresis. Negative β-globin samples were deemed insufficient for genotyping purposes. Conversely, the β-globin positive samples were amplified with PGMY09-PGMY11 PCR primers for HPV. Reverse hybridization and biotin-labeled probes were used for genotyping 36 HPV genotypes: 6, 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, and 89. These HPV genotypes infect oral, genital mucosal epithelium and belong to the alpha human papillomavirus (α-HPV) genus.

5.5.3 Quality Control Measures

Several steps were taken to ensure the best data quality. Dr Nicolau's research team used audiovisual aids to train all interviewers to conduct the life grid-based interviews; an interviewer

reference guide was also maintained at the interview site. The team also conducted in-person site visits to supervise recruitment and interview processes. All participants' interview responses were reviewed twice: first by the interviewers (on the day of the interview) and again by the research coordinator. In case of inconsistencies, phone calls were made to clarify the answers. Data were inputted into the central database and reviewed for quality. The biological samples collected were stored at 4°C until they were analyzed. Where required, the research assistants personally transferred biological materials between institutions.

5.6 Definition of Variables

5.6.1 Outcome (dependent) variable-

HNC is the outcome for this study. Cases were individuals with incident, histologically confirmed HNC (oral cavity, larynx, and pharynx). This variable was treated as binary, indicating HNC presence or absence.

5.6.2 Independent variable

α-HPV co-infection is the main exposure variable. HPV genotyping was done for 36 α-HPV genotypes. If α-HPV was detected in none of the two specimens collected (mouthwash and oral brush), the participant was considered α-HPV negative for that specific genotype. If α-HPV was present in both or in either specimen collected, the participant was considered α-HPV positive for that genotype. Some participants were positive for more than one α-HPV genotype. Among α-HPV positive participants, those with one α-HPV genotype were labeled "single α-HPV infection," while those with more than one α-HPV genotype (≥2) were labeled "multiple α-HPV infections." Participants with no α-HPV genotype detected were labeled "No α-HPV infection."

Targeted HPV status is another exposure variable. Participants were categorized based on the presence or absence of α-HPV genotypes targeted by currently available HPV vaccines. They were grouped into four categories: those with at least one α-HPV genotype targeted by vaccines (vaccine-targeted HPV genotype), those that were α-HPV positive but without any α-HPV genotypes targeted by vaccines (Non-targeted HPV genotype), those with both vaccine-targeted and non-targeted HPV genotypes, and those that were α-HPV negative (no HPV genotype).

5.6.3 Other Variables

Covariates to be included in statistical models examining the relationship between the variables indicated above were identified a priori using a causal directed acyclic graph (DAG). In epidemiological literature, a priori model specification with DAGs is widely used to control for confounding. (202, 203) DAGs consist of a set of arrows drawn along a timeline describing causal and temporal relationships between variables (nodes). (204) They are directed, which means that each line has a single arrowhead extending from a variable to indicate its effect on another. They are also acyclic, meaning there are no feedback loops of arrows because a variable cannot be its own descendant in time. (204) Following identifying confounders, I chose the minimum sufficient set that closes the backdoor path from exposure to outcome (Figure 3)**.** The final adjustment set included: age (years), sex, age at sexual debut (years), number of lifetime sexual partners, history of oral sex, oral health status, total education years, lifetime smoking (pack-years), lifetime alcohol consumption (liter-years). All were self-reported. The detailed DAG is shown in the manuscript (Chapter 6).

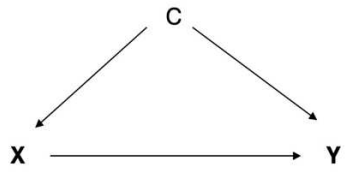

Figure 3: Hypothetical Directed Acyclic Graph

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

5.7 Statistical Analysis

All analyses were conducted in R Studio (version 4.2.2) using the R statistical programming language. (205) Data analyses began with calculating descriptive statistics to compare the characteristics and behaviours among the cases and controls. An overview of the major analytical techniques used in this thesis is described below:

5.7.1 Poisson Regression

Poisson distribution was developed to model discrete counts. (206) The model is based on two key assumptions. (206, 207) First, the occurrences of events are independent of each other. The second assumption is that the variance of the count outcome is equal to the mean. I used Poisson regression model to assess the independence of co-infecting α-HPV genotypes; under independence, expected frequencies for the number of co-infecting α-HPV types would arise from a Poisson distribution. I calculated observed/expected ratios and exact 95% Poisson confidence intervals (CIs). Because a Poisson distribution prescribes an equal mean and variance, I quantified the degree of departure from independence by calculating the dispersion parameter, variance inflation factor (VIF). VIF values >1 would indicate that multiple α-HPV infections occurred more than expected by chance, whereas values <1 would indicate less than expected multiple α-HPV infections.

5.7.2. Logistic regression

Logistic regression is employed when the dependent variable (outcome) is binary. (208, 209) It evaluates the relationship between an exposure or set of exposures and an outcome. (210) "This model works by fitting the probability of response to the proportions of responses observed." (209) Variables that may act as confounders are included in the models to account for their effect. (210) In studies where cases and controls are frequency-matched, unconditional logistic regression is typically used, (202) as is the case in this study. I used unconditional logistic regression to investigate the association between α-HPV co-infection and HNC, with HNC status (case/control) as the outcome (dependent) variable. In logistic regression, the odds ratio (OR), alongside its confidence interval (usually 95%), is the measure of association obtained. (208)

5.7.2 Interaction Analysis

In interaction analysis, the joint effect of two independent exposures on an outcome is assessed against their separate individual effects. (211) Interaction can be identified on different scales; the common ones in epidemiological research are the risk difference, risk ratio, and odds ratio scales. In this thesis, I used the risk difference scale because of its great public health significance (211, 212) and its associations with biologically based concepts of interaction. (202) On the risk difference scale, interaction is based on how much the joint effect differs from the sum of

individual exposures. (213) Most statistical modelling, however, estimates ORs from logistic regression models, which operate on a multiplicative scale. This approach is favored because linear risk and log-linear models frequently encounter convergence issues in the presence of covariates, thereby complicating the assessment of additive interaction. An alternative approach is to compute measures such as the relative excess risk due to interaction (RERI), attributable proportion (AP), and synergy index (S). They estimate additive interaction from multiplicative measures (RR or OR). (213, 214)

RERI is defined as a "departure from additivity of effects from two binary exposure variables". (202) It will be greater than zero if and only if the additive interaction is positive; it will be equal to zero if and only if there is no additive interaction; it will be less than zero if and only if the additive interaction is negative. AP is the proportion of the disease in the doubly exposed group due to the interaction between the two exposures. (202) It is essentially a derivative measure of the relative excess risk due to interaction: AP>0 if and only if RERI> 0; AP< 0 if and only if RERI< 0.

5.7.3 Causal inference Analysis

A portion of this thesis involves estimating the impact of a hypothetical intervention (removing all vaccine-targeted HPV genotypes) on an outcome (HNC). A randomized control trial would have been the ideal study design; however, where rare outcomes such as HNC, which require a lengthy follow-up time, are studied, case-control design is more practical. (215) In this study, therefore, I utilized causal inference methods as they can effectively emulate randomized experiments. In randomised experiments, association measures are interpreted as causal effect measures because the exposed (treated) and the unexposed (untreated) are exchangeable. (216) Numerous techniques exist for conducting causal inference analysis with case-control data, including inverse probability-of-treatment weighting (IPTW), parametric g-formula, and targeted maximum likelihood estimation (TMLE). (215, 217-219) The TMLE approach integrates aspects of both IPTW and parametric g-formula, rendering it doubly robust as it allows for two avenues of accurate model specification. (215) Case-control weighted targeted maximum likelihood estimation is a modification of TMLE appropriate for analyzing case-control data. (220) CCW-TMLE is a weighted analysis that takes into account prevalence estimates of the outcome in the study base to

eliminate the bias induced by the sampling design. (221) The parameters estimated under the assumptions of exchangeability, (216) consistency, (222) positivity, (223) include the average marginal treatment effect in the total population (ATE), marginal treatment effect in the subpopulation that received the treatment (ATT), marginal treatment effect in the subpopulation that did not receive treatment (ATU). (224)

5.7.4 Missing values

HeNCe contains a few missing data points as interviewers attempted to avoid missing values. The missing information distribution for variables included in the analyses of this thesis is as follows: number of years of education missing for one control, smoke pack-years missing for two cases, ethanol litre-years missing for two cases and one control, age at sexual debut missing for 12 cases and 11 controls, number of lifetime sexual partners missing for 14 cases and 14 controls. The proportion of missing values ranged from 0.1% to 3.4%. The rest of the variables had all values. Missing values were imputed using multivariate imputation by chained equations (MICE). MICE impute missing values in a dataset by iteratively imputing each variable with missing data conditional on the other variables in the dataset. (225)

6 MANUSCRIPT

Oral Co-infection with Multiple Alpha-Human Papillomavirus and Head and Neck Cancer Risk

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ABSTRACT

***Objectives**:* In Canada, the incidence of human papillomavirus (HPV)-related head and neck cancer (HNC) is increasing and has recently surpassed that of cervical cancer, making it the most common HPV-associated cancer. While multiple oral HPV infections have been observed in several studies, the role of these infections in HNC etiology remains unclear. Additionally, evidence of the effectiveness of HPV vaccination in reducing HNC incidence is limited. We, therefore, investigated HPV co-infection patterns, estimated the extent to which multiple HPV infections are associated with HNC risk, and estimated the effect of eliminating all vaccine-targeted HPV genotypes on HNC incidence in a sample of Canadians.

***Methods**:* We used data from a hospital-based case-control study. Incident HNC cases (n=460) and frequency-matched controls (n=458) by age and sex were recruited from four main referral hospitals in Montreal. In-person interviews collected information on an array of life course exposures, and exfoliated cells from the mouth and cancer site were analyzed by PCR to detect α-HPV genotypes. We assessed the independence of co-infecting α-HPV genotypes using a Poisson model and estimated the odds ratios (OR) and 95% confidence intervals (CI) for the association between multiple α-HPV infections and HNC using logistic regression. We also emulated a target trial and used targeted maximum likelihood estimation (TMLE) to evaluate the effect [average treatment effect (ATE), average treatment effect on the treated (ATT), average treatment effect on the untreated (ATU)] of HPV vaccination on HNC.

***Results**:* Of 225 HPV-positive individuals (164 cases, 61 controls), 34.76% of cases and 31.15% of controls had multiple α-HPV infections. The distribution of multiple α-HPV infections was considerably different than expected under a mutually independent model of infection. Participants infected with multiple α-HPV genotypes, including [OR= 22.09; 95%CI: 4.31, 404.74] and excluding co-infection with HPV 16 [OR= 1.90; 95%CI: 0.86, 4.28], had increased HNC risk, compared to those with no α-HPV infection. There was a 0.7%-point reduction in HNC risk [ATE= -0.007, 95% CI; -0.008, -0.005], 4%-point reduction in HNC risk [ATT= -0.04, 95% CI; -0.05, -0.03] and 5%-point increase in HNC risk [ATU= 0.05, 95% CI; -0.03, 0.14] in the entire population, among individuals with no vaccine-targeted HPV genotype and among those with at least one vaccine-targeted HPV genotype respectively.

***Conclusion**:* Multiple oral α-HPV infections are common and increase HNC risk, with this risk greatly heightened when HPV 16 is one of the infecting genotypes. Conversely, HPV vaccination holds promise in reducing the incidence of HNC. Future studies can elucidate mechanisms underlying codependence of oral α-HPV genotypes and assess which α-HPV genotypes are more or less likely to be involved in oral co-infection.

INTRODUCTION

Head and neck cancer (HNC) commonly refers to all carcinomas that arise from the epithelial lining of the sinonasal tract, oral cavity, pharynx, and larynx and show microscopic evidence of squamous differentiation. (1) Globally, the estimated burden of HNC is 5.3% of all cancers. (2) It is the seventh most common malignancy worldwide, accounting for more than 660,000 new cases and 325,000 deaths annually. (3) HNC is strongly associated with environmental and lifestyle risk factors, particularly tobacco use (smoked and smokeless), regular alcohol consumption, and chewing of areca nut (betel nut). (4) However, despite changes in behavioural exposure to these traditional HNC risk factors, (5-7) the incidence of a subset of these cancers has increased in recent decades in Canada (8, 9) and other high-income countries. (10-12) This rising incidence has been attributed to oral human papillomavirus (HPV) infection, the main driver of oropharyngeal cancers, a subset of HNC. (13-15) In fact, HPV-related HNC has surpassed the annual incidence and mortality of cervical cancer, the most well-known HPV-related malignancy in Canada and other high-income countries. (16) While it is now accepted that HPV infection is an etiological factor in head and neck carcinogenesis, some of the mechanisms underlying this role remain unclear.

The presence of multiple HPV genotypes at the same oral site within an individual has been observed. (17-19) However, their role in HNC etiology has remained unexplored. At the genital site, some studies revealed that people with multiple genital HPV infections have a low clearance frequency, (20) thus, more persistent infection, which is essential for carcinogenesis. (21-23) Other studies revealed an association between multiple HPV infections and cervical neoplasia. (24, 25). Similar investigations have not yet been conducted for HNC sites. However, evidence from genital sites supports that oral HPV infection by multiple genotypes may play a potential role in HNC development and progression.

Assessing the role of multiple oral HPV genotypes is also important in the context of vaccination. There are currently three Food and Drug Administration (FDA)- approved HPV vaccines: HPV 16 and 18 (Cervarix), HPV 16, 18, 6, and 11 (Gardasil), HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58 (Gardasil 9). Removing certain HPV genotypes by type-specific vaccination could either result in non-targeted genotypes occupying the niche vacated by the vaccine targets, thus increasing their prevalence, (26) or decrease the prevalence of non-targeted genotypes because of cross-type immunity. (27, 28) These concerns necessitate a solid understanding of the equilibrium in the distribution of oral HPV genotypes, an equilibrium that the introduction of vaccination into the population may modify. Studying clustering & co-infection patterns with samples retrieved in the pre-vaccination era may help better characterize this equilibrium, upon which the impact of type-specific vaccination on non-targeted oral HPV genotypes can be assessed.

Further, the evidence of the effectiveness of HPV vaccination in reducing HNC incidence is limited. Given the lack of an effective screening programs, the use of HPV vaccines in the prevention of HNC is especially important. (29-31) Currently, the only evidence of the utility of HPV vaccination in HNC prevention is the lower incidence of chronic oral HPV infection in vaccinated groups compared to unvaccinated groups. (29) However, while this is promising, the limitations of using a surrogate marker of efficacy, such as chronic oral HPV infection in this case, underscore the need to reassess the effectiveness of HPV vaccination in preventing HNC by exploring alternative methods.

We, therefore, aim to investigate the co-infection patterns of oral HPV genotypes and estimate the extent to which multiple oral HPV infections increase HNC risk among a sample of the Canadian population. We will also assess the impact of a hypothetical intervention eliminating all vaccine-targeted HPV genotypes on HNC incidence.

MATERIALS AND METHODS

Study Design

Data for this study come from the Head and Neck Cancer (HeNCe) Life Study; Laprise et al. (19) described its methodology in detail. Briefly, this hospital-based case-control study, conducted

between September 2005 and November 2013, recruited participants from four main referral hospitals in Montreal, Canada. The study was approved by McGill IRB, and all participants signed a consent form.

Cases ascertainment and control selection

Cases (n=460) were individuals with incident, untreated, primary, and histologically confirmed head and neck squamous cell carcinomas (HNSCC) identified based on relevant International Classification of Diseases 10th Revision (ICD-10) codes and included lesions of anatomical sites in the oral cavity, pharynx, and larynx. Controls (n=458) were frequency-matched to cases by sex and age within five years. To mitigate the possibility of Berkson's bias, (32) controls were selected from several outpatient clinics (at the same hospitals as cases) of diseases considered to be unrelated to major HNC risk factors (e.g., tobacco and alcohol). The participation of controls from each outpatient clinic was restricted to less than 20% to limit the overrepresentation of a single disease group.

Data Collection, HPV DNA detection and genotyping

Face-to-face interviews using the life grid technique, a technique suggested to improve recall, (33) collected information on several domains of exposure, including socio-demographic, environmental, and behavioural factors along the participants' lives. Cases and controls provided two oral samples collected with a rinse and a brush. Participants rinsed their mouths with an alcohol-based mouthwash solution and spat into a pre-labeled container. Oral CDx® brushes were firmly pressed against the lesion (oral cavity cases) or normal buccal mucosa (controls and cases) and rotated until pinpoint bleeding appeared. (19) HPV testing has been explained in detail in previous publications. (19) Briefly, HPV DNA detection and genotyping were done using Linear Array (Roche Molecular Diagnostics, Pleasanton, California) and tested for the presence of the β-globin gene using polymerase chain reaction (PCR). Genotyping was done for 36 alpha papillomavirus (α-HPV) genotypes - HPV 6, 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, and 89.

Confounding

Using a directed acyclic graph (DAG) (Figure 1) and *a priori* knowledge of common risk factors of both the exposure (oral α-HPV co-infection) and the outcome (HNC), (4, 34-37) we identified age (years), sex, age at sexual debut (years), number of lifetime sexual partners, history of oral sex, oral health status, total education years, lifetime smoking (pack-years), lifetime alcohol consumption (liter-years) as potential confounders.

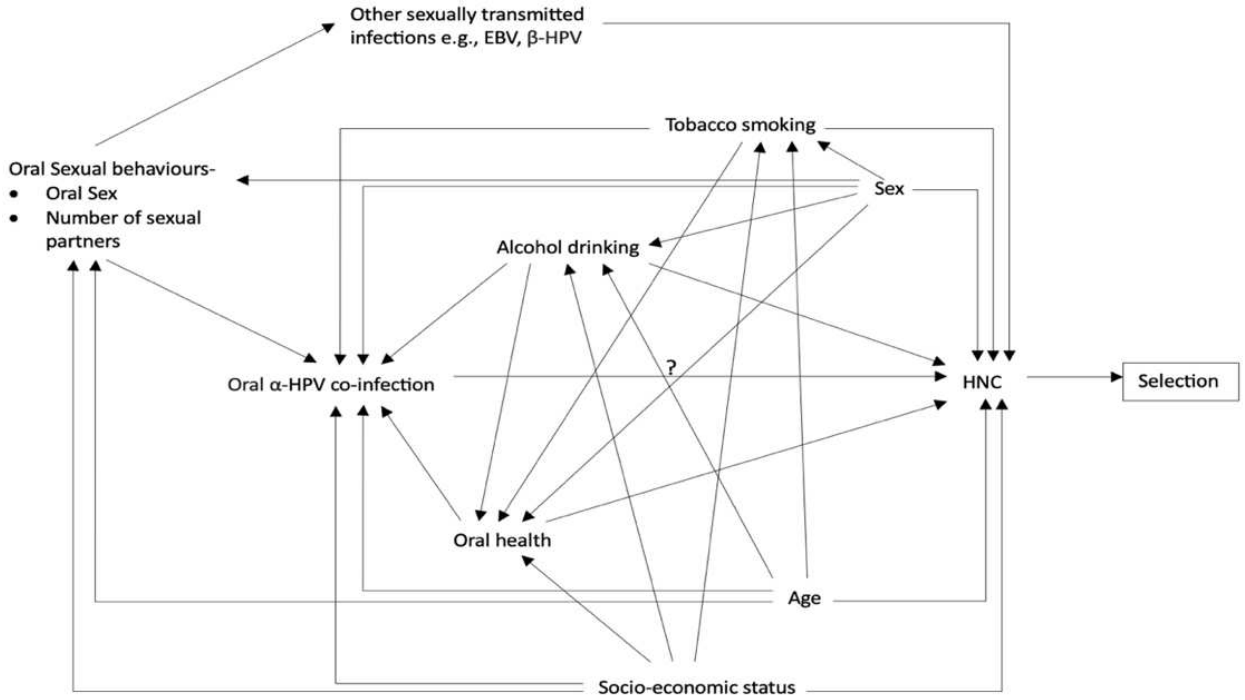

Figure 1. Directed acyclic graph presenting the associations between oral α-HPV co-infection and HNC

Statistical Analysis

Following descriptive statistics, we used the Poisson model to assess whether the number of co-infecting α-HPV types in individuals represent independent infections. Under independence, expected frequencies for the number of co-infecting α-HPV types would arise from a Poisson distribution (i.e., having one α-HPV infection would neither increase nor decrease the probability of another infection) and variance inflation factor (VIF) would equal 1. VIF measures the degree of departure of the observed frequencies of co-infecting α-HPV types from the assumption of independence.

Further, to assess the clustering patterns of α-HPV genotypes, we conducted latent class analysis (LCA), to classify individuals into mutually exclusive latent classes (LCs) based on their probability to test positive for α-HPV genotypes. We then used unconditional logistic regression to estimate the odds ratios (OR) and 95% confidence intervals (CI) for the associations between multiple α-HPV infections and HNC risk. We tested different scenarios, including co-infection with HPV 16 and other α-HPV genotypes, co-infection with vaccine-targeted and non-targeted α-HPV genotypes. We also assessed interaction between vaccine-targeted and non-targeted α-HPV genotypes and estimated relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP) and Synergy index (S). All models were adjusted for the confounders described above.

Lastly, we used a doubly robust, maximum likelihood-based causal inference method- case-control weighted targeted maximum likelihood estimation (CCW-TMLE) (38) to evaluate the treatment effect of HPV vaccination on HNC. We estimated the average treatment effect (ATE), average treatment effect on the treated (ATT), average treatment effect on the untreated (ATU), odds ratio (OR) and relative risk (RR). CCW-TMLE is a modification of TMLE appropriate for analyzing of case-control data; it allows for weighing cases and controls to eliminate the sampling bias of the case-control study design. (38) The weights were calculated as follows: each participant was placed in one of 20 groups based on their age (15–29 years, 30–49 years, 50–69 years, 70–84 years, 85+ years), sex (male, female) and disease status (case, control). For each case group, the number of incident HNC cases in Quebec from 2007 to 2013 were retrieved from the Quebec cancer registry. (39) The weight of each case group (thus each individual in that group) was calculated as the number of incident HNC cases in Quebec divided by the number of incident HNC cases in HeNCe study during the study period. Likewise, for control groups, mid-year population estimates in Quebec from 2007 to 2013 were retrieved from statistics Canada website. (40) The weight of each control group (thus each individual in that group) was calculated as the population of Quebec divided by the number of controls in HeNCe study during the study period. R software (version 4.2.2) was used for all analyses.

RESULTS

A total of 818 participants (389 cases and 429 controls) were included in the analyses, as they had at least one sample with β-globin-positive results. Table 1 presents the distribution of selected characteristics among participants. Controls generally had lower life-time consumption of tobacco and alcohol relative to cases. α-HPV infection was more common among cases (42.2%) than controls (14.2%). The overall prevalence of multiple oral α-HPV infections was 9.3%, higher among cases (14.7%) than controls (4.4%).

Table 2 summarizes the observed and expected frequencies of co-infecting α-HPV types. Among cases and controls, the frequency of co-infecting α-HPV genotypes did not conform to a Poisson distribution (figure 2) as indicated by variance inflation (VI) factors which were 1.51 and 1.31 (without and with adjustment of sexual behaviours, respectively) among cases and 1.61 and 1.54 among controls.

Results from latent class analysis show that a 2-class model, the model with the lowest Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) value, best fits our data. Notably, classes with HPV 16 had considerably lower prevalence [cases- 9.5%; controls- 5.6%], than classes with HPV 18 [cases- 90.5%; controls- 94.4% respectively]. Detailed information on the latent classes is presented in Supplementary Tables 1 (model fit statistics) and 2 (participants' clusters).

Compared to participants without any α-HPV, those infected with HPV 16 only [OR= 24.43; 95%CI: 10.45, 71.61], those with HPV 16 and one other α-HPV [OR= 8.91; 95%CI: 3.15, 32.04], and those with multiple infection that included HPV 16 (HPV 16 and two or more other α-HPV) [OR= 22.09; 95%CI: 4.31, 404.74] had increased HNC risk (Table 3). Also, participants without HPV 16 but with one [OR= 1.36; 95%CI: 0.81, 2.30] or several [OR= 1.90; 95%CI: 0.86, 4.28] α-HPV infection had increased HNC risk compared to those without any α-HPV infection (Table 3).

Further analyses exploring the relationship between the nine HPV genotypes targeted by the vaccines and HNC risk showed that participants infected with targeted HPV only [OR= 8.35; 95%CI: 4.77, 15.46], those infected with both targeted and non-targeted α-HPV genotypes [aOR=

4.92; 95%CI: 2.49, 10.38], and those infected with non-targeted α-HPV genotypes only [aOR= 1.65; 95%CI: 0.95, 2.86] had an increased HNC risk when compared to individuals without α-HPV (Table 4). The measures of interaction [RERI= -3.3, AP= -0.58 and S= 0.59] indicate a sub-additive interaction between targeted and non-targeted α-HPV genotypes (Table 5).

Causal inference analysis assessing the treatment effect of eliminating all vaccine-targeted HPV genotypes on HNC revealed a 0.7%-point reduction in HNC risk [ATE= -0.007, 95% CI; -0.008, -0.005] and 4%-point reduction in HNC risk [ATT= -0.04, 95% CI; -0.05, -0.03] in the entire population and among individuals with no vaccine-targeted HPV genotype, respectively. A 5%-point increase in HNC risk [ATU= 0.05, 95% CI; -0.03, 0.14] among those with at least one vaccine-targeted HPV genotype was also observed. Individuals with no vaccine-targeted HPV genotype had a 96% lower risk of HNC compared to individuals with at least one vaccine-targeted HPV genotype [RR= 0.04, 95% CI; 0.03, 0.05] (Table 6).

DISCUSSION

This study investigated the HNC risk conferred by multiple oral α-HPV infections, co-infection patterns of oral α-HPV genotype and the potential effect of HPV vaccination on HNC using a sample of Canadians. To our knowledge, no prior studies have explored the associations between multiple α-HPV infections and HNC risk. Similarly to cervical neoplasia (25, 41), our findings show that multiple α-HPV infections increase HNC risk. HPV 16, the type to which the greatest carcinogenic potential is most commonly ascribed, accounts for 90% of HPV-positive HNC. (42) Hence, our study further examined the association between multiple α-HPV infections and HNC risk by taking into account the risk conferred by HPV 16. Indeed, multiple α-HPV infections increase HNC risk. However, this risk greatly heightens when HPV 16 is one of the infecting genotypes, suggesting that HPV 16 interacts with other α-HPV genotypes, substantially increasing the baseline HNC risk observed with multiple α-HPV infections (without HPV 16). Additionally, infection with non-vaccine-targeted α-HPV genotypes increases HNC risk, indicating that albeit, HPV vaccination might prevent HNC, considerable risk from non-vaccine-targeted α-HPV genotypes remains.

Similar to previous studies (41, 43), we found a deviation of the observed number of individuals with co-infecting genotypes from a Poisson distribution. This lack of conformity was anticipated because HPV infections share common risk factors and transmission modes. However, even after controlling for sources of correlation between HPV types, multiple α-HPV infections still occurred more often than would be expected by chance. Further, upon assessing clustering patterns, participants with HPV 16 and 18 were grouped into separate classes with disparate prevalences. This may be a pointer to certain implicit interactions within the "HPV 16-based cluster" and the "HPV 18-based cluster," which should be investigated further.

Additionally, our study revealed that HPV vaccination should be efficacious in preventing HNC. Unlike previous studies (44-47) that used persistent oral infection as a surrogate of efficacy (i.e., outcome measured was a persistent oral infection), we assessed efficacy using causal inference statistical methods (exposure being HPV vaccination and outcome being HNC). Under the assumption that participants infected with no targeted HPV genotype were vaccinated and those infected with at least one targeted HPV genotype were unvaccinated, we simulated what would happen if all vaccine-targeted HPV genotypes were removed from the population. Our findings indicate decreased HNC risk with the eradication of all vaccine-targeted HPV genotypes both across the entire population and among those who had no vaccine-targeted HPV genotypes. Surprisingly, however, we also observed that eliminating all vaccine-targeted HPV genotypes increases HNC risk among individuals with at least one vaccine-targeted HPV genotype had they had none. One possible explanation is that the presence of non-targeted HPV genotypes among individuals with at least one vaccine-targeted HPV genotype might have been sufficient to drive carcinogenesis. Additionally, the reduced sample size in this subgroup may have limited the statistical power to accurately estimate causality. Further research is necessary to investigate this finding.

Some methodological limitations should be considered. Due to the case-control design, we cannot draw conclusions about the time of infection, and therefore we were not able to ascertain whether long-term co-infection raises the probability of HNC. Additionally, the small subgroup sizes precluded providing some conditional estimates. Our study also has strengths, however. This is one of the largest case-control studies on oral HPV infection and risk of HNC in Canada. Data

were collected on a considerable number of domains of exposure along the individual life span. We were, therefore, able to control for possible confounding effects of major HNC risk factors, in the association between multiple α-HPV infections and HNC risk. In addition, we used TMLE- a statistical method used to establish causal association in observational studies to identify the treatment effect of HPV vaccination, which, unlike Inverse Probability of Treatment Weighting (IPTW) and parametric g-formula, is doubly robust. Also, TMLE can remove biases due to model misspecification compared with ordinary logistic regression. (48)

CONCLUSION

Our findings indicate that multiple oral α-HPV infections are not uncommon and increase HNC risk. However, this risk is contingent upon the specific α-HPV genotypes infecting an individual. Our results also substantiated current evidence that HPV vaccination reduces HNC risk, supporting the implementation of prevention strategies targeting oral α-HPV infections to reduce HNC incidence in the Canadian population. Future studies can elucidate mechanisms underlying codependence of oral α-HPV genotypes and assess which α-HPV genotypes are more or less likely to be involved in oral co-infection.

DECLARATION OF COMPETING INTEREST

None declared

GRANT SUPPORT

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TABLES AND FIGURES

Table 1: Frequency distribution of selected characteristics among study participants (n= 818)

Variable	Case- n=389 (%)	Control- n=429 (%)
Sex		
Female	101 (26.0)	132 (30.8)
Male	288 (74.0)	297 (69.2)
Age [Mean, SD]	61.7 [10.4]	61.1 [10.9]
No. of educational years [Mean, SD]	12.1 [3.9]	13.9 [4.4]
Cigarette smoking [Mean pack-year, SD]	40.9 [46.2]	25.3 [39.1]
Smoker	84 (21.6)	88 (20.5)
Former smoker	235 (60.4)	213 (49.7)
Never Smoked	68 (17.5)	128 (29.8)
Alcohol drinking [Mean liter-year, SD]	1.84 [3.9]	1.03 [2.3]
Drinker	200 (51.4)	281 (65.5)
Former drinker	124 (31.9)	75 (17.5)
Never drank	63 (16.2)	73 (17.0)
Age at sexual debut [Mean, SD]	18.4 [4.1]	19.4 [4.7]
Lifetime number of sexual partners		
0-3	151 (38.8)	209 (48.7)
>3-7	176 (45.2)	154 (35.9)
>7	48 (12.3)	52 (12.1)
Ever practiced oral sex		
No	52 (13.4)	95 (22.1)
Yes	325 (83.5)	325 (75.8)
α-HPV status		
α-HPV positive	164 (42.2)	61 (14.2)
α-HPV negative	225 (57.8)	368 (85.8)
α-HPV Co- infection		
Multiple α-HPV infection	57 (14.7)	19 (4.4)
Single α-HPV infection	107 (27.5)	42 (9.8)
Tumor site (ICD-10 code)		
Pharynx	188 (48.3)	-
Larynx	128 (32.9)	-
Oral cavity	73 (18.8)	-

*Abbreviations: SD, standard deviation; HPV, human papillomavirus; ICD, International Classification of Diseases

Table 2: Number of co-infecting α-HPV types among cases (n= 389) and controls (n= 429)

	Number of Coinfecting α-HPV types	**Observed number of individuals (O)**	**Poisson Expected number of individuals (E)**	**O/E (95% CI)**
CASES	0	225	201.42	1.12 (1.03 – 1.21)
	1	107	104.30	1.03 (0.86 – 1.18)
	2	35	27.00	1.30 (0.89 – 1.74)
	3	16	4.66	3.43 (1.93 – 5.15)
	4	1	0.60	1.67 (0.00 – 5.00)
	5	4	0.062	64.52 (16.13 – 129.03)
	6	-	0.0054	-
	7	1	0.000040	25000.00 (0.00 – 75000.00)
CONTROLS	0	368	349.46	1.05 (1.01 – 1.09)
	1	42	71.67	0.59 (0.44 – 0.75)
	2	12	7.34	1.63 (0.82 – 2.59)
	3	6	0.50	12.00 (4.00 – 22.00)
	4	1	0.026	38.46 (0.00 – 115.38)
	5	-	0.00105	-
	6	-	0.000036	-
	7	-	0.0000011	-

*Abbreviations: HPV, human papillomavirus; CI, confidence interval.

Table 3: Associations between multiple α-HPV infections and HNC risk (n= 818) by HPV-16 status

		HPV 16					
		Positive (116)			Negative (702)		
		Case	Control	aOR (95% CI)	Case	Control	aOR (95% CI) [a]
α-HPV genotypes other than HPV 16	None (664)	66	5	24.43 (10.45 – 71.61)	225	368	1.00
	One α-HPV (105)	23	4	8.91 (3.15 – 32.04)	41	37	1.36 (0.81 – 2.30)
	Several α-HPV (49)	17	1	22.09 (4.31 – 404.74)	17	14	1.90 (0.86 – 4.28)

*Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.
[a]OR: Odds ratios adjusted for age, sex, tobacco smoking (cigarette pack years), alcohol drinking (ethanol litre years), sexual behaviours (age at sexual debut, no of lifetime sexual partners, history of oral sex), socio-economic status, and oral health status

Table 4: Associations between α-HPV genotypes based on the nine-valent HPV vaccines and HNC risk (n= 818)

Variable	Cases	Controls	OR (95% CI)	aOR (95% CI) [a]
No α-HPV	225	368	1.00	1.00
Non-targeted α-HPV	38	32	1.94 (1.18 - 3.21)	1.53 (0.90- 2.61)
Both targeted & non-targeted α-HPV	42	12	5.72 (3.04 - 11.58)	5.32 (2.75 - 11.03)
Targeted α-HPV	84	17	8.08 (4.79 - 14.40)	9.03 (5.18- 16.67)

*Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; HPV, human papillomavirus; OR, odds ratio.
[a]aOR: Odds ratios adjusted for age, sex, tobacco smoking (cigarette pack years), alcohol drinking (ethanol litre years), sexual behaviours (age at sexual debut, no of lifetime sexual partners, history of oral sex), socio-economic status, and oral health status

Table 5: Interaction of Vaccine-Targeted and Non-targeted α-HPV genotypes

	Non-targeted HPV absent	**Non-targeted HPV present**	**Effect of Non-targeted HPV within the Strata of Targeted HPV**
	OR [95% CI]	**OR [95% CI]**	**OR [95% CI]**
Targeted HPV absent	1 [Reference]	1.94 [1.18, 3.2]	1.94 [1.18, 3.2]
Targeted HPV present	8.08 [4.68, 13.97]	5.72 [2.95, 11.1]	0.71 [0.31, 1.62]
Effect of Targeted HPV within the strata of non-targeted HPV	8.08 [4.68, 13.97]	2.95 [1.33, 6.53]	
Multiplicative scale	0.36 [0.14, 0.96]		
RERI	-3.3 [-9.01, 2.41]		
AP	-0.58 [-1.84, 0.68]		
S	0.59 [0.23, 1.51]		

*Abbreviations: HPV, human papillomavirus; OR, odds ratio; CI, confidence interval; RERI, Relative excess risk due to interaction; AP, Attributable proportion due to interaction; S, Synergy index.

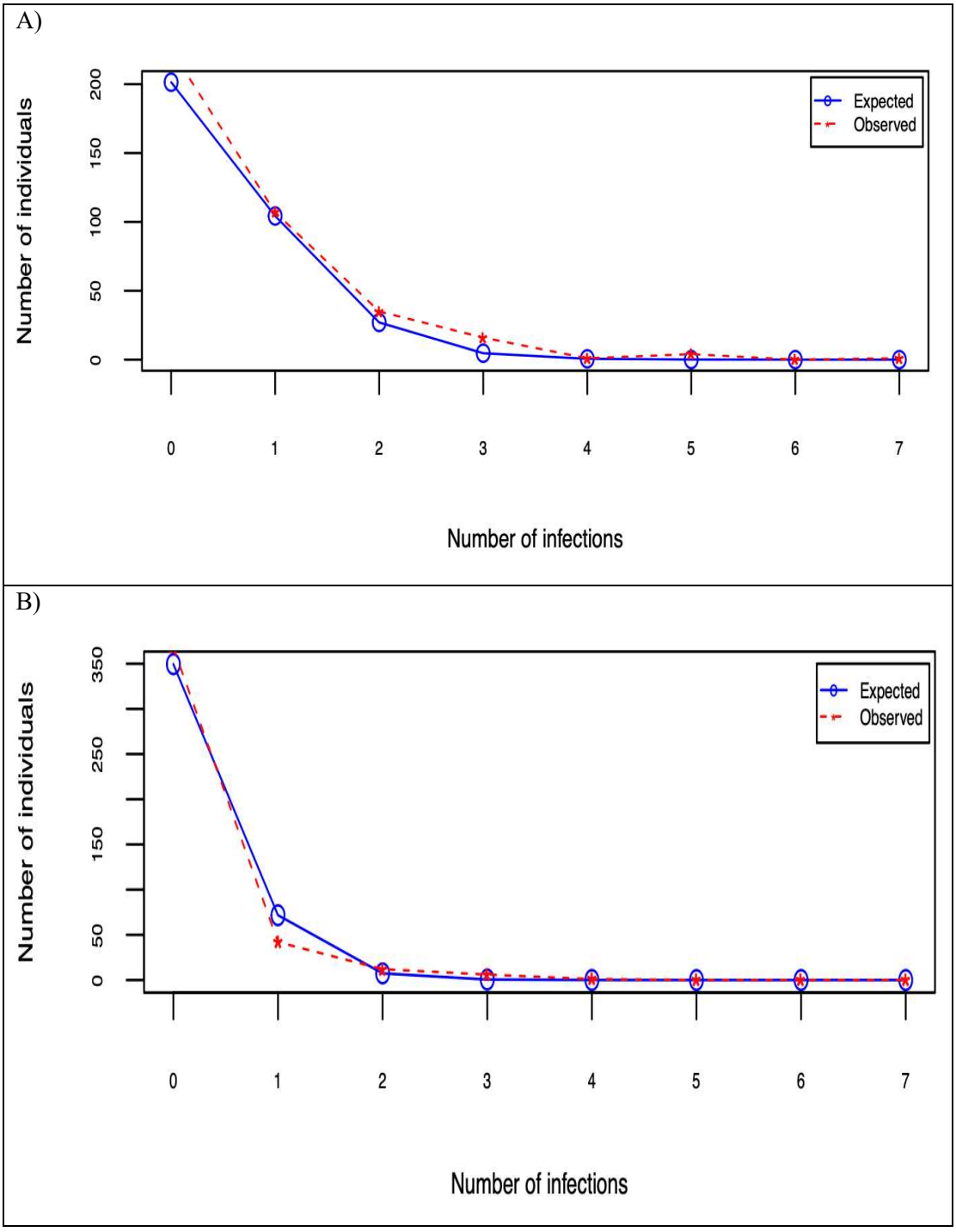
Table 6: Causal relationship between vaccine-targeted HPV elimination and HNC risk (n= 818)

Parameter	**Point Estimate**	**Variance[a]**	**95% CI[a]**
Average Treatment Effect (ATE)	-0.007	3.6e-7	-0.008, -0.005
Average Treatment Effect on the Treated (ATT)	-0.04	1.7e-5	-0.05, -0.03
Average Treatment Effect on the Untreated (ATU)	0.05	0.0019	-0.04, 0.14
Relative Risk (RR)	0.04	0.019	0.03, 0.05
Odds Ratio (OR)	0.04	0.019	0.03, 0.05

*Abbreviations: CI, confidence interval

[a] Variance and the 95% confidence intervals were based on 1000 bootstrap samples.

Figure 2: Poisson Distribution showing the frequencies of the number of co-infecting HPV types among A) Cases and B) Controls



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7 DISCUSSION

In line with the objectives of the study, this chapter presents a summary of the results and their comparison with existing literature, along with potential explanations for the findings. Furthermore, I address the strengths and limitations of this thesis work, future research directions, implications for public health and knowledge translation.

7.1 Summary of Research

The principal motivations behind this thesis were twofold: firstly, the gap in HNC literature as it relates to multiple oral α-HPV infections, and secondly, the potential deleterious implication of multiple oral α-HPV infections on these cancers.

The primary goal of this thesis was to elucidate the role of multiple oral α-HPV infections in HNC aetiology. Initially, I examined the patterns of oral α-HPV co-infection to understand the equilibrium in the distribution of oral HPV genotypes and how they interact. Subsequently, I assessed the amount of HNC risk conferred by multiple oral α-HPV infections compared to single or no oral α-HPV infections. Further, I assessed the risk of HNC conferred by co-infection of vaccine-targeted and non-vaccine targeted HPV genotypes, vaccine-targeted HPV genotypes only, and non-vaccine-targeted HPV genotypes only. Lastly, by emulating a target trial on the efficacy of HPV vaccination, I evaluated the impact of eliminating all vaccine-targeted HPV genotypes on HNC incidence.

Examining oral α-HPV co-infection patterns is the first step in understanding the role of multiple oral α-HPV infections in HNC aetiology. In this pioneering study within the Canadian context, in comparison to controls, a higher proportion of cases were infected with more than one α-HPV genotype. Cases also had a higher number of co-infecting HPV genotypes compared to controls. Notably, even after controlling for sexual behaviours, α-HPV genotypes involved in an oral co-infection were not independent of each other. Infections with multiple α-HPV genotypes occurred more often than expected by chance alone, which might suggest cooperative interactions among the HPV genotypes, wherein they facilitate each other's infectivity. It is possible that HPV genotypes facilitate co-existence within the same tissue by influencing each other's replication,

cellular entry or simultaneously infecting a cell. McLaughlin-Drubin et al. (226) found that HPV types can coexist episomally in the same population of cells or in the same cell, with interactions that could impact disease progression. Unlike studies from cervical literature (159, 227, 228), I did not conduct further analysis to identify types that were more likely to be detected together. This is important because the oncogenicity (high risk, low risk) or phylogenetic relationship (α-9, α-7 etc.) between two HPV genotypes may impact their ability to coexist and interact. (226)

Further, I found a positive association between multiple oral α-HPV infections and HNC risk. Indeed, in agreement with McLaughlin-Drubin et al., (226) it is plausible that these genotypes do not merely depend on each other to infect and coexist but may also interact and influence the development or progression of HNC carcinogenesis. Given the knowledge that most multiple oral α-HPV infections include at least one high-risk type, (155) with HPV 16 being the most oncogenic genotype, I conducted a stratified analysis with and without HPV 16 to ascertain the influence of multiple infections on HNC risk (229). The results shows that HNC risk increases exponentially when HPV 16 is one of the co-infecting genotypes. This not only reaffirms the strong oncogenic potential of HPV 16 but also indicates that the influence of α-HPV multiple infections is contingent upon the specific genotypes implicated.

I also investigated associations between α-HPV genotypes targeted by the nine-valent HPV vaccines and the risk of HNC. As anticipated, infection with HPV genotypes targeted by the vaccine (HPV 16, 18, 31, 33, 45, 52, 58) was associated with an increased HNC risk. Unexpectedly, infection with non-targeted α-HPV genotypes, although with some imprecision around the effect estimate, showed a positive association with HNC risk. This finding is contrary to that reported by Senapati et al. (230), who observed that women infected with genotypes non-targeted by the nonvalent vaccine were at a lesser risk for cervical cancer, suggesting the maximum effectiveness of the nonavalent vaccine. Although only from one study, our finding holds significance for the development of the upcoming generation of vaccines, particularly for HNC prevention. Co-infection of vaccine-targeted and vaccine-non-targeted α-HPV genotypes, while increasing HNC risk, exhibited a joint effect lower than the sum of individual exposures, indicating a sub-additive interaction. This finding emphasizes the need to assess further type-specific interactions between HPV genotypes (co-infection with the same species, different species, vaccine targeted, non-vaccine targeted, high risk, low risk) in relation to HNC development.

Indeed, multiple infections with α-9 genotypes conferred 5.3-fold higher risk while co-infection with α-7 genotypes conferred 2.5-fold risk of cervical cancer. (230)

Lastly, using causal inference methodology and data from HeNCe (observational study), I emulated a target trial to assess the impact of eliminating all vaccine-targeted HPV genotypes on the risk of developing HNC. Essentially, this technique imitates the effect of HPV vaccination on HNC risk as if a randomized controlled trial (RCT) had been conducted. The findings suggest a reduced HNC risk in the population upon eradication of all vaccine-targeted HPV genotypes. This supports and expands upon prior research demonstrating the efficacy of HPV vaccination, albeit indirectly on HNC, but rather on its surrogate, persistent oral infection. (188, 190, 191, 231) The analysis also revealed a counterintuitive finding that eliminating all vaccine-targeted HPV genotypes appears to increase the HNC risk among individuals who had at least one vaccine-targeted HPV genotype had they had none. This finding prompt consideration of two potential explanations. Firstly, the presence of non-targeted HPV genotypes among individuals with at least one vaccine-targeted HPV genotype might still drive HNC carcinogenesis, thus waning the effect "HPV vaccination" in this subgroup. However, when I restricted the analysis to only those without non-targeted HPV genotypes, I still observed increased HNC risk among those who had at least one vaccine-targeted HPV genotype. This highlights the need to explore alternative explanations. Secondly, the subgroup with at least one vaccine-targeted HPV genotype comprised approximately one-fifth of the total sample, which may have reduced statistical power to detect accurate causal estimates, leading to this unexpected finding. Hence, the observed counterintuitive trend could partly be attributed to limitations in sample size. Considering these, further investigations are warranted.

7.2 Strengths and Limitations

In this section, the strengths of our study and limitations asides those already identified, are described below.

In HeNCe life study, only newly diagnosed HNC cases were recruited, ensuring that the diagnosis did not influence their lifestyle behaviours and habits. Moreover, the study gathered comprehensive data on various exposures spanning the life course of participants. These encompassed living conditions, socioeconomic indicators, habits (e.g., smoking, drinking, sexual

behaviour, and dietary patterns), previous medical history, and more. The study also used the life-grid technique to help in the questionnaire administration, improving participants' ability to remember past life events with greater precision. Additionally, strict quality control procedures were in place, including data quality, reliability, and validity. HPV detection and testing were conducted using state of the art methodology in an internationally recognized laboratory accredited by WHO. Together, these procedures help to reduce measurement errors in the assessment of variables included in this analysis.

Furthermore, the extensive collection of information facilitated the construction of a more comprehensive direct acyclic graph to select the confounding variables, ensuring an appropriate adjustment of confounders in the analysis. However, we cannot rule out the presence of unknown confounders that were not included in the analysis.

Some limitations should be considered when interpreting the findings of the study. First, because of the small subgroup sizes, I did not assess multiple oral α-HPV infections by HNC subsites (oral cavity, oropharynx, larynx). Given the predominant association of HPV with oropharyngeal cancers, it would have been advantageous to stratify the analysis by subsite. This approach would have provided a comprehensive understanding of the implications of multiple oral α-HPV infections across various oral sites. Also, as I previously mentioned, I did not conduct an analysis to identify the oral α-HPV genotypes that were more likely to be detected together. However, to the best of our knowledge, this is one of the earliest studies and the first in the Canadian population to investigate the role of multiple oral α-HPV infections in HNC aetiology; hence, it opens the door for more studies in the future to explore multiple oral α-HPV infections.

Another limitation is the possible measurement error of HPV. Using exfoliated cells for HPV detection in HeNCe instead of tissue biopsies (considered the gold standard), (232) might have resulted in in exposure misclassification. The brush method used in HeNCe, however, is simple, relatively inexpensive, safe and well-tolerated by patients. (233) It obtains samples of the oral epithelium down to the basal layer cells. (234) The mouthwash method also used is economical, easy to apply, more readily accepted by participants, and yields a high number of and a more representative sample of HPV DNA-containing cells than tissue and brush biopsies. (235, 236)

These two efficient methods of sample collection were combined in HeNCe and used the same way in both cases and controls. If any misclassification bias occurred, it would be non-differential, which would bias the results towards the null. However, a prominent effect of HPV infection on HNC was present, which is generally in agreement with the literature. For example, the reported stronger association for HPV with oropharyngeal cancers, and the most prevalent HPV type being HPV 16.

In every case-control study, recall bias is a concern. In HeNCe life study, the life grid technique (as described in the methodology section) (200) was used to improve the participants' recall of prior life events and exposures, and thus reduced the bias. In addition, a validation study was carried out, which showed a very good agreement among several variables used in this study.
Potential exposure misclassification for some exposures is plausible. For example, sexual behaviour measures. Participants may feel uncomfortable answering questions on sexual behaviour and could also provide inaccurate information (reporting bias). However, this misclassification might not be different between cases and controls.

Hospital-based controls were used in HeNCe rather than population-based controls. If controls are not selected from the study base that produced the cases, selection bias could arise. (192) However, investigators in HeNCe recruited participants living within 50 km of the hospital making it more likely that cases and controls came from the same catchment area. Further, the disproportionate representation of a single disease category among controls might introduce bias stemming from unmeasured shared risk factors for both the disease and HNC. To address this potential bias, no outpatient clinic contributed more than 20% of the entire pool of control participants.

Another type of selection bias that could have ensued is Berkson's bias. It occurs when the exposure under investigation correlates with the likelihood of being hospitalized. (237, 238) To mitigate Berkson's bias, controls were selected from a specific list of non-chronic conditions not linked to the major risk factors of HNC, tobacco, alcohol, HPV. (238, 239) This also implies that the possibility that the distribution of exposure (HPV genotypes) differs from the study base is minimal i.e. the exposure distribution in the study most likely reflects the study base.

7.3 Implications for Public Health

The rise in HPV-related HNC is a significant public health challenge, requiring prompt and solid preventive strategies. This study investigated the role of multiple oral α-HPV infections in the aetiology of HNC, and its results suggest some potential preventive strategies. It is imperative to consider expanding current vaccination strategies to encompass HPV genotypes that are presently not targeted. Additionally, alongside HPV vaccination initiatives, there is need for the development of interventions aimed at preventing co-infections. These measures should aim to intercept the mechanisms facilitating the co-existence of oral HPV genotypes, thereby reducing the prevalence of co-infections and ultimately mitigating the burden of HPV-related HNC.

7.4 Knowledge Translation Plan

I aim to disseminate these findings to the Faculty of Dental Medicine and Oral Health Sciences at McGill University, the University of Montreal, and the National Institute of Scientific Research, Laval, through the positions of my supervisors, Drs. Nicolau and Madathil, both at McGill, and the members of my supervisory committee, Dr. Laprise and Dr. Rousseau. They teach cancer epidemiology in their respective institutions. This dissemination effort will increase awareness of the importance of multiple oral α-HPV infections among researchers.

Furthermore, I have and will continue disseminating these findings at several research events. The first was an oral presentation at the prestigious Canadian Cancer Research Conference, which took place in Halifax, Nova Scotia. This project was also awarded first place in the CADR-NCOHR Student Research Award competition and selected for entry into the IADR Hatton Competition.

Lastly, I will submit the findings of this study to a peer-reviewed scientific journal to further enhance the widespread knowledge translation and dissemination of this research.

7.5 Future directions of research

Future studies can:

1. Elucidate mechanisms underlying codependence of oral α-HPV genotypes

2. Investigate which α-HPV genotypes are more or less likely to occur in multiple oral infections
3. Assess type specific interactions between α-HPV genotypes in relation to HNC development

8 CONCLUSION

The following conclusions could be made from this thesis:

1. Multiple oral α-HPV infections are common
2. Multiple oral α-HPV infections increase HNC risk; however, contingent upon the genotypes involved
3. HPV vaccination holds promise in reducing the incidence of HNC

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10 APPENDIX

10.1 Supplementary Tables

10.1.1 Supplementary Table 1: Latent class analysis model fit statistics

Cases				Controls			
Number of classes	**AIC**	**BIC**	**Log-Likelihood**	**Number of classes**	**AIC**	**BIC**	**Log-Likelihood**
2	2017.04	2266.74	338.52	2	1067.94	1307.56	152.47
3	2035.22	2411.76	292.70	3	1091.13	1452.60	115.66
4	2081.23	2584.61	274.71	4	1132.86	1616.18	97.39
5	2101.55	2731.76	231.03	5	1171.40	1776.56	75.93

* Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion

10.1.2 Supplementary Table 2: Clusters of participants with α-HPV genotypes - Class 2 model fit

			Description
	Latent class	**Class prevalence**	**α-HPV genotype**
Cases	Class 1	90.5%	HPV 18, 68, 70, 84
	Class 2	9.5%	HPV 6, 16, 31, 33, 35, 39, 42, 45, 51, 52, 53, 54, 44, 56, 58, 59, 61, 62, 34, 66, 67, 71, 72, 73, 81, 82, 89
Controls	Class1	94.4%	HPV 11, 18, 35, 67, 69, 70, 71, 73, 89
	Class 2	5.6%	HPV 6, 16, 33, 39, 42, 45, 51, 52, 53, 44, 56, 58, 59, 61, 62, 34, 66, 72, 82, 84

*Abbreviations: HPV, human papillomavirus.

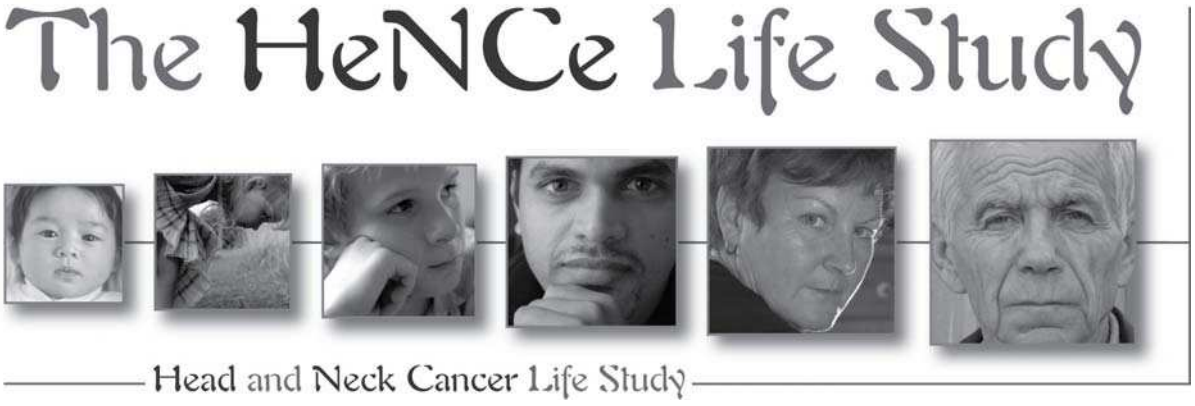
10.1.3 Supplementary Table 3: Distribution of the clinics where controls were recruited

Control recruitment: Outpatients clinics n (%)			
Ophthalmology	106 (23.3)	ENT	24 (5.3)
Stomatology	79 (17.3)	Urology	18 (3.9)
Gastroenterology	56 (11.4)	Rheumatology	8 (1.8)
Nephrology	48 (10.5)	Endocrinology	6 (1.3)
Family medicine	46 (10.1)	Dentistry	2 (0.4)
Neurology	40 (8.7)	Other	3 (0.7)
Orthopaedics	24 (5.3)		

10.2 Study Questionnaire

CONFIDENTIAL

MULTI CENTER STUDY OF ORAL CANCER: A LIFE COURSE APPROACH



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

2010

TABLE OF CONTENTS

A. MEDICAL INFORMATION

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

Identification Number..... 0 2 - [ ][ ][ ] - [ ]
Country **Participant**

Country: (01) Brazil (04) United Kingdom
(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

D8 Please describe your job / different positions (LC)..... **FIRST** ___ **LAST** ___

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)

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

D34 Please describe your job / different positions (LC)..... **FIRST** ___ **LAST** ___

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 in general, regardless of the different positions you may have occupied.

D68 Did your work often 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)
(99) Don't know
(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: _____ ☐☐

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)
(07) Other, specify: _____ ☐☐
(99) Don't know

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

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

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 (03) Less than once a week (06) More than 5 times a week
(01) Less than once a month (04) Once or twice a week (99) Don't know
(02) Once a month (05) 3 to 5 times a week

H42 On average, how often did you eat barbecued food in the winter?..... ___

(00) I never eat BBQ (03) Less than once a week (06) More than 5 times a week
(01) Less than once a month (04) Once or twice a week (99) Don't know
(02) Once a month (05) 3 to 5 times a week

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)
(01) Yes, bottom only (GO TO I3)
(02) Yes, top only
(03) Yes, top AND bottom (GO TO I10)

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 I5.

I4 Did you wear partial dentures?..... [ ][ ]
(00) No
(01) Yes, top only
(02) Yes, bottom 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
(01) Less than once a week
(02) 1-2 time a week
(03) Every other day (3-4 times a week)
(04) Once a day
(05) Twice or more a day

I6 Did you use dental floss?..... [ ][ ]
(00) No
(01) Yes, daily
(02) Yes, once a week
(03) Rarely

I7 Did you use toothpicks / sticks?..... [ ][ ]
(00) No
(01) Yes, daily
(02) Yes, once a week
(03) Rarely

I8 Did you use any kind of substance to clean your teeth?..... [ ][ ]
(00) No
(01) Toothpaste
(02) Other, specify: _____ [ ][ ]

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
(02) 1-2 times a week
(03) Every other day (3-4 times a week)
(04) Once a day
(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
(01) Every 6 months
(02) Every year
(03) Every 2 –5 years
(04) Once every 5 years
(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

Country: 0 2 - ID N°: ___ - ___

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 natural 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 natural 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

Interviewer Reminder: 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) 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?.....	_ _	_ _
L7 How old were you when the relationship ended?.....	_ _	_ _

(00) Still ongoing! (GO TO L8)
(01) Divorce
(02) Separation
(03) Partner deceased

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 (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			