Abnormal glucose metabolism and metabolic syndrome in the etiology of head and neck squamous cell carcinoma (HNSCC)

Marni Stott-Miller

A dissertation
submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington
2012

Reading Committee:
Stephen M Schwartz, Chair
Chu Chen
Mario Kratz
Brian LeRoux

Program Authorized to Offer Degree:
Public Health - Epidemiology

University of Washington

Abstract

Abnormal glucose metabolism and metabolic syndrome in the etiology of head and neck squamous cell carcinoma (HNSCC)

Marni Stott-Miller

Chair of the Supervisory Committee:
Professor Stephen M Schwartz
Department of Epidemiology

Abnormalities of glucose metabolism, such as impaired glucose tolerance and diabetes, have been associated with increased risk of multiple types of cancers. Similarly, metabolic syndrome, which is a cluster of health abnormalities including impaired fasting glucose, has been found to increase the risk of various cancers. Whether abnormal glucose metabolism is associated with risk of head and neck squamous cell carcinoma (HNSCC) has received little attention.

To address this, we conducted three studies. First, we examined whether self-reported history of diabetes was associated with head and neck cancer in a pooled analysis of case-control studies. Second, we tested whether medical administrative records of type II diabetes or metabolic syndrome were associated with HNSCC using data from the SEER-Medicare database. Third, we compared gene expression profiles of oral squamous cell carcinoma (OSCC) and normal oral tissue from controls with respect to pathways and genes involved in glucose metabolism.

In the pooled analysis, we observed a weak association between diabetes and head and neck cancer overall (OR=1.09; 95% CI: 0.95-1.24), but a modest association among never smokers (OR=1.59; 95% CI: 1.22-2.07) and no association among ever smokers. In the second study, we observed a marginal inverse association between type II diabetes and HNSCC (OR=0.92; 95% CI: 0.88-0.96) and a moderate inverse association for metabolic syndrome (OR=0.81; 95% CI: 0.78-0.85). We observed differential expression between normal tissue and OSCC, as well as dysplastic tissue, for pathways and genes involved in glucose metabolism.

Contrasting results from the first two studies make conclusions about the role of diabetes and/or metabolic syndrome in HNSCC difficult. However, it is likely that results from the pooled study were closest to the truth, as we were better able to stratify by risk factors

and adjust for important potential confounders. The third study provided some molecular evidence that glucose metabolism abnormalities play a role in HNSCC. Prospective studies incorporating biomarkers are needed to improve our understanding of the relationship between diabetes and HNSCC, possibly providing new prevention strategies. As rates of glucose metabolism abnormalities increase worldwide, even a small impact on HNSCC risk is of public health concern.

TABLE OF CONTENTS

List of Figures

Figure Number Page

List of Tables

Acknowledgments

I sincerely thank my dissertation committee members, Stephen M. Schwartz, Chu Chen, Mario Kratz and Brian LeRoux for all of their advice, attention, and mentorship. They all gave freely of their time and expertise, and provided me with invaluable guidance through all stages of this process. I am deeply grateful to Dr. Schwartz and Dr. Chen for encouraging me to pursue my research interests, and providing me with all the support and expertise I needed to achieve these goals. I sincerely thank Dr. Kratz for all the time he spent tutoring me in biochemistry and molecular biology. I would also like to express my gratitude to Dr. LeRoux for his advice and support in helping me achieve my research goals.

I thank everyone in the Chen Laboratory at the Fred Hutchinson Cancer Research Center, in particular Pawadee Lohavanichbutr and John Houck, who provided direction and assistance with all the lab work that I conducted as part of this research. I am also grateful to my colleagues at the Fred Hutchinson Cancer Research Center, as well as the University of Washington Epidemiology faculty and doctoral students, for all their time and assistance. I would especially like to thank Jacqueline R. Starr for her mentorship and encouragement.

I would like to thank the members of the International Head and Neck Cancer Epidemiology (INHANCE) Consortium for providing me with advice, scientific insight and assistance.

I am deeply thankful to my husband, Jeff Miller, who encouraged me and supported me every step of the way.

Finally, I wish to acknowledge the financial support that I received. This research was supported by NIH 5T32DE007132-28 (Comprehensive training in inter-disciplinary oral health research), U.S. NIH R01CA095419 from the National Cancer Institute, NCI R03CA113157 (INHANCE Pooled Data Project), and institutional funds from the Fred Hutchinson Cancer Research Center.

CHAPTER 1

Introduction: Abnormal glucose metabolism and metabolic syndrome in the etiology of head and neck squamous cell carcinoma (HNSCC)

Abnormal glucose metabolism, diabetes and HNSCC risk

HNSCC is comprised of cancers arising from the oral cavity, oropharynx, hypopharynx or larynx. These cancers are among the most common worldwide, with over 500,000 new cases and approximately 300,000 deaths in 2008 worldwide (1). Survival is very poor, and patients often suffer orofacial dysfunction and severe disfigurement. Tobacco and alcohol use and, more recently, infection with oncogenic HPV, are established risk factors for HNSCC (2,3). However, approximately 28% of head and neck cancers are not attributed to tobacco or alcohol use (4), and HPV infection is strongly associated with oropharyngeal SCC, but not cancers in other head and neck sites (2,3). We sought to investigate whether abnormal glucose metabolism plays a role in the development of HNSCC.

As early as the 1920's, Otto Warburg observed that tumor cells have an inherently higher need for glucose (the "Warburg effect") (5). In particular, HNSCC cell lines have been observed to be highly dependent on glucose for energy production and survival (6). This increased requirement for glucose raises the possibility that abnormal glucose metabolism may contribute to tumor growth (7).

Conditions such as diabetes and impaired glucose tolerance are well-known abnormalities of glucose metabolism. Diabetes is associated with an increased risk of multiple types of cancer. Results of a meta-analysis analyzing this relationship indicated two-fold or higher relative risks for liver, pancreatic and endometrial cancer, and lesser elevated risks for colorectal, breast and bladder cancers (8). However, diabetes was not found to be associated with lung cancer, and was inversely associated with prostate cancer

(8). The decreased risk of developing prostate cancer has been attributed to decreased testosterone levels in diabetic patients (9). It has been suggested that shorter life expectancy or inadequate control for confounding by cigarette smoking may explain the lack of association between diabetes and lung cancer risk (10).

Some of the mechanisms that have been proposed to explain the increased risk of various cancers among diabetics are: (i) increased generation of reactive oxygen species and greater oxidative damage to DNA (11), (ii) increased formation of advanced glycation endproducts with resultant cellular dysfunction (12, 13), (iii) exposure to high levels of insulin and insulin-like growth factors resulting in increased cellular proliferation (14,15), and (iv) chronic inflammation, which is hypothesized to underlie disturbances in glucose metabolism, resulting in a pro-angiogenic, anti-apoptotic microenvironment (16-18).

Metabolic syndrome and HNSCC risk

Metabolic syndrome is a cluster of health abnormalities, including hypertension, excess abdominal body fat, dyslipidemia, and abnormal glucose metabolism in the form of hyperinsulinemia/hyperglycemia,. The presence of metabolic syndrome is known to increase the risk of developing diabetes and cardiovascular disease (19). A large body of evidence also implicates metabolic syndrome as an important risk factor in the development of several different cancers, including colorectal (20,21), breast (22,23), pancreatic (24), prostate (25), endometrial (26), and liver (27). However, the role of metabolic syndrome in the development of HNSCC cancers has not been investigated.

Metabolic syndrome has also been referred to as “insulin resistance syndrome,” (28) and abnormal glucose metabolism in the form of hyperinsulinemia is a hallmark of the syndrome. Similar potentially carcinogenic mechanisms as for abnormal glucose metabolism are expected to operate in metabolic syndrome, which is also characterized by dysglycemia and a state of chronic systemic inflammation (19). Components of metabolic syndrome,

such as obesity, insulin resistance, dysglycemia, and elevated triglycerides are postulated to promote cancer by generating ROS, increasing hormone production/bioavailability (including estrogen, IGF-1, insulin, and adipokines), and creating an energy-rich, proinflammatory environment (29).

Previous Research

Suba et al. conducted a hospital-based case-control study in Hungary in 2,660 in-patients with confirmed OSCC and 2,980 "complaint-free" controls who volunteered to participate in oral cancer screenings during the same period, and observed that repeatedly elevated (>5.5 mmol/l) fasting glucose over a period of 4 days was strongly associated with oral cancer in females (OR=1.61; no 95% CI reported; $p<0.05$), but that no such association existed in males (OR=0.97; $p>0.05$) (30). Cases and controls were matched on age, but no adjustment was made for, or effect modification examined with, known OSCC risk factors. It has been suggested that hormonal factors may play a role in the observed effect modification by sex (30); however biological mechanisms for this explanation are unclear. Men are more likely than women to be heavy users of tobacco and alcohol in Hungary (31,32), and lack of adjustment for these important risk factors may have influenced the results.

In a study on the risk of multiple cancers in a nationwide cohort of diabetics in Denmark, Wideroff et al. reported increased risk of mouth/pharynx cancer associated with diabetes (33). However, there were only 30 cases in those analyses. Additionally, the association was only observed in subjects less than 50 years old at diabetes diagnosis (standardized incidence ratios (SIR) based on age, sex and calendar year, 1.8; 95% CI, 1.2-2.6). The estimates were similar for males and females.

Diabetics also appear to have an increased prevalence of oral lesions such as erythroplakia and leukoplakia (34,35). While these types of oral lesions are not necessarily

preneoplastic, their presence is associated with increased oral cancer risk (36). In a study of participants in the US National Health and Nutrition Examination Survey (NHANES III), diabetics were found to be 3 times more likely to have oral leukoplakia than non-diabetics (95% CI, 1.28-7.21) (34). Dikshit et al. observed a 2-fold and 3-fold increased risk of leukoplakia and erythroplakia respectively among women, but not men, with a history of diabetes, adjusting for age, pack-years of smoking, duration of chewing, alcohol drinking, education and BMI (35). An association between metabolic syndrome and oral premalignancy (leukoplakia, erythroplakia, and oral submucous fibrosis) was recently observed in a study of subjects undergoing general health and oral examinations in Taiwan; OR=1.68; 95%CI, 1.39–2.04 after adjustment for education, areca nut chewing, smoking, and alcohol drinking (37). The presence of oral lesions predisposing to oral cancer has been associated with low plasma antioxidant status and markers of oxidative stress (38,39); thus, increased levels of reactive oxygen species and oxidative stress observed in diabetes and metabolic syndrome (11,29) may play a role in development of these lesions.

All of these results point to a potential role of impaired glucose metabolism in the development of HNSCC, but are limited by small sample sizes (33), and the use of oral lesions predisposing to cancer risk instead of oral cancer as the endpoint (34,35). The only study to date on glucose abnormalities and HNSCC risk used fasting glucose as the measurement (30), which would identify subjects with impaired fasting glucose (IFG), but may exclude subjects with other glucose metabolism abnormalities, such as postprandial glucose intolerance or insulin resistance. A person may be insulin resistant for many years without experiencing an increase in fasting glucose. Increasing evidence suggests that metabolic syndrome may be an important risk factor for various types of cancers (20-27); however this risk factor has not been investigated in HNSCC.

Public health significance

This investigation has the potential to reveal novel factors that may be important in the development of HNSCC, and may help to explain etiology for the proportion of patients presenting with no or minimal history of tobacco and alcohol use risk factors (4,40). If an association between abnormal glucose metabolism and HNSCC exists, several treatment avenues could be explored. Patients presenting with oral lesions predisposing them to future risk for HNSCC could be tested for impaired glucose tolerance or insulin resistance, and given information or treatment to address these conditions.

In addition, there has recently been great interest in usage of the safe and well-tolerated anti-diabetic drug, metformin, as a potential cancer treatment. A lower incidence of cancer has been noted in diabetics treated with metformin compared with diabetics not treated with metformin or those using other treatments (41,42), and metformin inhibits growth of cancer cells of the breast, colon, and prostate (43-45). The anti-cancer effect of metformin may be due to alleviation of hyperglycemia/hyperinsulinemia (41). Other proposed mechanisms include activation of AMP-activated protein kinase (AMPK) and suppression of the mammalian Target Of Rapamycin (mTOR), a key regulator of cell growth in response to energy state, nutrient status, and growth factor stimulation (46,47). Metformin has a long history of safe use with minimal side effects (48), and preliminary results from prospective clinical trials in breast cancer indicate favorable effects on insulin metabolism and tumor cell proliferation and apoptosis in diabetics as well as non-diabetics (49-51). Therapies that address glucose metabolism abnormalities could thus potentially be important adjuvant treatments to standard HNSCC treatment, as well as provide early preventative strategies for those patients presenting with premalignant oral lesions.

Overview of dissertation research

The purpose of this dissertation project was to test the hypothesis that abnormal glucose metabolism and/or metabolic syndrome are associated with risk of HNSCC. Three separate studies were conducted that examined different aspects of the predictions that would be expected if the hypothesis was true. We sought to determine whether a history of type II diabetes or metabolic syndrome plays a role in the etiology of HNSCC, and whether abnormal glucose metabolism is associated with HNSCC on a molecular level.

In Chapter 2 ("History of diabetes and risk of head and neck cancer: a pooled analysis from the international head and neck cancer epidemiology consortium"), we conducted a pooled analysis of case-control studies participating in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium (52). We included data for 6,448 cases and 13,747 controls from 12 studies for which data on self-reported diabetic status, as well as on head and neck cancer risk factors and other characteristics were available. We estimated associations between self-reported history of diabetes and head and neck cancer, adjusted for age, education level, sex, race/ethnicity, study center, cigarette smoking, alcohol use, and body mass index.

In Chapter 3 ("Type II diabetes and metabolic syndrome and the association with head and neck squamous cell carcinoma: a study in the SEER-Medicare database"), we conducted a nested case-control study using data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare database (53). Cases (n=14,022) were all persons aged 68-99 years diagnosed with HNSCC between 1994 and 2007. Controls (n=42,066) were selected from a similarly-aged 5% sample of individuals residing in the same regions as the SEER registries of the cases. We estimated associations between type II diabetes and HNSCC as well as metabolic syndrome and HNSCC, matched on sex and time enrolled in

Medicare and adjusted for age, race, income, tobacco use, alcohol use, overweight, and SEER registry.

Finally, in Chapter 4 (“The association between oral squamous cell carcinoma and genes and pathways involved in dysregulated glucose metabolism”), we investigated differential expression of pathways and genes involved in glucose metabolism between oral tumor tissue and normal oral epithelium. We used data and specimens from the ORALCHIP study, an ongoing case-control study of gene expression profiling of tumor tissue from oral squamous cell carcinoma (OSCC) patients and normal oral tissue from controls (54). ORALCHIP is based in three medical centers affiliated with the University of Washington. We compared primary OSCC, oral dysplasia and control oral epithelial tissue with respect to differential expression of 7 pathways involved in abnormal glucose metabolism using Affymetrix U133 2.0 Plus GeneChip arrays. We identified 4 genes from the most highly differentially expressed pathways and confirmed differential expression of these genes by qRT-PCR.

References for Chapter 1

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010.
2. Schwartz SM, Daling JR, Doody DR, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst. 1998;90(21):1626-1636.
3. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125-9.
4. Hashibe M, Brennan P, Chuang SC, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev. 2009;18(2):541-50.
5. Warburg O. The Metabolism of Tumors. Constable Press, London, UK. 1930.
6. Sandulache VC, Ow TJ, Pickering CR, Frederick MJ, Zhou G, Fokt I, Davis-Malesevich M, Priebe W, Myers JN. Glucose, not glutamine, is the dominant energy source required for proliferation and survival of head and neck squamous carcinoma cells. Cancer. 2011.
7. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. CA Cancer J Clin. 2010;60(4):207-21.
8. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer. 2009;16(4):1103-23.

9. Betancourt-Albrecht M, Cunningham GR. Hypogonadism and diabetes. Int J Impot Res. 2003;15 Suppl 4:S14-20.
10. Hall GC, Roberts CM, Boulis M, Mo J, MacRae KD. Diabetes and the risk of lung cancer. Diabetes Care. 2005;28(3):590-4.
11. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.
12. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004;63:582-92.
13. Schmidt AM, Hori O, Brett J, et al. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb. 1994;14:1521-1528.
14. Giovannucci E. Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res. 2003;35: 694-704.
15. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol. 2000. 183(1):1-9.
16. De Rekeneire N, Peila R, Ding J, et al. Diabetes, Hyperglycemia, and Inflammation in Older Individuals: The Health, Aging and Body Composition study. Diabetes Care. 2006; 29:1902-1908.
17. Pickup JC, Chusney GD, Thomas SM, et al. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci. 2000; 67:291–300.
18. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001; 85(4):473-83.
19. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28.

20. Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. World J Gastroenterol. 2009;15(41):5141-8.
21. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven F, et al. Metabolic Syndrome and Risks of Colon and Rectal Cancer: the European Prospective Investigation into Cancer and Nutrition Study. Cancer Prev Res (Phila). 2011.
22. Kabat GC, Kim M, Chlebowski RT, Khandekar J, Ko MG, McTiernan A, Neuhouser ML, Parker DR, Shikany JM, Stefanick ML, Thomson CA, Rohan TE. A longitudinal study of the metabolic syndrome and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2009;18(7):2046-53.
23. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr. 2007;86(3):s823-35.
24. Johansen D, Stocks T, Jonsson H, Lindkvist B, Björge T, Concin H, et al. Metabolic factors and the risk of pancreatic cancer: a prospective analysis of almost 580,000 men and women in the Metabolic Syndrome and Cancer Project. Cancer Epidemiol Biomarkers Prev. 2010;19(9):2307-17.
25. Hsing AW, Sakoda LC, Chua S Jr. Obesity, metabolic syndrome, and prostate cancer. Am J Clin Nutr. 2007;86(3):s843-57.
26. Rosato V, Zucchetto A, Bosetti C, Dal Maso L, Montella M, Pelucchi C, Negri E, Franceschi S, La Vecchia C. Metabolic syndrome and endometrial cancer risk. Ann Oncol. 2011;22(4):884-9.
27. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: A study in the SEER-medicare database. Hepatology. 2011;54(2):463-71.
28. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes. 1992;41(6):715-22.

29. Cowey S, Hardy RW. The metabolic syndrome: A high-risk state for cancer? Am J Pathol. 2006;169(5):1505-22.
30. Suba Z. Gender-related hormonal risk factors for oral cancer. Pathol Oncol Res. 2007;13(3):195-202.
31. Brennan P, Bray I. Recent trends and future directions for lung cancer mortality in Europe. Br J Cancer. 2002;87(1):43-8.
32. Kopp M, Csoboth C. Self-destructive behaviour in the Hungarian population. Magy Onkol. 2001;45(2):139-142.
33. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89(18):1360-5.
34. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncol. 2004;40(2):158-63.
35. Dikshit RP, Ramdas K, Hashibe M, et al. Association between diabetes mellitus and premalignant oral diseases: a cross sectional study in Kerala, India. Int J Cancer. 2006;118(2):453–7.
36. Silverman S, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53:563-568.
37. Yen AM, Chen SL, Chiu SY, Chen HH. Association between metabolic syndrome and oral pre-malignancy: a community- and population-based study (KCIS No. 28). Oral Oncol. 2011;47(7):625-30.
38. Bose SC, Singh M, Vyas P, Singh M. Plasma zinc antioxidant vitamins, glutathione levels and total antioxidant activity in oral leukoplakia. Dent Res J (Isfahan). 2012;9(2):158-61.
39. Ma N, Tagawa T, Hiraku Y, Murata M, Ding X, Kawanishi S. 8-Nitroguanine formation in oral leukoplakia, a premalignant lesion. Nitric Oxide. 2006;14(2):137-43.

40. Shiboski CH, Shiboski SC, Silverman S, Jr. Trends in oral cancer rates in the United States, 1973-1996. Community Dent Oral Epidemiol. 2000;28(4):249-25.
41. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010;3(11):1451-61.
42. Evans JM, Donnelly LA, Emslie-Smith AM, et al. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330:1304–5.
43. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 2007;67:6745-6752.
44. Phoenix KN, Vumbaca F, Claffey KP. Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. Breast Cancer Res Treat. 2009;113(1):101-11.
45. Sahra IB, Laurent K, Loubat A, et al. The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. Oncogene. 2008;27:3576-3586.
46. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006; 66:10269-73.
47. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res 2007; 67:10804-12.
48. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes

Association and the European Association for the Study of Diabetes. Diabetes Care. 2009;32(1):193-203.

49. Niraula S, Stambolic V, Dowling RJO, Ennis M, Chang MC, Done SJ, Hallak S, Hood N, Goodwin PJ. Clinical and biologic effects of metformin in early stage breast cancer. Cancer Res. 2010;70(24, Suppl):104s.
50. Hadad SM, Dewar JA, Elseedawy E, Jordan LB, Purdie C, Bray SE, Thompson AM. Gene Signature of metformin actions on primary breast cancer within a window of opportunity randomized clinical trial. J Clin Oncol. 2010;28(Suppl):560.
51. Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. BMC Med. 2011;9:33.
52. Stott-Miller M, Chen C, Chuang SC, Lee YC, Boccia S, Brenner H, et al. History of diabetes and risk of head and neck cancer: a pooled analysis from the international head and neck cancer epidemiology consortium. Cancer Epidemiol Biomarkers Prev. 2012;21(2):294-304.
53. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.
54. Chen C, Méndez E, Houck J, Fan W, Lohavanichbutr P, Doody D, Yueh B, Futran ND, Upton M, Farwell DG, Schwartz SM, Zhao LP. Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2008;17(8):2152-62.

CHAPTER 2

History of Diabetes and risk of head and neck cancer: a pooled analysis from the International Head and Neck Cancer Epidemiology (INHANCE) Consortium

Introduction

Diabetes and/or abnormal glucose metabolism are associated with an increased risk of various types of cancers, including colorectal (1), pancreatic (2), breast (3), liver (4) and endometrial cancer (5). There are several mechanisms through which diabetes may drive the carcinogenic process. Neoplasms have an inherently high need for glucose to fuel proliferation, raising the possibility that untreated hyperglycemia may contribute to tumor growth (6). Diabetics also exhibit increased generation of reactive oxygen species and greater oxidative damage to DNA (7,8). Exposure to high levels of insulin and insulin-like growth factors (IGFs), a hallmark of type II diabetes, results in increased cellular proliferation. The IGF receptor additionally activates the oncogenic epidermal growth factor receptor (EGFR) (9). In addition, IGFs also appear to exert anti-apoptotic effects (10-12).

Head and neck cancers (HNC) are among the most common worldwide, with more than 500,000 new cases and approximately 300,000 deaths in 2008 worldwide (13). While tobacco, alcohol use and, infection with oncogenic HPV are established risk factors for HNC (14,15), emerging evidence suggests that abnormalities of glucose metabolism and diabetes may also play a role (16-19). Several studies have reported that diabetics have an increased prevalence of oral lesions such as erythroplakia and leukoplakia that predispose to oral cancer (17-19). A Danish population-based study comparing individuals hospitalized with a diagnosis of diabetes to the general population observed an increased risk of mouth/pharynx cancer associated with diabetes in subjects less than 50 years old but not in older persons (20); however, these results were based on only 30 cases. A hospital-based

case-control study of 2,660 patients and 2,980 controls observed that elevated fasting glucose was strongly associated with oral cancer in females but not males (16).

We used pooled data from multiple studies from different countries to investigate whether a history of diabetes is associated with HNC overall, as well as within subgroups defined by known HNC risk factors.

Methods

Overview and Design

We conducted a pooled analysis of case-control studies participating in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium. Data pooling methods for the INHANCE consortium have been previously described (21). The following 12 INHANCE studies collected data on diabetic status: Milan (22), Aviano (23), Italy Multicenter (24), Switzerland-Vaud (25), Germany-Saarland (26), Seattle (OralGen) (27), Seattle (LEO) (28), Tampa (29), Los Angeles (30), Rome (31), Japan (32), and North Carolina (33). The 12 studies comprised 6,448 cases and 13,747 controls for which data on diabetic status, as well as on HNC risk factors and other characteristics were available.

Study Population

Cases were patients with tumors classified by the original studies as invasive tumors of the (i) oral cavity, (ii) oropharynx, (iii) hypopharynx, (iv) larynx, (v) oral cavity or pharynx not otherwise specified or (vi) HNC unspecified, as defined previously (21). The pooled studies were all hospital-based, except for both Seattle studies, the North Carolina study and the Los Angeles study. For these four studies, cases were identified from population-

based cancer registries. For all of the included studies, controls were frequency-matched on age and sex, with the exception of the Los Angeles study, for which controls were individually matched on age, sex and neighborhood. The Italy Multicenter study additionally matched on center, and the Tampa and North Carolina studies additionally matched on race/ethnicity. The date of reference was defined as the date of diagnosis for cases and the date of selection for controls, except for the Seattle (OralGen) study (27) where the reference date for a particular control subject was assigned at random from among the possible case subject diagnosis dates (27). The North Carolina, Tampa and Rome studies restricted eligibility to case subjects with squamous cell carcinomas (SCC). For the other studies, SCC was identified by ICD-O-2 or ICD-0-1 histologic codes, with the exception of the Milan, Aviano and Italy Multicenter study, for which no data were available on histologic type. We excluded all known non-SCC cases (n=205).

Measures and Data Collection

Data collection procedures regarding the data pooling and harmonization have been described in detail (21). All interviews for the studies used in this pooled analysis were face-to-face interviews, with the exception of the Germany-Saarland study, for which a self-administered questionnaire was used. Blank questionnaires were collected from the studies to assess comparability and wording of interview questions. Data from each study were received at the INHANCE Data Coordination Center with personal identifiers removed. Each data item was checked for illogical or missing values and queries were sent to the investigators to resolve inconsistencies.

We classified diabetic status as a binary variable (yes/no). Studies from Tampa, Los Angeles, Rome, North Carolina, Seattle (OralGen) (27), Seattle (LEO) (28), Germany, and Japan had a specific question in the interview that asked whether the subject had ever been

diagnosed with diabetes (yes/no). The interview from the Milan, Aviano, Italy, and Switzerland-Vaud studies asked for the age at diabetes, coded as zero for no history of diabetes. Nine out of 12 studies had data on age or date at diagnosis, and this variable was used to estimate duration of diabetes (continuous). Only the Rome study collected information on whether subjects with a history of diabetes were diagnosed with type I or type II diabetes, or had used insulin or oral hypoglycemic agents.

Other relevant subject characteristics, including ethnicity, education, tumor site and histology, cigarette smoking, other tobacco habits, alcohol consumption, height, and weight were harmonized across studies, as described previously (21). Pack-years of cigarette smoking was calculated by multiplying packs (defined as 20 cigarettes) of cigarettes per day and number of years smoking. Alcohol consumption was standardized across studies by first converting beverage-specific number of drinks to ethanol volume in milliliters. The average daily number of ethanol-standardized drinks was then calculated as frequency of consumption of each alcoholic beverage type weighted by the corresponding duration, with the exception of the Tampa, Rome and Germany-Saarland studies in which the average of the frequency of all alcoholic beverage type was used (due to missing data for duration) (34).

Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in square meters). Height and weight at the reference date were self-reported. One study also collected data on height and weight 2-5 years prior to diagnosis, and three studies collected data on height and weight between ages 20-30 years. In a previous INHANCE study on the relationship between BMI and HNC, results were similar for analyses using BMI at these differing time periods (35). We thus used BMI at reference date in the analyses for simplicity and completeness.

A number of subjects were missing data on education level (13% of cases and 26% of controls) and BMI (10% of cases and 5% of controls). There were also a small amount of

missing data for smoking (1.8% of cases and 1.6% of controls) and alcohol use (3.5% of cases and 2.0% of controls). We thus imputed data on these characteristics conditional upon covariates by using a 'MICE' procedure (multiple imputation by chained equations), developed for use in STATA as 'ICE' (36). This algorithm uses a sequence of regression equations to impute missing data conditional on other predictors, cycling through the equations until all variables have complete data. We used age, sex, race/ethnicity, study, case/control status, education level, BMI, smoking status, pack years of smoking, alcohol drinking status, and alcohol drinks per day (excluding the variable to be imputed) to impute the missing data.

Statistical Analyses

We estimated adjusted odds ratios (OR) and 95% CI using unconditional logistic regression models. We performed three levels of covariate adjustment: (i) a minimally adjusted model that controlled for age (categorical), sex, education level (categorical), race/ethnicity (categorical), and study center; (ii) a model that adjusted for age, sex, education level, race/ethnicity, study center, pack-years of cigarette smoking (continuous), and alcohol drinks per day (continuous); and (iii) a model controlling for all the previously listed covariates as well as BMI (continuous). We did not adjust for pipe or cigar smoking because of a substantial amount of missing data for these covariates. Based on previous research that suggested an association between diabetic status and oral cancer risk among women, but not among men (16), we calculated adjusted odds ratios for men and women separately in all primary analyses.

It has been suggested that pack years of cigarette smoking is not an optimal way to model exposure to cigarette smoking, and that duration and dose rate should be modeled as separate variables (37). We thus repeated primary analyses after adjusting for (i) number of

cigarettes smoked per day and (ii) total years of smoking, instead of pack years of cigarette smoking.

To determine whether a history of diabetes is a risk factor for HNC for those cases not associated with excess tobacco/alcohol, we stratified by cigarette smoking and alcohol drinking status. To statistically assess departures from multiplicative effects on the odds scale we included product terms in these stratified analyses, and used a log-likelihood ratio test to compare logistic models with and without the product terms.

To attempt to disentangle the effects of obesity and diabetes on HNC risk and to explore possible interactions, we also stratified by BMI using categories recommended by the World Health Organization (obese ≥ 30; overweight ≥ 25 and < 30; normal, ≥ 18.5 and < 25; and underweight, < 18.5). The previous INHANCE study on the relationship between BMI and HNC reported effect modification by tobacco alone and by tobacco/alcohol (35). We therefore stratified by BMI in analyses that additionally dichotomized subjects according to tobacco use (ever/never).

To determine whether duration of diabetes is associated with HNC risk, we estimated adjusted ORs for the following exposure categories: (i) no history of diabetes; (ii) duration of diabetes less than 10 years; and (iii) duration of diabetes greater than 10 years. Based on a previous study showing differential results according to age at diabetes diagnosis (20), we estimated adjusted ORs for the categories: (i) no history of diabetes; (ii) diabetes diagnosis before age 50; and (iii) diabetes diagnosis after age 50. We additionally examined diabetes diagnosis before or after age 50 stratified by duration of diabetes.

To address possible selection bias due to control participants being systematically healthier than non-participants, we repeated the main analyses after dichotomizing the pooled studies according to participation percentage (<90% (five studies) vs, ≥90% (five studies)). These participation rate percentages were available for all studies, with the exception of the Germany-Saarland study.

We categorized cases by tumor site and performed polytomous logistic regression to assess how the association with diabetes varied across tumor sites. We derived study-specific and summary estimates and evaluated the extent of between-study heterogeneity using the Stata “Metan” command for random effects meta-analyses (38). Finally, we used the “Metainf” module (39) for “leave-one-out” influence analyses to determine whether the associations were dependent on any one study. We used Stata statistical software (version 10.0, Stata Corp., College Station, TX) for all analyses.

Results

Cases were more likely to be male, non-Hispanic White, cigarette smokers, alcohol drinkers, and to have lower BMI compared to controls (Table 1). Of the cases, 18.9% had cancer of the oral cavity, 26.3% had oropharyngeal cancer, and 33.1% had cancer of the larynx. The majority of cases (64.4%) were known to be squamous cell carcinomas, with a sizeable proportion of cases having unknown histologic type (32.5%).

Diabetes was not associated with HNC overall in models adjusted for age, race, sex, study center, education level, pack-years of cigarette smoking, and alcohol drinks per day (OR=0.95; 95% CI, 0.83-1.08; Table 2) and was weakly associated in models that additionally adjusted for BMI (OR=1.09; 95% CI, 0.95-1.24). Results for minimally adjusted models were similar to results obtained with adjustment for age, race, sex, study center, education level, pack-years of cigarette smoking, and alcohol drinks per day. ORs were slightly higher for women than for men (Table 2), but a comparison of models with and without a product term for sex and diabetes yielded p=0.09. Adjustment for BMI reported at age 20 to 30, versus BMI reported at the reference date, did not materially affect the estimates (results not shown).

The overall association between diabetes and HNC was slightly stronger if duration and dose rate of cigarette smoking were modeled as separate variables instead of as pack years of smoking (fully adjusted OR=1.15; 95% CI, 1.00-1.31). The adjusted association between diabetes and HNC was stronger among never smokers (fully adjusted OR=1.59; 95% CI, 1.22-2.07) than among ever smokers (fully adjusted OR=0.96; 95% CI, 0.83-1.11; p=0.001 for homogeneity of the OR; Table 3). The pattern of results among never smokers and never alcohol drinkers versus ever smokers and drinkers was similar to those obtained by stratification on smoking status alone, but ORs were not as high in the never smoking-drinking category as those obtained in the never smoking category. There was an interaction by smoking status in comparisons of models that did and did not include a product interaction term for smoking and diabetes (likelihood ratio p=0.001), but not in models with and without an interaction term for alcohol and diabetes (p=0.36). In analyses that classified smoking status as never, former or current, the OR's were highest for never smokers (fully adjusted OR=1.61; 95% CI, 1.24-2.10; Table 4), lower for former smokers (fully adjusted OR=1.14; 95% CI, 0.94-1.39) and lowest for current smokers (fully adjusted OR=0.91; 95% CI, 0.73-1.12).

We observed little departure from multiplicativity of effects for diabetes and BMI (likelihood ratio p=0.25), and there was no discernible pattern in the results of analyses stratified by BMI, either overall or dichotomized by sex (results not shown). Among never smokers, there was a slight indication of an elevated risk of HNC associated with diabetes among normal weight and overweight subjects (fully adjusted OR=1.64; 95% CI, 0.92-2.93 and fully adjusted OR=1.42, 95% CI, 0.86-2.33 respectively), but not underweight or obese subjects (fully adjusted OR=1.13; 95% CI, 0.37-3.49 and fully adjusted OR=1.12, 95% CI, 0.66-1.92 respectively).

There was no strong evidence of heterogeneity by tumor site in the association between diabetes and HNC (Table 5). In sex-specific strata, results for women were close to

unity for all sites except the hypopharynx; however, wide confidence intervals limit interpretation. There was some indication of heterogeneity by tumor site when analyses were stratified by smoking status (Table 6). Among never smokers, ORs appeared to be greatest for oral cavity and larynx cancers, with null results for oropharyngeal cancers. However, small sample sizes within strata limited our ability to draw meaningful conclusions. In a fully-adjusted polytomous logistic regression model, we did not observe associations between history of diabetes and HNC risk across tumor sites.

Compared to subjects with no history of diabetes, those with diabetes for less than 10 years had a small elevated OR (fully adjusted OR=1.15; 95% CI, 0.95-1.39; Table 7), with similar results obtained for those with diabetes greater than 10 years (fully adjusted OR=1.16; 95% CI, 0.90-1.49). Compared to non-diabetics, we observed an elevated association between HNC and diabetes diagnosed before age 50 (fully adjusted OR=1.37; 95% CI, 1.07-1.74), and no association for those diagnosed after age 50 (fully adjusted OR=1.00; 95% CI, 0.83-1.20). We observed the same pattern for men, but ORs for women diagnosed before and after age 50 were both similarly elevated. These analyses excluded the Seattle (LEO) (28), Rome (31) and Japan (32) studies, due to lack of data on duration of diabetes or age at diabetes diagnosis; however the overall adjusted association between diabetes and HNC for these 9 studies was similar to results for all 12 studies (OR=1.13; 95% CI, 0.97-1.31 and OR=1.09; 95% CI, 0.95-1.24, respectively). An analysis of diabetes diagnosis before or after age 50 stratified by duration of diabetes did not provide meaningful results due to small stratum specific numbers, and did not yield evidence of a particularly unique subgroup (results not shown).

Among the eleven studies with information on control participation proportions, the fully adjusted OR was 1.09 (95% CI, 0.96-1.24).The estimate from five studies with control participation rates below 90% (OR=1.11; 95% CI, 0.89-1.38) was similar to the estimate from six studies with control participation rates above 90% (OR=1.09; 95% CI, 0.92-1.29).

There was evidence of heterogeneity in a meta-analyses of the study-specific ORs (chi-squared p=0.002; Figure 1). “Leave-one-out” influence analyses indicated that the North Carolina study had a large impact on results, due to its unique inverse relationship between diabetes and HNC (Figure 2). The association between history of diabetes and HNC in fully adjusted models was greater when the North Carolina study was excluded (OR=1.19; 95% CI, 1.02-1.38). Similarly, the association among never smokers was appreciably increased after exclusion of the North Carolina study (OR=1.91; 95% CI, 1.39-2.62); with the association among ever smokers increasing slightly after exclusion, but remaining close to unity (OR=1.07; 95% CI, 0.90-1.26).

Discussion

In this large pooled analysis of 12 international studies, we observed a weak association between history of diabetes and risk of HNC overall. However, we observed a stronger association between history of diabetes and HNC in never smokers. In addition, we observed a positive association between diabetes diagnosed before age 50 and HNC.

An association between history of diabetes and HNC only in never smokers may exist if the diabetic condition affects an, as yet, unknown causal pathway for HNC among never smokers. Alternatively, a substantial proportion of people who are both heavy smokers and diabetic and who would have developed HNC in the future, may be at particular risk for early death or illness, and may have died before developing HNC. A third possibility is that adjustment for pack years of smoking is not sufficient to remove all confounding among smokers, and that examining the association between a history of diabetes and HNC among never smokers circumvents this source of residual confounding. This possibility is supported by results from studies on the association between HNC and BMI, which is strongly associated with diabetes (40,41). A recent INHANCE pooled analysis

observed an etiologically improbable reduced risk of HNC associated with overweight and obesity even after adjustment for duration and intensity of smoking (35). However, when analyses were confined to never smokers, the reduced risk associated with overweight and obesity was attenuated to the null. In addition, we observed a slightly stronger association between diabetes and HNC in our data if we modeled duration and dose rate of cigarette smoking as separate variables, which has recently been suggested as a more accurate measure than pack years of smoking (37), and may thus have reduced some of the confounding from this source.

We observed a positive association between HNC and diabetes diagnosed before age 50, and no association for those diagnosed after age 50. Only 5 cases and 9 controls were diagnosed with diabetes before age 20, making it unlikely that these results were due to the inclusion of type I diabetics. Cases diagnosed as diabetic before age 50 had a mean age of 55 at HNC diagnosis and a mean duration of diabetes of 15 years, while cases with a diabetes diagnosis over age 50 had a mean age of 64 at HNC diagnosis and a mean diabetes duration of 6 years. These data suggest that a younger age of type II diabetes onset may confer particular risk for subsequent development of HNC, and that these cancers may develop at a relatively young age. The difference according to age may partly be explained by the observation that younger HNC patients are less likely to have extensive histories of tobacco and alcohol use (42,43). The longer duration of exposure to the diabetic condition could also explain the increased risk for younger patients. In addition, it has been suggested that adults diagnosed with diabetes at a younger age may represent a more aggressive phenotype than people diagnosed late in life (44), and thus the diabetic condition in older people may not predispose to HNC to the same degree as in younger diabetics. The exclusion of several studies due to lack of diabetes diagnosis age data and the small numbers in each of the strata limit interpretation of these results.

Results from the majority of studies in these pooled analyses indicated a positive relationship between history of diabetes and HNC, with the notable exception of the North Carolina study, for which an inverse relationship was observed. Subjects from the North Carolina study made up 14% of the total pooled sample, resulting in a relatively heavy influence of this study on the overall results. A notable difference in the North Carolina study is the high prevalence of diabetes among controls (17%) (45) compared with controls from other US studies and other countries (mean prevalence of 6% in controls for all other studies). The North Carolina study population had a larger proportion of African Americans than other studies; however race is unlikely to play a role because cases and controls were frequency matched on race and estimates were adjusted for race.

Our results support previous research suggesting involvement of abnormal glucose metabolism in HNC. Suba et al. conducted a hospital-based case-control study in Hungary in 2,660 in-patients with confirmed OSCC and 2,980 “complaint-free” controls who volunteered to participate in oral cancer screenings during the same period, and observed that repeatedly elevated (>5.5 mmol/l) fasting glucose over a period of 4 days was strongly associated with oral cancer in females (OR=1.61; no 95% CI reported; $p<0.05$), but that no such association existed in males (OR=0.97; $p>0.05$) (16). Cases and controls were matched on age, but no adjustment was made for, or effect modification examined with, known OSCC risk factors. In a study on the risk of multiple cancers in a nationwide cohort of diabetics in Denmark, Wideroff et al. reported increased risk of mouth/pharynx cancer associated with diabetes (20). However, there were only 30 cases in those analyses. Additionally, the association was only observed in subjects less than 50 years old at diabetes diagnosis (standardized incidence ratios (SIR) based on age, sex and calendar year, 1.8; 95% CI, 1.2-2.6). The estimates were similar for males and females.

In a previous pooled INHANCE study, it was observed that HNC risk is elevated among lean people and reduced among overweight or obese people (35). If overweight and

obesity are negatively associated with HNC, it could be argued that this makes a positive association between diabetes and HNC less likely since obesity is strongly associated with conditions such as metabolic syndrome, and an increased risk of developing insulin resistance, followed by glucose intolerance and type II diabetes (46,47). However, glucose intolerance can also occur independently of insulin resistance (47-49). Diabetes is emerging as more of a heterogeneous disease than initially thought, with subtypes of people who are classified as type II diabetics, but who exhibit defects in insulin secretion with no evidence of insulin resistance. Examples include maturity-onset diabetes of the young (MODY) (50) and mitochondrial diabetes (51). There are also populations that have type II diabetes, especially in Asia, who are not overweight or obese by Western criteria. For example, in a study of type II diabetics in Taiwan, only 43% of women and 48% of men had a BMI greater than 25 kg/m^2 (52). These observations suggest that, although there is an association of overweight with diabetes, the diabetic condition is a distinct disease state that frequently also develops in people who are not overweight.

Hyperglycemia and associated biochemical consequences, independent of obesity-linked characteristics of diabetes, may be a mechanism by which diabetes increases the risk of cancer. Interestingly, several prospective cohort studies indicate that cancer risk starts to increase at blood glucose levels even below the diabetic range. Studies in Korea (53), Austria (54) and Sweden (55) found a linear increase in risk for multiple cancers across the entire spectrum of glucose values, regardless of weight.

A limitation of this study is that we were only able to examine self-reported diabetic status, which may have resulted in exposure misclassification. In the United States, it is estimated that one third of type II diabetics are undiagnosed (56). However, we have no reason to believe that this misclassification would differ between cases and controls; thus any error from this source is most likely to bias results toward the null. The heterogeneity of HNC may also hinder the ability to adequately examine whether diabetes is a risk factor.

Weak or inconsistent associations with all HNC may result if the subtypes of HNC are etiologically distinct. Although we performed analyses for separate sites (larynx, hypopharynx, oropharynx, oral cavity, and non-specific pharynx), small sample sizes prevented meaningful interpretation of the results. The inability to control for HPV infection is a further limitation. However, cancers occurring in the oropharynx, as opposed to other HNC sites, are most strongly associated with HPV infection (27,57,58), and we are not aware of any studies that suggest that diabetics are more likely to be infected with HPV.

We did not have sufficient data to adjust for factors such as diabetic medication use or extent of glycemic control in this study. Many diabetics are able to maintain good glycemic control and/or lowered insulin levels by oral hypoglycemic agents, diet, appropriate use of exogenous insulin, etc. In addition, recent evidence suggests that some oral hypoglycemic agents used to treat diabetes, such as metformin, may reduce incidence of a wide variety of cancers (59). Researchers have observed an association of use of exogenous insulin with increased risk of cancer of the breast, colon, pancreas, prostate, or any solid tumor (60), and increased risk of death from any type of cancer (61). Among 710 subjects who reported a history of diabetes and who had data on insulin use in the present study, 33% were insulin users. Between the years 1997 to 2008 in the United States, the proportion of diabetics aged 65-74 using any diabetes medication (pills, insulin or both) ranged from 83.2% to 90.0% (62). Even if rates of medication use are not this high in our international pooled data, it is nonetheless likely that a large percentage of diabetics were taking oral hypoglycemic agents.

Selection bias may have influenced results in a positive or negative direction. Diabetics are more likely to have multiple hospitalizations than non-diabetics (63,64), creating a selection bias when controls are recruited in hospital-based studies. The possible influence of bias due to controls in hospital-based studies was difficult to evaluate because exclusion of the hospital-based studies increased the proportional influence of the North

Carolina study on the results, attenuating the odds ratio toward the null. An alternative source of selection bias may occur if control participants are systematically healthier than control non-participants, thus spuriously raising the OR. However, this source of bias is unlikely because the pooled OR for studies with control participation rates less than 90% was almost identical to the OR for studies with control participation above 90%.

Conclusion

In this large pooled analysis of 12 case-control studies, we observed a weak association between diabetes and HNC in all subjects, adjusting for several potential confounders; however, we did find a modest association among never smokers. Prospective studies, with data that more accurately captures potential confounding relationships, may provide insight into a possible relationship between glucose metabolism abnormalities and HNC.

Table 1. Characteristics of cases and controls with data on history of diabetes, INHANCE Pooled Case-Control Study of Head and Neck Cancer.[a]

Characteristic	Cases (n=6,448)		Controls (n=13,747)	
	N	%	n	%
Age (years)				
17–39	237	3.7	920	6.7
40–44	300	4.7	787	5.7
45–49	641	9.9	1235	9.0
50–54	1024	15.9	2089	15.2
55–59	1253	19.4	2408	17.5
60–64	1222	19.0	2314	16.8
65–69	953	14.8	1946	14.2
70–74	596	9.2	1464	10.7
75–93	222	3.4	584	4.3
Sex				
Men	5152	79.9	9810	71.4
Women	1296	20.1	3937	28.6
Race				
Non-Hispanic White	5417	84.0	9805	71.3
Black	445	6.9	479	3.5
Hispanic	82	1.3	255	1.9
Asian	471	7.3	3179	23.1
Other	33	0.5	29	0.2
Study center				
Milan	416	6.5	1531	11.1
Aviano	470	7.3	821	6.0
Italy Multicenter	1208	18.7	2545	18.5
Switzerland-Vaud	560	8.7	820	6.0
Seattle (OralGen)	381	5.9	607	4.4
Tampa	203	3.2	893	6.5
Los Angeles	414	6.4	1005	7.3
Rome	321	5.0	389	2.8
Seattle (LEO)	587	9.1	546	4.0
Germany-Saarland	92	1.4	92	0.7
Japan	433	6.7	3102	22.6
North Carolina	1363	21.1	1396	10.2

Table 1 continued

Study design				
Hospital-based	3611	56.0	10101	73.5
Population-based	2837	44.0	3646	26.5
Pack-years of cigarette smoking[b]				
Never	779	12.1	5429	39.5
1–10	351	5.4	1830	13.3
11–20	579	9.0	1560	11.4
21–30	888	13.8	1420	10.3
31–40	975	15.1	1212	8.8
41–50	873	13.5	835	6.1
>50	2003	31.1	1461	10.6
Number of alcohol drinks per day[b]				
Never	710	11.0	3352	24.4
>0 to <1	1077	16.7	3752	27.3
1 to <3	1260	19.5	3244	23.6
3 to <5	896	13.9	1645	12.0
≥5	2505	38.9	1754	12.8
Body mass index (in kg/m^2)				
<18.5	299	4.6	287	2.1
18.5 to <25	3233	50.1	6396	46.5
25 to <30	2109	32.7	5208	37.9
≥30	807	12.5	1856	13.5
Tumor site				
Oral cavity	1218	18.9		
Oropharynx	1693	26.3		
Hypopharynx	558	8.7		
Oral cavity/pharynx NOS	838	13.0		
Larynx	2135	33.1		
Overlapping head and neck sites	6	0.1		
Tumor histology				
Squamous cell	4287	64.4		
Unknown	2161	32.5		

[a] Percentages have been rounded and may not total 100.
[b] Data from first imputed dataset (out of 4 imputations) used for missing data.

Table 2. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and diabetes (yes/no), INHANCE Pooled Case-Control Study of Head and Neck Cancer

Diabetes (yes/no)	Cases exposed/ unexposed	Controls exposed/ unexposed	Covariate Set 1[a]		Covariate Set 2[b,c]	
	N	N	OR	(95% CI)	OR	(95% CI)
All	533/5915	1024/12723	0.95	(0.83-1.08)	1.09	(0.95-1.24)
Women	118/1178	249/3688	1.06	(0.82-1.38)	1.33	(1.02-1.73)
Men	415/4737	775/9035	0.91	(0.79-1.06)	1.03	(0.89-1.19)

[a] Adjusted for age, race, sex, study center, education level, pack-years of cigarette smoking, and alcohol drinks per day.

[b] Adjusted for age, race, sex, study center, education level, pack-years of cigarette smoking, alcohol drinks per day, and BMI.

[c] Likelihood ratio test for interaction by sex: p=0.09.

Table 3. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and diabetes (yes/no) by cigarette smoking and alcohol drinking status,[a] INHANCE Pooled Case-Control Study of Head and Neck Cancer

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	Covariate Set 1[b]		Covariate Set 2[c,d]	
			OR	(95% CI)	OR	(95% CI)
Never cigarette smokers						
All	82/694	366/5047	1.38	(1.06-1.79)	1.59	(1.22-2.07)
Women	39/309	164/2370	1.39	(1.02-1.89)	1.70	(1.25-2.32)
Men	43/385	202/2677	1.36	(1.00-1.84)	1.49	(1.10-2.03)
Ever cigarette smokers						
All	451/5221	658/7676	0.85	(0.73-0.98)	0.96	(0.83-1.11)
Women	79/869	85/1318	0.86	(0.64-1.16)	1.07	(0.79-1.45)
Men	372/4352	573/6358	0.84	(0.73-0.98)	0.94	(0.81-1.10)
Never cigarette smokers and never alcohol drinkers						
All	33/242	172/1827	1.04	(0.69-1.56)	1.26	(0.83-1.91)
Women	22/162	103/1157	1.00	(0.65-1.54)	1.26	(0.81-1.96)
Men	11/80	69/670	1.12	(0.68-1.83)	1.26	(0.76-2.06)
Ever cigarette smokers and ever alcohol drinkers						
All	398/4866	534/6516	0.84	(0.72-0.98)	0.98	(0.84-1.14)
Women	57/732	54/933	0.76	(0.52-1.12)	0.98	(0.66-1.45)
Men	341/4134	480/5583	0.85	(0.73-1.00)	0.98	(0.83-1.15)

[a] Numbers of never smokers/drinkers do not match numbers for zero pack years/drinks per day because missing data for these variables were simultaneously imputed.

[b] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), and alcohol drinks per day (if ever drinker).

[c] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), alcohol drinks per day (if ever drinker), and BMI.

[d] Likelihood ratio test for interaction by smoking status: p=0.001; and by alcohol drinking status: p=0.36.

Table 4. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and diabetes (yes/no) by cigarette smoking status (never, former, current),[a] INHANCE Pooled Case-Control Study of Head and Neck Cancer

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	Covariate Set 1[b]		Covariate Set 2[c]	
			OR	(95% CI)	OR	(95% CI)
Never cigarette smokers						
All	82/694	366/5047	1.43	(1.10-1.86)	1.61	(1.24-2.10)
Women	39/309	164/2370	1.47	(1.08-2.00)	1.74	(1.28-2.38)
Men	43/385	202/2677	1.39	(1.02-1.88)	1.51	(1.11-2.04)
Former cigarette smokers						
All	214/1922	418/4468	1.03	(0.85-1.24)	1.14	(0.94-1.39)
Women	26/231	53/766	1.08	(0.77-1.50)	1.30	(0. 93-1.81)
Men	188/1691	365/3702	1.02	(0.84-1.24)	1.12	(0.92-1.37)
Current cigarette smokers						
All	237/3299	240/3208	0.83	(0.67-1.02)	0.91	(0.73-1.12)
Women	53/638	32/552	0.87	(0.62-1.21)	1.02	(0.73-1.43)
Men	184/2661	208/2656	0.82	(0.66-1.02)	0.88	(0.71-1.10)

[a] Numbers of never smokers do not match numbers for zero pack years because missing data for these variables were simultaneously imputed.

[b] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), and alcohol drinks per day.

[c] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), alcohol drinks per day, and BMI.

Table 5. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and diabetes (yes/no) stratified by location of tumor, INHANCE Pooled Case-Control Study of Head and Neck Cancer[a]

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	Covariate Set 1[b]		Covariate Set 2[c]	
			OR	(95% CI)	OR	(95% CI)
Oral cavity						
All	73/945	984/12156	0.81	(0.61-1.06)	1.03	(0.78-1.37)
Women	20/287	239/3524	0.69	(0.40-1.19)	0.89	(0.51-1.56)
Men	53/658	745/8632	0.87	(0.63-1.20)	1.11	(0.80-1.54)
Oropharynx						
All	114/1411	984/12156	0.90	(0.72-1.12)	1.06	(0.84-1.34)
Women	15/248	239/3524	0.70	(0.37-1.30)	0.83	(0.44-1.56)
Men	99/1163	745/8632	0.96	(0.75-1.22)	1.13	(0.88-1.45)
Hypopharynx						
All	35/523	984/12156	0.73	(0.49-1.07)	0.98	(0.66-1.45)
Women	8/72	239/3524	1.38	(0.58-3.25)	1.96	(0.79-4.83)
Men	27/451	745/8632	0.63	(0.40-0.97)	0.83	(0.53-1.29)
Oral cavity/pharynx NOS						
All	61/764	984/12156	0.75	(0.56-1.00)	0.87	(0.65-1.16)
Women	19/225	239/3524	0.91	(0.53-1.55)	0.99	(0.58-1.71)
Men	42/539	745/8632	0.71	(0.51-1.01)	0.85	(0.60-1.20)
Larynx						
All	215/1920	984/12156	0.98	(0.81-1.18)	1.08	(0.89-1.31)
Women	37/248	239/3524	1.03	(0.62-1.73)	1.08	(0.63-1.85)
Men	178/1672	745/8632	0.95	(0.77-1.17)	1.05	(0.86-1.30)

[a] Analyses do not include Seattle (OralGen)[27] because two sites were not included in this study (hypopharynx and larynx).

[b] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking, and alcohol drinks per day.

[c] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking, alcohol drinks per day, and BMI.

Table 6. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and diabetes (yes/no) by cigarette smoking status[a] and stratified by site,[b] INHANCE Pooled Case-Control Study of Head and Neck Cancer

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	Covariate Set 1[c] OR	(95% CI)	Covariate Set 2[d] OR	(95% CI)
Never cigarette smokers						
Oral cavity	14/130	356/4840	1.30	(0.72-2.36)	1.45	(0.80-2.64)
Oropharynx	19/206	356/4840	1.11	(0.66-1.85)	1.00	(0.59-1.68)
Hypopharynx	1/34	356/4840	0.26	(0.03-1.94)	0.34	(0.05-2.63)
Oral cavity/pharynx NOS	23/191	356/4840	1.46	(0.90-2.35)	1.42	(0.87-2.30)
Larynx	16/85	356/4840	1.92	(1.07-3.46)	1.93	(1.06-3.50)
Ever cigarette smokers						
Oral cavity	59/815	628/7316	0.74	(0.54-1.01)	0.95	(0.69-1.31)
Oropharynx	95/1205	628/7316	0.87	(0.68-1.12)	1.09	(0.84-1.41)
Hypopharynx	34/489	628/7316	0.77	(0.52-1.15)	1.03	(0.69-1.54)
Oral cavity/pharynx NOS	38/573	628/7316	0.58	(0.40-0.83)	0.71	(0.49-1.02)
Larynx	199/1835	628/7316	0.94	(0.77-1.15)	1.04	(0.85-1.27)

[a] Numbers of never smokers do not match numbers for zero pack years because missing data for these variables were simultaneously imputed.

[b] Analyses do not include Seattle (OralGen)[27] because two sites were not included in this study (hypopharynx and larynx).

[c] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), and alcohol drinks per day (if ever drinker).

[d] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking (if ever smoker), alcohol drinks per day (if ever drinker), and BMI.

Table 7. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck cancer and age at diabetes diagnosis,[a] INHANCE Pooled Case-Control Study of Head and Neck Cancer.[b]

Measure	Cases N	Controls N	Covariate Set 1[c] OR	Covariate Set 1[c] (95% CI)	Covariate Set 2[d] OR	Covariate Set 2[d] (95% CI)
Diabetes diagnosis age						
All:						
No history of diabetes	4686	9006	ref	ref	ref	ref
< 50 years old	158	234	1.14	(0.90-1.44)	1.37	(1.07-1.74)
≥ 50 years old	258	463	0.87	(0.73-1.04)	1.00	(0.83-1.20)
Women:						
No history of diabetes	868	2767	ref	ref	ref	ref
< 50 years old	39	72	1.16	(0.73-1.83)	1.43	(0.90-2.28)
≥ 50 years old	55	119	1.13	(0.78-1.63)	1.44	(0.99-2.09)
Men:						
No history of diabetes	3818	6239	ref	ref	ref	ref
< 50 years old	119	162	1.13	(0.86-1.49)	1.35	(1.02-1.79)
≥ 50 years old	203	344	0.81	(0.66-0.99)	0.90	(0.73-1.11)
Diabetes duration						
All:						
No history of diabetes	4686	9006	ref	ref	ref	ref
> 0 to ≤10 years	250	393	0.97	(0.80-1.18)	1.15	(0.95-1.39)
> 10 years	139	229	1.02	(0.80-1.31)	1.16	(0.90-1.49)
Women:						
No history of diabetes	868	2767	ref	ref	ref	ref
> 0 to ≤10 years	53	107	1.05	(0.71-1.54)	1.39	(0.94-2.05)
> 10 years	36	63	1.35	(0.83-2.18)	1.58	(0.97-2.57)
Men:						
No history of diabetes	3818	6239	ref	ref	ref	ref
> 0 to ≤10 years	197	286	0.95	(0.77-1.18)	1.09	(0.88-1.35)
> 10 years	103	166	0.93	(0.70-1.24)	1.05	(0.78-1.40)

[a] Score test for trend of odds: p<0.001.

[b] Based on data from the following studies: Milan, Aviano, Italy Multicenter, Switzerland-Vaud, Seattle (OralGen),[27] Tampa, Los Angeles, Germany-Saarland, North Carolina.

[c] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking, and alcohol drinks per day.

[d] Adjusted for age, race, sex, study centers, education level, pack-years of cigarette smoking, alcohol drinks per day, and BMI.

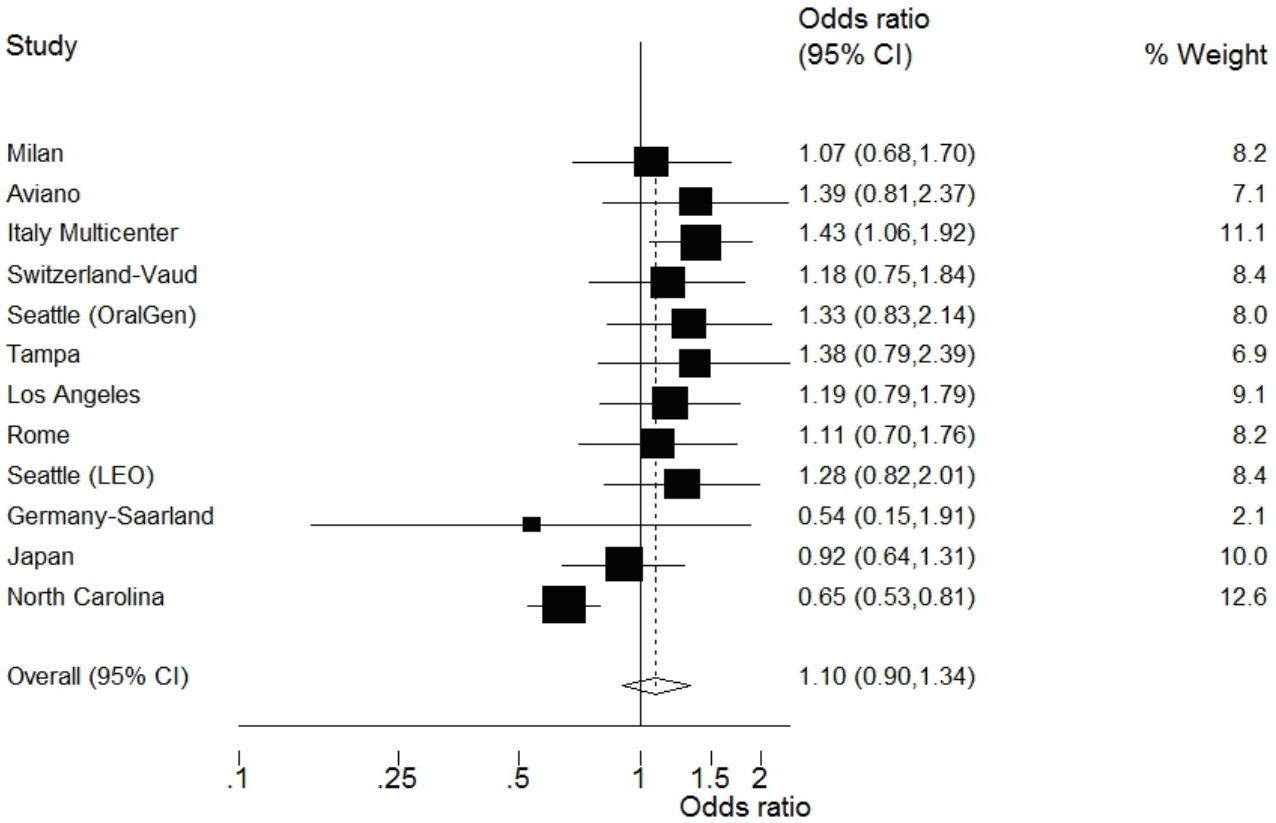

Figure 1. Forest plot of study-specific ORs and 95% CIs for the association between history of diabetes and HNC risk, INHANCE Pooled Case-Control Study of Head and Neck Cancer. The squares represent the OR estimates and the horizontal lines represent the 95% CIs for each study. The area of the square reflects the weight that the study contributes. This random-effects model incorporates an estimate of between-study heterogeneity in the weighting. The diamond at the center indicates the random-effects estimate and the width of the diamond indicates the 95% CI.

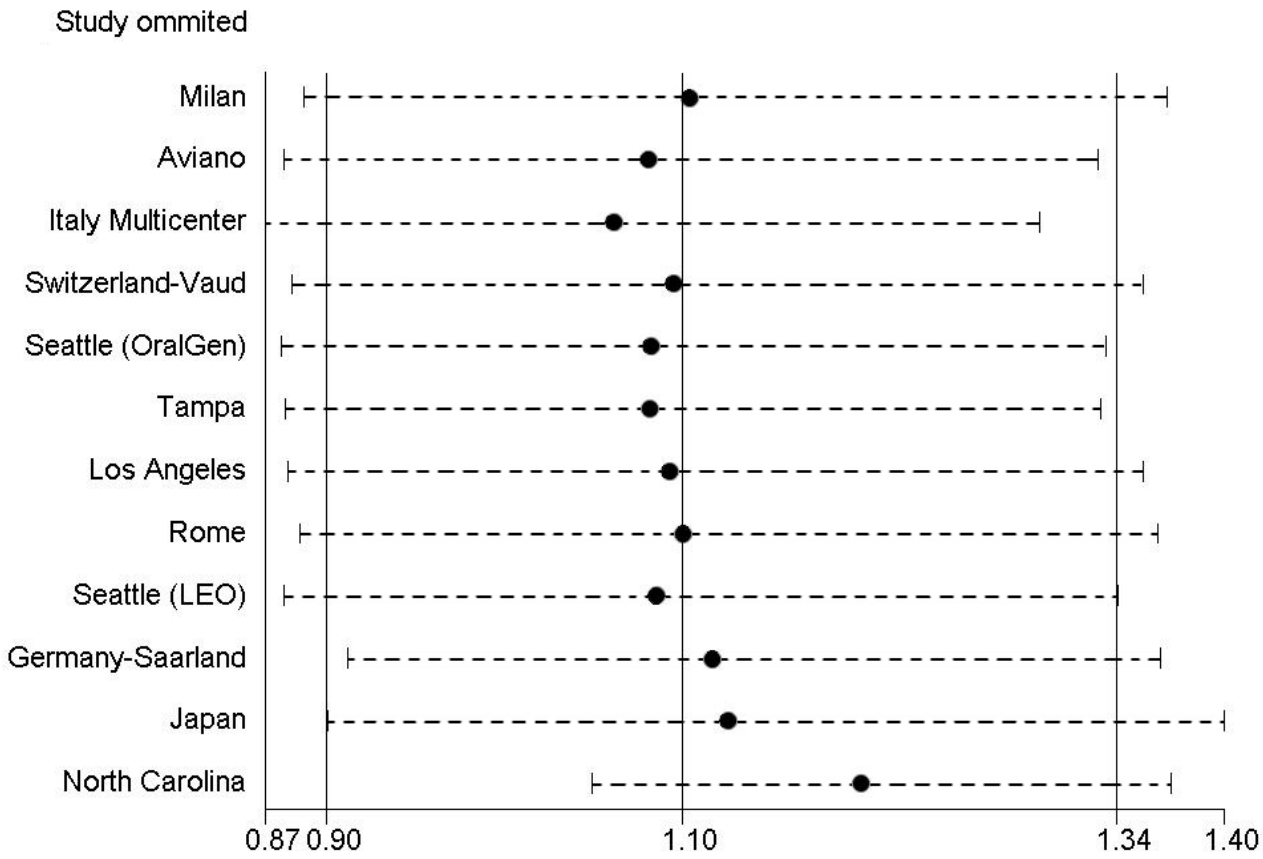

Figure 2. Influence analyses of single studies on the overall estimate for the association between history of diabetes and HNC risk, INHANCE Pooled Case-Control Study of Head and Neck Cancer. The circle for each study represents the OR estimate and the horizontal line represents the 95% CI from the pooled data after excluding that study. The three vertical lines represent the random-effects pooled point estimate and associated 95% CI.

References for Chapter 2

1. Nilsen TI and Vatten LJ. Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyperinsulinaemia hypothesis. Br J Cancer 2001;84:417-22.
2. Gapstur SM, Gann PH, Lowe W, Liu K, Colangelo L, Dyer A. Abnormal glucose metabolism and pancreatic cancer mortality. JAMA 2000;283:2552-8.
3. Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, Colditz GA, et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care 2003;26:1752-8.
4. Jee SH, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. JAMA 2005;293:194-202.
5. Stattin P, Bjor O, Ferrari P, Lukanova A, Lenner P, Lindahl B, et al. Prospective study of hyperglycemia and cancer risk. Diabetes Care 2007;30:561-7.
6. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, et al. Diabetes and cancer: a consensus report. CA Cancer J Clin 2010;60(4):207-21.
7. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.
8. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004;63:582-92.
9. Adams TE, McKern NM, Ward CW. Signalling by the type 1 insulin-like growth factor receptor: interplay with the epidermal growth factor receptor. Growth Factors 2004;22(2):89-95.

10. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol 2000;183:1-9.
11. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346-53.
12. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. Mol Pathol 2001;54:133-7.
13. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010.
14. Hennessey PT, Westra WH, Califano JA. Human papillomavirus and head and neck squamous cell carcinoma: recent evidence and clinical implications. J Dent Res 2009;88:300-6.
15. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125-9.
16. Suba Z. Gender-related hormonal risk factors for oral cancer. Pathol Oncol Res 2007;13(3):195-202.
17. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncology 2004;40:158-63.
18. Dikshit RP, Ramadas K, Hashibe M, Thomas G, Somanathan T, Sankaranarayanan R. Association between diabetes mellitus and pre-malignant oral diseases: A cross sectional study in Kerala, India. Int J Cancer 2006;118:453-7.
19. Albrecht M, Bánóczy J, Dinya E, Tamás G Jr. Occurrence of oral leukoplakia and lichen planus in diabetes mellitus. J Oral Pathol Med 1992;21(8):364-6.

20. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89(18):1360-5.
21. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst 2007;99:777–89.
22. Bosetti C, Gallus S, Trichopoulou A, Talamini R, Franceschi S, Negri E, et al. Influence of the Mediterranean diet on the risk of cancers of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 2003;12(10):1091-4.
23. Franceschi S, Talamini R, Barra S, Barón AE, Negri E, Bidoli E, et al. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. Cancer Res 1990;50(20):6502-7.
24. Baron AE, Franceschi S, Barra S, Talamini R, La Vecchia C. A comparison of the joint effects of alcohol and smoking on the risk of cancer across sites in the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 1993;2(6):519-23.
25. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, Monnier P. Food groups and risk of oral and pharyngeal cancer. Int J Cancer 1998;77(5):705-9.
26. Haug U, Hillebrand T, Bendzko P, Löw M, Rothenbacher D, Stegmaier C, et al. Mutant-enriched PCR and allele-specific hybridization reaction to detect K-ras mutations in stool DNA: high prevalence in a large sample of older adults. Clin Chem 2007;53(4):787-90.
27. Schwartz SM, Daling JR, Doody DR, Wipf GC, Carter JJ, Madeleine MM, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst 1998;90:1626-36.

28. Vaughan TL, Stewart PA, Davis S, Thomas DB. Work in dry cleaning and the incidence of cancer of the oral cavity, larynx, and oesophagus. Occup Environ Med 1997;54(9):692-5.
29. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 2002;23(7):1229-34.
30. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, et al. Polymorphism of Xeroderma Pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer 2006;118(3):714-20.
31. Boccia S, Cadoni G, Sayed-Tabatabaei FA, Volante M, Arzani D, De Lauretis A, et al. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. J Cancer Res Clin Oncol 2008;134(1):93-100.
32. Sugimura T, Kumimoto H, Tohnai I, Fukui T, Matsuo K, Tsurusako S, et al. Gene-environment interaction involved in oral carcinogenesis: molecular epidemiological study for metabolic and DNA repair gene polymorphisms. J Oral Pathol Med 2006;35(1):11-8.
33. Divaris K, Olshan AF, Smith J, Bell ME, Weissler MC, Funkhouser WK, et al. Oral health and risk for head and neck squamous cell carcinoma: the Carolina Head and Neck Cancer Study. Cancer Causes Control 2010;21(4):567-75.
34. Purdue MP, Hashibe M, Berthiller J, La Vecchia C, Dal Maso L, Herrero R, et al. Type of alcoholic beverage and risk of head and neck cancer--a pooled analysis within the INHANCE Consortium. Am J Epidemiol 2009;169(2):132-42.
35. Gaudet MM, Olshan AF, Chuang SC, Berthiller J, Zhang ZF, Lissowska J, et al. Body mass index and risk of head and neck cancer in a pooled analysis of case-control

studies in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium. Int J Epidemiol 2010;39(4):1091-102.

36. Royston P. Multiple imputation of missing values. Stata J 2004;4:227-241.
37. Peto J. That the effects of smoking should be measured in pack-years: misconceptions 4. Br J Cancer. 2012;107(3):406-7.
38. Harris R, Deeks J, Altman DG, Bradburn MJ, Harbord R, Sterne JAC. Metan: fixed and random effects meta-analysis. Stata J 2008;8:3.
39. Tobias A. sbe26: assessing the influence of a single study in the meta-analysis estimate. Stata Tech Bull 1999;47:15-17.
40. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol 1997;146:214-222.
41. Narayan KM, Boyle JP, Thompson TJ, Gregg EW, Williamson DF. Effect of BMI on lifetime risk for diabetes in the U.S. Diabetes Care 2007;30(6):1562-6.
42. Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev 2009;18(2):541-50.
43. Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for squamous cell carcinoma of the oral cavity in young people–a comprehensive literature review. Oral Oncol 2001;37(5):401-18.
44. Hillier TA, Pedula KL. Complications in young adults with early-onset type 2 diabetes: losing the relative protection of youth. Diabetes Care 2003;26(11):2999-3005.
45. North Carolina Diabetes Prevention and Control Fact Sheet, September 2009. http://www.ncdiabetes.org/factsFigures/index.aspx. Accessed June 8, 2011.

46. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444(7121):840-6.

47. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365(9467):1333-46.

48. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocr Rev 1998;19(4):491-503.

49. Arner P, Pollare T, Lithell H. Different aetiologies of type 2 (noninsulin-dependent) diabetes mellitus in obese and nonobese subjects. Diabetologia 1991;34:483-487.

50. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345(13):971-80.

51. Maassen JA, 'T Hart LM, Van Essen E, Heine RJ, Nijpels G, Jahangir Tafrechi RS, et al. Mitochondrial diabetes: molecular mechanisms and clinical presentation. Diabetes 2004;53 Suppl 1:S103-9.

52. Tseng CH. Body mass index and waist circumference as determinants of coronary artery disease in Taiwanese adults with type 2 diabetes mellitus. Int J Obes (Lond) 2006;30: 816–21.

53. Jee SH, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. JAMA 2005;293(2):194-202.

54. Rapp K, Schroeder J, Klenk J, Ulmer H, Concin H, Diem G, et al. Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria. Diabetologia 2006;49(5):945-52.

55. Stattin P, Björ O, Ferrari P, Lukanova A, Lenner P, Lindahl B, et al. Prospective study of hyperglycemia and cancer risk. Diabetes Care 2007;30(3):561-7.

56. Harris MI, Eastman RC. Early detection of undiagnosed diabetes mellitus: a US perspective. Diabetes Metab Res Rev 2000;16(4):230-6.

57. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125-9.

58. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944-56.

59. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010;3(11):1451-61.

60. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009;52(9):1766-77.

61. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 2006; 29:254-8.

62. Centers for Disease Control and Prevention: Diabetes Data and Trends. http://www.cdc.gov/diabetes/statistics/treating_national.htm. Accessed June 8, 2011.

63. Jiang HJ, Stryer D, Friedman B, Andrews R. Multiple hospitalizations for patients with diabetes. Diabetes Care 2003;26(5):1421-6.

64. American Diabetes Association. Economic costs of diabetes in the U.S. In 2007. Diabetes Care 2008;31(3):596-615.

CHAPTER 3

Type II diabetes and metabolic syndrome and the association with head and neck squamous cell carcinoma: a study in the SEER-Medicare database

Introduction

Diabetes has been associated with an increased risk of multiple types of cancer. Results of a meta-analysis analyzing this relationship indicated two-fold or higher relative risks for liver, pancreatic and endometrial cancer, and lesser elevated risks for colorectal, breast and bladder cancers (1). On the other hand, diabetes was not found to be associated with lung cancer, and was inversely associated with prostate cancer (1). Metabolic syndrome is a cluster of health abnormalities, including hyperinsulinemia/hyperglycemia, hypertension, excess abdominal body fat and dyslipidemia. The presence of metabolic syndrome is known to increase the risk of developing type II diabetes and cardiovascular disease (2). A large body of evidence also implicates metabolic syndrome as an important risk factor in the development of similar cancers as those associated with diabetes, including colorectal (3,4), breast (5,6), pancreatic (7), endometrial (8), and liver cancer (9).

Metabolic syndrome has been referred to as “insulin resistance syndrome” (10), and both diabetes and metabolic syndrome are characterized by dysglycemia and a state of chronic systemic inflammation (2). The mechanisms by which diabetes and metabolic syndrome may influence the carcinogenic process are similar. These include hyperglycemia-induced formation of reactive oxygen species (ROS) and damage to DNA (11), exposure to high levels of insulin and insulin-like growth factors (IGF’s) (12,13), and/or chronic inflammation associated with dysglycemia resulting in a pro-angiogenic, anti-apoptotic microenvironment which may fuel malignant growth (14,15). Components of metabolic syndrome, such as obesity and insulin resistance, are additionally postulated to promote

cancer by increasing production/bioavailability of hormones such as estrogen and adipokines (16).

Whether type II diabetes or metabolic syndrome is associated with head and neck squamous cell carcinoma (HNSCC) has received little attention. In a pooled analysis of 12 international case-control studies from the International Head and Neck Cancer Epidemiology Consortium, we recently reported a positive association between a history of diabetes and the incidence of head and neck cancers among non-smokers (17). A Danish population-based study comparing individuals hospitalized with a diagnosis of diabetes to the general population observed an increased risk of mouth/pharynx cancer associated with diabetes in subjects less than 50 years old but not in older persons (18). A hospital-based case-control study of 2,660 patients and 2,980 controls in Hungary observed that elevated fasting glucose was strongly associated with oral cancer in females but not males (19). Whether metabolic syndrome is associated with risk of development of HNSCC has not been investigated.

Our goal was to investigate whether type II diabetes or metabolic syndrome is associated with HNSCC using a very large data source that was representative of the US population. The use of claims data additionally provided an opportunity to rely on records other than self-report, and allowed us to attempt to exclude type I diabetes and thus focus on type II diabetes.

Methods

Overview and Design

We conducted a nested case-control study using data from the Surveillance, Epidemiology, and End Results (SEER)-Medicare database. Cases were all persons aged 68-99 years diagnosed with HNSCC between 1994 and 2007. Controls were selected from

a similarly-aged 5% sample of individuals residing in the same regions as the SEER registries of the cases. We compared the prevalence of metabolic syndrome and type II diabetes among persons who developed HNSCC and those who did not.

Data Source

The SEER-Medicare database links population-based cancer registry data and Medicare enrollment and claims files. Medicare is the primary health insurer for 97% of the US population aged 65 years and older (20). The linkage is based on a deterministic algorithm using name, Social Security number, sex, and date of birth, and successfully matches 94% of SEER cancer cases aged 65 and older with Medicare recipients (21). We obtained data for the study period 1991-2007 from 16 SEER registries (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, Atlanta, San Jose, Los Angeles, Rural Georgia, Greater California, Kentucky, Louisiana, and New Jersey) (22). While SEER areas are generally representative of the overall US population, urban areas, higher income persons and racial/ethnic minorities are overrepresented (21). Claims data were available for Medicare parts A (hospital inpatient care to which all Medicare beneficiaries are entitled) and B (physician and outpatient services, to which approximately 96% of participants subscribe) (21). These data contained International Classification of Diseases, ninth revision, Clinical Modification (ICD-9-CM) diagnosis codes and dates of service.

Study Population

We identified all persons between the ages of 68 and 99 diagnosed with histologically confirmed HNSCC between 1994 and 2007 and no other prior cancers. We obtained a diagnosis month and year from the SEER data, and assigned the 15th of each

month to create a diagnosis date. The classification of malignancies in SEER is based on the International Classification of Diseases for Oncology, Version 3 (ICD-O3) (23). HNSCC cases included cancers of the tongue (C02.0, C02.1, C02.2, C02.3, C02.4, C02.8, C02.9), floor of mouth (C04.0, C04.1, C04.8, C04.9), gum (C03.0, C03.1, C03.9), palate (C05.0, C05.1, C05.2, C05.8, C05.9), other and unspecified parts of mouth (C06.0, C06.1, C06.2, C06.8, C06.9), tonsil (C09.0, C09.1, C09.8, C09.9), oropharynx (C01.9, C10.0, C10.1, C10.2, C10.3, C10.4, C10.8, C10.9), hypopharynx (C13.0, C13.1, C13.2, C13.8, C13.9), larynx (C32.0, C32.1, C32.2, C32.3, C32.8, C32.9), and other oral cavity and pharynx (C14.0, C14.2, C14.8). We only included cases enrolled in Medicare parts A and B continuously for at least three years immediately prior to diagnosis to ensure adequate time for diagnoses of diabetes and metabolic syndrome, as well as other aspects of medical history, to be captured in the claims data. Thus, although data were available for persons aged ≥65, all subjects in the present study were 68 years of age or older. In addition, the following subjects were excluded: (i) persons enrolled in Medicare only because of disabilities or end-stage renal disease, (ii) persons with unknown diagnostic confirmation of HNSCC, (iii) persons with cancers identified solely by autopsy or death certificate, and (iv) subjects enrolled in a health maintenance organization (HMO) in the three years prior to diagnosis (because these plans are not required to submit claims to Medicare).

We identified controls as persons aged 68-99 with no prior cancer diagnoses selected from a 5% random sample of Medicare beneficiaries residing in the regions of the SEER registries. We randomly assigned each potential control an index date between the years 1994 to 2007. As for cases, controls were enrolled in Medicare parts A and B continuously but not enrolled in an HMO for at least three years immediately prior to their index date, and could not have been enrolled in Medicare because of disabilities or end-stage renal disease. We excluded subjects with a date of death prior to the index date. We

assigned a registry region to each control, based on the state and county of the last residence that person lived in during their index year, and excluded controls for which a valid registry could not be determined.

We frequency matched controls to cases in a ratio of 3:1 on sex and the estimated total number of months of Medicare enrollment prior to index date. We estimated the amount of Medicare enrollment prior to the index date for cases and controls by adding 1 for each month of enrollment in both Part A and Part B, and adding ½ for each month of enrollment in Part A only or Part B only. This calculation was done so as to create a measure of duration of Medicare enrollment that was reflective of the likelihood of capturing claims event data. We calculated the proportion of cases who were male and female, and the proportion with each unique number of months of Medicare enrollment prior to the index date (a minimum of 37 and maximum of 204, rounded to the nearest whole number), and then conducted frequency matching.

Definition of metabolic syndrome and type II diabetes

We assigned a diagnosis of type II diabetes or metabolic syndrome if these conditions were identified in the claims data prior to the HNSCC diagnosis date for cases and index date for controls. We focused on type II diabetes because hypothesized mechanisms for an increased risk of cancer associated with diabetes included elevated levels of insulin/IGFs and a state of chronic systemic inflammation, which are hallmarks of type II diabetes (12-15). Type II diabetes was defined by ICD-9-CM code 250 (Table 8). The ICD-9-CM codes 250.X0 and 250.X2 specifically indicate type II diabetes, while codes 250.X1 and 250.X3 indicate type I diabetes, and were thus not included. We elected to include codes 250 and 250.X in the definition of type II diabetes, where type may be

unknown, because there are relatively few people with type 1 diabetes among the Medicare population (24).

We assigned a diagnosis of metabolic syndrome based on criteria suggested by the US National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). These criteria are the presence of at least 3 of the following conditions: elevated waist circumference/central obesity, dyslipidemia, hypertension, and impaired fasting glucose or diabetes (25). We used overweight/obesity as a surrogate for elevated waist circumference. The specific ICD-9-CM codes used are listed in Table 8. The occurrence of any of these codes was used to identify each condition.

Definition of relevant covariates

We obtained data on age, gender, race/ethnicity, and SEER registry region for cases from SEER data and from Medicare data for controls. Income was determined from the median household income for the zip code of residence at index date and was based on the year 2000 census bureau survey. We classified subjects as to the presence or absence of HNSCC risk factors using ICD-9-CM codes in the claims data prior to the index date. These included tobacco use: V15.82, 305.1, 989.84; and alcohol use: 303, 305.0, V11.3, V79.1, 291. We also inferred smoking and alcohol use from the presence of medical conditions that may arise from smoking (chronic bronchitis (490, 491) and emphysema (492)) or alcohol drinking (alcoholic fatty liver disease (571.0), alcoholic hepatitis (571.1), alcoholic cirrhosis of the liver (571.2), and alcoholic liver damage (571.3)).

A small number of subjects had missing data for median household income (4.6% of cases and 2.8% of controls), and we therefore imputed data using a ‘MICE’ procedure (multiple imputation by chained equations) (26). We generated four imputed datasets, and used the following predictors to impute missing income data: case/control status, registry

region, sex, age, race, tobacco use, alcohol drinking status, overweight, hypertension, dyslipidemia, impaired fasting glucose and diabetes.

Statistical Analyses

We estimated odds ratios and 95% confidence intervals using unconditional logistic regression, adjusting for all confounding and matching factors. We used SAS, version 9.3 (SAS Institute, Cary, NC) and Stata statistical software (version 11.0, Stata Corp., College Station, TX) for analyses.

Metabolic syndrome/diabetes as risk factors for HNSCC

We evaluated the risk of HNSCC associated with a history of type II diabetes and metabolic syndrome as dichotomous variables (yes/no). Covariates used for adjustment were age (continuous), sex (dichotomous), race/ethnicity (categorical), SEER geographic region (categorical), median income (continuous), duration of Medicare membership prior to index date (continuous), tobacco use (yes/no) and alcohol use (yes/no). In analyses of the relationship between diabetes and HNSCC, we additionally adjusted for overweight/obesity (yes/no). In additional analyses, we also evaluated associations with individual metabolic syndrome components.

Secondary analyses

In secondary analyses, we examined the association between diabetes/metabolic syndrome and HNSCC after stratifying by tobacco use and sex. To statistically assess departures from multiplicative effects on the odds scale, we included product terms in these stratified analyses and used a log-likelihood ratio test to compare logistic models with and without the product terms. We categorized cases by tumor site and performed polytomous

logistic regression to assess whether, and to what extent, the association with diabetes varied across tumor sites. To examine whether detection bias due to increased diagnostic workup of cases during the time leading up their diagnosis may have been present, we repeated primary analyses after excluding all diagnoses that occurred within one year of diagnosis date for cases and index date for controls.

Results

We identified 46,531 subjects with a first HNSCC. We excluded: 114 cases with unknown diagnostic confirmation or who were identified solely by autopsy or death certificate; 8,237 cases enrolled in an HMO in the three years prior to diagnosis; 19,919 cases who were not enrolled in Medicare Part A and Part B continuously during the 3 years prior to diagnosis; 4,227 cases enrolled in Medicare only because of disabilities or end-stage renal disease; and 12 cases who were not between the ages of 68 and 99. After these exclusions, 14,022 cases remained for analysis. Controls included 42,066 persons, matched to cases in a 3:1 ratio, without any prior cancer diagnosis who met the same inclusion criteria as for cases.

Cases and controls were matched on sex and amount of time enrolled in Medicare, and were similar with respect to age, race, registry region and median household income (Table 9). Cases were more likely to be tobacco users and alcohol drinkers compared with controls, and controls were more likely to be overweight/obese. Of the cases, 36.5% had cancer of the oral cavity, 18.4% had oropharyngeal cancer, and 40.4% had cancer of the larynx.

The prevalence of type II diabetes and metabolic syndrome observed prior to diagnosis/index date were similar for cases and controls: 32.6% of cases and 33.1% of

controls had a diagnosis of type II diabetes; 27.4% of cases and 29.3% of controls had a diagnosis of metabolic syndrome. For the individual metabolic syndrome components, 7.0% of cases and 9.1% of controls were considered overweight/obese; 61.6% of cases and 65.6% of controls had dyslipidemia; 75.5% of cases and 75.1% of controls had hypertension; and 33.8% of cases and 34.3% of controls had impaired fasting glucose / type II diabetes.

Type II diabetes was slightly inversely associated with HNSCC in models matched on sex and duration of Medicare enrollment and adjusted further for age, race, income, tobacco use, alcohol use, overweight, and registry (OR=0.92; 95% CI, 0.88-0.96; Table 10). We observed effect modification by tobacco use ($p<0.001$ for the interaction), with a null association between diabetes and HNSCC among non-users (OR=1.00; 95% CI, 0.95-1.06; Table 11) and an inverse association among tobacco users (OR=0.80; 95% CI, 0.75-0.86). There was no effect modification by sex ($p=0.22$ for the interaction; Table 11). The association between type II diabetes and HNSCC did not differ materially after exclusion of all diagnoses within the year prior to diagnosis for cases and index date for controls (OR=0.91; 95% CI, 0.87-0.95).

Metabolic syndrome was inversely associated with HNSCC in models matched on sex and duration of Medicare enrollment and additionally adjusted for age, race, income, tobacco use, alcohol use, and registry (OR=0.81; 95% CI, 0.78-0.85; Table 10). This inverse association was strongest for overweight (OR=0.69; 95% CI, 0.64-0.74) and dyslipidemia (OR=0.75; 95% CI, 0.72-0.79), and closer to null for impaired fasting glucose (OR=0.90; 95% CI, 0.86-0.94) and hypertension (OR=0.95; 95% CI, 0.90-0.99).

As with type II diabetes, tobacco use was an effect modifier of the association between metabolic syndrome and HNSCC ($p<0.001$). The inverse association was strongest among tobacco users (OR=0.72; 95% CI, 0.67-0.77), but was closer to the null among

never-users (OR=0.88; 95% CI, 0.84-0.94; Table 12). There was no effect modification by sex (p=0.80 for the interaction).

There was no strong evidence of heterogeneity by tumor site in the association between diabetes and HNSCC (p=0.33; Table 13). The inverse association between diabetes and HNSCC appeared to be slightly stronger for tumors arising in the hypopharynx compared with other sites; however this is likely due to chance as the number of cases in this category was very small.

Discussion

In this large, population-based study, we observed a marginal inverse association between type II diabetes and HNSCC (OR=0.92; 95% CI, 0.88-0.96). This result was modified by tobacco use, with a null association among non-users and an inverse association among tobacco users. We observed a modest inverse association between metabolic syndrome and HNSCC (OR=0.81; 95% CI, 0.78-0.85).

An inverse association between diabetes and HNSCC was unexpected as observations from previous studies had indicated a positive association among subgroups of the population (17-19). In a pooled analysis of 12 international case-control studies, we observed a weak association between diabetes and the incidence of head and neck cancer overall (OR=1.09; 95% CI, 0.95-1.24) (17). However, we observed a modest association among never smokers (OR=1.59; 95% CI, 1.22-2.07), and no association among ever smokers (OR=0.96; 95% CI, 0.83-1.11). Suba et al. conducted a hospital-based case-control study on 2,660 in-patients with confirmed OSCC and 2,980 “complaint-free” controls participating in oral cancer screenings, and observed that repeatedly elevated fasting glucose was strongly associated with oral cancer in females (OR=1.61; no 95% CI reported;

$p<0.05$), but not males (OR=0.97; $p>0.05$) (19). In a cohort of diabetics in Denmark, Wideroff et al. reported increased risk of mouth/pharynx cancer associated with diabetes (18). However, there were only 30 cases in those analyses and the association was only observed in subjects less than 50 years old at diabetes diagnosis.

To the best of our knowledge, no previous studies have examined the association between metabolic syndrome and HNSCC. An association between metabolic syndrome and oral premalignancy was recently observed in a study of subjects undergoing general health and oral examinations in Taiwan (27). This association remained after accounting for areca nut chewing and other confounding factors. Diabetics also appear to be at increased risk of developing oral predisposing lesions such as leukoplakia and erythroplakia (28-30). However, oral predisposing lesions as endpoints is limited by the fact that oral lesions of this type do not necessarily transform to malignancy (31,32).

Obesity is strongly associated with metabolic syndrome, and an increased risk of developing insulin resistance, followed by glucose intolerance and type II diabetes (33,34). In a previous pooled case-control study, it was observed that head and neck cancer risk is elevated among lean people and reduced among overweight or obese people, and that these findings remained for overweight/obesity 2-5 years prior to case diagnosis date (35). The strong relationship between obesity and type II diabetes/metabolic syndrome may thus be a factor accounting for the inverse association observed with HNSCC in the present study. Although we adjusted for overweight/obesity in all analyses, our ability to capture overweight/obese persons from claims data was very poor. We categorized 9.1% of controls as overweight/obese. However, according to data from the Medicare Current Beneficiary Survey (MCBS), a survey on medical care use and health status of a representative sample of Medicare beneficiaries in the United States, 63% of Medicare beneficiaries were overweight or obese in the year 2000 (36).

We observed a null association between type II diabetes and HNSCC among non-users of tobacco and an inverse association among tobacco users. In our previous analysis of pooled case-control studies (17), we also observed effect modification by smoking; however in these analyses, we observed a positive association between history of diabetes and HNSCC among non-smokers and no association among smokers. As a possible explanation, we suggested that adjustment for smoking was not sufficient to remove all confounding among smokers, and that the association between a history of diabetes and HNSCC among never smokers may be closer to the true association as it circumvented this source of residual confounding. Another possibility is that a proportion of people who are both heavy smokers and diabetic and who would have developed HNSCC in the future, may be at particular risk for early death, and may have died before developing HNSCC.

In our previous pooled analysis (17), we observed a positive association between head and neck cancer and diabetes diagnosed before age 50, and no association for those diagnosed after age 50. Considering that the minimum age in the present study was 68, this factor might be an additional partial explanation for differing results between that study and the present study. However, an analysis of the pooled data after excluding subjects less than 68 years old did not materially change the estimates for that study. Nevertheless, it has been proposed that adults diagnosed with diabetes at a younger age may represent a more aggressive phenotype than people diagnosed late in life (37). More people with diabetes diagnoses before the age of 50 may thus have died at an earlier age than those diagnosed over the age of 50, and may thus not be included in the SEER-Medicare data.

There are several limitations of the SEER-Medicare data in conducting a case-control study of this nature. Only conditions recorded by a health-care provider are available, and asymptomatic or underdiagnosed conditions will often not be recorded (21). As previously noted, claims data particularly lack sensitivity for conditions such as obesity.

Tobacco and alcohol use, which are both very important risk factors for HNSCC, are also often substantially underreported, and there are no data on duration or intensity of tobacco or alcohol use. In addition, these risk factors could only be identified indirectly for some individuals by conditions resulting from use, such as emphysema or alcoholic hepatitis. This limitation of the data source may be an explanation for the observed inverse association between diabetes/metabolic syndrome and HNSCC, as all prior evidence had suggested that a positive association might be expected (17-19). Nonetheless, it should be noted that previous research using the SEER-Medicare data resulted in associations that were in the expected direction. A recent meta-analysis indicated a positive association between diabetes and hepatocellular carcinoma in 18 out of 25 cohort studies (summary relative risk 2.01; 95% CI, 1.61-2.51) (38), and this positive association was confirmed in the SEER-Medicare data (39).

Part D data, which covers medication use, was only available from 2007 onwards, and thus we were not able to include these data in our analyses. Medication data would be useful in identifying subjects with metabolic syndrome and diabetes, particularly the more serious cases. In addition, medication use may modify associations between diabetes and/or metabolic syndrome; for example, the diabetes drug metformin is associated with reduced risk of various types of cancers, whereas the use of exogenous insulin is associated with increased risk (40,41).

Lack of sensitivity of claims data is not expected to differ between cases and controls; thus misclassification from this source is likely to be non-differential. Nevertheless, to make sure that increased medical evaluation of cases did not lead to heightened ascertainment of medical conditions, we repeated analyses after excluding all diagnoses in the year preceding diagnosis date, and observed no material change in the results.

An important strength of the present study is the very large population-based nature of the data source. SEER registries have a 99% completeness rate of case ascertainment, and participating registries are required to meet strict standards for data quality (41). In addition, the 5% random subcohort of controls is reflective of the source population. Although there is some overrepresentation of urban areas, higher income persons and racial/ethnic minorities, the SEER-Medicare population is a good representation of the general US elderly population (21).

Conclusion

In this large population-based case-control study, we observed a very slight inverse association between type II diabetes and HNSCC, and a modest inverse association between metabolic syndrome and HNSCC, adjusted for several potential confounders. These results were contrary to the evidence to date, which had suggested that a positive association between diabetes and HNSCC may exist in subgroups of the population (17-19). It is possible that inadequate control for important confounding factors, such as overweight/obesity and duration/intensity of tobacco use, may have resulted in the observed inverse association in the present study.

Table 8. ICD-9-CM codes used to determine type II diabetes and metabolic syndrome in the SEER-Medicare database, 1991-2007.[a]

ICD-9-CM	Description
Type II diabetes	
250	Diabetes mellitus
250.0	Diabetes mellitus without mention of complication
250.00	Diabetes mellitus without mention of complication, type II/unspecified, not stated as uncontrolled
250.02	Diabetes mellitus without mention of complication, type II or unspecified type, uncontrolled
250.1	Diabetes with ketoacidosis
250.10	Diabetes with ketoacidosis, type II or unspecified type, not stated as uncontrolled
250.12	Diabetes with ketoacidosis, type II or unspecified type, uncontrolled
250.2	Diabetes with hyperosmolarity
250.20	Diabetes with hyperosmolarity, type II or unspecified type, not stated as uncontrolled
250.22	Diabetes with hyperosmolarity, type II or unspecified type, uncontrolled
250.3	Diabetes with other coma
250.30	Diabetes with other coma, type II or unspecified type, not stated as uncontrolled
250.32	Diabetes with other coma, type II or unspecified type, uncontrolled
250.4	Diabetes with renal manifestations
250.40	Diabetes with renal manifestations, type II or unspecified type, not stated as uncontrolled
250.42	Diabetes with renal manifestations, type II or unspecified type, uncontrolled
250.5	Diabetes with ophthalmic manifestations
250.50	Diabetes with ophthalmic manifestations, type II or unspecified type, not stated as uncontrolled
250.52	Diabetes with ophthalmic manifestations, type II or unspecified type, uncontrolled
250.6	Diabetes with neurological manifestations
250.60	Diabetes with neurological manifestations, type II or unspecified type, not stated as uncontrolled
250.62	Diabetes with neurological manifestations, type II or unspecified type, uncontrolled
250.7	Diabetes with peripheral circulatory disorders
250.70	Diabetes with peripheral circulatory disorders, type II or unspecified type, not stated as uncontrolled
250.72	Diabetes with peripheral circulatory disorders, type II or unspecified type, uncontrolled
250.8	Diabetes with other specified manifestations
250.80	Diabetes with other specified manifestations, type II or unspecified type, not stated as uncontrolled
250.82	Diabetes with other specified manifestations, type II or unspecified type, uncontrolled
250.9	Diabetes with unspecified complication
250.90	Diabetes with unspecified complication, type II or unspecified type, not stated as uncontrolled
250.92	Diabetes with unspecified complication, type II or unspecified type, uncontrolled
Overweight/Obesity	
278.0	Overweight and obesity
278.1	Localized adiposity
278.00	Obesity unspecified
278.01	Morbid obesity
278.02	Overweight
783.1	Abnormal weight gain
V77.8	Screening for obesity
Dyslipidemia	
272.0	Pure hypercholesterolemia
272.1	Pure hyperglyceridemia
272.2	Mixed hyperlipidemia
272.4	Other and unspecified hyperlipidemia
272.5	Lipoprotein deficiencies
272.9	Unspecified disorder of lipid metabolism

Table 8 continued

Hypertension	
401	Essential hypertension
401.0	Malignant essential hypertension
401.1	Benign essential hypertension
401.9	Unspecified essential hypertension
402	Hypertensive heart disease
402.0	Malignant hypertensive heart disease
402.1	Benign hypertensive heart disease
402.9	Unspecified hypertensive heart disease
403	Hypertensive renal disease
403.0	Malignant hypertensive renal disease
403.1	Benign hypertensive renal disease
403.9	Unspecified hypertensive renal disease
404	Hypertensive heart and chronic kidney disease
404.0	Malignant hypertensive heart and renal disease
404.1	Benign hypertensive heart and renal disease
404.9	Unspecified hypertensive heart and renal disease

Table 9. Characteristics of HNSCC cases and controls, SEER-Medicare database, 1991-2007.[a]

Characteristic	Cases (n=14,022)		Controls (n=42,066)	
	N	%	n	%
Age (years)				
68-72	4,302	30.7	11,724	27.9
73-77	4,079	29.1	12,076	28.7
78-82	3,002	21.4	9,265	22.0
83-99	2,639	18.8	9,001	21.4
Sex				
Men	8,976	64.0	26,928	64.0
Women	5,046	36.0	15,138	36.0
Race				
White	12,223	87.2	35,919	85.4
Black	987	7.0	2,432	5.8
Asian	356	2.5	1,606	3.8
Hispanic	206	1.5	1,050	2.5
American Native	30	0.2	131	0.3
Other	195	1.4	850	2.0
Unknown	25	0.2	78	0.2
SEER Registry				
San Francisco	657	4.7	1,606	3.8
Connecticut	1,256	9.0	2,929	7.0
Detroit	1,395	10.0	3,081	7.3
Hawaii	290	2.1	722	1.7
Iowa	1,078	7.7	3,189	7.6
New Mexico	379	2.7	1,145	2.7
Seattle	996	7.1	2,394	5.7
Utah	288	2.1	1,300	3.1
Atlanta	502	3.6	1,280	3.0
San Jose	375	2.7	1,026	2.4
Los Angeles	1,266	9.0	3,355	8.0
Rural Georgia	60	0.4	108	0.3
Greater California	1,907	13.6	7,522	17.9
Kentucky	969	6.9	3,377	8.0
Louisiana	843	6.0	2,701	6.4
New Jersey	1,761	12.6	6,331	15.1
Median Household Income[b]				
≤ $35,000	3,501	25.0	10,505	25.0
$35,001-$45,000	3,403	24.3	10,166	24.2
$45,001-$60,000	3,805	27.1	11,169	26.6
≥ $60,000	3,313	23.6	10,226	24.3
Amount of Enrollment in Medicare (years)[c]				
3-5	2,939	21.0	8,817	21.0
5 - <8	3,792	27.0	11,376	27.0
8 - <11	3,486	24.9	10,458	24.9
≥11	3,805	27.1	11,415	27.1
Tobacco use				
Yes	5,378	38.4	10,962	26.1
No	8,644	61.7	31,104	73.9
Alcohol use				
Yes	575	4.1	424	1.0
No	13,447	95.9	41,642	99.0

Table 9 continued

Overweight/obesity				
Yes	985	7.0	3,811	9.1
No	13,037	93.0	38,255	90.9
Tumor site				
Oral cavity	5,118	36.5		
Oropharynx	2,579	18.4		
Hypopharynx	385	2.8		
Larynx	5,666	40.4		
Ill-defined site	274	2.0		

[a] Reference dates start from 1994, but claims diagnoses are ascertained from 1991 onwards. Percentages have been rounded and may not total 100.
[b] Based on zip code of residence at diagnosis/index date.
[c] If enrollment was in Part A or Part B only, we counted this as half a month instead of a full month.

Table 10. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck squamous cell carcinoma and diabetes and metabolic syndrome, SEER-Medicare database, 1991-2007[a]

Exposure (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	OR	(95% CI)
Diabetes [b]	4574/9448	13939/28127	0.92	(0.88-0.96)
Metabolic syndrome [c]	3835/10187	12306/29760	0.81	(0.78-0.85)
Overweight	985/13037	3811/38255	0.69	(0.64-0.74)
Hypertension	10592/3430	31596/10470	0.95	(0.90-0.99)
Dyslipidemia	8630/5392	27599/14467	0.75	(0.72-0.79)
Impaired fasting glucose	4737/9285	14434/27632	0.90	(0.86-0.94)

[a] Reference dates start from 1994, but claims diagnoses are ascertained from 1991 onwards.
[b] Matched on sex and time enrolled in Medicare and adjusted for age, race, income, tobacco use, alcohol use, overweight, and registry.
[c] Matched on sex and time enrolled in Medicare and adjusted for age, race, income, tobacco use, alcohol use, and registry.

Table 11. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck squamous cell carcinoma and diabetes (yes/no) by tobacco use (yes/no) and sex, SEER-Medicare database, 1991-2007.[a]

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	OR[b]	(95% CI)
Tobacco user[c]	1985/3393	4619/6343	0.80	(0.75-0.86)
Non-tobacco user	2589/6055	9320/21784	1.00	(0.95-1.06)
Women[d]	1653/3393	4983/10155	0.95	(0.89-1.02)
Men	2921/6055	8956/17972	0.90	(0.86-0.95)

[a] Reference dates start from 1994, but claims diagnoses are ascertained from 1991 onwards.
[b] Matched on sex and time enrolled in Medicare and adjusted for age, race, income, alcohol use, obesity/overweight, and registry.
[c] Likelihood ratio test for interaction by tobacco use: p=0.00001.
[d] Likelihood ratio test for interaction by sex: p=0.22.

Table 12. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck squamous cell carcinoma and metabolic syndrome (yes/no) by tobacco use (yes/no) and sex, SEER-Medicare database, 1991-2007.[a]

Metabolic syndrome (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	OR[b]	(95% CI)
Tobacco user[c]	1780/3598	4389/6573	0.72	(0.67-0.77)
Non-tobacco user	2055/6589	7917/23187	0.89	(0.84-0.94)
Women[d]	1450/3596	4722/10416	0.86	(0.80-0.93)
Men	2385/6591	7584/19344	0.87	(0.82-0.93)

[a] Reference dates start from 1994, but claims diagnoses are ascertained from 1991 onwards.
[b] Matched on sex and time enrolled in Medicare and adjusted for age, race, income, alcohol use, and registry.
[c] Likelihood ratio test for interaction by tobacco use: p<0.001.
[d] Likelihood ratio test for interaction by sex: p=0.80

Table 13. Adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between head and neck squamous cell carcinoma and diabetes, stratified by location of tumor, SEER-Medicare database, 1991-2007.[a]

Diabetes (yes/no)	Cases exposed/ unexposed N	Controls exposed/ unexposed N	OR[b]	(95% CI)
Oral cavity	1691/3427	13939/28127	0.97	(0.91-1.03)
Oropharynx	824/1755	13939/28127	0.92	(0.84-1.00)
Hypopharynx	110/275	13939/28127	0.74	(0.59-0.93)
Larynx	1865/3801	13939/28127	0.90	(0.85-0.96)
Ill-defined site	84/190	13939/28127	0.86	(0.66-1.13)

[a] Reference dates start from 1994, but claims diagnoses are ascertained from 1991 onwards.
[b] Matched on sex and time enrolled in Medicare and adjusted for age, race, income, tobacco use, alcohol use, overweight, and registry. P=0.33 for heterogeneity by site.

References for Chapter 3

1. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer. 2009;16(4):1103-23.
2. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28.
3. Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. World J Gastroenterol. 2009;15(41):5141-8.
4. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven F, et al. Metabolic Syndrome and Risks of Colon and Rectal Cancer: the European Prospective Investigation into Cancer and Nutrition Study. Cancer Prev Res (Phila). 2011;4(11):1873-83.
5. Kabat GC, Kim M, Chlebowski RT, Khandekar J, Ko MG, McTiernan A, et al. A longitudinal study of the metabolic syndrome and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2009;18(7):2046-53.
6. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr. 2007;86(3):s823-35.
7. Johansen D, Stocks T, Jonsson H, Lindkvist B, Björge T, Concin H, et al. Metabolic factors and the risk of pancreatic cancer: a prospective analysis of almost 580,000 men and women in the Metabolic Syndrome and Cancer Project. Cancer Epidemiol Biomarkers Prev. 2010;19(9):2307-17.
8. Rosato V, Zucchetto A, Bosetti C, Dal Maso L, Montella M, Pelucchi C, et al. Metabolic syndrome and endometrial cancer risk. Ann Oncol. 2011;22(4):884-9.

9. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: A study in the SEER-medicare database. Hepatology. 2011;54(2):463-71.
10. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes. 1992;41(6):715-22.
11. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414(6865):813-20.
12. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4(8):579-91.
13. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet. 2004;363(9418):1346-53.
14. de Rekeneire N, Peila R, Ding J, Colbert LH, Visser M, Shorr RI, et al. Diabetes, hyperglycemia, and inflammation in older individuals: the health, aging and body composition study. Diabetes Care. 2006;29(8):1902-8.
15. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001;85(4):473-83.
16. Cowey S, Hardy RW. The metabolic syndrome: A high-risk state for cancer? Am J Pathol. 2006;169(5):1505-22.
17. Stott-Miller M, Chen C, Chuang SC, Lee YC, Boccia S, Brenner H, et al. History of diabetes and risk of head and neck cancer: a pooled analysis from the international head and neck cancer epidemiology consortium. Cancer Epidemiol Biomarkers Prev. 2012;21(2):294-304.

18. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89(18):1360-5.
19. Suba Z. Gender-related hormonal risk factors for oral cancer. Pathol Oncol Res. 2007;13(3):195-202.
20. Warren JL, Klabunde CN, Schrag D, Bach PB, Riley GF. Overview of the SEER-Medicare data: content, research applications, and generalizability to the United States elderly population. Med Care 2002;40:IV-3-18.
21. Engels EA, Pfeiffer RM, Ricker W, Wheeler W, Parsons R, Warren JL. Use of surveillance, epidemiology, and end results-medicare data to conduct case-control studies of cancer among the US elderly. Am J Epidemiol. 2011;174(7):860-70.
22. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.
23. Percy C. International Classification of Diseases for Oncology (ICD-O-3). 3rd ed. Geneva, Switzerland: World Health Organization; 1990.
24. Harris MI, Cowie CC, Eastman R. Health-insurance coverage for adults with diabetes in the U.S. population. Diabetes Care. 1994;17(6):585-91.
25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486-2497.
26. Royston P. Multiple imputation of missing values. Stata J 2004;4:227-241.

27. Yen AM, Chen SL, Chiu SY, Chen HH. Association between metabolic syndrome and oral pre-malignancy: a community- and population-based study (KCIS No. 28). Oral Oncol. 2011;47(7):625-30.
28. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncology 2004;40:158-63.
29. Dikshit RP, Ramadas K, Hashibe M, Thomas G, Somanathan T, Sankaranarayanan R. Association between diabetes mellitus and pre-malignant oral diseases: A cross sectional study in Kerala, India. Int J Cancer 2006;118:453-7.
30. Albrecht M, Bánóczy J, Dinya E, Tamás G Jr. Occurrence of oral leukoplakia and lichen planus in diabetes mellitus. J Oral Pathol Med 1992;21(8):364-6.
31. Silverman SJ, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53:563-8.
32. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med, 2003;14:47-62.
33. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444(7121):840-6.
34. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365(9467):1333-46.
35. Gaudet MM, Olshan AF, Chuang SC, Berthiller J, Zhang ZF, Lissowska J, et al. Body mass index and risk of head and neck cancer in a pooled analysis of case-control studies in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium. Int J Epidemiol 2010;39(4):1091-102.
36. Wilkins TL, Rust GS, Sambamoorthi U. Changing BMI Categories and Healthcare Expenditures Among Elderly Medicare Beneficiaries. Obesity (Silver Spring). 2011.

37. Hillier TA, Pedula KL. Complications in young adults with early-onset type 2 diabetes: losing the relative protection of youth. Diabetes Care 2003;26(11):2999-3005.
38. Wang C, Wang X, Gong G, Ben Q, Qiu W, Chen Y, Li G, Wang L. Increased risk of hepatocellular carcinoma in patients with diabetes mellitus: a systematic review and meta-analysis of cohort studies. Int J Cancer. 2012;130(7):1639-48.
39. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut. 2005;54(4):533-9.
40. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010;3(11):1451-61.
41. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia. 2009;52(9):1766-77.
42. Surveillance, Epidemiology and End Results (SEER). http://seer.cancer.gov. Accessed June 1, 2012.

CHAPTER 4 The association between oral squamous cell carcinoma and genes and pathways involved in dysregulated glucose metabolism

Introduction

Oral squamous cell carcinoma (OSCC) is associated with substantial mortality and morbidity, with an estimated 400,000 new cases and 200,000 deaths in 2008 worldwide (1). Tobacco and alcohol use are established risk factors, and are responsible for a substantial proportion of OSCC. More recently, infection with oncogenic human papillomavirus has been identified as a cause of OSCC, particularly oropharyngeal tumors (2,3). Nevertheless, there remains a proportion of OSCC that cannot be attributed to the usual risk factors, particularly for oral cavity cancer, among women and among those under age 45 (4).

A growing body of evidence points to the crucial role that the tumor microenvironment and tumor cell metabolism play in cancer development and growth. The essential hallmarks of cancer are closely linked to altered programming of cancer cell metabolism (5,6). As early as the 1920's, Otto Warburg observed that tumor cells have an inherently higher need for glucose (the "Warburg effect") (7). The fact that glucose metabolism is altered in a wide range of cancers is used to visualize and detect malignant tumors by the use of [18F] flouro-2-deoxyglucose (FDG) positron emission tomography (PET). Specifically, head and neck squamous tumor cells are highly dependent on glucose for energy production and survival (8). The increased requirement for glucose in neoplastic tumors raises the possibility that abnormal glucose metabolism may fuel tumor proliferation (9).

In a pooled analysis of 12 international case-control studies, we recently observed an association between a history of diabetes and the incidence of head and neck cancers

among non-smokers (Chapter 2) (10). However, in an analysis using SEER-Medicare data, this finding was not confirmed, and instead we observed a marginal inverse association between type II diabetes and HNSCC (Chapter 3). Several other studies suggest that a positive relationship may exist between OSCC and abnormalities of glucose metabolism or the diabetic condition. Diabetics have an increased prevalence of oral lesions such as erythroplakia and leukoplakia that predispose to oral cancer (11,12). Mouth/pharynx cancer was found to be associated with diabetes in subjects less than 50 years old (13), and in a large hospital-based case-control study, researchers observed that elevated fasting glucose was strongly associated with oral cancer in females (14).

These results motivated us to investigate whether pathways involved in glucose metabolism were differentially expressed in primary OSCC compared with non-neoplastic oral epithelium. Such differential expression may be an expected consequence of malignancy. However, dysplastic oral tissue would not be expected to induce dysregulation of glucose metabolism in the same manner. Thus, to evaluate the hypothesis that impaired glucose metabolism may play a role in the cause or promotion of OSCC, we sought to determine whether pathways involved in glucose metabolism were similarly differentially expressed in dysplastic oral tissue compared with control tissue. In addition, we sought to stratify results by site, HPV status and two-year survival, in an attempt to examine whether tumors with different characteristics may be more metabolically active, and how this might impact prognosis.

Methods

Study Population

These analyses were performed on a population that is part of an ongoing prospective study (the ORALCHIP study) to study the association between genome-wide gene expression profiles and the progression and outcome of OSCC. As described by Chen et al. (15), cases were subjects over the age of 18 with an incident primary cancer or dysplasia of the oral cavity or oropharynx scheduled for biopsy or surgery at one of three medical centers affiliated with the University of Washington. Controls were patients who received oral surgery, such as uvulopalatopharyngoplasty or tonsillectomy, for a non-malignant or non-pre-malignant condition at the same institutions and during the same time period. Subjects were enrolled between December 2003 and April 2007.

Tumor tissue from cases was obtained at time of resection prior to any chemo/radiotherapy. Tissue from dysplasia patients and controls was obtained at time of biopsy or surgery. Oral epithelial tissue from controls was collected from the uvula or anterior tonsillar pillar, avoiding contamination with surrounding lymphoid tissue. For controls with tonsillitis or tonsil hypertrophy, only mucosal tissue from the tonsillar pillar was obtained to avoid potential influence of inflammation on the results. After removal, the tissue was immersed in RNALater (Applied Biosystems, Inc.) for a minimum of 12 h at 4℃ before being transferred to long-term storage at -80℃ until use.

Study participants were interviewed using a structured questionnaire on demographic, medical, functional, quality of life, and lifestyle history, including tobacco and alcohol use. Diabetic status was not recorded during the interviews. We thus reviewed medical records of cases and controls to identify patients with a diagnosis of diabetes. Data

on tumor stage and other tumor characteristics were abstracted from medical records. Patients were followed through telephone contact as well as by review of medical records and linkage to cancer registry data and the U.S. Social Security Death Index. Participants gave informed consent and study procedures were approved by the institutional review boards of the Fred Hutchinson Cancer Research Center, University of Washington, and VA Puget Sound Health Care System.

DNA microarray and HPV testing

Total RNA was extracted from tumor and non-neoplastic oral tissue using a TRIzol method (Invitrogen), purified with an RNeasy mini kit (Qiagen), processed using a GeneChip Expression 3'-Amplification Reagents Kit (Affymetrix), and examined with Affymetrix GeneChip Human Genome U133 Plus 2.0 Arrays, as previously documented (15). Several quality control checks were performed on the microarray data. We followed Affymetrix recommendations to determine if any GeneChips needed to be excluded, and used the "affyQCReport" and "affyPLM" software in the Bioconductor package within the R statistical programming language to determine if there were poor-quality chips that needed to be excluded(www.bioconductor.org). In total, GeneChips from 167 cases, 17 dysplasia, and 45 controls passed all rounds of quality control procedures. Gene expression values for ~54,000 probe sets were extracted from probe intensity values (CEL files) and normalized using the RMA algorithm in Partek Genomics Suite (www.partek.com).

Tumor and non-neoplastic oral tissue were screened for the presence of HPV DNA using a nested polymerase chain reaction (PCR) protocol. All samples that showed a positive PCR result were tested for HPV DNA presence using the LINEAR ARRAY HPV Genotyping Test (Roche, Indianapolis, Indiana) containing complementary sequences to the

PCR products for 37 HPV genotypes, under a research-use only agreement. The HPV genotypes included the 13 “high risk” genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). To verify type calls, a subset of the samples were amplified and sequenced using HPV-16–specific primers, and compared against a known HPV-16 sequence (GenBank 333031).

Pathway-based differential gene expression

We compared 167 OSCC and 45 normal oral epithelium samples for differential expression with respect to seven pathways related to dysregulated glucose metabolism from the KEGG (Kyoto Encyclopedia of Genes and Genomes) (www.genome.jp/kegg/) database: (1) insulin signaling (pathway:04910, 358 probe sets), (2) type 2 diabetes (pathway:04930, 121 probe sets), (3) peroxisome proliferator-activated receptor (PPAR) signaling (pathway:03320, 149 probe sets), (4) glycolysis/gluconeogenesis (pathway:00010, 119 probe sets), (5) fatty acid metabolism (pathway:00071, 87 probe sets), (6) pyruvate metabolism (pathway:00620, 75 probe sets), and (7) oxidative phosphorylation (pathway:00190, 218 probe sets).

We used the global test (16) in R statistical programming language, adjusting for age and sex, and accounting for multiple comparisons by the Benjamini and Hochberg false discovery rate (FDR) method (17). The global test is based on an empirical Bayesian generalized linear model, and provides a score statistic for an entire pathway based on random-effects modeling of parameters corresponding to the coefficients of the individual genes in the pathway (16). We chose this pathway-based tool because it allows for covariate adjustment and does not depend on individual gene analyses. As described by Goeman and Bühlmann (18), the global test evaluates the "self-contained" null hypothesis:

that genes in the pathway are not differentially expressed. Thus, only the pathways of interest are analyzed instead of the entire microarray (18).

To determine whether differential expression of diabetes-related genes was due to differing numbers of cases and controls with diabetes, we repeated these pathway-based analyses after additionally adjusting for diabetic status (164 OSCC and 38 controls for which diabetic status was known). We also performed analyses for the same seven pathways after excluding 23 controls that underwent surgery for sleep apnea. This additional analysis was performed to address possible bias from an association of sleep apnea with obesity, type II diabetes, insulin resistance, and chronic systemic inflammation (19,20). Finally, we used the same pathway-based analyses in a comparison of 17 dysplasias with 45 control tissue samples.

To investigate whether differential expression of the seven pathways was similar in an independent dataset, we downloaded a publicly-available external data set from Gene Expression Omnibus (GEO; GSE6791) of 42 OSCC cases and 14 controls. We normalized the CEL files using the RMA algorithm in Partek Genomics Suite, and repeated the same pathway-based analyses as were conducted on our own data.

Pathway-based differential gene expression after stratification

We compared the expression of the seven pathways among primary OSCC after stratifying by high-risk HPV status (37 high-risk HPV positive oropharyngeal cancers versus 15 high-risk HPV negative oropharyngeal cancers) and adjusting for age and sex. We also examined differential expression of the seven pathways after stratifying by tumor site (115 oral cavity cancers versus 52 oropharyngeal cancers) and adjusting for age and sex. We repeated these analyses after additional adjustment for pack-years of smoking.

To examine whether pathways involved in dysregulated glucose metabolism might be associated with survival, we stratified cases into two groups based on whether they died from any cause within two years or survived beyond two years, and compared differential expression of the seven pathways. We repeated these analyses for cases who died from OSCC.

Individual gene analyses from differentially expressed pathways

To perform individual gene analyses, we identified the two pathways that were most significantly differentially expressed between OSCC and normal oral epithelium, based on the FDR-adjusted p-value. We then created datasets for each of these two pathways that only contained the probe sets associated with the relevant pathway. We then compared oral cancers and normal epithelium controls using ANCOVA (Partek Genomics Suite) with respect to expression of genes in the two pathways. We adjusted for age and sex and controlled the FDR at 0.05. From this analysis, we produced a list of differentially expressed individual probe sets. We then identified four probe sets, corresponding to four genes, from the two selected pathways, with the lowest p-values that also exhibited at least a three-fold difference.

To obtain association estimates and to conduct various levels of adjustment for the four genes, we compared log2 expression levels in primary OSCC with expression levels in normal oral epithelium from control subjects by fitting adjusted linear regression models and estimating 95% confidence intervals (CI) for all association estimates. We adjusted the models using several different covariate sets: (i) age (continuous) and sex, (ii) age, sex and diabetic status (yes/no), and (iii) age, sex, pack years of smoking (continuous), and alcoholic drinks per day in the year prior to date of diagnosis for OSCC cases or recruitment for controls (categorical).

Increased expression of pro-inflammatory cytokines or activated immune cells in the tumor microenvironment may affect glucose metabolism (21-23).To investigate whether the four genes associated with glucose dysregulation might be differentially expressed only as a consequence of greater numbers of immune cells in tissue samples or because of the resulting inflammation, in secondary analyses we adjusted for the expression of several key genes associated with inflammation. The genes selected for adjustment were: (i) tumor necrosis factor (*TNF*), a major pro-inflammatory cytokine (24) (ii) lipopolysaccharide-binding protein CD14 and macrosialin CD68, two commonly-used macrophage markers (25), and (iii) CD4, a glycoprotein expressed on T-cells. To explore the influence of diabetic status on gene expression, we compared log2 expression levels of the four genes in diabetics with expression levels in non-diabetics among cases as well as among controls. In all analyses for which software is not specified, we used STATA statistical software (version 10.0, Stata Corp., College Station, TX)

Platform verification of gene expression by quantitative reverse transcription-PCR

For the four genes that we found to be differentially expressed, qRT-PCR was performed in triplicate in 10 μL reaction volumes using the QuantiTect SYBR Green RT-PCR kit (Qiagen) and bioinformatically validated QuantiTect primers (Qiagen) on a ABI 7900HT Sequence Detection System (Applied Biosystems, Inc., Foster City, CA). We performed this verification on a subset of 30 cases and 22 controls. We selected only those controls not treated for sleep apnea. Standard curves were generated using Universal Human Reference RNA (Stratagene) for all genes, with the linear correlation coefficient (R2) ≥ 0.99 for all runs. The mean threshold cycle (Ct) values were calculated from the triplicate Ct values. We repeated qRT-PCR on the samples if Ct values had a standard deviation >

0.3 in the triplicate run. Mean Ct values were standardized to the mean Ct value of the reference gene, β-actin. The cycling conditions were as follows: 30 min incubation at 50℃, 15 min incubation at 95℃, and 40 cycles each of 15 s at 94℃, 30 s at 55℃, and 30 s at 72℃. We compared mean Ct values of OSCC with control tissue by performing linear regression, adjusting for age and sex, and estimating 95% confidence intervals.

Results

Characteristics of study participants are summarized in Table 14. OSCC cases tended to be older and male and more likely to be current smokers compared with controls. The majority of cases had advanced-stage disease (American Joint Committee on Cancer (AJCC) stage III and IV). Controls were more likely to have a diabetes diagnosis than cases (20.0% of controls versus 8.4% of cases).

Pathway-based differential gene expression

All seven pathways that were identified as involved in glucose metabolism were significantly differentially expressed in primary OSCC compared with normal epithelium from control subjects (p-values adjusted for multiple comparisons all $<1.17 \times 10^{-36}$, Table 15). The pathways observed to be the most significantly different were those involved in PPAR signaling and type II diabetes. Controlling the FDR at 0.05 resulted in 75 significantly differentially expressed probe sets out of a total of 149 for the PPAR signaling pathway. The corresponding results for the type II diabetes pathway were 45 significant probe sets out of a total of 121.

Results for pathway-based analyses that additionally adjusted for diabetic status were very similar to the primary analyses (Table 15). Analyses that excluded controls who

received surgery for sleep apnea were almost identical to the primary analyses that included these controls, and the ranking of the seven pathways by p-value were similar to the primary analyses (Table 15).

All seven pathways were significantly differentially expressed in dysplasia compared with epithelium from control subjects. However, results were weaker than those obtained for primary OSCC versus control tissue (p-values adjusted for multiple comparisons ranged from 5.50×10^{-05} to 9.90×10^{-20}; Table 15). The top two pathways in these analyses were those involved in oxidative phosphorylation and insulin signaling.

The seven pathways were also substantially differentially expressed in the independent GEO dataset (GSE6791) consisting of 42 OSCC cases and 14 controls (data not shown). As in our data, the most differentially expressed pathways were also the PPAR signaling pathway ($p=9.79 \times 10^{-09}$) and the type II diabetes pathway ($p=9.72 \times 10^{-07}$).

Pathway-based differential gene expression after stratification

We observed differential expression of the seven pathways involved in dysregulated glucose metabolism in a comparison of oral cavity SCC and oropharyngeal SCC (Table 16). There was no differential expression after stratification by high-risk HPV status among oropharyngeal SCC (Table 16). Results were similar in analyses that additionally adjusted for pack-years of smoking (results not shown). There was a marginal amount of differential expression between OSCC cases that within two years or survived beyond two years (Table 16). Results were similar for cases who died of OSCC (results not shown).

Individual gene analyses from differentially expressed pathways

Based on p-value and fold-difference, and adjusted for age and sex, the two most influential genes in the PPAR signaling pathway were *MMP1* (probe set 204475_at) and *SLC27A6* (probe set 219932_at). *MMP1* was overexpressed (absolute fold-difference of 219.4) and *SLC27A6* was underexpressed (fold-difference of -3.2) in OSCC compared to normal tissue. We previously reported on the strongly elevated overexpression of *MMP1* in OSCC (26). The two most influential genes in the type II diabetes pathway were *SOCS3* (probe set 227697_at) and *IRS1* (probe set 204686_at). Both *SOCS3* and *IRS1* were overexpressed in OSCC compared with normal control tissue (fold-difference of 7.5 and 3.6 respectively.) *SOCS3* was also the most influential gene in the insulin signaling pathway.

Adjusting for age and sex, the difference in mean log2 expression levels between primary OSCC and normal epithelium from cancer-free controls was 7.76 (95% CI, 7.26, 8.26; Table 17) for *MMP1*, 1.82 (95% CI, 1.55, 2.09) for *IRS1*, 2.91 (95% CI, 2.49, 3.33) for *SOCS3*, and -1.50 (95% CI, -1.74, -1.26) for *SLC27A6*. Results were similar in models that were additionally adjusted for diabetic status and in analyses that additionally adjusted for pack years of smoking and alcoholic drinks per day.

Adjustment for inflammation and immune-related genes in addition to age and sex did not affect the results materially. The difference in mean log2 expression levels between primary OSCC and epithelium from cancer-free controls for *MMP1* was 7.67 (95% CI, 7.18-8.17) adjusted for *TNF*, 7.02 (95% CI, 6.25-7.79) adjusted for *CD14*, 7.67 (95% CI, 7.14-8.21) adjusted for *CD68*, and 7.90 (95% CI, 7.34-8.46) adjusted for *CD4*. Results were similar for *IRS1*, *SOCS3* and *SLC27A6* (Table 17).

There were no significant differences in expression levels of *MMP1*, *IRS1*, *SOCS3* or *SLC27A6* between diabetics and non-diabetics, either among OSCC cases or among controls.

Verification by quantitative reverse transcription-PCR

Results obtained by qRT-PCR on 30 cases and 22 controls confirmed the substantial differential expression of *MMP1*, with an adjusted difference in mean threshold cycle (Ct) value for control epithelium versus primary OSCC of 10.43 (95% CI, 9.31-11.55, p<0.001). qRT-PCR results for the other three genes were similarly confirmed, with an adjusted difference in mean Ct for control epithelium versus OSCC of 2.10 (95% CI, 1.54-2.66, p<0.001) for *IRS1*, 2.36 (95% CI, 1.58-3.13, p<0.001) for *SOCS3*, and 4.67 (95% CI, 3.73-5.62, p<0.001) for *SLC27A6*. As observed in the microarray data, *MMP1*, *IRS1* and *SOCS3* were overexpressed and *SLC27A6* was underexpressed in primary OSCC compared with normal oral epithelium.

Discussion

To the best of our knowledge, this is the first study to investigate pathways involved in dysregulated glucose metabolism with respect to OSCC. We observed the PPAR signaling, type II diabetes, insulin signaling, fatty acid metabolism, oxidative phosphorylation, glycolysis/gluconeogenesis, and pyruvate metabolism pathways to be significantly differentially expressed in OSCC compared with normal tissue from controls.

That dysregulated glucose metabolism may play a role in development and/or promotion of OSCC, instead of just being a consequence of malignancy, might be supported by our observation of significant differences between dysplasia and normal oral epithelium from controls. On the other hand, gene expression studies, including our own (15), indicate that there is a progression of transcriptional dysregulation from normal tissue to premalignancy to malignancy, with premalignant lesions displaying many of the same

genetic alterations as invasive cancers (27). Whether the “Warburg effect” is also part of such an incremental progression is unclear. Limited evidence suggests that this may be a possibility: increased glycolytic metabolism has been observed in precancerous Barrett's esophagus biopsy tissue compared with neighboring normal squamous esophagus and gastric cardia (28), and alterations in glucose metabolism have been observed in tissue from precancerous colorectal lesions in a rat model (29).

We observed differential expression of pathways related to glucose metabolism in a comparison of oral cavity SCC and oropharyngeal SCC, with oral cavity SCC generally displaying stronger overexpression or underexpression of genes related to glucose metabolism in comparison with control epithelium. The implications of this, if any, are unclear. The squamous epithelium of the oropharynx derives from endoderm, whereas the squamous epithelium of the oral cavity derives from ectoderm, and tumors arising from different sites might be expected to have different molecular signatures (30). However, consistent differences in gene expression patterns between OSCC sites have not been observed (31).

In contrast, we did not observe differential expression of pathways related to glucose metabolism in a comparison of high-risk HPV positive oropharyngeal cancers compared with high-risk HPV negative oropharyngeal cancers. We observed some differential expression in a comparison of OSCC from patients who died within two years compared with subjects who survived beyond two years; however p-values were not highly significant, especially in comparison with p-values obtained in other stratified analyses of similar samples size that we conducted. We had hypothesized that greater differential expression of pathways involved in glucose metabolism might indicate greater tumor metabolic activity and aggressiveness, which might have been evidenced by differential expression with respect to survival.

The top differentially expressed pathways between primary OSCC and non-neoplastic oral epithelium were the PPAR signaling and type II diabetes pathways. Pathways ranked less prominently were pathways involved in glycolysis/gluconeogenesis and pyruvate metabolism. While the latter pathways are integral for the regulation of cellular fuel use, the PPAR signaling and type II diabetes pathways involve more genes that affect systemic fuel utilization.

The PPARs are ubiquitously expressed ligand-activated transcription factors, acting as sensors of dietary and endogenous signals, and controlling the expression of a very large number of genes involved in energy homeostasis, glucose metabolism and lipid metabolism (32,33). The top two differentially expressed genes in the PPAR signaling pathway were *MMP1* (overexpressed in OSCC) and *SLC27A6* (underexpressed in OSCC).

*MMP*1 is the most ubiquitously expressed interstitial collagenase and plays a key role in degradation of the extracellular matrix and basement membranes, thereby promoting tumor expansion and metastasis (34,35). Increased anaerobic metabolism of glucose elevates lactate levels, causing acidification that results in deterioration of the extracellular matrix, and activating MMPs (36,37). The identification of *MMP1* as an exceptionally strong marker of OSCC motivated us to investigate the MMPs in greater depth, including the measurement of MMP1 protein levels in saliva, for evaluation as a potential non-invasive biomarker of OSCC (26). Salivary concentrations of MMP1 in OSCC patients were 6.2 times (95% CI: 3.32-11.73) higher than controls, and displayed an increasing trend with higher stage disease.

The *SLC27A6* gene encodes long-chain fatty acid transport protein 6, part of the fatty acid transport protein family (FATP), which facilitates cellular uptake of fatty acids. Some long-chain fatty acids have hormone-like functions, regulating key metabolic functions such as hepatic glucose production (38). Little is known about the *SLC27A6* gene, and whether

the observation of underexpression of *SLC27A6* in OSCC compared with control tissue is expected or is in line with anticipated biological function in relation to glucose metabolism is unclear.

The top two differentially expressed genes in the type II diabetes pathway were *IRS1* and *SOCS3* (both overexpressed in OSCC). *IRS1* encodes the insulin receptor substrate 1 protein, which is a major substrate of insulin, insulin-like growth factors, and cytokine signaling, and which mediates major metabolic, proliferative, and antiapoptotic functions of the insulin receptor and the insulin-like growth factor receptor (39,40). Constitutive activation of IRS-1 has been observed in several types of tumors, including breast cancer and leiomyoma (41). *SOCS3* is part of the suppressor of cytokine signaling family. Epigenetic silencing of *SOCS3* has been observed in OSCC (42), suggested that underexpression of this gene in OSCC might have been expected; however, elevated expression of *SOCS3* has been observed in other cancers, such as breast cancer (43).

The tumor microenvironment is rich in mediators and effectors of inflammation (21). In addition, immune cells are highly metabolically active (44,45) and comprise a larger proportion of tumor tissue than normal tissue. Activated immune cells require large amounts of glucose as their primary fuel, and cytokines such as TNF-α and IL-6 directly affect insulin sensitivity and glucose metabolism in neighboring cells (22,23). Increased presence of activated immune cells and increased expression of pro-inflammatory cytokines would therefore be likely to affect the expression of genes involved in glucose metabolism in a heterogeneous tissue sample. However, our results suggest that differential expression of the four genes associated with abnormal glucose metabolism may not be wholly due to these processes, based on the observation that adjustment for inflammation or immune-related gene expression did not modify the observed associations.

The results obtained from the pathway-based analyses as well as the individual gene analyses did not change materially after adjusting for diabetic status. This suggests that differences associated with dysregulated glucose metabolism observed between cases and controls are not merely reflective of differing proportions of diabetic subjects. We previously observed an association between a history of diabetes and head and neck cancer among non-smokers (10), and thus might have expected greater numbers of diabetics among OSCC cases. On the contrary, we observed a higher proportion of controls with a diabetes diagnosis than cases. This is likely because of the large proportion of controls who were recruited because they were having oral surgery for sleep apnea, which is associated with an elevated risk of type II diabetes (19,20).

The present study has several limitations. Although we had data on diabetic status for the majority of subjects, the lack of data on glucose tolerance status is a limitation, as various types of glucose intolerance, besides diabetes, are relatively common in the US population (46). A major limitation of the present study was the cross-sectional nature of the study design. We were able to report that genes and pathways involved in glucose metabolism were differentially expressed between OSCC/dysplasia and normal oral epithelium; however, the data did not provide direct insight into whether this is a cause or consequence of malignancy, or whether the differential expression indicates greater tumor metabolic activity.

In addition, it is unclear whether dysregulated glucose metabolism that is observed in gene expression data from tissue has any meaningful relationship with systemic processes. If systemic abnormalities of glucose metabolism involve alterations in genes associated with glucose metabolism, this would not necessarily imply that these alterations would be expressed in the oral mucosa. Furthermore, if differences are found in pathways/genes

involved in glucose metabolism in a comparison of tumor and non-neoplastic tissue, this does not necessarily indicate that a systemic abnormality exists.

To the best of our knowledge, the present study is the first attempt at an analysis of pathways related to abnormal glucose metabolism with respect to development of OSCC. Biological processes occur by concerted expression of multiple genes, and pathway models, which emphasize systems of functionally related components instead of the individual components, may thus be more biologically meaningful. However, pathway-based models are highly dependent on the validity of the genes that comprise the pathway, selected pathways may be highly correlated (which was not accounted for in our analyses), and biological processes usually involve several pathways, with complex interconnections in the network never fully accounted for (47). Some of our identified pathways had multiple genes in common; for example, the type II diabetes pathway (comprising 121 genes) and the insulin signaling pathway (358 genes) shared 31 common genes. On the other hand, the PPAR signaling pathway, comprising 149 genes, only had one gene in common with the type II diabetes pathway.

In addition, a highly influential gene in a pathway may drive an observation of large differential expression. Furthermore, large differences in gene expression exist between cancer and normal tissue for many different biological pathways, and the relative importance of the glucose metabolism pathways are difficult to characterize or quantify when analyses are confined only to pathways of interest, as was done in the present study. Gene set enrichment analyses (GSEA) may be able to overcome this disadvantage as these analyses test the hypothesis that genes in a pathway are at most as differentially expressed as genes not in the pathway across the entire microarray (48). However, these types of pathway-based analyses have limitations, including lack of power and assumptions that all genes of a

significantly differentially expressed pathway are differentially expressed in the same direction (18,47).

Conclusion

We observed that pathways and genes that are indicative of abnormal glucose metabolism were differentially expressed in OSCC as well as dysplasia, compared with non-neoplastic oral epithelium. The present study was motivated by our prior finding of an association between self-reported history of diabetes and head and neck cancer among subgroups of the population (10). Abnormal glucose metabolism may be a key event in malignant transformation in OSCC; however we were unable to determine whether a temporal relationship exists due to the cross-sectional nature of the study. Prospective studies that incorporate biomarkers indicative of diabetes, or other glucose metabolism abnormalities, may help to provide insight into the temporal relationship.

Table 14. Selected Characteristics of OSCC Patients and Controls, University of Washington Affiliated Hospitals, 2003-2007

Characteristic	OSCC case (n=167)		Control (n=45)	
	n	%	n	%
Age				
19-39	7	(4.2)	17	(37.8)
40-49	26	(15.6)	14	(31.1)
50-59	57	(34.1)	5	(11.1)
60-88	77	(46.1)	9	(20.0)
Sex				
Male	120	(71.9)	32	(71.1)
Female	47	(28.1)	13	(28.9)
Race				
White	146	(87.4)	30	(66.7)
Non-white	15	(9.0)	14	(31.1)
Unknown	6	(3.6)	1	(2.2)
Smoking status[a]				
Current	81	(48.5)	12	(26.7)
Former	50	(29.9)	15	(33.3)
Never	36	(21.6)	17	(37.8)
Unknown			1	(2.2)
Alcoholic drinking status[a]				
Current	109	(65.3)	33	(73.3)
Former	39	(23.4)	9	(20.0)
Never	16	(9.6)	2	(4.4)
Unknown	3	(1.8)	1	(2.2)
Diabetic status				
Yes	14	(8.4)	9	(20.0)
No	150	(89.8)	29	(64.4)
Unknown	3	(1.8)	7	(15.6)
AJCC Stage				
I/II	55	(32.9)		
III/IV	112	(67.1)		
Tumor Site				
Oral	115	(68.9)		
Oropharynx	52	(31.1)		
High risk HPV Status, by Tumor Site[b]				
Oral				
HPV+	19	(16.5)		
HPV-	96	(83.5)		
Oropharynx				
HPV+	37	(71.2)		
HPV-	15	(28.9)		

[a] As of the date of diagnosis (OSCC cases) or recruitment (controls)
[b] Percentages estimated within site

Table 15. Pathway analyses of differential expression for pathways involved in glucose metabolism, stratified by comparison group, and ranked by p-value, University of Washington Affiliated Hospitals, 2003-2007.

Pathway	167 Cases vs 45 Controls[a]		164 Cases vs 38 Controls[b]		167 Cases vs 22 Controls[a,c]		17 Dysplasia vs 45 Controls[a]	
	p-value[d]	rank	p-value[d]	rank	p-value[d]	rank	p-value[d]	rank
PPAR signaling pathway	$1.56x10^{-55}$	1	$3.19x10^{-49}$	1	$1.43x10^{-41}$	1	$3.75x10^{-06}$	3
Type II diabetes mellitus	$8.33x10^{-48}$	2	$2.71x10^{-42}$	2	$2.71x10^{-31}$	3	$3.41x10^{-05}$	6
Fatty acid metabolism	$1.05x10^{-45}$	3	$5.80x10^{-40}$	5	$3.65x10^{-30}$	5	$3.75x10^{-06}$	4
Insulin signaling pathway	$4.24x10^{-45}$	4	$7.51x10^{-41}$	4	$5.41x10^{-31}$	4	$5.52x10^{-07}$	2
Oxidative phosphorylation	$4.24x10^{-45}$	5	$3.23x10^{-41}$	3	$1.90x10^{-32}$	2	$9.90x10^{-20}$	1
Glycolysis/Gluconeogenesis	$6.21x10^{-40}$	6	$5.67x10^{-35}$	6	$9.42x10^{-28}$	6	$5.50x10^{-05}$	7
Pyruvate metabolism	$1.17x10^{-36}$	7	$6.85x10^{-33}$	7	$1.09x10^{-23}$	7	$2.64x10^{-05}$	5

[a] Adjusted for age and sex.

[b] Adjusted for age and sex and diabetic status (excludes 10 subjects with missing data for diabetic status).

[c] Excludes controls diagnosed with sleep apnea.

[d] Adjusted for multiple comparisons using Benjamini and Hochberg FDR method.

Table 16. Differential expression among cases for pathways involved in glucose metabolism, stratified by case comparison group, and ranked by p-value, University of Washington Affiliated Hospitals, 2003-2007

Pathway	37 HPV+ vs 15 HPV- (oropharynx)		115 oral cavity vs 52 oropharynx		50 cases dead within 2 yrs vs 117 alive after 2 yrs	
	p-value[a]	rank	p-value[a]	rank	p-value[a]	rank
PPAR signaling pathway	0.0589	-	2.91×10^{-15}	1	0.0009	7
Type II diabetes mellitus	0.0589	-	3.98×10^{-14}	2	0.0007	6
Fatty acid metabolism	0.0589	-	9.92×10^{-09}	6	0.0003	3
Insulin signaling pathway	0.0589	-	2.50×10^{-12}	3	0.0002	1
Oxidative phosphorylation	0.0589	-	1.92×10^{-11}	4	0.0006	5
Glycolysis/Gluconeogenesis	0.0589	-	4.22×10^{-08}	7	0.0002	2
Pyruvate metabolism	0.0848	-	1.82×10^{-10}	5	0.0003	4

[a] Adjusted for age and sex, and adjusted for multiple comparisons using Benjamini and Hochberg FDR method.

Table 17. Gene expression differences between primary OSCC (n=167) and non-neoplastic control epithelium (n=45), University of Washington Affiliated Hospitals, 2003-2007

Genes, variables used for adjustment	Difference in log2 means	95% Confidence Interval
MMP1		
Age and sex	7.76	7.26, 8.26
Age, sex, packyears of smoking, drinks per day	7.88	7.30, 8.47
Age, sex and diabetes	7.69	7.13, 8.26
Age, sex, *TNF* gene	7.67	7.18, 8.17
Age, sex, *CD14* gene	7.02	6.25, 7.79
Age, sex, *CD68* gene	7.67	7.14, 8.21
Age, sex, *CD4* gene	7.90	7.34, 8.46
IRS1		
Age and sex	1.82	1.55, 2.09
Age, sex, packyears of smoking, drinks per day	1.90	1.58, 2.21
Age, sex and diabetes	1.78	1.49, 2.08
Age, sex, *TNF* gene	1.85	1.59, 2.11
Age, sex, *CD14* gene	1.78	1.46, 2.11
Age, sex, *CD68* gene	1.83	1.56, 2.10
Age, sex, *CD4* gene	1.96	1.70, 2.23
SOCS3		
Age and sex	2.91	2.49, 3.33
Age, sex, packyears of smoking, drinks per day	2.91	2.45, 3.37
Age, sex and diabetes	2.92	2.46, 3.38
Age, sex, *TNF* gene	2.83	2.42, 3.23
Age, sex, *CD14* gene	2.40	1.93, 2.86
Age, sex, *CD68* gene	2.89	2.46, 3.31
Age, sex, *CD4* gene	2.89	2.46, 3.31
SLC27A6		
Age and sex	-1.50	-1.74, -1.26
Age, sex, packyears of smoking, drinks per day	-1.52	-1.78, -1.27
Age, sex and diabetes	-1.46	-1.74, -1.18
Age, sex, *TNF* gene	-1.50	-1.74, -1.26

Table 17 continued

Age, sex, *CD14* gene	-1.33	-1.64, -1.02
Age, sex, *CD68* gene	-1.44	-1.69, -1.20
Age, sex, *CD4* gene	-1.51	-1.76, -1.27

References for Chapter 4

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010.
2. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, Moller B, Pukkala E, Schiller JT, Youngman L, Lehtinen M and Dillner J. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med, 2001;344:1125-9.
3. Hennessey PT, Westra WH and Califano JA. Human papillomavirus and head and neck squamous cell carcinoma: recent evidence and clinical implications. J Dent Res, 2009;88:300-6.
4. Hashibe M, Brennan P, Chuang SC, et al. Interaction between Tobacco and Alcohol Use and the Risk of Head and Neck Cancer: Pooled Analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev, 2009;18:541-50.
5. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell. 2008;13(6):472-82.
6. Seyfried TN, Shelton LM. Cancer as a metabolic disease. Nutr Metab (Lond). 2010;7:7.
7. Warburg O. The Metabolism of Tumors. Constable Press, London, UK. 1930.
8. Sandulache VC, Ow TJ, Pickering CR, Frederick MJ, Zhou G, Fokt I, Davis-Malesevich M, Priebe W, Myers JN. Glucose, not glutamine, is the dominant energy source required for proliferation and survival of head and neck squamous carcinoma cells. Cancer. 2011.
9. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. CA Cancer J Clin. 2010;60(4):207-21.

10. Stott-Miller M, Chen C, Chuang SC, et al. History of diabetes and risk of head and neck cancer: a pooled analysis from the international head and neck cancer epidemiology consortium. Cancer Epidemiol Biomarkers Prev. 2012;21(2):294-304.
11. Dietrich T, Reichart PA and Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncology, 2004;40:158-63.
12. Dikshit RP, Ramadas K, Hashibe M, Thomas G, Somanathan T and Sankaranarayanan R. Association between diabetes mellitus and pre-malignant oral diseases: A cross sectional study in Kerala, India. Int J Cancer, 2006;118:453-7.
13. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, Borch-Johnsen K, Olsen JH. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst. 1997;89(18):1360-5.
14. Suba Z. Gender-related hormonal risk factors for oral cancer. Pathol Oncol Res, 2007;13(3):195-202.
15. Chen C, Méndez E, Houck J, Fan W, Lohavanichbutr P, Doody D, Yueh B, Futran ND, Upton M, Farwell DG, Schwartz SM, Zhao LP. Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2008;17(8):2152-62.
16. Goeman JJ, van de Geer SA, de Kort F, van Houwelingen HC. A global test for groups of genes: testing association with a clinical outcome. Bioinformatics. 2004;20(1):93-9.
17. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological. 1995; 57(1):289–300.
18. Goeman JJ, Bühlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics. 2007;23(8):980-7.

19. Tasali E, Mokhlesi B, Van Cauter E. Obstructive sleep apnea and type 2 diabetes: interacting epidemics. Chest. 2008;133(2):496-506.
20. Foster GD, Sanders MH, Millman R, Zammit G, Borradaile KE, Newman AB, Wadden TA, Kelley D, Wing RR, Sunyer FX, Darcey V, Kuna ST; Sleep AHEAD Research Group. Obstructive sleep apnea among obese patients with type 2 diabetes. Diabetes Care. 2009;32(6):1017-9.
21. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454(7203):436-44.
22. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115(5):1111-9.
23. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006;17(1):4-12.
24. Sethi G, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. Front Biosci 2008;13:5094-107.
25. Martinez-Pomares L, Platt N, McKnight AJ, da Silva RP, Gordon S. Macrophage membrane molecules: markers of tissue differentiation and heterogeneity. Immunobiology 1996;195(4-5):407-16.
26. Stott-Miller M, Houck JR, Lohavanichbutr P, Méndez E, Upton MP, Futran ND, Schwartz SM, Chen C. Tumor and salivary matrix metalloproteinase levels are strong diagnostic markers of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2011;20(12):2628-36.
27. Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, et al. A transcriptional progression model for head and neck cancer. Clin Cancer Res 2003;9:3058-64.

28. van Baal JW, Diks SH, Wanders RJ, Rygiel AM, Milano F, Joore J, Bergman JJ, Peppelenbosch MP, Krishnadath KK. Comparison of kinome profiles of Barrett's esophagus with normal squamous esophagus and normal gastric cardia. Cancer Res. 2006;66(24):11605-12.
29. Liao W, Wei H, Wang X, Qiu Y, Gou X, Zhang X, Zhou M, Wu J, Wu T, Kou F, Zhang Y, Bian Z, Xie G, Jia W. Metabonomic Variations Associated with AOM-induced Precancerous Colorectal Lesions and Resveratrol Treatment. J Proteome Res. 2012.
30. Trotta BM, Pease CS, Rasamny JJ, Raghavan P, Mukherjee S. Oral cavity and oropharyngeal squamous cell cancer: key imaging findings for staging and treatment planning. Radiographics. 2011;31(2):339-54.
31. Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, Butterfoss D, Xiang D, Zanation A, Yin X, Shockley WW, Weissler MC, Dressler LG, Shores CG, Yarbrough WG, Perou CM. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell. 2004;5(5):489-500.
32. Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. Pharmacol Res. 2005;51:85–94.
33. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, et al. International Union of Pharmacology. LXI. Peroxisome proliferator-activator receptors. Pharmacol Rev. 2006;58(4):726–41.
34. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827–39.
35. Kleiner DE, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. Cancer Chemother Pharmacol. 1999;43 Suppl:S42-51.
36. Pelicano H, Xu RH, Du M, Feng L, Sasaki R, Carew JS, Hu Y, Ramdas L, Hu L, Keating MJ, Zhang W, Plunkett W, Huang P. Mitochondrial respiration defects in cancer cells

cause activation of Akt survival pathway through a redox-mediated mechanism. J Cell Biol. 2006;175(6):913-23.

37. Masur K, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B, Zänker KS. Diabetogenic glucose and insulin concentrations modulate transcriptome and protein levels involved in tumour cell migration, adhesion and proliferation. Br J Cancer. 2011;104(2):345-52.
38. Hirsch D, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. Proc Natl Acad Sci U S A. 1998;95(15):8625-9.
39. White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab. 2002;283(3):E413-22.
40. Dearth RK, Cui X, Kim HJ, Hadsell DL, Lee AV. Oncogenic transformation by the signaling adaptor proteins insulin receptor substrate (IRS)-1 and IRS-2. Cell Cycle. 2007;6(6):705-13.
41. Chang Q, Li Y, White MF, Fletcher JA, Xiao S. Constitutive activation of insulin receptor substrate 1 is a frequent event in human tumors: therapeutic implications. Cancer Res. 2002;62(21):6035-8.
42. Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, Dietz A, Wittekind C, Tannapfel A. SOCS-3 is frequently methylated in head and neck squamous cell carcinoma and its precursor lesions and causes growth inhibition. Oncogene. 2005;24(44):6699-708.
43. Raccurt M, Tam SP, Lau P, Mertani HC, Lambert A, Garcia-Caballero T, Li H, Brown RJ, McGuckin MA, Morel G, Waters MJ. Suppressor of cytokine signalling gene expression is elevated in breast carcinoma. Br J Cancer. 2003;89(3):524-32.
44. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005;5(11):844-52.

45. Kubota R, Yamada S, Kubota K, Ishiwata K, Tamahashi N, Ido T. Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. J Nucl Med 1992;33(11):1972-80.

46. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care 2009;32(2):287-94.

47. Wu MC, Lin X. Prior biological knowledge-based approaches for the analysis of genome-wide expression profiles using gene sets and pathways. Stat Methods Med Res. 2009;18(6):577-93.

48. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-50.

Bibliography

1. Adams TE, McKern NM, Ward CW. Signalling by the type 1 insulin-like growth factor receptor: interplay with the epidermal growth factor receptor. Growth Factors 2004;22(2):89-95.
2. Albrecht M, Bánóczy J, Dinya E, Tamás G Jr. Occurrence of oral leukoplakia and lichen planus in diabetes mellitus. J Oral Pathol Med 1992;21(8):364-6.
3. Aleksandrova K, Boeing H, Jenab M, Bueno-de-Mesquita HB, Jansen E, van Duijnhoven F, et al. Metabolic Syndrome and Risks of Colon and Rectal Cancer: the European Prospective Investigation into Cancer and Nutrition Study. Cancer Prev Res (Phila). 2011.
4. American Diabetes Association. Economic costs of diabetes in the U.S. In 2007. Diabetes Care 2008;31(3):596-615.
5. Arner P, Pollare T, Lithell H. Different aetiologies of type 2 (noninsulin-dependent) diabetes mellitus in obese and nonobese subjects. Diabetologia 1991;34:483-487.
6. Baron AE, Franceschi S, Barra S, Talamini R, La Vecchia C. A comparison of the joint effects of alcohol and smoking on the risk of cancer across sites in the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 1993;2(6):519-23.
7. Barrett-Connor E, Laakso M. Ischemic heart disease risk in postmenopausal women: effects of estrogen use on glucose and insulin levels. Arteriosclerosis. 1990;10:531-534.
8. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovasc Res 2004;63:582-92.

9. Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw. 2006;17(1):4-12.
10. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B-Methodological. 1995; 57(1):289–300.
11. Betancourt-Albrecht M, Cunningham GR. Hypogonadism and diabetes. Int J Impot Res. 2003;15 Suppl 4:S14-20.
12. Boccia S, Cadoni G, Sayed-Tabatabaei FA, Volante M, Arzani D, De Lauretis A, et al. CYP1A1, CYP2E1, GSTM1, GSTT1, EPHX1 exons 3 and 4, and NAT2 polymorphisms, smoking, consumption of alcohol and fruit and vegetables and risk of head and neck cancer. J Cancer Res Clin Oncol 2008;134(1):93-100.
13. Bosetti C, Gallus S, Trichopoulou A, Talamini R, Franceschi S, Negri E, et al. Influence of the Mediterranean diet on the risk of cancers of the upper aerodigestive tract. Cancer Epidemiol Biomarkers Prev 2003;12(10):1091-4.
14. Bose SC, Singh M, Vyas P, Singh M. Plasma zinc antioxidant vitamins, glutathione levels and total antioxidant activity in oral leukoplakia. Dent Res J (Isfahan). 2012;9(2):158-61.
15. Bowker SL, Majumdar SR, Veugelers P, Johnson JA. Increased cancer-related mortality for patients with type 2 diabetes who use sulfonylureas or insulin. Diabetes Care 2006; 29:254-8.
16. Brennan P, Bray I. Recent trends and future directions for lung cancer mortality in Europe. Br J Cancer. 2002;87(1):43-8.
17. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813-20.

18. Buzzai M, Jones RG, Amaravadi RK, et al. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. Cancer Res. 2007;67:6745-6752.
19. Calle EE, Kaaks R. Overweight, obesity and cancer: epidemiological evidence and proposed mechanisms. Nat Rev Cancer. 2004;4(8):579-91.
20. Calle EE, Murphy TK, Rodriguez C, et al. Diabetes mellitus and pancreatic cancer mortality in a prospective cohort of United States adults. Cancer Causes Control. 1998;9:403–410.
21. Centers for Disease Control and Prevention: Diabetes Data and Trends. http://www.cdc.gov/diabetes/statistics/treating_national.htm. Accessed June 8, 2011.
22. Chang Q, Li Y, White MF, Fletcher JA, Xiao S. Constitutive activation of insulin receptor substrate 1 is a frequent event in human tumors: therapeutic implications. Cancer Res. 2002;62(21):6035-8.
23. Chen C, Méndez E, Houck J, Fan W, Lohavanichbutr P, Doody D, Yueh B, Futran ND, Upton M, Farwell DG, Schwartz SM, Zhao LP. Gene expression profiling identifies genes predictive of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2008;17(8):2152-62.
24. Chung CH, Parker JS, Karaca G, Wu J, Funkhouser WK, Moore D, Butterfoss D, Xiang D, Zanation A, Yin X, Shockley WW, Weissler MC, Dressler LG, Shores CG, Yarbrough WG, Perou CM. Molecular classification of head and neck squamous cell carcinomas using patterns of gene expression. Cancer Cell. 2004;5(5):489-500.
25. Cowie CC, Rust KF, Ford ES, Eberhardt MS, Byrd-Holt DD, Li C, et al. Full accounting of diabetes and pre-diabetes in the U.S. population in 1988-1994 and 2005-2006. Diabetes Care 2009;32(2):287-94.

26. Cowey S, Hardy RW. The metabolic syndrome: A high-risk state for cancer? Am J Pathol. 2006;169(5):1505-22.
27. Cui Y, Morgenstern H, Greenland S, Tashkin DP, Mao J, Cao W, et al. Polymorphism of Xeroderma Pigmentosum group G and the risk of lung cancer and squamous cell carcinomas of the oropharynx, larynx and esophagus. Int J Cancer 2006;118(3):714-20.
28. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia 2009;52(9):1766-77.
29. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut. 2005;54(4):533-9.
30. Dearth RK, Cui X, Kim HJ, Hadsell DL, Lee AV. Oncogenic transformation by the signaling adaptor proteins insulin receptor substrate (IRS)-1 and IRS-2. Cell Cycle. 2007;6(6):705-13.
31. Decensi A, Puntoni M, Goodwin P, Cazzaniga M, Gennari A, Bonanni B, et al. Metformin and cancer risk in diabetic patients: a systematic review and meta-analysis. Cancer Prev Res (Phila) 2010;3(11):1451-61.
32. De Rekeneire N, Peila R, Ding J, et al. Diabetes, Hyperglycemia, and Inflammation in Older Individuals: The Health, Aging and Body Composition study. Diabetes Care. 2006; 29:1902-1908.
33. Dietrich T, Reichart PA, Scheifele C. Clinical risk factors of oral leukoplakia in a representative sample of the US population. Oral Oncol. 2004;40(2):158-63.
34. Dikshit RP, Ramdas K, Hashibe M, et al. Association between diabetes mellitus and premalignant oral diseases: a cross sectional study in Kerala, India. Int J Cancer. 2006;118(2):453–7.

35. Divaris K, Olshan AF, Smith J, Bell ME, Weissler MC, Funkhouser WK, et al. Oral health and risk for head and neck squamous cell carcinoma: the Carolina Head and Neck Cancer Study. Cancer Causes Control 2010;21(4):567-75.
36. Dowling RJ, Goodwin PJ, Stambolic V. Understanding the benefit of metformin use in cancer treatment. BMC Med. 2011;9:33.
37. Dowling RJ, Zakikhani M, Fantus IG, Pollak M, Sonenberg N. Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells. Cancer Res 2007; 67:10804-12.
38. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med 2007;356:1944-56.
39. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. Lancet. 2005;365(9468):1415-28.
40. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 2002;23(7):1229-34.
41. Engels EA, Pfeiffer RM, Ricker W, Wheeler W, Parsons R, Warren JL. Use of surveillance, epidemiology, and end results-medicare data to conduct case-control studies of cancer among the US elderly. Am J Epidemiol. 2011;174(7):860-70.
42. Evans JM, Donnelly LA, Emslie-Smith AM, et al. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330:1304–5.

43. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285: 2486-2497.
44. Fajans SS, Bell GI, Polonsky KS. Molecular mechanisms and clinical pathophysiology of maturity-onset diabetes of the young. N Engl J Med 2001;345(13):971-80.
45. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010.
46. Ford ES, Williamson DF, Liu S. Weight change and diabetes incidence: findings from a national cohort of US adults. Am J Epidemiol 1997;146:214-222.
47. Foster GD, Sanders MH, Millman R, Zammit G, Borradaile KE, Newman AB, Wadden TA, Kelley D, Wing RR, Sunyer FX, Darcey V, Kuna ST; Sleep AHEAD Research Group. Obstructive sleep apnea among obese patients with type 2 diabetes. Diabetes Care. 2009;32(6):1017-9.
48. Fox CJ, Hammerman PS, Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005;5(11):844-52.
49. Franceschi S, Talamini R, Barra S, Barón AE, Negri E, Bidoli E, et al. Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. Cancer Res 1990;50(20):6502-7.
50. Gapstur SM, Gann PH, Lowe W, Liu K, Colangelo L, Dyer A. Abnormal glucose metabolism and pancreatic cancer mortality. JAMA 2000;283:2552-8.
51. Gaudet MM, Olshan AF, Chuang SC, Berthiller J, Zhang ZF, Lissowska J, et al. Body mass index and risk of head and neck cancer in a pooled analysis of case-control studies in the International Head and Neck Cancer Epidemiology (INHANCE) Consortium. Int J Epidemiol 2010;39(4):1091-102.

52. Gerich JE. The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. Endocr Rev 1998;19(4):491-503.

53. Giovannucci E. Nutrition, insulin, insulin-like growth factors and cancer. Horm Metab Res. 2003;35: 694-704.

54. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. CA Cancer J Clin. 2010;60(4):207-21.

55. Goeman JJ, Bühlmann P. Analyzing gene expression data in terms of gene sets: methodological issues. Bioinformatics. 2007;23(8):980-7.

56. Goeman JJ, van de Geer SA, de Kort F, van Houwelingen HC. A global test for groups of genes: testing association with a clinical outcome. Bioinformatics. 2004;20(1):93-9.

57. Goutzanis L, Vairaktaris E, Yapijakis C, Kavantzas N, Nkenke E, Derka S, Vassiliou S, Acil Y, Kessler P, Stavrianeas N, Perrea D, Donta I, Skandalakis P, Patsouris E. Diabetes may increase risk for oral cancer through the insulin receptor substrate-1 and focal adhesion kinase pathway. Oral Oncol. 2007;43(2):165-73.

58. Grimberg A, Cohen P. Role of insulin-like growth factors and their binding proteins in growth control and carcinogenesis. J Cell Physiol 2000;183:1-9.

59. Hadad SM, Dewar JA, Elseedawy E, Jordan LB, Purdie C, Bray SE, Thompson AM. Gene Signature of metformin actions on primary breast cancer within a window of opportunity randomized clinical trial. J Clin Oncol. 2010;28(Suppl):560.

60. Haffner SM, Valdez RA, Hazuda HP, Mitchell BD, Morales PA, Stern MP. Prospective analysis of the insulin-resistance syndrome (syndrome X). Diabetes. 1992;41(6):715-22.

61. Hall GC, Roberts CM, Boulis M, Mo J, MacRae KD. Diabetes and the risk of lung cancer. Diabetes Care. 2005;28(3):590-4.

62. Harris MI, Cowie CC, Eastman R. Health-insurance coverage for adults with diabetes in the U.S. population. Diabetes Care. 1994;17(6):585-91.

63. Harris MI, Eastman RC. Early detection of undiagnosed diabetes mellitus: a US perspective. Diabetes Metab Res Rev 2000;16(4):230-6.

64. Harris R, Deeks J, Altman DG, Bradburn MJ, Harbord R, Sterne JAC. Metan: fixed and random effects meta-analysis. Stata J 2008;8:3.

65. Ha PK, Benoit NE, Yochem R, Sciubba J, Zahurak M, Sidransky D, et al. A transcriptional progression model for head and neck cancer. Clin Cancer Res 2003;9:3058-64.

66. Hashibe M, Brennan P, Benhamou S, Castellsague X, Chen C, Curado MP, et al. Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. J Natl Cancer Inst 2007;99:777–89.

67. Hashibe M, Brennan P, Chuang SC, et al. Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. Cancer Epidemiol Biomarkers Prev. 2009;18(2):541-50.

68. Haug U, Hillebrand T, Bendzko P, Löw M, Rothenbacher D, Stegmaier C, et al. Mutant-enriched PCR and allele-specific hybridization reaction to detect K-ras mutations in stool DNA: high prevalence in a large sample of older adults. Clin Chem 2007;53(4):787-90.

69. Hennessey PT, Westra WH, Califano JA. Human papillomavirus and head and neck squamous cell carcinoma: recent evidence and clinical implications. J Dent Res 2009;88:300-6.

70. Hillier TA, Pedula KL. Complications in young adults with early-onset type 2 diabetes: losing the relative protection of youth. Diabetes Care 2003;26(11):2999-3005.

71. Hirsch D, Stahl A, Lodish HF. A family of fatty acid transporters conserved from mycobacterium to man. Proc Natl Acad Sci U S A. 1998;95(15):8625-9.

72. Hsing AW, Sakoda LC, Chua S Jr. Obesity, metabolic syndrome, and prostate cancer. Am J Clin Nutr. 2007;86(3):s843-57.

73. Hursting SD, Lavigne JA, Berrigan D, Perkins SN, Barrett JC. Calorie restriction, aging, and cancer prevention: mechanisms of action and applicability to humans. Annu Rev Med. 2003;54:131-52.

74. Jee SH, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. JAMA 2005;293:194-202.

75. Jiang HJ, Stryer D, Friedman B, Andrews R. Multiple hospitalizations for patients with diabetes. Diabetes Care 2003;26(5):1421-6.

76. Johansen D, Stocks T, Jonsson H, Lindkvist B, Björge T, Concin H, et al. Metabolic factors and the risk of pancreatic cancer: a prospective analysis of almost 580,000 men and women in the Metabolic Syndrome and Cancer Project. Cancer Epidemiol Biomarkers Prev. 2010;19(9):2307-17.

77. Kabat GC, Kim M, Chlebowski RT, Khandekar J, Ko MG, McTiernan A, Neuhouser ML, Parker DR, Shikany JM, Stefanick ML, Thomson CA, Rohan TE. A longitudinal study of the metabolic syndrome and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2009;18(7):2046-53.

78. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature 2006;444(7121):840-6.

79. Kleiner DE, Stetler-Stevenson WG. Matrix metalloproteinases and metastasis. Cancer Chemother Pharmacol. 1999;43 Suppl:S42-51.

80. Kopp M, Csoboth C. Self-destructive behaviour in the Hungarian population. Magy Onkol. 2001;45(2):139-142.

81. Kota BP, Huang TH, Roufogalis BD. An overview on biological mechanisms of PPARs. Pharmacol Res. 2005;51:85–94.

82. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell. 2008;13(6):472-82.

83. Kubota R, Yamada S, Kubota K, Ishiwata K, Tamahashi N, Ido T. Intratumoral distribution of fluorine-18-fluorodeoxyglucose in vivo: high accumulation in macrophages and granulation tissues studied by microautoradiography. J Nucl Med 1992;33(11):1972-80.

84. Levi F, Pasche C, La Vecchia C, Lucchini F, Franceschi S, Monnier P. Food groups and risk of oral and pharyngeal cancer. Int J Cancer 1998;77(5):705-9.

85. Liao W, Wei H, Wang X, Qiu Y, Gou X, Zhang X, Zhou M, Wu J, Wu T, Kou F, Zhang Y, Bian Z, Xie G, Jia W. Metabonomic Variations Associated with AOM-induced Precancerous Colorectal Lesions and Resveratrol Treatment. J Proteome Res. 2012.

86. Llewellyn CD, Johnson NW, Warnakulasuriya KA. Risk factors for squamous cell carcinoma of the oral cavity in young people–a comprehensive literature review. Oral Oncol 2001;37(5):401-18.

87. Lohavanichbutr P, Houck J, Fan W, Yueh B, Mendez E, Futran N, Doody DR, Upton MP, Farwell DG, Schwartz SM, Zhao LP, Chen C. Genomewide gene expression profiles of HPV-positive and HPV-negative oropharyngeal cancer: potential implications for treatment choices. Arch Otolaryngol Head Neck Surg. 2009;135(2):180-8.

88. Maassen JA, 'T Hart LM, Van Essen E, Heine RJ, Nijpels G, Jahangir Tafrechi RS, et al. Mitochondrial diabetes: molecular mechanisms and clinical presentation. Diabetes 2004;53 Suppl 1:S103-9.

89. Ma N, Tagawa T, Hiraku Y, Murata M, Ding X, Kawanishi S. 8-Nitroguanine formation in oral leukoplakia, a premalignant lesion. Nitric Oxide. 2006;14(2):137-43.

90. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454(7203):436-44.

91. Martinez-Pomares L, Platt N, McKnight AJ, da Silva RP, Gordon S. Macrophage membrane molecules: markers of tissue differentiation and heterogeneity. Immunobiology 1996;195(4-5):407-16.

92. Masur K, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B, Zänker KS. Diabetogenic glucose and insulin concentrations modulate transcriptome and protein levels involved in tumour cell migration, adhesion and proliferation. Br J Cancer. 2011;104(2):345-52.

93. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, et al. International Union of Pharmacology. LXI. Peroxisome proliferator-activator receptors. Pharmacol Rev. 2006;58(4):726–41.

94. Michels KB, Solomon CG, Hu FB, Rosner BA, Hankinson SE, Colditz GA, et al. Type 2 diabetes and subsequent incidence of breast cancer in the Nurses' Health Study. Diabetes Care 2003;26:1752-8.

95. Mork J, Lie AK, Glattre E, Hallmans G, Jellum E, Koskela P, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. N Engl J Med 2001;344:1125-9.

96. Narayan KM, Boyle JP, Thompson TJ, Gregg EW, Williamson DF. Effect of BMI on lifetime risk for diabetes in the U.S. Diabetes Care 2007;30(6):1562-6.

97. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, et al. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care. 2009;32(1):193-203.

98. Nilsen TI, Vatten LJ. Prospective study of colorectal cancer risk and physical activity, diabetes, blood glucose and BMI: exploring the hyperinsulinemia hypothesis. Br J Cancer. 2001; 84:417-422.

99. Niraula S, Stambolic V, Dowling RJO, Ennis M, Chang MC, Done SJ, Hallak S, Hood N, Goodwin PJ. Clinical and biologic effects of metformin in early stage breast cancer. Cancer Res. 2010;70(24, Suppl):104s.

100. North Carolina Diabetes Prevention and Control Fact Sheet, September 2009. http://www.ncdiabetes.org/factsFigures/index.aspx. Accessed June 8, 2011.

101. O'Byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001; 85(4):473-83.

102. Pais R, Silaghi H, Silaghi AC, Rusu ML, Dumitrascu DL. Metabolic syndrome and risk of subsequent colorectal cancer. World J Gastroenterol. 2009;15(41):5141-8.

103. Pelicano H, Xu RH, Du M, Feng L, Sasaki R, Carew JS, Hu Y, Ramdas L, Hu L, Keating MJ, Zhang W, Plunkett W, Huang P. Mitochondrial respiration defects in cancer cells cause activation of Akt survival pathway through a redox-mediated mechanism. J Cell Biol. 2006;175(6):913-23.

104. Percy C. International Classification of Diseases for Oncology (ICD-O-3). 3rd ed. Geneva, Switzerland: World Health Organization; 1990.

105. Phoenix KN, Vumbaca F, Claffey KP. Therapeutic metformin/AMPK activation promotes the angiogenic phenotype in the ERalpha negative MDA-MB-435 breast cancer model. Breast Cancer Res Treat. 2009;113(1):101-11.

106. Pickup JC, Chusney GD, Thomas SM, et al. Plasma interleukin-6, tumor necrosis factor alpha and blood cytokine production in type 2 diabetes. Life Sci. 2000; 67:291–300.

107. Proudler AJ, Felton CV, Stevenson JC. Ageing and the response of plasma insulin, glucose and C-peptide concentrations to intravenous glucose in postmenopausal women. Clin Sci (Lond). 1992;83(4):489-94.

108. Purdue MP, Hashibe M, Berthiller J, La Vecchia C, Dal Maso L, Herrero R, et al. Type of alcoholic beverage and risk of head and neck cancer--a pooled analysis within the INHANCE Consortium. Am J Epidemiol 2009;169(2):132-42.

109. Raccurt M, Tam SP, Lau P, Mertani HC, Lambert A, Garcia-Caballero T, Li H, Brown RJ, McGuckin MA, Morel G, Waters MJ. Suppressor of cytokine signalling gene expression is elevated in breast carcinoma. Br J Cancer. 2003;89(3):524-32.

110. Rapp K, Schroeder J, Klenk J, Ulmer H, Concin H, Diem G, et al. Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria. Diabetologia 2006;49(5):945-52.

111. Reibel J. Prognosis of oral pre-malignant lesions: significance of clinical, histopathological, and molecular biological characteristics. Crit Rev Oral Biol Med, 2003;14:47-62.

112. Reisser C, Eichhorn K, Herold-Mende C. Glucose uptake in malignant tumors of the head and neck. HNO. 1999;47(8):712-7.

113. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346-53.

114. Rosato V, Zucchetto A, Bosetti C, Dal Maso L, Montella M, Pelucchi C, Negri E, Franceschi S, La Vecchia C. Metabolic syndrome and endometrial cancer risk. Ann Oncol. 2011;22(4):884-9.

115. Royston P. Multiple imputation of missing values. Stata J 2004;4:227-241.

116. Sahra IB, Laurent K, Loubat A, et al. The antidiabetic drug metformin exerts an antitumoral effect *in vitro* and *in vivo* through a decrease of cyclin D1 level. Oncogene. 2008;27:3576-3586.

117. Sandulache VC, Ow TJ, Pickering CR, Frederick MJ, Zhou G, Fokt I, Davis-Malesevich M, Priebe W, Myers JN. Glucose, not glutamine, is the dominant energy source required for proliferation and survival of head and neck squamous carcinoma cells. Cancer. 2011.

118. Schmidt AM, Hori O, Brett J, et al. Cellular receptors for advanced glycation end products. Implications for induction of oxidant stress and cellular dysfunction in the pathogenesis of vascular lesions. Arterioscler Thromb. 1994;14:1521-1528.

119. Schwartz SM, Daling JR, Doody DR, et al. Oral cancer risk in relation to sexual history and evidence of human papillomavirus infection. J Natl Cancer Inst. 1998;90(21):1626-1636.

120. Sethi G, Sung B, Aggarwal BB. TNF: a master switch for inflammation to cancer. Front Biosci 2008;13:5094-107.

121. Seyfried TN, Shelton LM. Cancer as a metabolic disease. Nutr Metab (Lond). 2010;7:7.

122. Shiboski CH, Shiboski SC, Silverman S, Jr. Trends in oral cancer rates in the United States, 1973-1996. Community Dent Oral Epidemiol. 2000;28(4):249-25.

123. Silverman S, Gorsky M, Lozada F. Oral leukoplakia and malignant transformation. A follow-up study of 257 patients. Cancer. 1984;53:563-568.

124. Stattin P, Bjor O, Ferrari P, Lukanova A, Lenner P, Lindahl B, et al. Prospective study of hyperglycemia and cancer risk. Diabetes Care 2007;30:561-7.

125. Stott-Miller M, Chen C, Chuang SC, Lee YC, Boccia S, Brenner H, et al. History of diabetes and risk of head and neck cancer: a pooled analysis from the international head

and neck cancer epidemiology consortium. Cancer Epidemiol Biomarkers Prev. 2012;21(2):294-304.

126. Stott-Miller M, Houck JR, Lohavanichbutr P, Méndez E, Upton MP, Futran ND, Schwartz SM, Chen C. Tumor and salivary matrix metalloproteinase levels are strong diagnostic markers of oral squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2011;20(12):2628-36.

127. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. Lancet 2005;365(9467):1333-46.

128. Suba Z. Gender-related hormonal risk factors for oral cancer. Pathol Oncol Res. 2007;13(3):195-202.

129. Sugimura T, Kumimoto H, Tohnai I, Fukui T, Matsuo K, Tsurusako S, et al. Gene-environment interaction involved in oral carcinogenesis: molecular epidemiological study for metabolic and DNA repair gene polymorphisms. J Oral Pathol Med 2006;35(1):11-8.

130. Surveillance, Epidemiology, and End Results (SEER) Program (www.seer.cancer.gov) Research Data (1973-2008), National Cancer Institute, DCCPS, Surveillance Research Program, Cancer Statistics Branch, released April 2011, based on the November 2010 submission.

131. Surveillance, Epidemiology and End Results (SEER). http://seer.cancer.gov. Accessed June 1, 2012.

132. Tasali E, Mokhlesi B, Van Cauter E. Obstructive sleep apnea and type 2 diabetes: interacting epidemics. Chest. 2008;133(2):496-506.

133. Thissen JP, Ketelslegers JM, Underwood LE. Nutritional regulation of the insulin-like growth factors. Endocr Rev. 1994;15(1):80-101.

134. Tobias A. sbe26: assessing the influence of a single study in the meta-analysis estimate. Stata Tech Bull 1999;47:15-17.

135. Trotta BM, Pease CS, Rasamny JJ, Raghavan P, Mukherjee S. Oral cavity and oropharyngeal squamous cell cancer: key imaging findings for staging and treatment planning. Radiographics. 2011;31(2):339-54.

136. Tseng CH. Body mass index and waist circumference as determinants of coronary artery disease in Taiwanese adults with type 2 diabetes mellitus. Int J Obes (Lond) 2006;30: 816–21.

137. Valentinis B, Baserga R. IGF-I receptor signalling in transformation and differentiation. Mol Pathol 2001;54:133-7.

138. van Baal JW, Diks SH, Wanders RJ, Rygiel AM, Milano F, Joore J, Bergman JJ, Peppelenbosch MP, Krishnadath KK. Comparison of kinome profiles of Barrett's esophagus with normal squamous esophagus and normal gastric cardia. Cancer Res. 2006;66(24):11605-12.

139. Vaughan TL, Stewart PA, Davis S, Thomas DB. Work in dry cleaning and the incidence of cancer of the oral cavity, larynx, and oesophagus. Occup Environ Med 1997;54(9):692-5.

140. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer. 2009;16(4):1103-23.

141. Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 2003;92:827–39.

142. Warburg O. The Metabolism of Tumors. Constable Press, London, UK. 1930.

143. Warren JL, Klabunde CN, Schrag D, Bach PB, Riley GF. Overview of the SEER-Medicare data: content, research applications, and generalizability to the United States elderly population. Med Care 2002;40:IV-3-18.

144. Weber A, Hengge UR, Bardenheuer W, Tischoff I, Sommerer F, Markwarth A, Dietz A, Wittekind C, Tannapfel A. SOCS-3 is frequently methylated in head and neck

squamous cell carcinoma and its precursor lesions and causes growth inhibition. Oncogene. 2005;24(44):6699-708.

145. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115(5):1111-9.

146. Welzel TM, Graubard BI, Zeuzem S, El-Serag HB, Davila JA, McGlynn KA. Metabolic syndrome increases the risk of primary liver cancer in the United States: A study in the SEER-medicare database. Hepatology. 2011;54(2):463-71.

147. White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab. 2002;283(3):E413-22.

148. Wideroff L, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, et al. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. J Natl Cancer Inst 1997;89(18):1360-5.

149. Wilkins TL, Rust GS, Sambamoorthi U. Changing BMI Categories and Healthcare Expenditures Among Elderly Medicare Beneficiaries. Obesity (Silver Spring). 2011.

150. Wu MC, Lin X. Prior biological knowledge-based approaches for the analysis of genome-wide expression profiles using gene sets and pathways. Stat Methods Med Res. 2009;18(6):577-93.

151. Xue F, Michels KB. Diabetes, metabolic syndrome, and breast cancer: a review of the current evidence. Am J Clin Nutr. 2007;86(3):s823-35.

152. Yen AM, Chen SL, Chiu SY, Chen HH. Association between metabolic syndrome and oral pre-malignancy: a community- and population-based study (KCIS No. 28). Oral Oncol. 2011;47(7):625-30.

153. Zakikhani M, Dowling R, Fantus IG, Sonenberg N, Pollak M. Metformin is an AMP kinase-dependent growth inhibitor for breast cancer cells. Cancer Res 2006; 66:10269-73.

VITA

Marni Stott-Miller received a Bachelor of Science in Mathematics from the University of the Witwatersrand, Johannesburg, South Africa in 1994. After working as an analyst in financial markets for several years, she entered the University of Washington, School of Public Health in 2007 to study Epidemiology. She received a Master of Science in Epidemiology in 2009. During this time, Marni worked as a research associate at Seattle Children's Hospital, assisting with research studies on craniofacial birth defects. She entered the Epidemiology doctoral program in 2009, and began her dissertation research in the area of head and neck squamous cell carcinoma. During this time, she worked as a research associate at Fred Hutchinson Cancer Research Center in Seattle, Washington. She completed her Doctor of Philosophy in Epidemiology at the University of Washington in 2012.