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한국인 갑상선암과 관련된 생활습관, 의료방사선 노출 및 후성유전적 요인 평가

Evaluation of lifestyle, medical radiation exposure and epigenetic factors in relation to thyroid cancer in Koreans

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ABSTRACT

Introduction: The incidence of thyroid cancer has increased worldwide in recent decades, and the annual recorded percentage change of thyroid cancer was as high as 22.3% in Korea from 1999 to 2012. Although improved detection technologies and the relatively low cost of ultrasonography of the thyroid gland have been suggested as reasons for the increase in thyroid cancer in Korea, the possibility of a true increase has also been discussed. Particularly, except ionizing radiation exposure at early ages, environmental and genetic risk factors of thyroid cancer have not been established. The principle goal of the current study is to evaluate (1) alcohol consumption, (2) smoking, (3) obesity, (4) medical diagnostic radiation and (5) epigenetic profiles as risk factors of thyroid cancer in Korea.

Methods: We used two data sources. First, the Thyroid Cancer Longitudinal Study (T-CALOS), which enrolled thyroid cancer patients at Seoul National University Hospital, Korea from 2010 to 2014, was used. Eligible subjects were those aged 20 years or older who agreed to participate, signed a written informed consent form, and completed a face-to-face interview to

provide information regarding environmental and genetic factors and their general health condition. For thyroid cancer cases, we reviewed medical charts for clinical information, and this study included patients who were confirmed histologically by pathologists. For healthy controls, we conducted individual matching by age and sex from Health Examinees (HEXA), a community-based cohort study. For both cases and the controls, measured anthropometric information was collected, and obesity indicators including the body mass index (BMI), body surface area (BSA) and body fat percentage (BF%) were calculated. Secondly, data from the Korean Multi-center Cancer Cohort (KMCC) study, which collected epidemiologic information pertaining to general lifestyles, physical activity levels, and diet and reproductive factors in relation to the possible etiology of cancer, was used. The first enrollment of the KMCC was recorded in 1993, with follow-up investigations using a data link to the national cancer registry and the national death certificate system until December of 2013.

For the statistical analyses, we used t-tests and Chi-square tests of the descriptive statistics. Regarding the associations between potential risk factors and thyroid cancer, odds ratios

(ORs), hazard ratios (HRs) and their 95% confidence intervals (95%CIs) were calculated. We also evaluated the deoxyribonucleic acid (DNA) methylation levels of 96 selected gene sites in 90 blood DNA samples from both the subjects and the controls matched by age and sex from the hospital-based health examinees. Differences in the DNA methylation status between the two groups, the thyroid cancer cases and the controls, were compared.

Results: In the T-CALOS study, while light or moderate drinking behavior was related to a reduced risk of differentiated thyroid cancer (DTC), acute heavy alcohol consumption (151 g or more per event or on a single occasion) was associated with increased risks in men (OR=2.2, 95%CI=1.3-3.9) and women (OR=3.6, 95%CI=1.5-8.6) compared with never-drinkers. Drinking alcohol beverages for 31 or more years was a significant risk factor for DTC for both men (31-40 years: OR=1.6, 95%CI=1.1-2.3; 41+ years: OR=3.5, 95%CI=2.1-5.8) and women (31-40 years: OR=2.2, 95%CI=1.6-2.9; 41+ years: OR=2.7, 95%CI=1.4-5.1) compared with never-drinkers. The consumption of a large amount of alcohol on a single occasion was also a significant risk factor, even after

restricting DTC outcomes to tumor size, lymph node metastasis, extrathyroidal extension and TNM stage. For exposure to smoking, although active smoking itself was not associated with papillary thyroid cancer (PTC) risk, in subjects who had been exposed to early-age secondhand smoking at home during as they were growing up, smoking initiated before 20 years of age was associated with an increased level of PTC risk (OR=1.6, 95% CI=1.0-2.6) compared with never-smokers. Further, secondhand smoking among the never-smokers was also associated with increased PTC risk (men: OR=2.2, 95%CI=1.3-3.9; women: OR=1.5, 95%CI=1.3-1.7). Subjects with a high BMI (30kg/m2 or greater) at enrollment showed a significantly increased level of PTC risk in the men (OR=1.9, 95%CI=1.1-3.3) compared to those who were in the normal range of BMI (18.5-24.9kg/m2) among the men. The highest quartile of BSA at enrollment was also associated with PTC risk in both men and women (men: OR=2.2, 95%CI=1.5-3.1; women: OR=1.7, 95%CI=1.4-2.0) compared to those in the lowest quartile of BSA. Regarding obesity at 18-20 years of age, a higher BMI was associated with elevated PTC risk (BMI, 25.0-29.9: OR=4.0, 95%CI=3.2-5.0; BMI, 30.0+: OR=4.0,

95%CI=1.4−11.6) compared to the normal BMI range (18.5−24.9). A BMI of 25 or greater at age 18 was linked to a pronounced increase in PTC risk, particularly in men (men: OR=6.8, 95%CI=4.5−10.2; women: OR=3.2, 95%CI=2.4−4.2; p−heterogeneity=0.002). Regarding weight changes in middle−aged adults, subjects with a total weight gain of 10 kg or more after age 35 years were more likely to have PTC (men: OR=5.4, 95%CI=3.9–7.5; women: OR=3.4, 95%CI=2.9–3.9) compared with subjects with a stable weight (loss or gain <5 kg). A marked increase in BMI since age 35 (an annual average change of BMI ≥0.3 kg/m2/year) was related to elevated PTC risk, and the association was more pronounced for the larger PTC risk levels (<1 cm, OR, 2.3, 95%CI, 1.9–2.9; ≥1 cm, OR, 4.0, 95%CI, 2.9−5.5, p−heterogeneity=0.005) compared to the low PTC risk levels. Based on data from the KMCC study, medical diagnostic radiation including X−ray radiography, upper gastrointestinal series (UGI), computerized tomography (CT), and mammography was not associated with increased thyroid cancer risk in general. Compared to those who remain unexposed to any of the five types of medical diagnostic radiation, those who were exposed to four combined sources

(X-ray, UGI, CT, and mammography) showed a six-fold increase in their thyroid cancer risk level (HR=5.9, 95%CI=1.5–24.1). We observed distinct and unique patterns of methylation profiles for the two groups, i.e., the subjects and the controls.

Conclusions: The factors of alcohol consumption, passive smoking, obesity at 18-20 years of age, and weight gain after age 35 was found to be potential risk factors of thyroid cancer risk in Koreans. Furthermore, the results of this study indicate that differences in DNA methylation levels can be used a biomarker for thyroid cancer.

Keywords: thyroid cancer, risk factor, drinking, smoking, obesity, body mass index, medical diagnostic radiation, methylation.

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LIST OF ABBREVIATIONS

95%CI=95% confidence interval;

AJCC/UICC=the American Joint Committee on Cancer and the Union for International Cancer Control;

APC=annual percentage change;

BCDDP= Breast Cancer Detection Demonstration Project;

BF%=body fat percentage;

BMI=body mass index;

BSA=body surface area;

CpG=cytosine–guanine dinucleotide;

CT= computerized tomography;

DNA=deoxyribonucleic acid;

DTC=differentiated thyroid cancer;

EPIC=European Prospective Investigation into Cancer and Nutrition;

F=females;

FTC=follicular thyroid cancer;

HEXA= the Health Examinees study;

HPT= hypothalamic–pituitary–thyroid;

HR=hazard ratio;

IARC=The International Agency for the Research of Cancer;

ICD= International Classification of Diseases;

IRB= Institutional Review Board;

KMCC= the Korean Multi-center Cancer Cohort;

KoGeS= the Korean Genome and Epidemiology Cohort Study;

M=males;

MDS=multidimensional;

MTC=medullary thyroid cancer;

NA=not applicable;

NBSS=National Breast Screening Study;

NIH-AARP= National Institutes of Health-American association of Retired Persons;

OR=odds ratio;

PLCO= Prostate, Lung, Colorectal, and Ovarian Screening Study;

PTC=papillary thyroid cancer;

RR=relative ratio;

SD= standard deviation;

SE=Standard Error;

T-CALOS=Thyroid Cancer Longitudinal Study;

TNM=tumor-node-metastasis;

TSH=thyroid-stimulating hormone.

UGI=upper gastrointestinal series;

USRT= US Radiologic Technologists Study; AHS=Agricultural Health Study;

WHI=Women's Health Initiative;

WHO=World Health Organization;

I. Introduction

1. Background

The worldwide incidence of thyroid cancer has been rapidly and steeply upraised in the recent three decades, almost exclusively attributable to papillary thyroid cancers [1-3], and it mostly occurred in developed countries according to the International Agency for Research on Cancer (IARC) as in Figure 1. Suggested explanations for the increases of incidence are unsettled. Enhanced detection with increased use of diagnostic imaging of small tumors in the preclinical stage has contributed to this drastic increase of thyroid cancer [2-5]. Improved techniques and increased frequencies for ultrasound and cytology examinations have been demonstrated, and these allowed us to discover small and asymptomatic thyroid tumors [1, 6-8]. This increase might reflect the better access to health services can lead more opportunistic detection of thyroid cancer. On the other views, an increase in thyroid cancer has been demonstrated across the all tumor sizes, which had not been affected by early diagnosis. Additionally, a true increase by lifestyle factors, environmental carcinogens, medical

radiation and epigenetic profiles has been suggested [1, 9]. Pellegriti et al., summarized potential carcinogenic factors of thyroid cancer in exogenous (medical x-rays, nuclear medicine procedures, dietary Iodine intakes, westernized lifestyle and environmental pollutants) and endogenous factors (thyroid stimulation hormone, oxidative stress, obesity and insulin resistance) [1]. Marcello and colleagues also added new possibilities that living in volcanic areas with abundant lava, xenobiotic compounds and oncogenic virus can influence on thyroid cancer development [9]. Other unknown cancer-causing agent may also be contributing behind this global increasing thyroid cancer incidence. Therefore, potential risk factors of thyroid cancer need to be considered to plan prevention strategies and clinical practice of thyroid cancer.

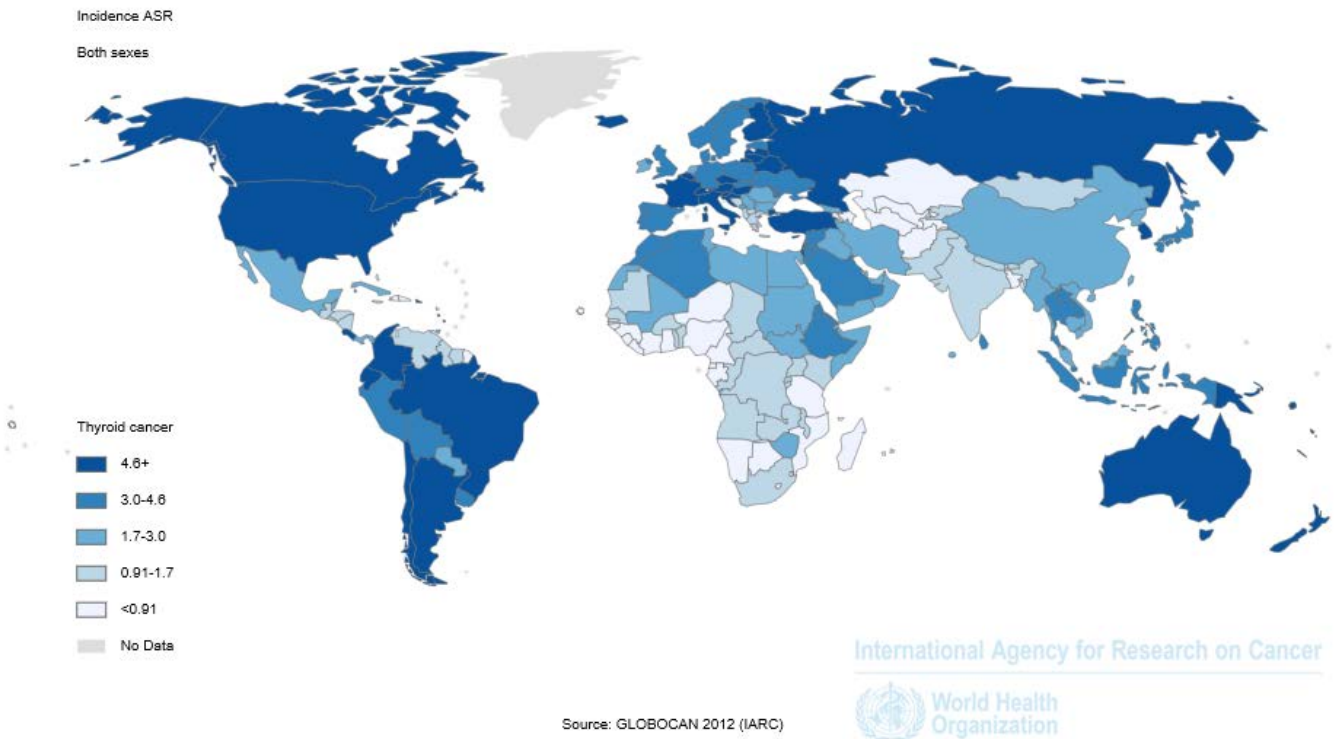

Figure 1 Age-standardized incidence of thyroid cancer (men and women), GLOBOCAN 2012, IARC, 2013.

2. Thyroid cancer in Korea

In Korea, thyroid cancer has been recorded as the most common cancer since 2009, with a notably high age-standardized incidence rate, 62.5 per 100,000 [10]. Thyroid cancer incidence in Korea is the highest in the world, and the age-standardized incidence rates of thyroid cancer in Korea are approximately 13-fold higher than in the rest of the world, the four-fold higher than in the United States and 10-fold higher than in the European Union (EU-28) based on the GLOBOCAN 2012 by the IARC.

Based on the incidence trends from 1993 to 2012, thyroid cancer increased by 22.3% of annual percentage change (APC) in total, as well as in both men (23.6%) and women (22.1%)of APC [10]. Thyroid cancer is much more likely to occur in women and those who were between the ages of 15 and 64 in Korea [10]. In addition, Jung et al., predicted that thyroid cancer alone would account for nearly 28% (34,255 cases) of total incidental cancer cases in Korean women in 2016 [11]. The reasons of this increase have been highly debated. Some experts presented that an extraordinary high incidence Korea's thyroid cancer in recent years could be attributed by a national

cancer screening program and more frequent health check-up procedures [12, 13]. The proportion of screening-detected thyroid cancer among the total cases showed an increasing trend, 13.0% in 1999, 42.5% in 2005 and 56.7% in 2008, which means a significant role of thyroid screening [14]. On the contrary, not all epidemiological and clinical data support this statement. Lee and Kwak reported that the cancer screening rate among women is relatively low, and there could be Korean-specific patterns of dietary habit, degree of obesity, environmental and cultural predictors related to thyroid cancer development [15]. In addition, thyroid cancer showed a high incidence rate among the adolescents and young adults between 15 and 34 years old, while this age range was not targeted for a national cancer screening program or routine health examinations [15].

Increasing incidence of thyroid cancer and its burden is currently one of the main public health concerns in Korea. Since it is not likely that genetic composition in Korean population has been changed in the recent decades, better understanding for environmental exposure and epigenetic alteration associated with the excessive risk for thyroid cancer is necessary.

3. Lifestyle factors of thyroid cancer

a) Alcohol consumption

The World Cancer Research Foundation and the American Institute for Cancer Research (2007) reported suggested that alcohol drinking was linked to various cancers [16]. For the association between alcohol consumption and thyroid cancer, the evidence from the previous results was inconsistent and several questions unanswered. An increased alcohol drinking rate from 1998 to 2005 was detected (men: 57.0% to 66.1%; women: 23.4 to 34.5%), based on the Korean National Health and Nutrition Examination Survey data [17]. Considering these sustainable and parallel increases of thyroid cancer in recent years, both alcohol consumption and thyroid cancer are serious public health concerns in Korea [11, 17].

Conflicting results have been reported. Abnormal functioning of the hypothalamic–pituitary–thyroid (HPT) axis has been observed in chronic alcoholics, indicating the potential involvement of chronic ethanol exposure in thyroid hormone metabolism [18]. On the other hand, recent studies demonstrated inverse relationship between alcohol drinking and development of thyroid cancer based on prospective cohort

studies [19-23] and case-control studies [24-28] as summarized in Table 1, which was updated from the meta-analysis results by Choi and Kim [29]. No significant association was found in cohort studies [30, 31] and case-control studies [32-35]. Results for male thyroid cancer were limited and few studies confirmed a positive association between alcohol intake and thyroid cancer considering an excess binge drinking or chronic exposure. Although the two cross-sectional studies were conducted in Korean population, the results showed an opposite direction of association between alcohol drinking and thyroid cancer [36, 37].

Binge alcohol exposure rather than the total volume of alcohol consumption, increased the risk of metabolic syndrome [38], obesity [39], and mortality caused by oropharyngeal and esophageal cancers [40] in Koreans. However, the potential effects of excess alcohol consumption per event or chronic lifetime exposure on the development of thyroid cancer have not been well understood. Therefore, this study evaluated the association between alcohol consumption and differentiated thyroid cancer (DTC) risk in both the T-CALOS and the KMCC study data. The associations between alcohol consumption and

clinicopathological features (thyroid tumor, such as tumor size, lymph node metastasis, multifocality, cancer stage, and BRAF (V600E) mutations) were also explored in the T-CALOS data.

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Cohort studies					
Iribarren, 2001, United States (enrollment: 1964-1973)	[30]	196 (73M+123F) / 204,964 after 20 years of follow-up	1-2 vs. 6+ drinks/day	RR=0.98 (0.59-1.61)	Subscribers of the Kaiser Permanente Medical Care Program of Northern California (San Francisco Bay area population)
Navarro Silvera, 2005, Canada (enrollment: 1980-1985)	[19]	169 / 89,835F after 15.9 years of follow-up	never vs. > 10g/day	HR=0.80 (0.45-1.42)	NBSS (Canadian women aged 40-59)
Allen, 2009, United Kingdom (enrollment: 1996-2001)	[20]	421/ 68,775F after 7 years of follow-up	≤2 vs. 15+ drinks/week	RR=0.54 (0.31-0.92)	The Million Women Study (middle-aged women in the breast cancer screening clinics in the United Kingdom)
Meinhold, 2010, United States (enrollment: 1995-1996)	[21]	370 (170M+200F) / 490,159 after 7.5 years of follow-up	never vs. ≥ 2 drinks per week	RR=0.57 (0.36-0.89)	The NIH-AARP Diet and Health Study.
Kitahara, 2012, United States (enrollment periods vary for each cohort, 1983-2009)	[22]	1,003 (335M+668F)/ 384,433M+361,664F after 10.5 years of follow-up	None vs. 7+drinks/week	HR=0.72 (0.58–0.90)	A pooled analysis of five prospective studies in the United States (NIH-AARP+ USRT+ AHS + PLCO+ BCDDP)

Abbreviations: HR=hazard ratio; RR=relative ratio; 95%CI=95% confidential interval; M=males; F=females; NBSS=National Breast Screening Study; NIH-AARP= National Institutes of Health-American Association of Retired Persons; USRT= US Radiologic Technologists Study; AHS=Agricultural Health Study; PLCO= Prostate, Lung, Colorectal, and Ovarian Screening Study; BCDDP= Breast Cancer Detection Demonstration Project)

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Kabat, 2012, United States (enrollment: 1993-1998)	[31]	331/ 159,340F after 13 years of follow-up	none vs. 7+drinks/week none vs. 4+g/day	HR=0.66 (0.44-1.01) HR=0.79 (0.60-1.05)	WHI study (Postmenopausal women)
Sen, 2015, 10 European countries including Denmark, France, Greece, Germany, Italy, the Netherlands, Norway, Spain, Sweden and United Kingdom (enrollment: 1992-2000)	[23]	556 (57M+499F)/ 477,263 after 7.2 years of follow-up	Intake at baseline: none vs. 15+g/day Average lifetime intake: none vs. 15+g/day	HR=0.77 (0.60-0.98) HR=0.90 (0.68-1.21)	EPIC study
Case-control studies					
Takezaki, 1996, Japan (enrollment: 1988-1993)	[32]	94F/22,666F	Sometimes/less vs. 4+ times/week	OR=0.7 (0.3-1.5)	Hospital-based case-referent study at Aichi Cancer Center Hospital in Nagoya, aged between 20-79 + controls were from the female outpatients without cancer

Abbreviations: HR=hazard ratio; OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females; WHI=Women's Health Initiative; EPIC=European Prospective Investigation into Cancer and Nutrition.

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Rossing, 2000, United States (enrollment: 1988-1994)	[24]	558F/574F	Never, ≤ 12 vs. 12+ drinks/ year	OR=0.7 (0.5-1.0)	Cases were from the Cancer Surveillance System, a population-based cancer registry in western Washington State and controls were from random-digit telephone dialing in case's residence area.
Mack, 2002, United States (enrollment: 1980-1983)	[33]	292F/292F	None vs. >3 per/week	OR=0.7 (0.3-1.5)	Cases were from the Los Angeles County population-based registry, the University of Southern California Cancer Surveillance Program and controls were recruited in case's neighborhood

Abbreviations: HR=hazard ratio; OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Mack, 2003, United States, Japan, China, Sweden, Norway, Northern Italy, Switzerland and Greece (enrollment: 1980-1997)	[25]	2725 (478M+2247F) / 4776 (1077M+3699 F)	Wine and beer: none vs. >14 drinks/week	OR=0.9 (0.7–1.1)	A pooled analysis of 14 case–control studies conducted in the United States, Europe, and Asia
Nagano, 2007, Japan (enrollment: 1970-1986)	[26]	362 (57M+305F)/ 435	Never vs. daily	OR=0.59 (0.35-1.01)	Cases were from the tumor registries in Hiroshima and Nagasaki and controls were from controls from members of the Life Span Study or offspring cohort in Hiroshima and Nagasaki areas
Guignard, 2007, New Caledonia (enrollment: 1993–1999)	[34]	332 (39M+293F) /412 (58M+354F)	Never vs. > 10	M: OR=0.32 (0.05-1.95); F: OR=0.92 (0.24-3.45)	Cases were from the two pathology laboratories of New Caledonia and confirmed with cancer registry and medical records, and controls were from the frequency matching in the community.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Xhaard, 2014, French Polynesia (enrollment: 1981-2003)	[35]	229 (26M+203F) /373	None vs. Regular	OR=1.2 (0.3-4.5)	Cases from the cancer incidence registry of French Polynesia and controls were matched based on the French Polynesian birth registry.
Lence-Anta, 2014, Cuba (enrollment: 2000-2011)	[27]	203 / 229	None vs. 6+ glasses/week	OR=0.4 (0.1 – 0.9)	Cases were selected from the National Cancer Registry Databases of Cuba and controls from the general population.
Stansifer, 2015, United States (enrollment: 2013)	[28]	467/ 255	<1 drink/day vs. 1-2 drink/day (current drinker)	OR=0.46 (0.29-0.73)	Cases were from the University of Nebraska Medical Center's Thyroid Tumor and Cancer Collaborative Registry and controls were from the Great Plains Health Informatics Database.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 1. The previous results regarding the association between alcohol consumption and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/RR (95%CI)	Notes
Cross-sectional studies					
Han, 2011, Korea (enrollment: 2009)	[36]	263 /2000	None vs. 1+ drinks/ month	OR=0.61 (0.44-0.86)	The 2009 Korea National Cancer Screening Survey
Choi, 2013, Korea (enrollment: 2010-2011)	[37]	71 / 12276	Non-drinker vs. drinker	OR=1.89 (1.08-3.32)	the Korean National Health and Nutrition Examination Survey

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval.

b) Cigarette smoking

The IARC reported that cigarette smoking is a risk factor for many cancers [41]. However, controversial results were found for smoking and thyroid cancer, showing negative [22, 25, 31, 34, 42–45], null [19, 30, 33, 35], and positive association [46]. While conflicting results including null associations were reported (Table 2), the recent meta-analysis suggested that current smoking, may have a protective effects related to thyroid cancer unlike other types of cancers based on 25 case–control studies and six cohort studies [47]. Particularly current smoking possibly influence susceptibility to thyroid cancer based on the findings [47]. Although there was a report that smoke exposure may influence metabolic and biological mechanisms in thyroid hormone and possibly associated with adverse effects on the thyroid gland [48], the reason for the preventive effects of smoking on thyroid cancer have not been explained.

Most of those studies have only focused on active smoking [49]. In addition, cancer risk was greater in those who were exposed to passive smoking than those who were not [50–52]. However, the combined effects of active and passive smoking on

developing thyroid cancer have not been well documented.

Therefore, we investigated the effect of both individual's smoking behaviors (active smoking) and secondhand or environmental smoking (passive smoking) exposure on risk of papillary thyroid cancer (PTC) which accounts for more than 90% of thyroid cancer. We hypothesized that the early-age secondhand smoking exposure could affect the association between cigarette smoking and PTC in both the T-CALOS and the KMCC data. The associations between smoking and clinicopathological features (thyroid tumor, such as tumor size, lymph node metastasis, multifocality, cancer stage, and BRAF (V600E) mutations) were also explored in the T-CALOS data.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Cohort studies					
Iribarren, 2001 United States	[30]	73M+123F / 94,549M+110,415F after 19.9 years of follow-up	Never vs. former Never vs. current	RR=1.13 (0.75–1.70) RR=1.01 (0.71–1.42)	Kaiser Permanente Multiphasic Cohort
Jee,2004, Korea	[53]	271M / 830,139M+382,767F after 9 years of follow-up	Never vs. former Never vs. current	RR=1.6 (0.6-4.4) RR=1.2 (0.2-3.0)	Korea Cancer Prevention Study
Navarro Silvera, 2005, Canada	[19]	169F / 89,835F after 15.9 years of follow-up	Never vs. ever Never vs. former Never vs. current	RR=1.04 (0.76–1.43) RR=1.07 (0.74–1.54) RR=1.01 (0.67–1.53) *Null associations was observed for age started smoking, number of years smoked, number of cigarettes/day and pack-years	Canadian National Breast Screening Study
Kabat, 2012, United States (enrollment: 1993-1998)	[31]	331/ 159,340F after 13 years of follow-up	Never vs. ever Never vs. former Never vs. current	HR=1.08 (0.87–1.34) HR=1.16 (0.93–1.44) HR=0.54 (0.29–1.00) *Null associations was observed for amount (cigarettes per day), age started smoking (years), Duration of smoking (years), years since quitting and pack-years of smoking.	WHI study (Postmenopausal women)

Abbreviations: OR=odds ratio; HR=hazard ratio; RR=relative ratio; 95%CI=95% confidential interval; M=males; F=females; WHI=Women's Health Initiative.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Kitahara, 2012, United States (enrollment periods vary for each cohort, 1983-2009)	[22]	335M+ 668F / 384,433M+ 361,664F after 12.7 years of follow-up	Never vs. former (<20 cigarettes/day) Never vs. former (20+ cigarettes/day) Never vs. current (<20 cigarettes/day) Never vs. current (20+ cigarettes/day)	HR=1.02 (0.88–1.19) HR=0.89 (0.73–1.09) HR=0.69 (0.53–0.88) HR=0.68 (0.48–0.97)	A pooled analysis of the five prospective studies in the United States (NIH-AARP+ USRT+ AHS + PLCO+ BCDDP)
Blakely, 2013, New Zealand (enrollment: 1981-1986, 1996-2001)	[54]	165M+ 615F cases	Never vs. current	HR=0.76 (0.60–0.96)	New Zealand 1981 & 1996 censuses and cancer registry record

Abbreviations: HR=hazard ratio; 95%CI=95% confidential interval; M=males; F=females; NIH-AARP= National Institutes of Health-American Association of Retired Persons; USRT= US Radiologic Technologists Study; AHS=Agricultural Health Study; PLCO= Prostate, Lung, Colorectal, and Ovarian Screening Study; BCDDP= Breast Cancer Detection Demonstration Project

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Case-control studies					
McTiernan, 1984, United States (enrollment: 1974-1979 for cases and 1980-1981 for controls)	[55]	183F / 394F	Never vs. current	46.4% for cases and 51.0% for controls	Cases were from the western Washington state (diagnosed 1974-1979) and controls were from the population control women (1980-1981)
Ron, 1987, United States (enrollment: 1978-2980)	[56]	50M +109F / 76M + 209F	Nontobacco users vs. cigarettes only Cigars ever Pipes ever	OR=0.9 (0.5-1.4) OR=1.6 (0.3-7.9) OR=1.4 (0.3-6.1)	Cases were from the Connecticut Tumor Registry and controls (frequency matching) were from the general population in two ways: random-digit dialing techniques and Medicare rosters
Kolonel, 1990 United States (Hawaii) (enrollment: 1980-1987)	[57]	51M+140F / 113M+328F	Never vs. ever	M: OR=1.3 (0.6 - 2.7) F: OR=0.7 (0.5 - 1.1)	Cases were from the Hawaii Tumor Registry and controls the Health Surveillance Program of the Hawaii Department of Health.
Hallquist, 1993 Sweden (enrollment: 1980-1989)	[58]	123F+48M / 240F+85M	Never vs. previous smoking Never vs. current smoking	OR=0.6 (0.3-1.0) OR=0.6 (0.3-1.0)	Cases were from the Swedish Cancer Registry and controls were from the National Population Registry.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Wingren, 1993 Sweden (enrollment 1977-1987)	[59]	26M+149F / 200M+187F	Combustion smoke (leisure time): no vs. yes	OR=3.6 (0.7-24.0)	Cases were from the regional cancer registry of the area and controls were from the regional population Registers.
Galanti, 1996 Norway/Sweden (enrollment: 1985-1993)	[60]	191F / 341F	Never vs. ever Never vs. 16+	OR=0.69 (0.47-1.01) OR=0.71 (0.33-1.51)	Cases were from the National and Regional Cancer Registers and controls were matched from the Population Register.
Takezaki, 1996 Japan (enrollment: 1988-1993)	[32]	94F / 22666F	Never vs. ever	OR=0.6 (0.3-1.2)	Hospital-based case-referent study at Aichi Cancer Center Hospital in Nagoya, aged between 20-79 + controls were from the female outpatients without cancer

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Kreiger, 2000 Canada (enrollment:	[61]	331M+893F / 706M+1953F	Never vs. ever	Total: OR=0.72 (0.63-0.83) M: OR=0.71 (0.60-0.83) F: OR=0.77 (0.58-1.02)	Cases were from the provincial cancer registries and controls were from the general population)
			Age started smoking (years)<15 vs. >24	M: OR=0.97 (0.49-1.93) F: OR=0.83 (0.56-1.21)	
			Number of years smoked ≥10 vs. >30	M: OR=0.49 (0.31- 0.78) F: OR=0.55 (0.40-0.76)	
			Number of cigarettes per day ≥10 vs. >25	M: OR=0.82 (0.53-1.26) F: OR=0.46 (0.30, 0.70)	
			Pack-years: ≥4 vs. >25	M: OR=0.55 (0.36-0.83) F: OR=0.46 (0.33-0.64)	
			Years since stopped smoking	M: OR=0.92 (0.57-1.48) F: OR=0.73 (0.51-1.06)	
Rossing, 2000 United States (enrollment: 1988–1994)	[24]	410F / 574F	Never vs. current Never vs. number of years smoked among current smokers *Null associations was observed for age first smoked, number of years smoked and total pack-years smoked among the former smokers.	OR=0.5 (0.4-0.7) OR=0.5 (0.3-0.9)	Cases were from the Cancer Surveillance System and controls were from the county of residence using random-digit telephone dialing.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Memon, 2002 Kuwait (enrollment: 1981-1996)	[62]	238F / 238F	Never vs. ever	OR=2.1 (0.9-5.3)	Cases were from the Kuwait Cancer Registry and controls were matched to each case based on year of birth (3 years), gender, nationality and district of residence.
Mack, 2002, United States (enrollment: 1980-1983)	[63]	292F / 292F	Never vs. ever	OR=1.1 (0.7–1.7)	Cases were from the Los Angeles County population-based registry, the University of Southern California Cancer Surveillance Program and controls were recruited in case's neighborhood.
Mack, 2003, United States, Japan, China, Sweden, Norway, Northern Italy, Switzerland and Greece (enrollment: 1980-1997)	[33]	2725 (478M+2247F) / 4776 (1077M+3699F)	Never vs. ever Never vs. former Never vs. current	OR=0.7 (0.7–0.8) OR=0.9 (0.8–1.1) OR=0.6 (0.6–0.7)	A pooled analysis of 14 case–control studies conducted in the United States, Europe, and Asia

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Zivaljevic, 2004 Serbia (enrollment: 1996-2000)	[64]	204F / 204F	Never vs. the initiation of smoking was at a younger age <20 years old	OR=0.66 (0.50-0.90)	Cases were diagnosed and histologically confirmed thyroid cancer that underwent surgery at the Center for Endocrine Surgery or in the Institute of Oncology in Belgrade, and controls were from the Institute of Rheumatology of Serbia in Belgrade.
Guignard, 2007, New Caledonia (enrollment: 1993-1999)	[34]	323 (39M+293F) / 412 (58M+354F)	Never vs. former Never vs. current Never vs. Pack-year (>30)	M: OR=0.76 (0.20-2.83) W: OR=1.10 (0.66-1.84) M: OR=1.36 (0.44- 4.26) W: OR=0.96 (0.63-1.45) M: OR=1.58 (0.43-5.76) W: OR=0.87 (0.37-2.02)	Cases were from the two pathology laboratories of New Caledonia and confirmed with cancer registry and medical records, and controls were from the frequency matching in the community.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

Table 2. The previous results regarding the association between smoking and thyroid cancer risk (Continued)

Author, year, country (enrollment period)	Reference	Number of study subjects -cases/ total subjects at baseline of the cohort -cases/controls	Exposure categories	Outcomes, OR/ HR/ RR (95%CI)	Notes
Nagano, 2007, Japan (enrollment: 1970-1986)	[26]	57M+305F / 57M+305F	Never vs. past Never vs. current (≤15 cigarettes/day) Never vs. current (16+ cigarettes/day)	OR=1.39 (0.58-3.34) OR=0.53 (0.27-1.02) OR=0.33 (0.16-0.69)	Cases were from the tumor registries in Hiroshima and Nagasaki and controls were from controls from members of the Life Span Study or offspring cohort in Hiroshima and Nagasaki areas
Lence-Anta, 2014, Cuba (enrollment: 2000-2011)	[27]	203 / 229	Never vs. ever smoking	OR=0.6 (0.4 – 0.9)	Cases were selected from the National Cancer Registry Databases of Cuba and controls from the general population.
Stansifer, 2015, United States (enrollment: 2013)	[28]	467/ 255	Any tobacco use Smoked .100 lifetime cigarettes	OR=0.52 (0.34, 0.78) OR=0.68 (0.50, 0.94) For secondhand smoking, only p-value=0.63 was provided.	Cases were from the University of Nebraska Medical Center's Thyroid Tumor and Cancer Collaborative Registry and controls were from the Great Plains Health Informatics Database.

Abbreviations: OR=odds ratio; 95%CI=95% confidential interval; M=males; F=females.

c) Obesity

Overweight (body mass index, BMI:25−29.9 kg/m^2) or obese (BMI: 30 kg/m^2 or greater) has been a recognized as a serious public health issue for cancers in comparison with a normal range of BMI [65]. The rises in both thyroid cancer incidence and prevalence of obesity were observed in recent years, and obesity in general were positively associated with thyroid cancer while effects of BMI vary by tumor histologic type [66−69].

However, there are additional questions that could be answered in a large sample size of study subjects. Only a few studies assessed (1) the other measures of adiposity rather than BMI [70, 71], (2) obesity at a young age [70, 72] and (3) weight management status [73, 74], and as potential risk factors for developing thyroid cancer. Based on the previous suggestion of body surface area (BSA) as an important predictor of thyroid volume [75], and one study reported a significant association between BSA and the risk of thyroid while BMI and body fat percentage (BF%) did not show the statistical significance [70]. Therefore, in both the T−CALOS and the KMCC data, the association of obesity (BMI, BSA and BF%) measured at

enrollment and thyroid cancer were examined. In addition, in T-CALOS data, obesity at age 18-20 and marked weight gain in middle-aged adults after the age of 35 years were examined as predictors of thyroid cancer. And the associations were compared in subgroups by assessing differences in the effects by age, sex, chronic diseases and reproductive factors. The associations between obesity and clinicopathological features (thyroid tumor, such as tumor size, lymph node metastasis, multifocality, cancer stage, and BRAF (V600E) mutations) were also explored in the T-CALOS data.

4. Medical radiation and thyroid cancer

Approximately 15% of the ionizing radiation exposures to the general population comes from man-made sources, and most of them is by medical radiation, particularly from medical diagnostic procedures [76]. Benefits of medical radiography have been noticed, but adverse health effects of radiation exposure from the procedures also have been addressed. In particular, thyroid gland is considered one of the radiosensitive organs.

Evaluating low-dose radiation exposures of x-ray radiography, there were no significant association was found for the increased risk of thyroid cancer [77-79]. Although radiation exposure amount from x-ray radiography is relatively low (a range from 0.04 to 0.54 mSv), 45% of x-rays can be a source of unnecessary radiation to the individual's neck when the Chest and abdomen X-ray examination were taken [80]. Only a few studies evaluated thyroid cancer risk related to computerized tomography (CT) scanning, which has a higher radiation dose (100 mSv) than that of conventional x-ray radiography [81].

In addition, a previous study reported that the population

exposed to medical diagnostic radiography, a major source of anthropogenic radiation, has increased worldwide [82, 83]. The annual per capita effective radiation dose from medical diagnostic radiography was apparently increased in the United States (0.53 mSv in 1980 and 3.0 mSv in 2006) [82]. Although the International Commission on Radiological Protection developed recommendations and guidance to protect individuals from radiation exposures of medical diagnosis [84-86], related health effects from low-dose-rate radiation have not been demonstrated reliably explained, especially for Asians. In Korea, the radiation-generating devices for X-rays imaging, CT, dental x-ray and mammography were increased 21.57% from 2008 to 2012, based on the report of the Korea Food and Drug Administration [87]. Correspondingly, an apparent increases in the annual frequency of diagnostic radiography (54.4%), collective effective dose (81.5%), and annual per caput effective dose (73.9%) of in Korea were observed in 2013 compared to that in 2006 [88].

Previous studies assessed the associations between medical radiation and thyroid cancer and provided contradictory findings [77, 78, 89-92], and these studies were conducted in the

United States [89, 90], Kuwait [91], Sweden [77, 78, 92] and Australia [93]. Additionally, the results of each study were based on a case-control study [77, 78, 89], recruited study subjects for radiologic technologists [90], restricted to dental x-ray exposure for the exposure variable [91] or assessed risks for only female papillary thyroid cancer [92].

To investigate long-term adverse effects of medical diagnostic radiography in developing thyroid cancer in Korea, we used the KMCC data to evaluate the risk of thyroid cancer from exposure to medical diagnostic radiographic procedures including X-ray radiography, UGI, CT and mammography. We also assessed the combined exposure to more than one type of diagnostic radiography in relation to thyroid cancer risk.

5. Epigenetic profiles and thyroid cancer

Biomarker analysis using a blood sample for thyroid cancer prediction has not been extensively explored. One of the important diagnostic and prognostic biomarkers for cancer can be DNA methylation profiles [94–98], as DNA methylation may be involved in gene expression [99] and carcinogenesis-related mechanisms [100, 101]. DNA methylation, as one type of epigenetic mechanism, can changes the interactions between DNA and histones, which influence the degree of condensation of chromatin as well as gene expression without altering the DNA sequence [102]. The DNA methylation level is defined by counting the number of cytosine–guanine dinucleotides (CpGs) islands present at the transcription start site of a gene [102]. Approximately 60% of all promoters contain CpG islands, and the possibility of the coordinated regulation of transcription and replication has been suggested [103]. Methylation at CpG sites serves to stabilize the chromosome structures and cell differentiation progress [104]. Abnormal epigenetic alterations, hypermethylation in promoter regions or hypomethylation in oncogenes may be associated with genomic instability and the inactivation of tumor repressor genes [105, 106]. An aberrant

DNA methylation level can cause problems related to the activity or inhibition of cell signaling pathways, which may then cause endocrine-related cancers [107].

While information pertaining to targeted genomic sites for DNA methylation and thyroid cancer risk is limited, the hypo-methylation of tumor-suppressor genes including CDH1, PTEN, RASSF1A, and FGFR2 may be linked to the pathology of thyroid cancer [102]. In addition, the methylation statuses on the RASSF1A, RASSF2, Ras-association domain family signaling protein (RASSF10) genes [108, 109], tumor suppressor genes (TIMP3 [110], SLC5A8 [111], and DAPK [112]) have been discussed as methylation sites related to thyroid cancer.

Among endocrine tumors, a possible link between thyroid cancer and breast cancer was recently suggested in Koreans considering the co-existence of common etiological factors in breast cancer and thyroid cancer [113]. In particular, methylation of the BRCA1 gene promoter was posited as an indicator of invasive forms of breast cancer based on a previous reviews and meta-analysis [114, 115], and BRCA1 mutations have been detected in the epithelia of various tissues including

breast and thyroid gland tissues [116]. Furthermore, associations between BRCA1 functional single-nucleotide polymorphisms and thyroid cancer risk have been reported [117].

Although DNA methylation was recently suggested as a critical biomarker for thyroid tumor features [118-121], it is also noted for aggressiveness [122] and survival inferences [123]. Most of these previous findings were based on DNA methylation as measured in cancer patients' tissue samples. Although using DNA in blood samples is somewhat limited, it can be useful as an informative biomarker for not only thyroid cancer patients but also for those who have not undergone surgery or a biopsy to gain a thyroid tissue sample.

Silencing of tumor-suppressor genes by hypermethylation of their promoter regions is thought to occur in the early stages of tumorogenesis [124-126], which suggests that aberrant DNA methylation could be a useful early biomarker in pre-diagnostic samples. Moreover, tumor DNA fragments exist in circulating blood mostly during tumor necrosis apoptosis [127]. Although the previous results were from breast cancer patients, we have evidence that matched tumor and blood samples from the same

patient were in good agreement [128-131]. Considering the possible links to the promotion of genomic instability, thyroid cell transformation, dynamic epigenetic changes in tumor features, and non-invasive sample collection as an early detection marker, DNA methylation in a blood sample is significant for thyroid cancer research. This study uses a case-control setting to assess methylation levels as a blood-based biomarker for thyroid cancer and its related modifiable risk factors to test for a potential association.

6. Study aim and objectives

The principle aim was to evaluate environmental and epigenetic risk factors of thyroid cancer in Korea. For the three study objectives related to lifestyle factors (alcohol drinking, cigarette smoking and obesity), medical radiation and DNA methylation were set up as presented in Figure 2. To achieve the objectives, the seven hypotheses were tested as following:

7. Hypotheses

The T-CALOS data

Hypothesis 1: Alcohol drinking is associated with thyroid cancer.

Hypothesis 2: Cigarette smoking (active and passive) is associated with thyroid cancer.

Hypothesis 3: Obesity at enrollment is associated with thyroid cancer.

Hypothesis 4: Obesity at age 18-20 is associated with thyroid cancer.

Hypothesis 5: Weight change in middle-aged adults is associated with thyroid cancer.

The KMCC study data

Hypothesis 6: Exposure to x-ray, upper gastrointestinal series (UGI), CT and mammography are associated with thyroid cancer risk.

The subset of the T-CALOS samples

Hypothesis 7: DNA methylation levels measured in blood samples are different in the two groups, thyroid cancer cases and controls.

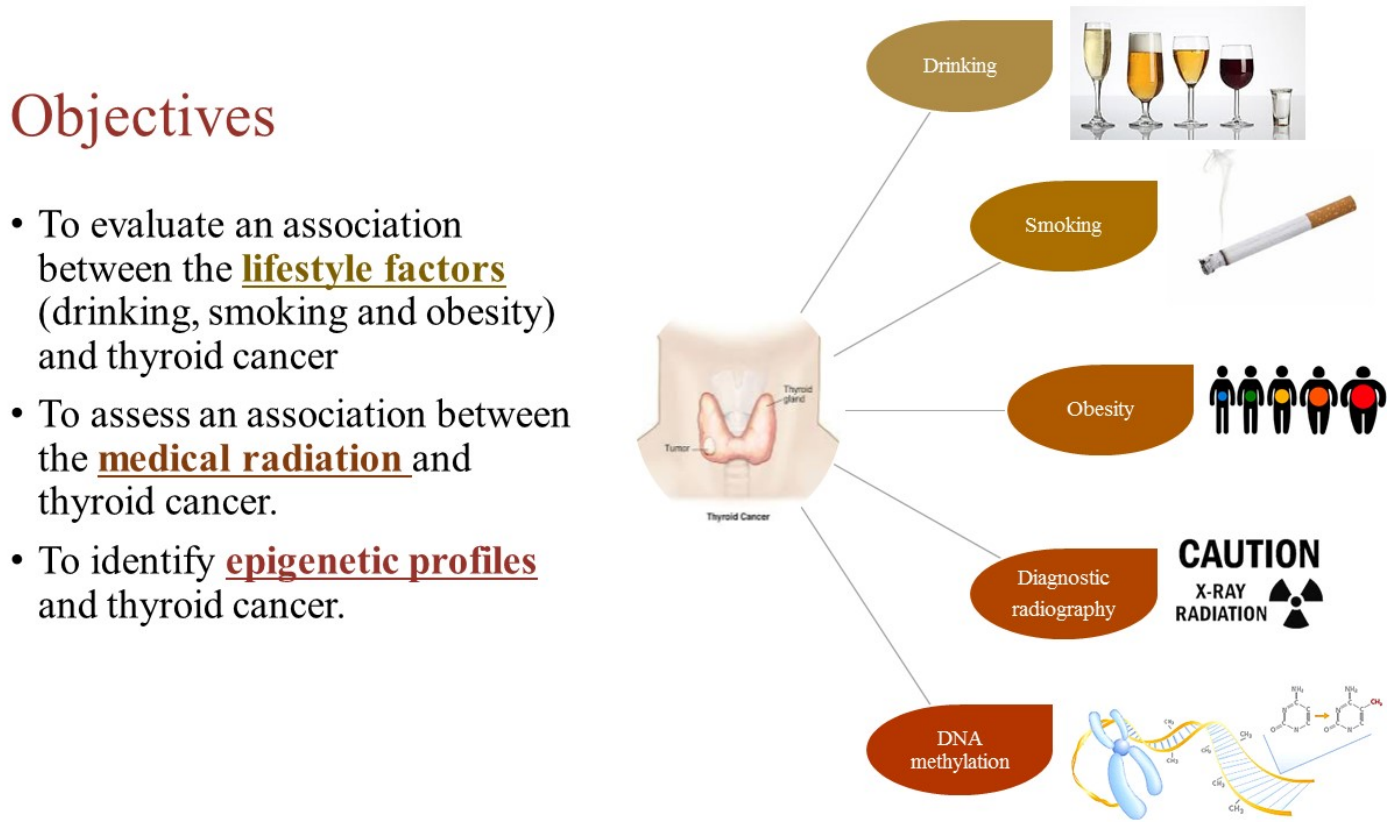

Figure 2 The objectives of thesis.

II. Materials and methods

1. The T-CALOS data

a) Study design

The Thyroid Cancer Longitudinal Study (T-CALOS) was initiated T-CALOS was established for the thyroid cancer research as previously described [132]. In 2010, epidemiologists and thyroid surgeons started this study at the Seoul National University College of Medicine and Seoul National University Hospital (SNUH). The outline of the T-CALOS data collection included in-person interviews for comprehensive epidemiologic data, sample collection of biospecimen, regular follow-ups in both active and passive manner and further clinical and pathologic data of thyroid cancer patients based one chart reviews (Figure 3).

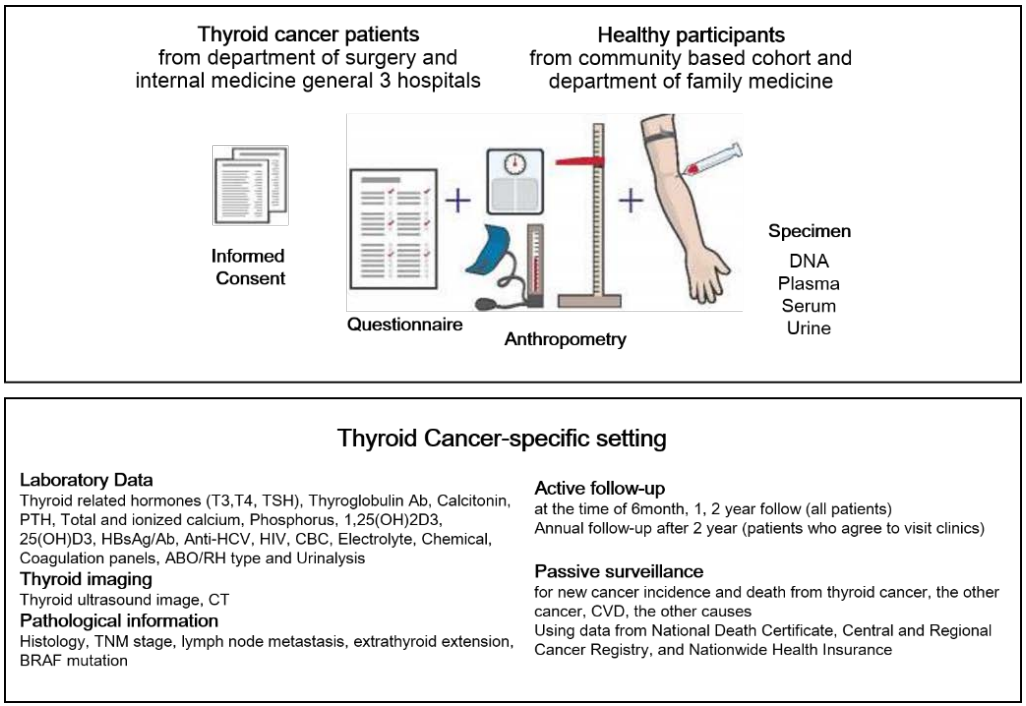

Figure 3 Study setting for data collection of T-CALOS study (Lee et al., 2015)

b) Eligible subjects for this study

Cases were thyroid cancer patients underwent surgical procedures at Department of Surgery in the participating hospitals or visited Department of Internal Medicine for treatments for thyroid cancer. The eligible criteria were those who were (i) diagnosed as thyroid cancer with clinico-pathologic information; (ii) at or over 20 years old; (iii) voluntarily agreed and signed an informed consent form for this study; (iv) agreed to donate blood and/or urine samples and (v) without any communicational difficulty for completing 30-minute interview. Controls, the healthy participants, were matched to each thyroid cancer patient using the community-based data, Health Examinee Study (HEXA) as a part of the Korean Genome and Epidemiology Study (KoGES) [133], and hospital-based health examines at Seoul National University Hospital and its affiliations. The entire study protocol was approved by the Institutional Review Board (IRB) of Seoul National University Hospital (IRB No. C-1001-067-307).

c) Selection of cases and controls

For the association between alcohol consumption, smoking and

DTC risks, we used the data from April, 2010 to April 2014, 2,529 newly diagnosed and pathologically confirmed thyroid cancer patients were included. After excluding those without information on drinking status, we included a total of 2,257 DTC patients (448 men and 1,809 women). We selected healthy controls from the HEXA data as a pool of controls examined from 2004 to 2013 (n=170,082). The HEXA is a part of Korean Genome and Epidemiology Study (KoGES), a large prospective population-based cohort by the Korean government (National Research Institute of Health (NIH), Centers for Disease Control and Prevention and the Ministry of Health and Welfare, Korea) for health research in Koreans [133]. The HEXA study data included health examinees under the National Health Insurance

Corporation at health examination centers, and they were aged at or over 35 years old for the study subjects. The two data sources for cases and controls were relatively comparable, because investors of T-CALOS designed and carried out study using an interview protocols and a standardized questionnaire as in the framework of the HEXA study. We excluded those subjects with a history of any type of cancer at enrollment

(n=795) or missing information for drinking status (n=1,552). We matched individuals at a 1:10 (cases: controls) ratio by age (no more than a 5-year difference), sex and enrollment year (no more than a 5-year difference), and selected 22,570 controls for this analysis (4,490 men and 18,090 women). For statistical analyses regarding impacts of drinking and smoking on thyroid cancer, we included 2,257 cases and 22,570 controls (Figure 4).

For the association between obesity and PTC risks, we used the data from April 10, 2010 to December 31, 2013. After excluding 25 PTC cases with missing information regarding their current and past body sizes, 1,551 PTC cases were included. Subjects with no available information regarding thyroid tumor size (n=15), multifocality (n=116), lymph node metastasis (n=116), TNM stage (n=91), BRAF (V600E) mutation (n=248) and chronic lymphocytic thyroiditis (n=385) were excluded from each association analysis. We included the subjects who provided a completed current measured body size and self-reported weight history. After excluding subjects with missing information for a weight history, we obtained a pool of potential controls (n=124,297). We performed 1:10 matching

by age at enrollment (within +/−5 years) and sex (women and men). Finally, we included 15,510 controls that matched to 1,551 PTC cases in the statistical analyses. The individual matching was performed by the greedy matching algorithm of the SAS program (GMATCH macro) [134].

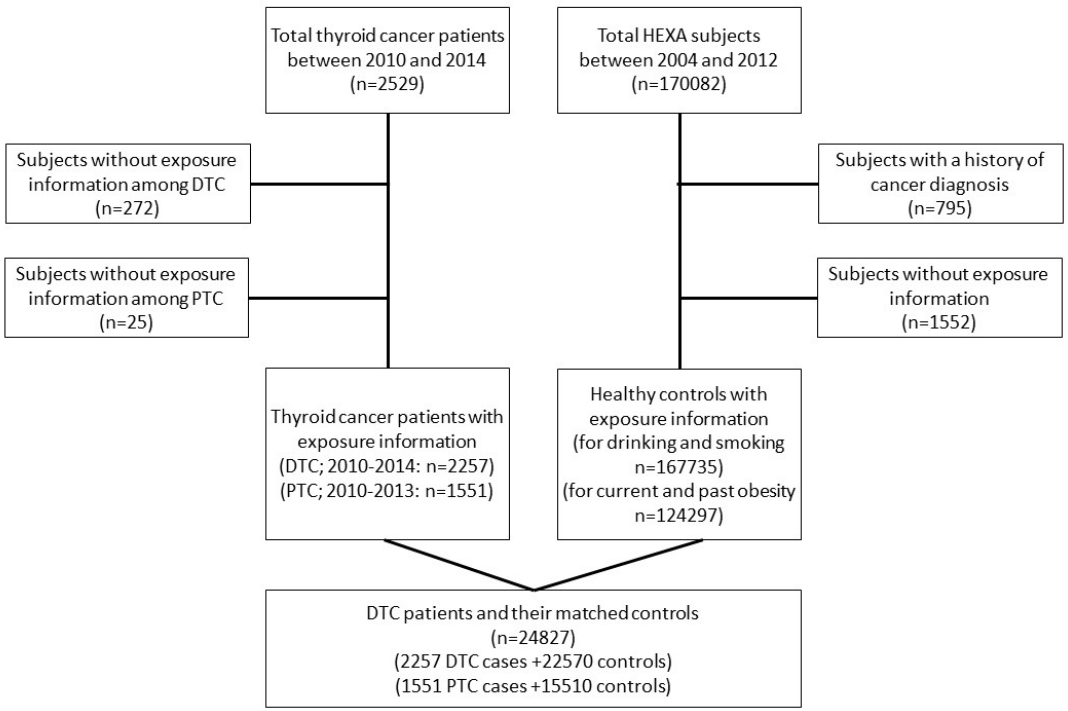

Figure 4 Study subjects of the T-CALOS study.

d) Data collection

Alcohol drinking

Status information for alcohol consumption was classified into two groups of "never-drinkers" and "ever-drinkers." The questionnaire included questions about the consumption of various types of alcoholic beverages, including beer, wine, gin, and four types of Korean traditional beverages (soju, rice wine, refined rice wine and fruit wine). The subjects selected one of 8 options for drinking frequency (almost never, once per month, 2-3 times per month, once per week, 2-3 times per week, 4-6 times per week, once per day and more than twice per day) for each type of beverage based on the alcohol consumption patterns of the previous year. The volume was defined for each type of drink as follows: beer (200 ml), wine (90 ml), hard liquor (30 ml), soju (50 ml), rice wine (250 ml), refined rice wine (50 ml) and fruit wine (50 ml). The amount (g) of alcohol consumption was calculated using an ethanol intensity of 0.79 and beverage-specific alcohol content (5% for beer, 12% for wine, 40% for hard liquor, 20% for soju, 6% for rice wine, 15% for refined rice wine and 15% for fruit wine). Alcohol consumption per event for the ever-drinkers was estimated as

the total amount consumed at a single occasion and was categorized into 5 groups (0–25 g, 26–50 g, 51–100 g, 101–150 g, and 151 g or more) or 3 groups (0–50 g, 51–150 g and 151 g or more). We further classified ever-drinkers by the drinking duration (0–10 years, 11–20 years, 21–30 years, 31–40 years, and 41+ years) and using generous cutoff values (0–20 years, 21–30 years, and 31+ years) because of the small number of subjects evaluated in subgroup analyses. After quantifying the reported alcohol consumption of the individuals, we divided the total alcohol consumption by the frequency of alcohol consumption to determine alcohol consumption per day (g/day). Binge drinking was defined as excessive alcohol consumption on a single occasion (5 drinks for men and 4 drinks for women), with a standard drink equal to 14 g of alcohol.

Smoking and thyroid cancer

For those who had ever smoked, information on the total duration of smoking (years), age of smoking initiation (years old), and number of cigarettes smoked per day was obtained. The smoking dose in terms of pack-years was then calculated

as follows: the number of packs (20 cigarettes per pack) per day multiplied by the duration of smoking (years). To assess secondhand smoking, we obtained information from the subjects on their current exposure to secondhand smoking at home or workplace (no or yes), exposure time (minutes/ day), and duration of secondhand smoking exposure (years). The age of exposure initiation (years old) was estimated using the subjects' age at enrollment and duration of exposure (years). Information was collected on early-age exposure to secondhand smoking: "Did you live with any member of the household who regularly smoked at home during your growing-up?" We restricted our analyses of secondhand smoking to never smokers to assess the effects of secondhand smoking alone.

To assess the association between smoking and thyroid risk, we classified subjects by exposure variables. Smoking dose (<20, 20+ person-years and unknown) and age of smoking initiation (25+, 20-24, <20 years old and unknown) were categorized with subjects who were never exposed to smoking as reference. For those who were exposed to secondhand smoking, the variable were categorized by daily exposure time

(<20, 20+ minutes and unknown) and smoking duration (1–9, 10+ years and unknown).

Obesity and thyroid cancer

Each subject’s BMI (kg/m^2) was calculated by dividing weight by height squared, while the BSA (m^2) was calculated using the equation: 0.007184 × height (cm)$^{0.725}$ × weight (kg)$^{0.425}$.[135, 136] Body fat percentage (BF%) was calculated using the equation: (1.20 × BMI) + (0.23 × age) − (10.8 × sex (1 for men and 0 for women)) − 5.40 [137]. The prediction formulas for BSA and BF were validated for effective physiological parameters in general [137–141], and specifically in Koreans [142, 143].

The self-reported past weights at age 18–20 were documented and BMI, BSA and BF% were calculated. Because the questionnaire used for the control subjects was revised from “weight at age 20 years” to “weight at 18 years” in 2007, 24% of the control subjects were imputed using their weight at age 20 years for their weight at age 18 years. The mean difference in the weight at age 18 years (52.83 kg) and the weight at age 20 years (52.86 kg) was evaluated (p-

value=0.85), and the imputation was evenly distributed between the men and the women (23.99% and 23.57%, respectively, p-value=0.63).

Weight at age 18 years were categorized into quartiles based on the distribution of the thyroid cancer patients. The cutoffs for weight (kg) at age 18 years were <63, 63-68, 69-73 and 74+ for males and <50, 50-63, 54-57, and 58+ for women. Overweight and obesity were classified based on their BMI (kg/m^2, <18.5, 18.5-24.9, 25.0-29.9, and 30+), which was defined by the World Health Organization (WHO) [144]. The cutoffs for BMI at age 18 years (kg/m2, men: 21.77, 23.42 and 25.10; women: 20.00, 21.45 and 23.00) were defined based on the quartile values of the thyroid cancer patients.

Current weight (kg) and height (m) were measured at enrollment, and the self-reported weight at the age of 35 years was recorded. The self-reported past weights at age 35 were documented and BMI, BSA and BF% were calculated. We used the cut-off value of 0.8 kg/year for the highest quartile of annual average weight change. The number was rounded to yield the cutoff, 1 kg/year in decreased or increased weight, and was doubled to yield the highest cutoff value, 2 kg/year.

Weight changes since the age of 35 years were categorized as the four groups (i. weight loss of 5 kg or more; ii. stable weight, with changes of less than 5 kg; iii. weight gain between 5.0 kg and 9.9 kg; and iv. weight gain of 10 kg or more). We calculated the annual average weight change by dividing the total weight change by the difference between the age at enrollment and 35 years as the annual average weight change. These calculated values classified subjects for the four groups (i. decreased weight of 1.0 kg/year or more; ii. stable weight, with changes of less than 1.0 kg/year, iii. increased weight between 1.0 kg/year and 1.9 kg/year; and iv. increased weight of 2.0 kg/year or more).

Under the assumption that middle-aged adult height is maintained, height at enrollment was used to calculate the BMI, BSA, and BF% at age 35 years. For each of these obesity indicators (BMI, BSA, and BF%), we calculated annual average changes using the following equation: obesity indicator value at enrollment − obesity indicator value at age 35 years) / difference between enrollment age and age 35 years. For the annual average BMI change, the subjects were assigned to one of the four groups (i. a decrease in BMI of 0.1 kg/m^2/year or

more; ii. stable BMI, with changes of less than 0.1 kg/m^2/year; iii. an increase in BMI between 0.1 kg/m^2/year and 0.2 kg/m^2/year; and iv. an increase in BMI of 0.3 kg/m^2/year or more). The annual average BSA changes were assigned to one of the four groups (i. a decrease in BSA of 0.005 m^2/year or more; ii. stable BSA, with changes of less than 0.005 m^2/year; iii. an increase in BSA of between 0.005 m^2/year and 0.009 m^2/year; and iv. an increase in BSA of 0.010 m^2/year or more), as were the annual average BF% changes (i. a decrease in BF% of 0.1%/year or more; ii. a stable BF%, with changes of less than 0.1%/year; iii. an increase in BF% of between 0.1 %/year and 0.2%/year; and iv. an increase in BF% of 0.3%/year or more). We defined the cutoffs for the three indicators, the annual average change in BMI, BSA and BF%; however, we used the median value to ensure a minimum of 5 subjects and the statistical power of the study. The associations between these indicators and the PTC incidence, the group with a stable average annual change (changed <0.1 kg/m^2/year for BMI; changed <0.005 m^2/year for BSA; and changed <0.1%/year for BF%) was used as the reference group.

Clinicopathologic features of thyroid cancer patients

For all cases, medical records were reviewed for determine clinicopathologic features. We used the American Joint Committee on Cancer and the Union for International Cancer Control (AJCC/UICC) TNM staging system (7th edition), which is based on age at diagnosis, tumor size, presence of an extrathyroidal extension, lymph node metastasis and distant metastasis [145]. Genetic testing for the BRAF (V600E) mutation was performed on PTC patients as previously addressed [132, 146].

e) Statistical analyses

For descriptive statistical analyses, differences in numerical and categorical variables were evaluated with the t-test and chi-square test, respectively. Conditional logistic regression models were used to compute odds ratios (ORs) and 95% confidence intervals (95%CIs) for both the univariate and multivariate models. The dependent variables included tumor size, lymph node metastasis, extrathyroidal extension, TNM stage, age at diagnosis and V600E BRAF mutation status in the three nominal categories (the controls, the cases with low

aggressive tumor features and the cases with high aggressive tumor features). The associations between drinking-related predictors and the clinicopathologic features were presented as ORs and 95%CIs based on polychotomous or unconditional logistic regression models.

To identify interactions, a multiplicative interaction model was used based on the likelihood ratio test, for which the main factor of alcohol exposure and the variables of education level, marital status, smoking and chronic diseases were included. Sensitivity analyses were conducted by restricting the age at diagnosis of the cases (40, 45 and 50 years old) to confirm the robustness of the results. We calculated p-trends for the dose-response associations by assigning increasing scores for the levels of the categorical variables, and these scores were used in the fully adjusted regression models. The p-heterogeneity for the comparisons of the associations in each group was calculated using Cochran's Q statistics. A p value of less than 0.05 was considered significant based on the 2-sided test. Statistical analyses were conducted with SAS software program (Version 9.4, SAS Institute, Cary, NC).

Covariates in the regression models

For the analyses of alcohol consumption and thyroid cancer, the regression models were adjusted for education level (high school graduation: yes, no, or unknown), marital status (single, married, or unknown), smoking (never, past, or current), regular exercise (yes, no, or unknown), and history of chronic diseases, including hypertension and dyslipidemia (diagnosed by a medical doctor: never, ever or unknown). No evidence of multicollinearity was found based on diagnostic analysis. In case of using polychotomous regression models, we used covariates for matching variables (age, sex and enrollment year), education level, marital status, smoking, regular exercise, and history of chronic diseases, including hypertension and dyslipidemia.

For the associations between smoking and thyroid cancer, differences of general characteristics between case and control were compared and we further evaluated associations with smoking behaviors and secondhand smoking (ever/never). The BMI (<25, ≥25, or unknown), education (high school graduation: no, yes, or unknown), marital status (single, married, or unknown) and history of chronic disease

(hypertension or dyslipidemia) were determined as covariates ($p< 0.05$).

For obesity and thyroid cancer, based on the significantly different distributions of the selected variables in the two groups (cases and controls, $p<0.05$), we adjusted the ORs for education (high school graduation: yes, no, and unknown) marital status (single, married, and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever-pregnancy (yes, no and unknown).

2. The KMCC study

a) Study design

The Korean Multi-center Cancer Cohort (KMCC) is a prospective cohort to examine the association between exposures to environmental factors, lifestyle factors, reproductive factors and previous health conditions in relation to cancer risk in Korea. The first enrollment started in 1993 and a total of 20636 study subjects were included in the baseline data until the year of 2005. Eligible criteria were defined as adults, male and female, aged over 35, and who were voluntary participants in an interview and health examination in the four regions (Choongju, Haman, Uljin and Pohang City). The entire research was based on a standardized cancer screening protocol and standardized questionnaire. The ascertainment of cancer occurrence was performed with a data linkage to the two sources of national database, Korea Central Cancer Registry provided by National Cancer Center and National Death Certificate databases provided by Statistics Korea, which is updated annually and covers 97.7% of all cancer cases in Korea [10, 147]. As of December 2013, we finally identified 3755 deaths and 2293 cancer cases.

b) Study subjects

Briefly, the KMCC study is a prospective, community-based, cohort study of subjects enrolled between 1993 and 2005 in Korea [148]. The cohort was designed to evaluate the etiology of cancer, as previously described [148, 149]. Eligible subjects were defined as adults aged over 35 in the four urban and rural regions (Haman, Chungju, Uljin, and Pohang) in Korea [148]. Providing cancer screening and general health checkups, the study recruited volunteers and included only those who had signed a written consent form. Comprehensive in-person interviews were conducted by using a structured questionnaire to determine demographic, lifestyle, and reproductive factors, as well as access to medical services and health condition [150, 151]. From the 20,636 possible study subjects (8235 men and 12401 women) at the baseline, we excluded 622 subjects who self-reported any type of cancer history at their enrollment (Figure 5). Using the data linkage to the national databases, we verified and excluded 37 subjects those who had a record of cancer (10 subjects for thyroid cancer and 24 subjects for other types of cancer) or those who had an error for data

linkage (3 subjects).

Our study protocol was reviewed and approved by the institutional review board (IRB) of Seoul National University Hospital (IRB number: 1405-018-577).

For the analyses of medical radiation and thyroid cancer risk, we excluded 973 subjects with no information on their medical diagnostic radiography status for X-ray, upper gastrointestinal series (UGI), CT and mammography. To focus on radiation exposures from diagnostic radiography, 11 subjects who had possibilities of occupational radiation exposure (4 workers in a nuclear power plant and 7 radiographers in a hospital) were excluded. Among a total of 18993 study subjects, we found 106 thyroid cancer cases (15 men and 91 women) as in Figure II-2-2. Nine deaths among the cases were identified, and 7 subjects (2 men and 5 women) had died as a result of thyroid cancer, and 2 subjects (2 women) died from other causes (Figure 5).

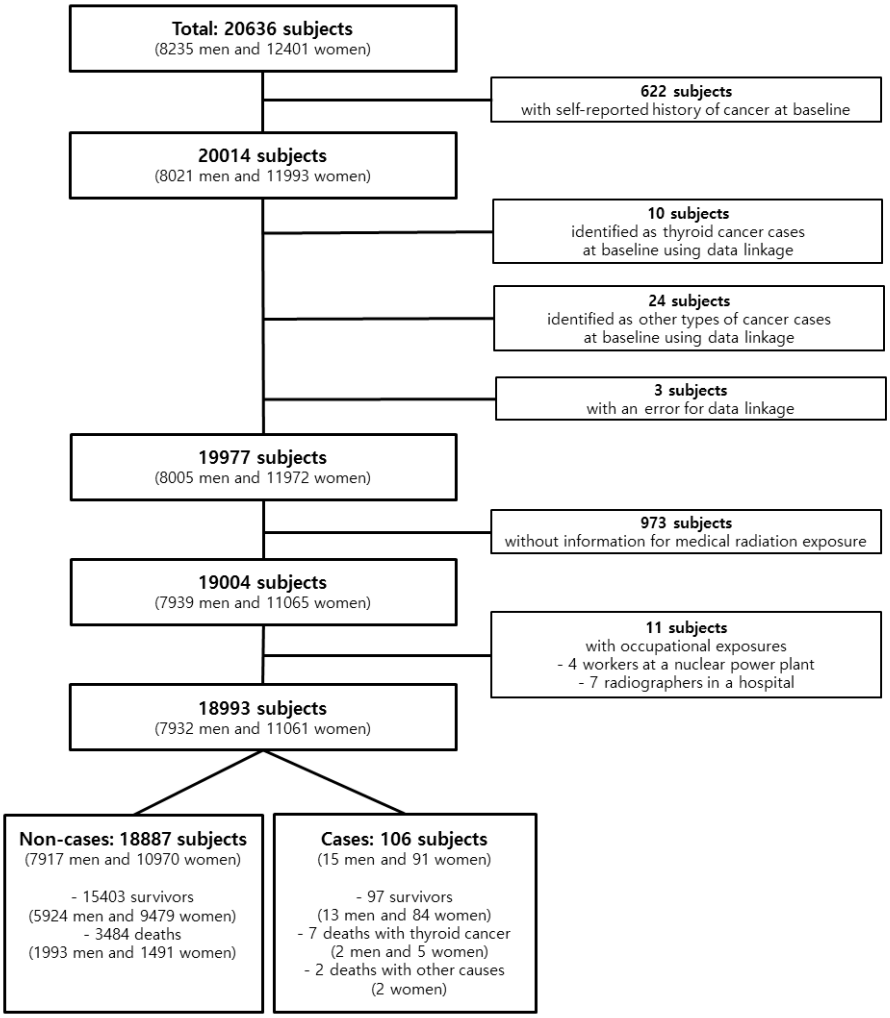

Figure 5 Study subjects for analyses for medical diagnostic radiography and thyroid cancer, the KMCC data.

c) Assessment of exposure status

The study subjects provided self-reported information on whether they had undergone medical diagnostic radiography including X-ray radiography, UGI, CT and mammography. The subjects who selected "exposed" to at least one type of medical diagnostic radiography were asked to indicate the total frequency of each medical diagnostic radiographic procedure during their lifetime (frequency of exposure: 1, 2-5, 6-10, 11-15, 16-20, 21+ and unknown). We re-categorized those responses into the 5 groups for X-ray (1, 2-5, 6-10, 11+ and unknown) and the 3 groups for UGI, CT and mammography (1, 2+ and unknown). After checking correlations among procedures, we categorized the exposed subjects into the 7 groups based on exposure combinations (exposed to X-ray only; exposed to X-ray and UGI; exposed to X-ray and CT; exposed to X-ray and mammography; exposed to X-ray and two additional types of radiation; exposed to X-ray, UGI, CT, and mammography; and other combinations of radiation exposure types).

d) Case ascertainment via data linkage

We have annually followed up the subjects using data linkage to the 2 sources of national database, Korea Central Cancer Registry provided by National Cancer Center and National Death Certificate databases provided by Statistics Korea, which is updated annually and covers 97.7% of all cancer cases in Korea [10, 147]. Cancer cases were classified according to the International Classification of Diseases (ICD), 10th edition (ICD-10). In this study, code C70 was used for thyroid cancer diagnosed as a primary cancer. To classify thyroid cancer by histologic type, we used ICD-O-3 codes to define papillary thyroid cancer (PTC; ICD-O-3 codes 8050, 8260, 8340, 8341, 8342, 8343, 8344, and 8450), follicular thyroid cancer (FTC; ICD-O-3 codes 8330, 8331, 8332, 8335, and 8290), medullary thyroid cancer (MTC; ICD-O-3 codes 8345, 8346, and 8510) and mixed medullary-papillary carcinoma (ICD-O-3 code 8347). The last follow-up date of these records for this study was December 31, 2013. There were106 cases of incidental thyroid cancer (15 men and 91 women) reported during 228691 person-years and the mean follow-up period was 12 years. We confirmed the histologic types of 90 of those cases

(82 PTC, 5 FTC, and 3 MTC; 4 cases had mixed medullary–papillary carcinomas). The remaining 12 cases were of an unspecified histologic type.

e) Potential confounders

We reviewed previous literatures for observed and hypothetical associations for thyroid cancer risk and the exposure of interest, medical diagnostic radiography. The selection of covariates for adjustment was based on an uneven distribution in the two exposure groups as well as the outcome groups.

For the analyses related to alcohol consumption, basic data (age, enrollment year, sex, and residential area), smoking (never, ever and unknown), history of benign thyroid disease (no, yes and unknown) and menopausal status (premenopausal, postmenopausal and unknown) were used as the adjustment variables for the regression models. For the analyses related to smoking and obesity, basic data (age, enrollment year, sex, and residential area), benign thyroid disease (no, yes and unknown) and menopausal status (premenopausal, postmenopausal and unknown) were used as the adjustment variables for the regression models. For potential confounding variables for the

analyses related to medical radiation, we considered the acquired basic data (age, enrollment year, sex, and residential area) and socioeconomic status information (education, occupation, and marital status) as potential confounders. Alcohol consumption (ever vs. never), smoking (ever vs. never), BMI, chronic disease history (benign thyroid disease, diabetes mellitus and hypertension) and female reproductive factors (pregnancy and menopausal status) were also used for covariates in the statistical analyses.

f) Statistical analyses

Mean and standard deviation (SD) values for age at enrollment and a follow-up duration were calculated for two groups: exposed and unexposed to medical diagnostic radiation. The mean difference between the two groups was assessed by using Student's t-test. Chi-square testing was used to compare distributions of other categorical variables in the two exposure status groups.

For the analyses related to medical radiation, the general characteristics of the study subjects were compared by sex, BMI (<25 kg/m2 vs. 25+ kg/m2), education (years of schooling:

<12 years vs. 12+ years), cigarette smoking (never vs. former and current), alcohol drinking (never vs. former and current), marital status (single vs. married). In addition, the subjects' disease histories (never- vs. ever-diagnosed by a medical doctor) of benign thyroid diseases, diabetes mellitus, hypertension were compared. We also compared family cancer history (no vs. yes) and female reproductive factors including pregnancy (never vs. ever) and menopausal status (premenopausal vs. postmenopausal). We tested each of those characteristics to determine their level of association with the outcome variable for decisions on the covariates to include only selected variables (p-value < 0.05 for both exposure and outcome) in the final regression model.

After checking that the proportional hazard assumption was satisfied by exhibiting parallel predictor curves on a log-log plot across the analysis period, we used Cox proportional hazard regression models to estimate hazard ratios (HRs) and 95% confidential intervals (95%CI) of thyroid cancer risk attributed to medical diagnostic radiography. For the follow-up duration, we used the time-to-event from the date of enrollment to the endpoint, the dates of cancer incidence, death

or the last date of follow-up (December 31, 2013). The HRs were robust for unadjusted, adjusted for two variables (age and sex), and fully adjusted for the potential confounders of age, enrollment year at baseline, sex, BMI, smoking, alcohol drinking, benign thyroid disease, menopausal status and the other procedures of medical diagnostic radiography. We adjusted models with medical radiation exposures in a mutually exclusive way for each main exposure variable. For example, when we examine the association between X-ray and thyroid cancer risk, we adjusted with other 3 types of exposure including UGI, CT and mammography. Similarly, we only present the results obtained from the final adjusted model in this paper. After arranging each categorized exposure variable as a continuous in the regression model, we calculated p-trends for the dose-response associations.

We also examined potential effect modifiers in the two steps. Firstly, we included an exposure variable and a potential effect modifier both individually and in combination as a multiplicative interaction term in Cox proportional hazard regression models, and checked a p-value of each interaction term. Secondly, we preformed subgroup analyses by baseline age (<50 vs. 50+

years old), sex (men vs. women) and menopausal status (premenopausal vs. postmenopausal women). Values of p-heterogeneity were calculated by using Chi-square tests to compare adjusted HRs and their 95%CIs between subgroups.

For sensitivity analyses, we considered different latent periods and the possibility that the reported radiation exposure was for diagnosis of thyroid cancer. Therefore, we estimated the associations of medical diagnostic radiography and thyroid cancer risk in different follow-up durations. Consistencies among results were checked for subjects with at least 2 years (102 cases), 5 years (81 cases), 7 years (75 cases), and 10 years (52 cases) of follow-up assessment. Secondly, the HRs for the two thyroid cancer subtypes were calculated for DTC and PTC, and we confirmed the consistency. Thirdly, we observed subjects without a frequency of each exposure variable did not significantly change the associations. To compare and confirm the robustness of the results, we assigned the subjects without frequency information into the low-frequency exposure group and the high-frequency exposure groups. To check possibilities of selection bias for defining study subjects, we compared the characteristics in the two

groups, included and excluded as study subjects. The statistical significance of p-value (0.05) was 2-sided. All data analyses were performed by using SAS version 9.4 (SAS Institute, Cary, NC, USA).

3. The subset for DNA methylation study

a) Study design

Histologically confirmed thyroid cancer patients were recruited at Seoul National University Hospital in Korea, which is a part of a longitudinal study of thyroid cancer (T-CALOS), with the ongoing project conducted since 2010, as previously described [132]. We used samples of thyroid cancer patients recruited at the Seoul National University Hospital between 2010 and 2011. For the controls, we recruited healthy subjects from the Kangwon University Hospital (April 2010 to December 2010) and Seoul National University Bundang Hospital in Korea (May 2010 to April 2011). The excluded subjects and the reason in each case are presented in Figure 6. From all health examinees, we defined subjects without a history of thyroid disease or thyroid cancer as the potential control group, and we matched them considering the age group (30-39, 40-49, 50-59 and 60+ years old) and sex (men and women) to a thyroid cancer patient a based on a 1:1 ratio. In this process, we included 90 cases and 90 controls for DNA methylation analyses.

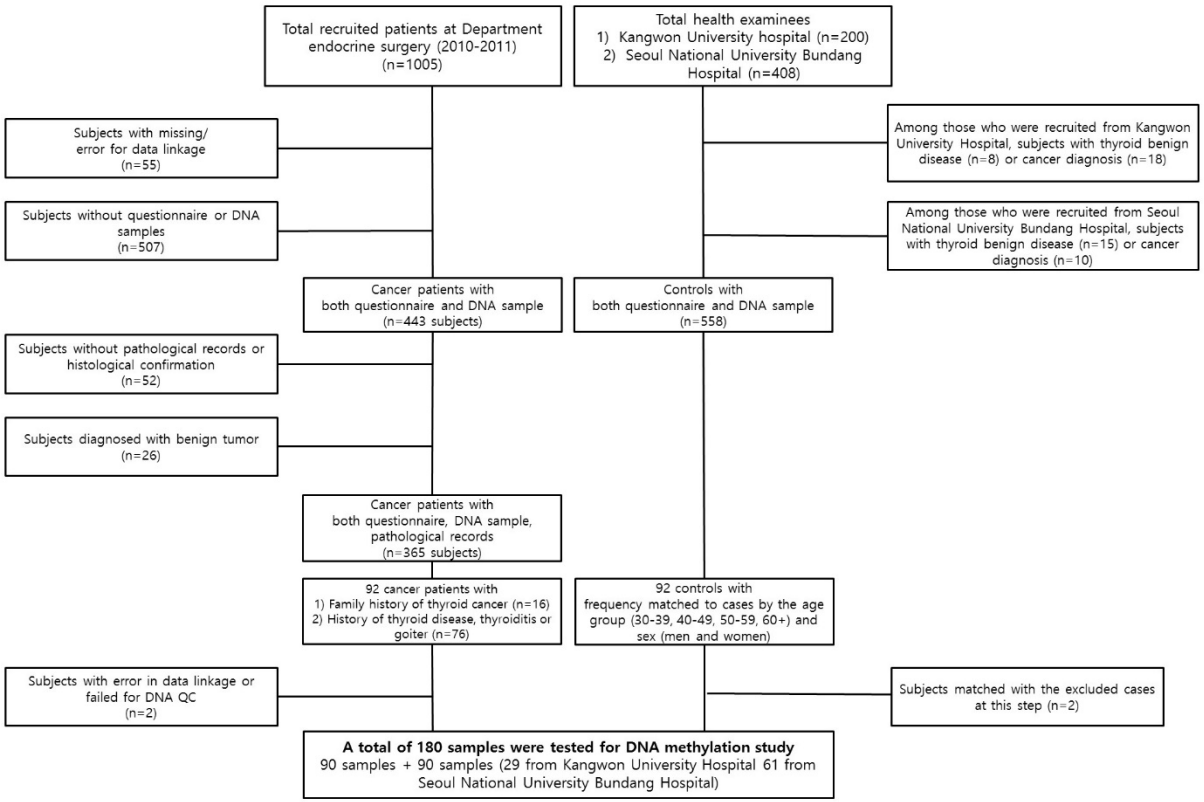

Figure 6 The selection process for the subset of T-CALOS for DNA methylation study.

b) Sample collection

Each study subject donated a fasting blood sample at enrollment. We extracted DNA from the collected blood samples within 24 hours and preserved them as an aliquot in a freezer maintained at −70℃. For DNA bisulfite conversion, we bisulfite−treated the NDA samples (500ng) with EZ DNA methylation kits (Zymo Research, Orange, CA). The bisulfite DNA was suspended in 20 μL of distilled water and stored at −20 ℃ until use. For the thyroid cancer patients, we reviewed medical records for detail information regarding clinical and pathological characteristics.

c) Loci selection and DNA methylation analyses

We undertook a comprehensive literature review and selected the target CpG sites from the most hyper or hypomethylated sites related to endocrine cancers. We used the results from a previous global methylation study of 24 samples (12 BRCA mutation careers and 12 non−careers: 4 hereditary breast cancer patients, 4 sporadic breast cancer patients and 4 familial controls for each group by mutation status) at the discovery stage. Briefly, the investigators in the previous study conducted multiple analyses to compare carriers and non−carriers (5

times within for the groups of hereditary and sporadic breast cancer patients) and sporadic and normal control groups(5 times), scoring and selecting the methylation sites for a customized methylation chip after sorting the results according to the p-values of less than 0.2, as follows: (i) score=1 for |Delta_mean| > 0.1 and 0.1<=p<0.2 for gene cites (ii) score=2: |Delta_mean| > 0.1 and 0.05<=p<0.1 for gene cites; and (iii) score=3: |Delta_mean| > 0.1 and p-value <0.05 for gene sites. In the next stop, the gene was selected based on a frequency of three or more significant p-values: ((i) score=1: 0.1<=p-value<0.2; (ii) score=2: 0.05<=p-value<0.10; and (iii) score=3: p-value<0.05. Based on the 96 selected sites, the DNA methylation level was measured using an Illumina GoldenGate assay with BeadArray technology, for which the arrays were customized to cover the 96 selected CpG sites and the scores were recorded with β values ranging from 0 to 1.

d) Statistical analyses

Quality checks of the DNA samples for the methylation analyses were confirmed and the data were normalized based on a box-plot, a density plot, a level plot and a

multidimensional (MDS) plot. The study subjects were grouped into the 2 different methylation groups, and the median values of the methylation level were compared for all control samples. The differences between the selected CpG sites were also assessed. We used the χ 2 test and/or student's t-test generally to assess the differences in the selected variables between the groups. Non-parametric methods including Mann-Whitney U test and Fisher's exact test were used when necessary. All statistical analyses were conducted with R software version 3.2.5 in conjuction with SAS software 9.4 (SAS Institute, Cary, NC).

III . Results

1. The T-CALOS data

a) Alcohol consumption and thyroid cancer

General characteristics

The general characteristics of the 24,827 total subjects including the 2,257 DTC cases and 22,570 controls were presented in Table 3. Among the total subjects, the mean age was similar (50.29 years old for cases and 50.71 for controls) and 80% were women. A higher percentage for the education level at or above high school graduation (83.70% vs. 74.41%; $p<0.05$) was observed. The proportion of postmenopausal women were not different between the cases and controls, but the cases were more likely to have chronic diseases (hypertension [22.86% vs. 16.70] and dyslipidemia [16.61% vs. 10.24%]) compared with the healthy controls. The patient group was less likely to be married, smoke, and exercise regularly ($p<0.05$) compared with the control group.

Table 3. Comparison of the general characteristics of cases and controls, T-CALOS April 2010–April 2014

	Cases (N=2,257)	Controls (N=22,570)	*p*-value[1]
Age (*Mean (SE)*)	50.29 (0.21)	50.71 (0.06)	0.055
BMI (*Mean (SE)*)	23.45 (0.07)	23.59 (0.02)	0.049
	n (%)	*n (%)*	
Gender			
Men	448 (19.85)	4,480 (19.85)	1.000
Women	1,809 (80.15)	18,090 (80.15)	
Education level			
Less than high school	361 (15.99)	5,636 (24.97)	<0.001
High school or more	1,889 (83.70)	16,794 (74.41)	
Unknown	7 (0.31)	140 (0.62)	
Marital status			
Not married	137 (6.07)	902 (4.00)	<0.001
Married	2,115 (93.71)	21,644 (95.90)	
Unknown	5 (0.22)	24 (0.11)	
Smoking			
Never	1,877 (83.16)	18,008 (79.79)	<0.001
Past	256 (11.70)	1,932 (8.56)	
Current	124 (5.49)	1,953 (8.65)	
Unknown	0 (0.00)	677 (3.00)	
Regular exercise			
No	1,279 (56.67)	11,258 (49.88)	<0.001
Yes	974 (43.15)	11,299 (50.06)	
Unknown	4 (0.18)	13 (0.06)	
History of hypertension			
No	1,739 (77.05)	18,799 (83.29)	<0.001
Yes	516 (22.86)	3,770 (16.70)	
Unknown	2 (0.09)	1 (0.00)	
History of dyslipidemia			
No	1,871 (82.90)	20,255 (89.74)	<0.001
Yes	375 (16.61)	2,311 (10.24)	
Unknown	11 (0.49)	4 (0.02)	
History of diabetes mellitus			
No	2,115 (93.71)	21,342 (94.56)	0.092
Yes	142 (6.29)	1,228 (5.44)	

Abbreviation: SE=Standard Error; BMI=Body Mass Index.

1. Comparison of healthy controls and thyroid cancer patients, and the *p*-values were based on *t*-test for continuous variables and Pearson's Chi-square test for categorical variables.

Alcohol intake and DTC

As observed in Table 4, not a significant association between drinking itself and DTC risk was found (men: OR=1.12; 95%CI=0.85−1.47; women: OR=1.10, 95%CI=0.99−1.22). J−shaped curves for the associations between alcohol intake per event and DTC risk were found. In other words, this indicated a decreased DTC risk for light to moderate alcohol consumption and an elevated risk for heavy alcohol consumption. Heavy alcohol consumption per event (151+ g) was a significant risk factor for DTC in men (OR=2.21, 95%CI=1.27−3.85) and women (OR=3.61, 95%CI=1.52−8.58) compared with the never−drinkers (Table 4). Stratification by histologic type revealed that the association between PTC risk and alcohol consumption on one occasion (151+g, men: OR=2.17, 95%CI=1.23−3.83; women: OR=3.56, 95%CI=1.50−8.46) was consistent with the results for total DTC, as shown in (table not shown). A trend of increasing FTC risk in association with alcohol intake on one occasion was observed, but the results were not statistically significant (table not shown).

Table 4. Alcohol consumptions and differentiated thyroid cancer in men and women, T-CALOS April 2010–April 2014

	Men			Women			
	Case (N=448)	Control (N=4,480)	OR (95%CI)[1]	Case (N=1,809)	Control (N=18,090)	OR (95%CI)[1]	*p*-heterogeneity[2]
Drinking status							
Never	78	828	1.00 (Reference)	1,111	11,322	1.00 (Reference)	
Ever	370	3,652	1.12 (0.85-1.47)	698	6,768	1.10 (0.99-1.22)	0.884
Alcohol intake (g) per event							
Never	78	828	1.00 (Reference)	1,111	11,322	1.00 (Reference)	
0-25	46	636	0.74 (0.50-1.11)	310	3,832	0.86 (0.75-0.99)	0.502
26-50	44	619	0.77 (0.52-1.15)	119	1,389	0.90 (0.74-1.10)	0.503
51-100	170	1,599	1.25 (0.92-1.69)	109	937	1.29 (1.03-1.60)	0.911
101-150	35	338	1.18 (0.76-1.84)	13	70	2.27 (1.23-4.21)	0.091
151+	20	108	2.21 (1.27-3.85)	7	24	3.61 (1.52-8.58)	0.353
p-trend[2]			*0.005*			*0.044*	
Duration (years)							
Never	78	828	1.00 (Reference)	1,111	11,322	1.00 (Reference)	
0-10	10	172	0.46 (0.22-0.98)	121	1,853	0.71 (0.58-0.86)	0.282
11-20	107	1174	0.84 (0.59-1.19)	245	3,020	0.81 (0.69-0.94)	0.853
21-30	106	1295	0.81 (0.58-1.13)	195	1,436	1.43 (1.21-1.69)	0.003
31-40	95	767	1.57 (1.09-2.26)	61	308	2.18 (1.62-2.92)	0.183
41+	43	224	3.44 (2.05-5.78)	13	45	2.71 (1.40-5.24)	0.571
p-trend[2]			*<0.001*			*<0.001*	

Abbreviations: OR=odds ratio; 95% CI=95% confidence interval.

1. Conditional logistic regression models adjusted for education level, marital status, smoking, regular exercise, and history of hypertension and dyslipidemia.

2. *p*-heterogeneity was calculated to compare of the ORs and their 95% CI for the men and women.

Drinking duration and DTC

The DTC risk was generally lower for those subjects reporting 10 years or less of alcohol consumption (men: OR=0.46, 95%CI=0.22−0.98; women: OR=0.71, 95% CI=0.58−0.86) as in Table 4. However, those reporting 31−40 years of alcohol consumption showed a 2−fold increased risk of DTC (men: OR=1.57, 95%CI=1.09−2.26; women: OR=2.18, 95%CI=1.62−2.92) compared with the never−drinkers. A 3−fold increased risk of DTC was observed in subjects reporting 41 years or more of alcohol consumption (men: OR=3.44, 95%CI=2.05−5.78; women: OR=2.71, 95%CI=1.40−5.24) (p−trend<0.05) compared to the never−drinkers (Table 4). Our results presented that women were more susceptible to drinking duration compared to men (p−heterogeneity for 21−30 years of drinking=0.003), whereas both men and women showed similar patterns for the association of alcohol consumption per event with drinking duration (Table 4). The general trend of these results was consistent across the age groups 40, 45 and 50 years (data not shown). A relatively long duration of alcohol consumption was associated with PTC risk (31+ years, men: OR=1.75, 95%CI=1.23−2.48; women: OR=2.38, 95%CI=1.81−

3.12) as well as male FTC risk (OR=13.27, 95%CI=1.11-158.28); and there were no significant differences in the PTC or FTC risk according to the *p*-heterogeneity values (table not shown).

Associations considering clinicopathologic features

Associations of alcohol consumption and DTC risk were classified according to patient age at diagnosis and clinicopathologic features, such as tumor size, lymph node metastasis, extrathyroidal extension, BRAF mutation and TNM staging. Alcohol consumption per event (≥151 g) was associated with a 2.2-fold increased risk of DTC with a tumor size of 1 cm or greater (OR=2.23, 95%CI=1.09-4.53) and a 1.1-fold increased risk of DTC with a tumor size of smaller than 1 cm (OR=1.11, 95%CI=0.29-2.11) compared to the never-drinkers, as shown in Table 5. There was also a positive association between long-term drinking (31 years or longer) and DTC with a tumor size of 1 cm or greater (OR=2.16, 95%CI=1.57-2.96) and DTC with a tumor size of 1 cm or smaller (OR=1.79, 95%CI=1.41-2.28) compared to the never-drinkers (data not shown). The effects of alcohol intake

per event (≥151 g) were consistent after the cases were stratified according to the presence of lymph node metastasis, extrathyroidal extension, and advanced TNM stage (Table 5). The BRAF mutation statuses of the PTC patients and the ages at diagnosis of the DTC patients (<45 years old and ≥45 years old) were also estimated in comparison with the never-drinkers (Table 6).

Table 5. Alcohol consumptions and differentiated thyroid cancer by clinicopathologic features, T-CALOS April 2010–April 2014

	OR (95% CI)[2]	OR (95% CI) [2]	OR (95% CI) [2]	OR (95% CI) [2]
	Tumor size≤1cm	Tumor size>1cm	LN metastasis (-)	LN metastasis (+)
Alcohol intake (g) per event				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-50	0.80 (0.69-0.92)	0.81 (0.65-1.00)	0.85 (0.73-0.98)	0.68 (0.56-0.83)
51-150	1.09 (0.89-1.32)	1.18 (0.89-1.57)	1.08 (0.87-1.35)	1.14 (0.89-1.46)
151+	1.11 (0.59-2.11)	2.23 (1.09-4.53)	1.22 (0.60-2.47)	1.87 (0.99-3.50)
p-trend[4]	*0.469*	*0.570*	*0.822*	*0.746*
Duration (years)				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-20	0.76 (0.65-0.88)	0.84 (0.67-1.05)	0.79 (0.67-0.93)	0.72 (0.58-0.88)
21-30	0.93 (0.78-1.12)	0.90 (0.68-1.18)	1.00 (0.82-1.22)	0.83 (0.65-1.06)
31+	1.80 (1.41-2.29)	2.14 (1.56-2.95)	1.95 (1.50-2.54)	1.89 (1.39-2.57)
p-trend[4]	*0.077*	*0.012*	*0.011*	*0.258*
	ETE (-)	ETE (+)	TNM stage I	TNM stage II-IV
Alcohol intake (g) per event				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-50	0.98 (0.83-1.16)	0.69 (0.59-0.81)	0.80 (0.70-0.92)	0.75 (0.58-0.95)
51-150	1.28 (1.01-1.63)	0.97 (0.78-1.21)	1.08 (0.89-1.32)	1.17 (0.84-1.62)
151+	1.51 (0.75-3.08)	1.39 (0.74-2.62)	1.39 (0.77-2.51)	1.87 (0.81-4.33)
p-trend[4]	*0.119*	*0.051*	*0.538*	*0.767*
Duration (years)				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-20	0.92 (0.77-1.10)	0.67 (0.56-0.79)	0.81 (0.70-0.94)	0.53 (0.39-0.73)
21-30	1.15 (0.92-1.43)	0.79 (0.64-0.97)	0.93 (0.78-1.12)	1.08 (0.80-1.44)
31+	2.15 (1.61-2.87)	1.73 (1.34-2.23)	1.81 (1.40-2.36)	1.90 (1.38-2.62)
p-trend[4]	*<0.001*	*0.564*	*0.138*	*0.013*

Abbreviations: OR=odds ratio; 95% CI=95% confidence interval; LN metastasis=lymph node metastasis; ETE (-)=absence of extrathyroidal extension; ETE (+)=presence of extrathyroidal extension.

1. Subjects with missing information for alcohol intake per event with FTC (5 men and 4 women) and PTC (50 men and 136 women) were identified. DTC patients with information on tumor size (<1 cm=1,567 cases; ≥1 cm=644 cases), LN metastasis (No=1,311 cases; Yes=764 cases) and TNM staging (stage I=1,606 cases; stage II=41 cases; stage III=406 cases; and stage IV=79 cases), and ETE (No=977 cases; Yes=1,198 cases) and the controls were included.
2. Polychotomous logistic regression models (controls vs. less advanced cases; and controls vs. more advanced cases) were adjusted for matching variables (age, sex and enrollment year), education level, marital status, smoking, regular exercise, and history of chronic diseases, including hypertension and dyslipidemia.

Table 6. Alcohol consumptions and differentiated thyroid cancer by diagnosis age and BRAF mutation, T-CALOS April 2010–April 2014

	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]	OR (95% CI)[2]
	Age at diagnosis <45	Age at diagnosis ≥45	$BRAF^{wt}$ in PTC	$BRAF^{V600E}$ in PTC
Alcohol intake (g) per event				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-50	0.79 (0.62-0.99)	0.69 (0.60-0.79)	0.91 (0.74-1.12)	0.83 (0.72-0.96)
51-150	1.23 (0.91-1.67)	1.21 (0.97-1.50)	1.12 (0.82-1.54)	1.49 (1.23-1.81)
151+	1.79 (0.72-4.48)	1.88 (1.02-3.47)	2.59 (1.03-6.54)	2.89 (1.72-4.86)
p-trend[4]	*0.735*	*0.389*	*0.280*	*0.017*
Duration (years)				
Never	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)	1.00 (Reference)
0-20	0.73 (0.58-0.91)	0.58 (0.48-0.70)	0.69 (0.54-0.87)	0.82 (0.70-0.95)
21-30	2.83 (2.05-3.90)	1.23 (1.02-1.48)	1.41 (1.07-1.85)	1.18 (0.98-1.43)
31+	0.19 (0.01-61.69)	2.08 (1.68-2.59)	2.87 (1.91-4.34)	2.03 (1.58-2.61)
p-trend[4]	*<0.001*	*<0.001*	*<0.001*	*<0.001*

Abbreviations: OR=odds ratio; 95% CI=95% confidence interval; $BRAF^{wt}$ in PTC=negative for BRAF (V600E) by mutation testing; $BRAF^{V600E}$ in PTC=positive for BRAF (V600E) by mutation testing; PTC=papillary thyroid cancer;.

1. Subjects with missing information for alcohol intake per event with FTC (5 men and 4 women) and PTC (50 men and 136 women) were identified.
2. ORs and 95%CIs were calculated for the PTC patients with information on BRAF mutation status (2,092 cases) and their matched controls using conditional logistic regression models adjusted for education level, marital status, smoking, regular exercise, and history of chronic diseases, including hypertension and dyslipidemia (diagnosed by a medical doctor: never, ever and unknown).

b) Smoking and thyroid cancer

General characteristics

Among the total study population consisting of 2142 PTC cases (417 men and 1725 women) and 21,420 matched controls (4170 men and 17250 women), 53.2% were 50 years old or older both among the case and control groups (P = 1.00). The PTC patients were more likely to be unmarried (married: 94.2% vs. 96.0%; P < .01), more educated (high school graduation: 83.9% vs. 75.1%; P < .01), and have a previous history of hypertension (23.0% vs. 16.6%; P < .01) and dyslipidemia (16.5% vs. 10.3%; P < .01) compared with control. Other factors including BMI of 25 or over, history of diabetes mellitus and menopausal status were not different between the case and control groups (data not shown).

Smoking and PTC

Table 7 demonstrates the association between smoking behaviors and the PTC. Compared to the never smokers, the current smokers exhibited an inverse association among both men and women (men: OR = 0.57, 95% CI = 0.43−0.76; and women: OR = 0.49, 95% CI = 0.33−0.75). Although our

analyses could not present sufficient evidence, there is a trend of increased PTC risk and smoking 20 pack-years or more (men: OR = 0.89 , 95% CI = 0.68-1.17; and women: OR = 1.69, 95% CI = 0.86-3.32) and a relatively younger age (<20 years old) of smoking initiation (men: OR=1.07, 95%CI=0.80-1.43; and women: OR=1.48, 95% CI=0.70-3.14) compared with the never smokers (Table 7). The results were confirmed by sensitivity analyses conducted after stratifying by enrollment year (before 2012 and during or after 2012). The associations were also consistent in the subgroups by age at enrollment (<50 and 50+ years) and BMI (<25 and 25+kg/m^2) (data not shown). The associations between smoking and PTC in subjects who had not been exposed to secondhand smoking at early-age during their growing up and who had been exposed at early-age were presented in Table 8. Although we did not find the evidence that smoking initiated at 20 years old increased the PTC risk, in subjects with early-age exposure to secondhand smoking, smoking initiated before 20 years of age was associated with elevated PTC risk (OR = 1.64, 95% CI = 1.02-2.61). We observed the consistent results in men and women (data not shown).

Table 7. Associations between smoking and papillary thyroid cancer, T-CALOS April 2010–April 2014

	Men (417 cases / 4170 controls)		Women (1725 cases / 17,250 controls)	
	Cases / Controls	OR[1] (95% CI)	Cases / Controls	OR[1] (95% CI)
Smoking status				
Never	122 / 1115	1.00	1664 / 16,515	1.00
Past	202 / 1598	1.15 (0.91-1.47)	36 / 260	1.27 (0.88-1.82)
Current	93 / 1457	0.57 (0.43-0.76)	25 / 475	0.49 (0.33-0.75)
Never	122 / 1115	1.00	1664 / 16,515	1.00
Ever	295 / 3055	0.89 (0.71-1.11)	61 / 735	0.77 (0.59-1.02)
Smoking dose (pack-year)				
Never smoker	122 / 1115	1.00	1664 / 16,515	1.00
<20	159 / 1720	0.81 (0.63-1.04)	44 / 659	0.65 (0.47-0.88)
20+	125 / 1307	0.89 (0.68-1.17)	11 / 63	1.69 (0.86-3.32)
P-trend		*.446*		*.137*
Age of smoking initiation (years old)				
Never smoker	122 / 1115	1.00	1664 / 16,515	1.00
25+	32 / 440	0.67 (0.45-1.01)	27 / 462	0.56 (0.38-0.83)
20-24	167 / 1816	0.82 (0.64-1.05)	24 / 210	1.00 (0.64-1.55)
<20	92 / 795	1.07 (0.80-1.43)	8 / 55	1.48 (0.70-3.14)
P-trend		*.952*		*.388*

Abbreviation: OR= odds ratio; CI= confidential interval; BMI= body mass index.

1. ORs and 95% CIs of smoking and thyroid cancer were calculated by conditional logistic regression model (matching variables: age at enrollment and sex) adjusted for BMI (<25, ≥25 and unknown), education (high school graduation: no, yes and unknown), marital status (single, married and unknown) and history of chronic disease (hypertension and dyslipidemia).

Table 8. Associations between smoking and papillary thyroid cancer, T-CALOS April 2010–April 2014

	No exposure to secondhand smoking[1]			Early-age exposure to secondhand smoking at home			
	902 cases	10903 controls	OR[2] (95% CI)	889 cases	7070 cases	OR[2] (95% CI)	*P*-heterogeneity
Smoking status							
Never	779	8966	1.00	734	6100	1.00	
Past	102	1066	0.87 (0.61-1.22)	104	480	1.82 (1.29-2.55)	.003
Current	21	871	0.25 (0.15-0.41)	51	490	0.75 (0.49-1.13)	.001
Never	779	8966	1.00	734	6100	1.00	
Ever	123	1937	0.59 (0.43-0.80)	155	970	1.28 (0.95-1.73)	<.001
Smoking dose (pack-year)							
Never smoker	779	8966	1.00	734	6100	1.00	
<20	69	1156	0.55 (0.39-0.78)	88	643	1.09 (0.78-1.52)	.005
20+	52	773	0.65 (0.42-1.00)	55	320	1.48 (0.95-2.28)	.008
P-trend			*0.007*			*0.078*	
Age of smoking initiation (years old)							
Never smoker	779	8966	1.00	734	6100	1.00	
25+	12	457	0.27 (0.14-0.49)	29	241	1.09 (0.67-1.76)	<.001
20-24	79	1068	0.75 (0.52-1.09)	81	523	1.20 (0.83-1.74)	.081
<20	31	408	0.73 (0.44-1.18)	41	203	1.64 (1.02-2.61)	.019
P-trend			*0.085*			*0.055*	

Abbreviation: OR=Odds Ratio; 95% CI=95% Confidence Interval; BMI=body mass index.

1. Subjects with neither current exposure nor early-age exposure to secondhand smoking at home during their growing up were included.
2. ORs and 95% CIs of smoking and thyroid cancer were calculated by conditional logistic regression model (matching variables: age at enrollment and sex) adjusted for BMI (<25, ≥25, and unknown), education (high school graduation: no, yes and unknown), marital status (single, married and unknown) and history of chronic disease (hypertension and dyslipidemia).

Secondhand smoking and PTC

Secondhand smoking among the non-smokers was related to an increased risk of PTC, as summarized in Table9. In terms of exposure status, subjects with secondhand smoking exposure had an elevated risk of PTC compared with those with no exposure (men: OR = 2.23, 95% CI = 1.27-3.91; and women: OR = 1.50, 95% CI = 1.33-1.70). In those who with secondhand smoking, the ORs for daily exposure time (20 minutes/ day) in related to PTC risk were 1.77 (95% CI = 1.46-2.14) for women only. Subjects exposed to secondhand smoking at least 10 years had a 2.7-fold higher PTC risk (men: OR = 2.71; and 95% CI = 1.35-5.44; women: OR = 1.58, 95% CI = 1.37-1.82) compared with subjects who were not exposed to secondhand smoking. The risk of PTC were elevated as the age of exposure initiation was lower (men: p-trend = .003; women: p-trend < .001). Exposure to secondhand smoking at 20 years old or younger (OR = 4.72, 95% CI = 3.70-6.00) or 18 years old or younger (OR = 6.14, 95% CI = 4.74-7.97) were also related with increased PTC risk in the women compared with those who responded "no" for secondhand smoking at enrollment.

Table 9. Associations between secondhand smoking and papillary thyroid cancer among never-smokers, T-CALOS April 2010–April 2014

	Men (122 cases/ 1115 controls)		Women (1664 cases/ 16515 controls)	
	Cases / Controls	OR[1] (95% CI)	Cases/ Controls	OR[1] (95% CI)
Current exposure to secondhand smoking				
No	89 / 905	1.00	1253 / 13526	1.00
Yes	33 / 210	2.23 (1.27-3.91)	411 / 2989	1.50 (1.33-1.70)
Exposure time (minutes/day)				
No	89 / 905	1.00	1253 / 13526	1.00
<20	12 / 96	1.84 (0.79-4.24)	138 / 1571	0.96 (0.80-1.16)
20+	11 / 68	2.15 (0.84-5.51)	141 / 899	1.77 (1.46-2.14)
P-trend		*.068*		*<.001*
Exposure duration (years)				
No	89 / 905	1.00	1253 / 13526	1.00
1-9	6 / 52	1.83 (0.59-5.66)	62 / 489	1.38 (1.05-1.82)
10+	22 / 118	2.71 (1.35-5.44)	291 / 2048	1.58 (1.37-1.82)
P-trend		*.007*		*<.001*
Age of exposure initiation (years old)				
Never	89 / 905	1.00	1253 / 13526	1.00
30+	10 / 97	1.54 (0.64-3.71)	106 / 1056	1.09 (0.88-1.35)
25-29	11 / 30	3.85 (1.19-12.50)	78 / 589	1.42 (1.11-1.82)
<25	7 / 43	4.09 (1.30-12.88)	168 / 892	2.21 (1.84-2.64)
P-trend		*.003*		*<.001*

Abbreviation: OR=Odds Ratio; 95% CI=95% Confidence Interval; BMI=body mass index.

1. ORs and 95% CIs of smoking and thyroid cancer were calculated by conditional logistic regression model (matching variables: age at enrollment and sex) adjusted for BMI (<25, ≥25 and unknown), education (high school graduation: no, yes and unknown), marital status (single, married and unknown) and history of chronic disease (hypertension and dyslipidemia).

Smoking and clinicopathological features of PTC

The association of smoking and BRAF (V600E) mutation among the PTC patients was presented in Table 10. Smoking 20 pack-years or more was related to BRAF (V600E) mutation in male PTC patients (OR = 1.81, 95% CI = 1.02-3.22) compared with the never smokers, whereas no clear association was observed in women. Among PTC patients who had reported early-age exposure to secondhand smoking, male PTC patients who had smoked for 20 pack-years or more tend to have PTC with BRAF (V600E) mutation (OR = 2.17, 95% CI = 0.86-5.46) compared to never smokers (data not shown). However, evidence for the association between secondhand smoking and PTC was not found (men: OR = 0.71, 95% CI = 0.27-1.85; and women: OR = 0.88, 95% CI = 0.69-1.12) (data not shown). For PTC with a tumor size of 1 cm or greater, the results were similar (data not shown). No association was found for smoking or other clinicopathological features including lymph node metastasis, multifocality and TNM stage (data not shown).

Table 10. Associations between smoking and BRAF mutation among papillary thyroid cancer patients, T-CALOS April 2010–April 2014

	Men[1]			Women[1]		
	$BRAF^{wt}$ (90 cases)	$BRAF^{V600E}$ (306 cases)	OR[2] (95% CI)	$BRAF^{wt}$ (532 cases)	$BRAF^{V600E}$ (1131 cases)	OR[2] (95% CI)
Smoking status						
Never	24	90	1.00	512	1094	1.00
Past	50	146	1.62 (0.96-2.74)	9	24	0.62 (0.30-1.28)
Current	16	70	0.81 (0.43-1.56)	11	13	1.53 (0.68-3.42)
Never	24	90	1.00	512	1094	1.00
Ever	66	216	1.32 (0.81-2.17)	20	37	0.91 (0.53-1.57)
Smoking dose (pack-year)						
Never smoker	24	90	1.00	512	1094	1.00
<20	28	125	1.08 (0.61-1.91)	13	29	0.82 (0.43-1.56)
20+	37	81	1.81 (1.02-3.22)	5	4	1.10 (0.32-3.72)
P-trend			*.048*			*.726*
Age of smoking initiation (years old)						
Never smoker	24	90	1.00	512	1094	1.00
25+	5	23	0.58 (0.22-1.52)	8	17	0.78 (0.34-1.77)
20-24	40	120	1.51 (0.87-2.61)	10	12	1.29 (0.56-2.96)
<20	20	70	1.41 (0.75-2.65)	1	7	0.41 (0.08-2.02)
P-trend			*.148*			*.590*

Abbreviation: OR=Odds Ratio; 95% CI=95% Confidence Interval; BMI=body mass index.

1. Subjects with missing information for BRAF mutation (21 men and 62 women) were excluded.

2. ORs and 95% CIs of smoking and thyroid cancer were calculated by conditional logistic regression model (matching variables: age at enrollment and sex) adjusted for BMI (<25, ≥25 and unknown), education (high school graduation: no, yes and unknown), marital status (single, married and unknown) and history of chronic disease (hypertension and dyslipidemia).

c) Obesity and thyroid cancer

General characteristics

In total, 1,551 PTC patients (300 men and 1,251 women) and 15,510 controls (3,000 men and 12,510 women) were studied in this analysis. The age range (years) was 35 to 76 for the PTC cases and 35-74 for the controls, and no significant difference in the age distribution by 5-year intervals was found between the cases and the controls (Table 11). The subjects in the case group were more likely to graduate high school and less likely to get married than those in the control group (Table 11). A higher frequency of chronic diseases, including diabetes (6.96% vs. 5.19%), hypertension (23.79% vs. 15.80%, respectively) and dyslipidemia (16.25% vs. 7.95%, respectively) was observed in the PTC cases compared to the controls (Table 11). We observed a similar distribution of smoking and drinking among the cases and controls ($p>0.05$). The women in the case group were less likely to have ever been pregnant compared with those in the control group (Table 11).

Table 11. Selected characteristics of total study subjects, T-CALOS April 2010–December 2013

	Cases (N=1,551)	Controls (N=15,510)	*p*-value[1]
Age (mean, SD)	50.8 (9.4)	50.8 (9.1)	0.715
Height at enrollment (mean, SD)	161.0 (7.5)	159.1 (7.4)	<0.001
Weight at enrollment (mean, SD)	61.5 (10.2)	60.1 (9.4)	<0.001
BMI at enrollment (mean, SD)	23.6 (3.1)	23.7 (2.9)	0.539
Women	1,251 (80.7)	12,510 (80.7)	1.000
High school graduation or more education	1,318 (85.0)	10,772 (69.5)	<0.001
Married	1,481 (95.5)	14,411 (92.9)	<0.001
Cigarette smoking	260 (16.8)	2,679 (17.3)	0.070
Alcohol drinking	678 (43.7)	6,974 (45.0)	0.071
Regular exercise	675 (43.5)	7841 (50.6)	<0.001
Family history of cancer	569 (36.7)	2,154 (13.9)	<0.001
Diabetes mellitus	108 (7.0)	805 (5.2)	0.011
Hypertension	369 (23.8)	2,450 (15.8)	<0.001
Dyslipidemia	252 (16.3)	1,233 (8.0)	<0.001
The ever-pregnant[2]	1168 (93.4)	12037 (96.2)	<0.001
The menopausal among the women	652 (52.1)	6171 (49.3)	<0.001

Abbreviation: SD=standard deviation; BMI=body mass index.

1. P-values were based on Chi-square test for categorical variables and t-test for continuous variables.

2. A total of 13761 women (1251cases and 12510 controls) were included.

Obesity at enrollment

Height and weight were significantly associated with an increased risk of PTC, as shown in Table 12. The subjects in the highest quartile group for height exhibited a substantially increased risk of PTC (men: OR=3.40, 95% CI=2.39-4.83; women: OR=2.66, 95% CI=2.23-3.17) compared with the lowest quartile group (p-trend <0.001). We also observed that high weight was a risk factor for PTC in both the men (OR=2.03, 95%CI=1.42-2.92) and the women (OR=1.48, 95%CI=1.25-1.76), which presented a dose-response association (p-trend<0.001). Male obesity (BMI $\geq$30 kg/m^2) was related to an approximately 1.9-fold increase in the risk of PTC (OR=1.87, 95%CI=1.07-3.27) compared with the reference category (BMI=18.5-24.9 kg/m^2), while no significant association was observed in the women (Table 13). It was estimated that there was an elevated risk of PTC for the highest quartile of current BSA compared with the lowest quartile in both men (OR=2.18, 95%CI=1.53-3.11) and women (OR=1.71, 95%CI=1.44-2.02). The associations between BSA and thyroid cancer in men and women were not statistically different (p-heterogeneity=0.226).

Table12. Anthropometric measures at enrollment and the risk for papillary thyroid cancer, T-CALOS April 2010–December 2013

	Males			Females			
	Cases[1] (N=300)	Controls[1] (N=3,000)	OR (95% CI)[2]	Cases[1] (N=1,251)	Controls (N=12,510)	OR (95% CI)[2]	*p*-heterogeneity
Height[3]							
Quartile 1	69	1,171	Reference	285	4,481	Reference	
Quartile 2	77	796	1.64 (1.17-2.32)	325	3,673	1.38 (1.17-1.64)	0.375
Quartile 3	57	494	2.06 (1.40-3.02)	267	2,173	1.96 (1.63-2.35)	0.819
Quartile 4	97	539	3.40 (2.39-4.83)	374	2,183	2.66 (2.23-3.17)	0.221
p-trend			*<0.001*			*<0.001*	
Weight[4]							
Quartile 1	69	1,100	Reference	270	3,412	Reference	
Quartile 2	67	679	1.34 (0.98-1.99)	331	3,525	1.17 (0.99-1.39)	0.498
Quartile 3	88	725	1.66 (1.18-2.32)	317	2,835	1.41 (1.19-1.68)	0.399
Quartile 4	76	496	2.03 (1.42-2.92)	333	2,738	1.48 (1.25-1.76)	0.121
p-trend			*<0.001*			*<0.001*	

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval.

1. Papillary thyroid cancer and their 1:10 matched healthy controls in the analyses.
2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown) marital status (single, married and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. The cutoffs of the current height were based on the quartile values of thyroid cancer patients (men: 168cm, 172cm and 175cm; women: 155cm, 159cm and 162cm).
4. The cutoffs of the current weight were based on the quartile values of thyroid cancer patients (men: 67kg, 72kg and 79kg; women: 53kg, 58kg and 63kg).

Table 13. Body mass index, body surface area and the risk for papillary thyroid cancer, T-CALOS April 2010–December 2013

	Males			Females			
	Cases[1] (N=300)	Controls[1] (N=3,000)	OR (95% CI)[2]	Cases[1] (N=1,251)	Controls (N=12,510)	OR (95% CI)[2]	*p*-heterogeneity
BMI[3] (kg/m^2)							
<18.5	2	31	0.74 (0.17-3.14)	36	284	1.21 (0.84-1.74)	0.514
18.5-24.9	170	1739	Reference	893	8,807	Reference	
25-29.9	110	1,151	0.83 (0.64-1.08)	289	3,063	0.95 (0.82-1.09)	0.383
30+	18	79	1.87 (1.07-3.27)	33	356	0.85 (0.58-1.24)	0.021
p-trend			*0.851*			*0.169*	
BSA[4]							
Quartile 1	74	1,198	Reference	313	4,244	Reference	
Quartile 2	73	715	1.52 (1.07-2.15)	312	3,164	1.32 (1.11-1.55)	0.465
Quartile 3	76	585	1.89 (1.34-2.68)	313	2,774	1.48 (1.26-1.75)	0.216
Quartile 4	77	502	2.18 (1.53-3.11)	313	2,328	1.71 (1.44-2.02)	0.226
p-trend			*<0.001*			*<0.001*	

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index; BSA=body surface area.

1. Papillary thyroid cancer and their 1:10 matched healthy controls in the analyses.
2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown) marital status (single, married and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. BMI= $(\text{weight (kg)})^2$/ height (m).
4. BSA=0.007184 x height (cm) $^{0.725}$ x weight (kg) $^{0.425}$. Based on the quartile values of thyroid cancer patients, the cutoffs of BSA at enrollment (men: 1.77, 1.84 and 1.93; women: 1.52, 1.59 and 1.66) were defined.

The results related to tumor size, lymph node metastasis, multifocality, and BRAF (V600E) mutation status are presented in Table 14. Among the PTC patients in the highest quartile group of BSA were marginally related to have a larger tumor size (≥1 cm; OR=1.32, 95%CI=0.97−1.82) compared with those who were in the lowest quartile group of BSA. Similarly, Among the PTC patients in the highest quartile group of BSA were marginally related to have a multifocality (OR=1.30, 95%CI=0.97−1.75) and BRAF mutation (OR=1.39, 95%CI=0.99−1.95) compared with those who were in the lowest quartile group of BSA. The relation between BSA and lymph node metastasis were observed, which was statistically significant (OR=1.41, 95%CI=1.03−1.94).

We regrouped subjects by the quartiles for BMI and BSA to perform the combined analyses for the detectability of PTC. No significant effect modification on PTC risk was noticed in the subjects in the highest quartile for BMI and BSA at enrollment compared with those in the lowest quartile for both BMI and BSA at enrollment (men: OR=2.06, 95%CI=1.28−3.30; women: OR=1.56, 95%CI=1.24−1.95; table not shown).

Table 14. Body mass index, body surface area and the risk for high aggressiveness among papillary thyroid cancer cases, T-CALOS April 2010–December 2013

BSA[2] at enrollment	Low aggressive PTC	High aggressive PTC	OR (95% CI)[1]	Low aggressive PTC	High aggressive PTC	OR (95% CI)[1]
	Tumor size[3]			Multifocality[3]		
	<1cm	1cm+		No	Yes	
Quartile 1	275	109	Reference	230	151	Reference
Quartile 2	278	104	0.96 (0.70-1.32)	234	146	0.97 (0.72-1.31)
Quartile 3	284	102	0.98 (0.71-1.35)	242	140	0.95 (0.70-1.28)
Quartile 4	255	129	1.32 (0.97-1.82)	213	170	1.30 (0.97-1.75)
p-trend			*0.087*			*0.107*
	Lymph node metastasis[3]			BRAF (V600E) mutation[3]		
	No	Yes		$BRAF^{wt}$	$BRAF^{V600E}$	
Quartile 1	239	113	Reference	119	214	Reference
Quartile 2	216	141	1.44 (1.05-1.97)	109	211	1.05 (0.76-1.45)
Quartile 3	220	141	1.38 (1.01-1.90)	82	248	1.63 (1.16-2.29)
Quartile 4	220	145	1.41 (1.03-1.94)	90	230	1.39 (0.99-1.95)
p-trend			*0.056*			*0.009*

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BSA=body surface area.

1. Unconditional logistic regression models were adjusted for age, sex (men and women), education (high school graduation: yes, no, unknown) marital status (single, married, unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
2. BSA=0.007184 x height $(cm)^{0.725}$ x $weight(kg)^{0.425}$. Based on the quartile values of thyroid cancer patients, the cutoffs of BSA at enrollment (men: 1.77, 1.84 and 1.93; women: 1.52, 1.59 and 1.66) were defined.
3. Subjects with missing information for thyroid tumor size (n=15), multifocality (n=116), Lymph node metastasis (n=116) and BRAF (V600E) mutation (n=248) were excluded.

Obesity at age 18-20

The highest quartile group for weight at age 18 years was associated with an approximately 6-fold increased PTC risk in general (OR=5.92, 95%CI=4.95-7.08) compared with the lowest quartile group. BMI at or higher than 25 kg/m2 at age 18 years was positively associated with PTC risk (OR=4.66, 95%CI=3.89-5.57) compared to the normal range of BMI (18.5-24.9 kg/m2), as shown in Table 15. When we defined the subjects for BMI at age 18 by WHO criteria, obesity (BMI; 30+) were associated with PTC risk (OR=2.99, 95%CI=1.01-8.86) compared to those who were in the normal BMI at age 18 (BMI: 18.5-24.9). In addition, the consistently elevated PTC risk was estimated for WHO criteria for Asians (OR=3.46, 95%CI=1.16-10.32) compared to the normal BMI at age 18 (BMI: 18.5-22.9) as in Table 15.

We observed that the highest quartile of weight at age 18 years was a risk factor of PTC for both the men and women (Table 16). The magnitudes of the ORs for weight at age 18 years and PTC risk were generally stronger for the men than the women (men: OR=11.69, 95%CI=7.33-18.64; women: OR=5.22, 95%CI=4.30-6.34).

Table 15. Weight and BMI at age 18 in relation with the risk of papillary thyroid cancer, T-CALOS April 2010–December 2013

	Cases (N=1551) n (%)	Controls (N=15510) n (%)	OR (95% CI)[1]
Weight at age 18 (kg)[2]			
Quartile 1	334 (21.53)	7,118 (45.89)	1.00
Quartile 2	390 (25.15)	4,406 (28.41)	1.98 (1.67-2.33)
Quartile 3	386 (24.89)	2,264 (14.60)	4.07 (3.42-4.85)
Quartile 4	441 (28.43)	1,722 (11.10)	5.92 (4.95-7.08)
p-trend			*<0.001*
BMI at age 18 (kg)[2]			
Quartile 1	386 (24.89)	6676 (43.04)	1.00
Quartile 2	388 (25.02)	4088 (26.36)	1.89 (1.60-2.22)
Quartile 3	390 (25.15)	2824 (18.21)	2.94 (2.48-3.48)
Quartile 4	387 (24.95)	1922 (12.39)	4.66 (3.89-5.57)
p-trend			*<0.001*
BMI at age 18 (kg/m^2)			
- WHO criteria			
<18.5	115 (7.41)	2,234 (14.40)	0.44 (0.36-0.55)
18.5-24.9	1251 (80.66)	12622 (81.38)	1.00
25.0-29.9	176 (11.35)	630 (4.06)	3.35 (2.68-4.19)
30.0+	9 (0.58)	24 (0.15)	2.99 (1.01-8.86)
p-trend			*<0.001*
BMI at age 18 (kg/m^2)			
- WHO criteria for Asians			
<18.5	115 (7.41)	2,234 (14.40)	0.50 (0.40-0.62)
18.5-22.9	958 (61.77)	10,753 (69.33)	1.00
23.0-24.9	293 (18.89)	1,869 (12.05)	2.10 (1.78-2.48)
25.0-29.9	176 (11.35)	630 (4.06)	3.99 (3.17-5.01)
30.0+	9 (0.58)	24 (0.15)	3.46 (1.16-10.32)
p-trend			*<0.001*

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index; WHO=World Health Organization.

1. Conditional logistic regression models were adjusted for age, sex, education, marital status, regular exercise, family history of cancer, history of chronic diseases (diabetes, hypertension and dyslipidemia), pregnancy and menopausal status.
2. The cut points of weight at age 18 (men: 63, 69 and 74kg; women: 50, 54 and 58kg) and BMI at age 18 (men: 21.8, 23.4 and 25.1kg/m^2; women: 20.0, 21.5 and 23.0kg/m^2) were used based on quartile values of thyroid cancer patients.

Table 16. Weight and BMI at age 18 in relation with the risk of papillary thyroid cancer (men and women), T-CALOS April 2010–December 2013

	Men			Women			*P* for heterogeneity
	Cases (N=300) n (%)	Controls (N=3,000) n (%)	OR (95% CI)[1]	Cases (N=1,251) n (%)	Controls (N=12,510) n (%)	OR (95% CI)[1]	
Weight at age 18 (kg)[2]							
Quartile 1	65 (21.67)	1,616 (53.87)	1.00	269 (21.50)	5,502 (43.98)	1.00	
Quartile 2	81 (27.00)	857 (28.57)	2.52 (1.71-3.72)	309 (24.70)	3,549 (28.37)	1.86 (1.55-2.24)	0.169
Quartile 3	72 (24.00)	323 (10.77)	5.64 (3.69-8.63)	314 (25.10)	1,941 (15.52)	3.76 (3.10-4.55)	0.087
Quartile 4	82 (27.33)	204 (6.80)	11.69 (7.33-18.64)	359 (28.70)	1,518 (12.13)	5.22 (4.30-6.34)	0.002
p-trend			*<0.001*			*<0.001*	
BMI at age 18 (kg/m^2)[3]							
<18.5	5 (1.67)	176 (5.87)	0.42 (0.15-1.18)	110 (8.79)	2,058 (16.45)	0.50 (0.40-0.62)	0.743
18.5-22.9	130 (43.33)	2,021 (67.37)	1.00	828 (66.19)	8,732 (69.80)	1.00	
23.0-24.9	85 (28.33)	585 (19.50)	2.57 (1.83-3.63)	208 (16.63)	1,284 (10.26)	2.02 (1.68-2.45)	0.228
25+	80 (26.67)	218 (7.27)	7.17 (4.74-10.83)	105 (8.39)	436 (3.49)	3.03 (2.30-4.00)	0.001
p-trend			*<0.001*			*<0.001*	

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index; WHO=World Health Organization.

1. Conditional logistic regression models were adjusted for age, sex, education, marital status, regular exercise, family history of cancer, history of chronic diseases (diabetes, hypertension and dyslipidemia), pregnancy and menopausal status.
2. The cut points of BMI at age 18 (men: 21.8, 23.4 and 25.1kg/m^2; women: 20.0, 21.5 and 23.0kg/m^2) were used based on quartile values of thyroid cancer patients.
3. The 9 cases (6 men and 3 women) and 24 controls (8 men and 16 women) were included in this group with at or higher than 30 kg/m^2 for BMI at age 18.

The relatively strong associations of the highest quartile of BMI at 18 years and PTC risk in subjects were overweight or obese at the enrollment (BMI <25 at enrollment: OR=4.78 95%CI=3.73−6.11; BMI 25+: OR=13.11, 95%CI=7.59−22.63), the difference was not statistically significant (Table 17). The BMI at age 18 years and PTC risk in those who were overweight or obese at enrollment (BMI <25 at enrollment: OR=1.98 95%CI=1.30−3.03; BMI 25+: OR=7.55, 95%CI=5.66−10.06), which the ORs between the two groups were significantly different (p−heterogeneity<0.001).

The detailed results related to BRAF (V600E) mutation status were presented in Table 18. The results were not statistically significant in terms of weight at age 18 (OR=1.40, 95%CI=1.00−1.97) and BMI at age 18 (OR=0.83, 95%CI=0.55−1.25). The results related to clinicopathological features, including tumor size, multifocality, extrathyroidal extension, lymphovascular invasion and lymph node metastasis were shown in Table 19. Among the PTC patients, subjects with BMI at or greater than 25 were more likely to have extrathyroidal extension.

Table 17. Weight and BMI at age 18 in relation with the risk of papillary thyroid cancer (BMI at enrollment: <25 and 25+ kg/m^2), T-CALOS April 2010–December 2013

	BMI at enrollment <25 kg/m^2			BMI at enrollment 25+ kg/m^2			
	Cases (N=1101) n (%)	Controls (N=10861) n (%)	OR (95% CI)[1]	Cases (N=450) n (%)	Controls (N=4649) n (%)	OR (95% CI)[1]	*P* for heterogeneity
Weight at age 18 (kg)[2]							
Quartile 1	287 (26.07)	5503 (50.67)	1.00	47 (10.44)	1615 (34.74)	1.00	
Quartile 2	320 (29.06)	3025 (27.85)	2.06 (1.70-2.51)	70 (15.56)	1381 (29.71)	1.39 (0.81-2.37)	0.172
Quartile 3	289 (26.25)	1463 (13.47)	4.39 (3.55-5.42)	97 (21.56)	801 (17.23)	4.74 (2.73-8.23)	0.796
Quartile 4	205 (18.62)	870 (8.01)	4.78 (3.73-6.11)	236 (52.44)	852 (18.33)	13.11 (7.59-22.62)	0.001
p-trend			*<0.001*			*<0.001*	
BMI at age 18 (kg/m^2)[3]							
<18.5	105 (9.54)	1928 (17.75)	0.48 (0.38-0.59)	10 (2.22)	306 (6.58)	0.52 (0.26-1.01)	0.823
18.5-22.9	788 (71.57)	7778 (71.61)	1.00	170 (37.78)	2975 (63.99)	1.00	
23.0-24.9	176 (15.99)	946 (8.71)	2.13 (1.75-2.60)	117 (26.00)	923 (19.85)	2.55 (1.94-3.35)	0.306
25+	32 (2.91)	209 (1.92)	1.98 (1.30-3.03)	153 (34.00)	445 (9.57)	7.55 (5.66-10.06)	<0.001
p-trend			*<0.001*			*<0.001*	

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index; WHO=World Health Organization.

1. Conditional logistic regression models were adjusted for age, sex, education, marital status, regular exercise, family history of cancer, history of chronic diseases (diabetes, hypertension and dyslipidemia), pregnancy and menopausal status.
2. The cut points of BMI at age 18 (men: 21.8, 23.4 and 25.1kg/m^2; women: 20.0, 21.5 and 23.0kg/m^2) were used based on quartile values of thyroid cancer patients.
3. The 9 cases (0 for <25kg/m^2 and 9 for 25+kg/m^2 of BMI at enrollment, respectably) and 24 controls (4 for <25kg/m^2 and 20 for 25+kg/m^2 of BMI at enrollment, respectably) were included in this group with at or higher than 30 kg/m^2 for BMI at age 18.

Table18. Weight and BMI at age 18 in relation with BRAF mutation among the case-only subgroup of the papillary thyroid cancer patients, T-CALOS April 2010–December 2013

	$BRAF^{wt}$ (N=400)	$BRAF^{V600E}$ (N=903)	
	n (%)	n (%)	OR (95% CI)[1]
Weight at age 18 (kg)[2]			
Quartile 1	103 (25.75)	196 (21.71)	1.00
Quartile 2	109 (27.25)	222 (24.58)	1.07 (0.76-1.50)
Quartile 3	91 (22.75)	225 (24.92)	1.28 (0.90-1.81)
Quartile 4	97 (24.25)	260 (28.79)	1.40 (1.00-1.97)
p-trend			*0.030*
BMI at age 18 (kg/m^2)[3]			
<18.5	36 (9.00)	74 (8.19)	0.95 (0.62-1.47)
18.5-22.9	249 (62.25)	557 (61.68)	1.00
23.0-24.9	69 (17.3)	170 (18.83)	0.96 (0.69-1.33)
25+	46 (11.5)	102 (11.30)	0.83 (0.55-1.25)
p-trend			*0.500*

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index.

1. Unconditional logistic regression models were adjusted for age, sex, education, marital status, regular exercise, family history of cancer, history of chronic diseases (diabetes, hypertension and dyslipidemia), pregnancy and menopausal status.
2. The cut points of BMI at age 18 (men: 21.8, 23.4 and 25.1kg/m^2; women: 20.0, 21.5 and 23.0kg/m^2) were used based on quartile values of thyroid cancer patients.
3. The 2 cases with $BRAF^{wt}$ and 5 cases with $BRAF^{V600E}$ were identified in this group with at or higher than 30 kg/m^2 for BMI at age 18

Table 19. Weight and BMI at age 18 in relation with aggressive indicators among the case-only subgroup of the papillary thyroid cancer patients, T-CALOS April 2010–December 2013

BMI at age 18 (kg/m^2)	PTC without aggressive indicators	PTC with aggressive indicators	
	n (%)	n (%)	OR (95% CI)[1]
Tumor size[2]	**< 2cm**	**≥2cm**	
<18.5	110 (7.57)	4 (4.88)	0.74 (0.26-2.13)
18.5-22.9	905 (62.24)	43 (52.44)	1.00
23.0-24.9	271 (18.64)	19 (23.17)	1.38 (0.78-2.45)
25.0+	168 (11.55)	16 (19.51)	1.78 (0.94-3.36)
p-trend			*0.042*
Multifocality[2]	**No**	**Yes**	
<18.5	66 (7.18)	46 (7.58)	1.16 (0.78-1.74)
18.5-22.9	593 (64.53)	348 (57.33)	1.00
23.0-24.9	151 (16.43)	137 (22.57)	1.51 (1.15-1.99)
25.0+	109 (11.86)	76 (12.52)	1.22 (0.87-1.72)
p-trend			*0.093*
Extrathyroidal extension (ETE)[2]	**No**	**Yes**	
<18.5	50 (7.86)	63 (7.07)	0.97 (0.65-1.44)
18.5-22.9	406 (63.84)	535 (60.04)	1.00
23.0-24.9	118 (18.55)	170 (19.08)	1.04 (0.79-1.37)
25.0+	62 (9.75)	123 (13.80)	1.49 (1.05-2.12)
p-trend			*0.052*
Lymphovascular (LV) invasion[2]	**No**	**Yes**	
<18.5	83 (7.15)	16 (9.76)	1.44 (0.80-2.59)
18.5-22.9	735 (63.31)	100 (60.98)	1.00
23.0-24.9	217 (18.69)	25 (15.24)	0.80 (0.50-1.30)
25.0+	126 (18.69)	23 (14.02)	1.10 (0.64-1.88)
p-trend			*0.521*
Lymph node (LN) metastasis[2]	**No**	**Yes**	
<18.5	71 (7.93)	34 (6.30)	0.87 (0.57-1.35)
18.5-22.9	559 (62.46)	326 (60.37)	1.00
23.0-24.9	167 (18.66)	105 (19.44)	0.94 (0.70-1.26)
25.0+	98 (10.95)	75 (13.89)	0.99 (0.70-1.42)
p-trend			*0.920*

Abbreviation: OR=odd ratio; 95% CI=95% confidential interval; BMI=body mass index; ETE: extrathyroidal extension; LV invasion: Lymphovascular invasion; LN metastasis: lymph node metastasis.

1. Unconditional logistic regression models were adjusted for age, sex, education, marital status, regular exercise, family history of cancer, history of chronic diseases (diabetes, hypertension and dyslipidemia), pregnancy and menopausal status.
2. The cut points of BMI at age 18 (men: 21.8, 23.4 and 25.1kg/m^2; women: 20.0, 21.5 and 23.0kg/m^2) were used based on quartile values of thyroid cancer patients.

Obesity in middle-aged adults

The weight change since age 35 years were associated with increased PTC risk (Table 20). Compared to subjects who maintained a stable total weight (<5 kg), the ORs for total weight gain since age 35 years in men were 2.01 (95%CI, 1.48-2.74) for those with a total weight gain of 5-9.9 kg and 5.39 (95%CI, 3.88-7.49) for those with a total weight gain of ≥10 kg (p-trend <0.01). Compared to subjects who maintained a stable total weight (<5 kg), the ORs for total weight gain since age 35 years in women were 1.68 (95%CI, 1.45-1.94) for those with a total weight gain of 5-9.9 kg and 3.36 (95%CI, 2.87-3.93) for those with a total weight gain of ≥10 kg). Table 20 also shows that compared with a stable weight, annual average increases in weight since age 35 years (increases of 1.0-1.9 kg/year) were associated with an increased PTC risk in men (OR=2.93, 95%CI=1.79-4.81) and women (OR=2.30, 95%CI, 1.85-2.85). The associations between weight increase and PTC risk were more pronounced when the annual average weight increase was equal to or greater than 2.0 kg/year (men: OR=12.57, 95%CI, 6.76-23.37; women: OR=6.05, 95%CI=4.45-8.24).

Table 20. Changes in weight since age 35 years and the risk for papillary thyroid cancer, T-CALOS April 2010–December 2013

	Men			Women			
Changes since age 35 years	Cases[1]	Controls[1]	OR (95%CI)[2]	Cases[1]	Controls[1]	OR (95%CI)[2]	*p*-heterogeneity[3]
Total weight changes (kg)							
Lost ≥5	14	273	0.80 (0.45-1.43)	49	621	1.17 (0.86-1.60)	0.256
Lost or gain <5	118	1873	Reference	535	7628	Reference	
Gained 5-9.9	81	599	2.01 (1.48-2.74)	336	2861	1.68 (1.45-1.94)	0.295
Gained ≥10	87	255	5.39 (3.88-7.49)	331	1400	3.36 (2.87-3.93)	0.011
Annual average changes[4]							
Weight (kg)							
Decreased ≥ 1.0	5	102	1.37 (0.51-3.68)	16	154	1.83 (1.06-3.17)	0.619
Changed < 1.0	223	2584	Reference	1013	11279	Reference	
Increased 1.0-1.9	27	180	2.93 (1.79-4.81)	132	803	2.30 (1.85-2.85)	0.377
Increased ≥ 2.0	45	134	12.57 (6.76-23.37)	90	274	6.05 (4.45-8.24)	0.039

Abbreviation: OR=odds ratio; 95%CI=95% confidential interval; BMI=body mass index; BSA=body surface area; BF%=body fat percentage.

1. Papillary thyroid cancer and their 1:10 matched healthy controls in the analyses.
2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown), marital status (single, married and unknown), regular exercise (yes, no and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. *p*-heterogeneity was to compare ORs in the men and women.
4. Annual average change= (obesity indicators at enrollment - obesity indicators at age 35 years)/ age difference since age 35 years.

Change in Obesity indicators since age 35 and PTC risk

Table 21 showed the associations between changes in obesity indicators since age 35 years and the risk for PTC. Among the subjects with annual average increase in BMI >0.3 kg/m^2/year, the PTC risk was elevated in both men (OR=4.42, 95%CI=2.93−6.66) and women (OR=2.51, 95%CI=2.09−3.01). There were similar trends in the associations between an annual average increase in BSA (increase ≥0.010 m^2/year) and PTC risk in men (OR=5.23, 95%CI=3.43−9.97) and women (OR=3.18, 95%CI=2.63−3.84) compared with those with a stable BSA. The association between BF% (increase ≥0.3 %/year) and PTC risk were estimated in men (OR=3.82, 95%CI=2.58−5.65) and women (OR=2.26, 95%CI=1.90−2.68) compared with those with a stable BF%. In general, weight gain and increased obesity, including weight change (p−heterogeneity=0.011) and annual average change in obesity indicators (p−heterogeneity<0.05), had a greater effect on PTC risk in men than in women.

Table 21. Changes in obesity indicators since age 35 years and the risk for papillary thyroid cancer, T-CALOS April 2010–December 2013

	Men			Women			
Changes since age 35 years	Cases[1]	Controls[1]	OR (95%CI)[2]	Cases[1]	Controls[1]	OR (95%CI)[2]	*p*-heterogeneity[3]
BMI[4] (kg/m^2)							
Decreased ≥ 0.1	14	384	0.58 (0.32-1.04)	77	1068	1.02 (0.79-1.32)	0.086
Changed < 0.1	116	1542	Reference	460	5724	Reference	
Increased 0.1-0.2	91	713	1.77 (1.30-2.40)	412	3975	1.29 (1.12-1.49)	0.065
Increased ≥ 0.3	79	361	4.42 (2.93-6.66)	302	1743	2.51 (2.09-3.01)	0.014
BSA[5] (m^2)							
Decreased ≥ 0.005	8	236	0.62 (0.29-1.32)	47	573	1.41 (1.02-1.96)	0.050
Changed < 0.005	158	2020	Reference	651	8289	Reference	
Increased 0.005-0.009	57	410	2.00 (1.41-2.83)	296	2282	1.77 (1.52-2.06)	0.532
Increased ≥ 0.010	77	334	5.23 (3.43-7.97)	257	1366	3.18 (2.63-3.84)	0.035
BF%[6] (%)							
Decreased ≥ 0.1	18	438	0.65 (0.38-1.11)	90	1257	0.98 (0.76-1.25)	0.180
Changed < 0.1	100	1371	Reference	403	4922	Reference	
Increased 0.1-0.2	92	743	1.71 (1.25-2.34)	385	4070	1.14 (0.98-1.32)	0.021
Increased ≥ 0.3	90	448	3.82 (2.58-5.65)	373	2261	2.26 (1.90-2.68)	0.016

Abbreviation: OR=odds ratio; 95%CI=95% confidential interval; BMI=body mass index; BSA=body surface area; BF%=body fat percentage.

1. Papillary thyroid cancer and their 1:10 matched healthy controls in the analyses.
2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown), marital status (single, married and unknown), regular exercise (yes, no and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. *p*-heterogeneity was to compare ORs in the men and women.
4. BMI= $(\text{weight (kg)})^2$/ height (m).

5. Using Dubois and Dubois's formula, BSA=0.007184 x height (cm) $^{0.725}$ x weight (kg) $^{0.425}$.
6. Using Deurenberg et al.,'s formula, BF%= (1.20 × BMI) + (0.23 × age) − (10.8 × sex (1 for men and 0 for women)) − 5.40.

Subgroup analyses by tumor size (<1cm and ≥1cm)

When stratified for thyroid tumor size, the effect of an increased annual average BMI (≥0.3 kg/m^2/year) for patients with large-sized PTC (tumors ≥1 cm) was greater than the effect for those with a relatively small tumor (tumor size ≥1 cm, OR, 4.00, 95%CI, 2.91-5.49; tumor size <1 cm, OR, 2.34, 95%CI, 1.92-2.85; p-heterogeneity=0.005; Table 22). The results for the annual average change in BSA and BF% were consistent (p-heterogeneity<0.01). We also estimated the association between the annual average change in obesity indicators and the PTC risk related to the clinicopathological features of lymph node metastasis, TNM stage, tumor multifocality, BRAF (V600E) mutation, and chronic lymphocytic thyroiditis; however, no significant difference in these associations were detected (data not shown).

Table 22. The associations between changes in weight and obesity indicators since age 35 years and the risk for papillary thyroid cancer stratified by patients' tumor size and their matched controls, T-CALOS April 2010–December 2013

	Tumor size[1] <1cm			Tumor size[1] ≥1cm			
Annual average changes[4]	Cases (N=148)	Controls (N=1326)	OR (95%CI)[2]	Cases (N=148)	Controls (N=1326)	OR (95%CI)[2]	*p*-heterogeneity[3]
Weight (kg)							
Decreased ≥ 1.0	16	176	1.69 (0.97-2.92)	5	76	1.50 (0.56-4.01)	0.837
Changed < 1.0	879	9719	Reference	346	4009	Reference	
Increased 1.0-1.9	106	721	2.07 (1.63-2.63)	51	252	3.33 (2.30-4.82)	0.034
Increased ≥ 2.0	91	304	5.99 (4.32-8.29)	42	103	10.01 (6.00-16.68)	0.096
BMI[5] (kg/m^2)							
Decreased ≥ 0.1	71	1034	0.93 (0.71-1.22)	20	400	0.85 (0.52-1.39)	0.749
Changed < 0.1	413	5012	Reference	159	2197	Reference	
Increased 0.1-0.2	352	3339	1.26 (1.08-1.47)	146	1293	1.63 (1.27-2.08)	0.088
Increased ≥ 0.3	256	1535	2.34 (1.92-2.85)	119	550	4.00 (2.91-5.49)	0.005
BSA[6] (m^2)							
Decreased ≥ 0.005	43	573	1.23 (0.88-1.74)	12	225	1.04 (0.55-1.95)	0.635
Changed < 0.005	574	7194	Reference	229	3024	Reference	
Increased 0.005-0.009	251	1902	1.73 (1.46-2.04)	98	756	1.98 (1.52-2.59)	0.386
Increased ≥ 0.010	224	1251	2.94 (2.40-3.61)	105	435	5.06 (3.63-7.04)	0.006
BF%[7] (%)							
Decreased ≥ 0.1	85	1204	0.95 (0.73-1.22)	23	473	0.76 (0.48-1.21)	0.421
Changed < 0.1	355	4322	Reference	144	1921	Reference	
Increased 0.1-0.2	334	3417	1.16 (0.99-1.37)	138	1342	1.38 (1.07-1.78)	0.267
Increased ≥ 0.3	318	1977	2.19 (1.82-2.63)	139	704	3.29 (2.45-4.43)	0.021

Abbreviation: OR=odds ratio; 95%CI=95% confidential interval; BMI=body mass index; BSA=body surface area; BF%=body fat percentage

1. Thyroid cancer patients with missing information for thyroid tumor size (n=15) and their matched controls were excluded.

2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown), marital status (single, married and unknown), regular exercise (yes, no and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. *p*-heterogeneity was to compare ORs in the subgroups by thyroid tumor size.
4. Annual average change= (obesity indicators at enrollment – obesity indicators at age 35 years)/ age difference since age 35 years.
5. BMI= (weight (kg))^2/ height (m).
f. Using Dubois and Dubois's formula, BSA=0.007184 x height (cm) $^{0.725}$ x weight (kg) $^{0.425}$.
6. Using Deurenberg et al.,'s formula, BF%= (1.20 × BMI) + (0.23 × age) − (10.8 × sex (1 for men and 0 for women)) − 5.40.
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Subgroup analyses by menopausal status

In Table 23, the associations between annual average BMI increases of 0.3 kg/m^2 or greater and PTC risk in the women were estimated according to menopausal status (post-menopausal women, OR=3.01, 95%CI=2.13-4.25; pre-menopausal women, OR=2.03, 95%CI=1.60-2.58). We observed significant differences between the associations for the subgroups in terms of BSA (annual average increase ≥0.010: post-menopausal women, OR=5.04, 95%CI=3.29-7.72; pre-menopausal women, OR=2.58, 95%CI=2.04-3.26; p-heterogeneity=0.007) and BF% (annual average increase ≥0.3: post-menopausal women, OR=1.82, 95%CI=1.43-2.31; pre-menopausal women, OR=2.67, 95%CI=2.01-3.53; p-heterogeneity=0.042) compared with those who maintained stable obesity indicators. Menopausal status and annual average changes in BSA had significant interaction effects for the development of PTC (p-value for interaction=0.035).

Table 23. The associations between changes in weight and obesity indicators since age 35 years and the risk for papillary thyroid cancer stratified by menopausal status, T-CALOS April 2010–December 2013

	Pre-menopausal women			Menopausal women			*p*-value for	*p*-value for
Annual average changes[4]	Cases[1]	Controls[1]	OR (95%CI)[2]	Cases[1]	Controls[1]	OR (95%CI)[2]	heterogeneity[3]	interaction[3]
Weight (kg)								
Decreased ≥ 1.0	15	143	1.72 (0.97-3.04)	0	5	NA	NA	0.682
Changed < 1.0	398	4648	Reference	611	6067	Reference		
Increased 1.0-1.9	99	660	1.92 (1.48-2.48)	33	88	3.38 (2.22-5.15)	0.024	
Increased ≥ 2	82	249	5.32 (3.84-7.37)	8	11	6.95 (2.66-18.13)	0.606	
BMI[5] (kg/m^2)								
Decreased ≥ 0.1	60	679	1.17 (0.84-1.62)	16	345	0.62 (0.37-1.05)	0.048	0.140
Changed < 0.1	160	1868	Reference	298	3592	Reference		
Increased 0.1-0.2	144	1812	0.86 (0.67-1.10)	266	1940	1.65 (1.38-1.99)	<0.001	
Increased ≥ 0.3	230	1341	2.03 (1.60-2.58)	72	294	3.01 (2.13-4.25)	0.066	
BSA[6] (m^2)								
Decreased ≥ 0.005	44	453	1.48 (1.03-2.12)	2	93	0.29 (0.07-1.20)	0.030	0.035
Changed < 0.005	218	2885	Reference	429	5008	Reference		
Increased 0.005-0.009	130	1242	1.29 (1.01-1.64)	166	911	2.21 (1.79-2.72)	0.001	
Increased ≥ 0.010	202	1120	2.58 (2.04-3.26)	55	159	5.04 (3.29-7.72)	0.007	
BF%[7] (%)								
Decreased ≥ 0.1	65	749	1.07 (0.78-1.49)	24	451	0.69 (0.44-1.08)	0.115	0.276
Changed < 0.1	143	1576	Reference	259	3120	Reference		
Increased 0.1-0.2	119	1741	0.67 (0.51-0.88)	263	2115	1.49 (1.23-1.80)	<0.001	
Increased ≥ 0.3	267	1634	1.82 (1.43-2.31)	106	485	2.67 (2.01-3.53)	0.042	

Abbreviation: OR=odds ratio; 95%CI=95% confidential interval; BMI=body mass index; ; BSA=body surface area; BF%=body fat percentage; NA=not applicable.

1. Papillary thyroid cancer and their 1:10 matched healthy controls in the analyses.
2. Conditional logistic regression models were adjusted for education (high school graduation: yes, no and unknown), marital status (single, married and unknown), regular exercise (yes, no and unknown), history of chronic diseases (diabetes, hypertension and dyslipidemia) and ever pregnancy (yes, no and unknown).
3. *p*-value for heterogeneity was to compare ORs in the pre-menopausal women and post-menopausal women: and *p*-value for interaction was derived from cross product term of obesity indicators and menopausal status in logistic regression models.
4. Annual average change= (obesity indicators at enrollment – obesity indicators at age 35 years)/ age difference since age 35 years.
5. BMI= (weight (kg))^2/ height (m).
6. Using Dubois and Dubois's formula, BSA=0.007184 x height (cm)$^{0.725}$ x weight (kg)$^{0.425}$.
7. Using Deurenberg et al.,'s formula, BF%= (1.20 × BMI) + (0.23 × age) − (10.8 × sex (1 for men and 0 for women)) − 5.40.

2. The KMCC study data

a) Medical radiation and thyroid cancer

General characteristics

The general characteristics of the study subjects within the two radiation exposure groups are summarized in Table 24. The mean age for the unexposed and exposed groups was 53.85 and 54.14, respectively (p= 0.18). Among the thyroid cancer cases only, the mean age at diagnosis was 47.13 and 49.19 for the unexposed group and the exposed group, respectively (p=0.416). The median follow-up duration in years was longer in the unexposed group (13.24 years) than that in the exposed group (11.24 years). In addition, there were significant differences BMI, education, and marital status between the two groups. With regard to lifestyle factors, a relatively high proportion of smokers (41.82% vs. 35.16%) and drinkers (44.34% vs. 41.16%) was observed in the unexposed groups (p < 0.001) compared to the exposed group. On the other hands, a lower proportion of subjects were observed for a previous diagnosis of benign thyroid disease, diabetes mellitus, hypertension and family history of cancer than that in the

unexposed group. The women in the exposed groups were more likely to be ever-pregnant and postmenopausal.

Medical diagnostic radiography with thyroid cancer risk

Exposure to at least one diagnostic radiography source was not clearly associated with thyroid cancer risk (HR=1.87, 95%CI=0.83-3.56), in general (Figure 7). Although the magnitude of the associations were elevated with an increasing follow-up period, the estimated HRs were not statistically significant (2-year: HR=1.81, 95%CI=0.73-3.38; 5 years: HR=2.13, 95%CI=0.74-4.50; 7 years: HR=2.46, 95%CI=0.74-5.40; 10 years: HR=3.29, 95%CI=0.72-9.94). Exposure to each procedure and thyroid cancer risk was not clearly associated considering statistical significance. X-ray radiography was associated with an increased risk of thyroid cancer (frequency of 2-5 times: HR=1.85, 95%CI=0.85-4.03; 6-10 times: HR=2.31, 95%CI = 0.97-5.49; 11+ times: HR=2.08, 95%CI=0.78-5.54) compared to those who were unexposed to any medical radiation including X-ray, UGI, CT and mammography (Table 24). The HRs related to UGI (once: HR=1.18, 95%CI=0.18-7.74; 2+ times: HR=1.73,

95%CI=0.27−11.24), CT (once: HR=1.54, 95%CI=0.26−9.36; 2+ times: HR=2.09, 95%CI=0.31−4.15) and mammography (once: HR=3.09, 95%CI=0.53−18.04; 2+times: 0.53−19.54) were not statistically significant (Table 25). Considering a latency period, the results of our sensitivity analyses after excluding the first 2 years, 5 years, 7 years and 10 years of follow−up (Table 26). Although there was a trend that the point estimates were higher in a cohort with a longer latency period, all p−values from heterogeneity test were not statistically significant (data not shown).

We found more enhanced associations in women compared to that in men, the sex difference was not statistically significant (all p−values for heterogeneity>0.05; data not shown). The effects of diagnostic radiography on developing the histologic types of thyroid cancer, DTC and PTC, similar HRs with slightly weakened associations were estimated (data not shown). Due to a small number of the cases, we did not analyze HRs for FTC and MTC. The results, whether the groups were stratified by age at baseline (<50 vs. 50+ years old) and menopausal status (premenopausal vs. postmenopausal) did not indicate any significant modifying effect (data not shown).

Table 24. General characteristics of the total study subjects according to the exposure status of medical diagnostic radiography based on the KMCC study

	Exposure status to any type of diagnostic radiography		
	Unexposed (N=7582)	Exposed (N=11411)	p-value[1]
Age (mean, SD)	53.85 (14.31)	54.14 (14.47)	0.176
Follow-up duration (mean, SD)	12.34 (4.37)	10.70 (4.42)	<0.001
Sex			
Men	3587 (47.31)	4345 (38.08)	<0.001
Women	3995 (52.69)	7066 (61.92)	
BMI (kg/m^2) [2,3]			
<25	4803 (63.35)	7439 (65.19)	<0.001
25+	1890 (24.93)	3569 (31.28)	
Years of schooling [2]			
<12	6583 (86.82)	9736 (85.32)	<0.001
12+	919 (12.12)	1633 (14.31)	
Marital status [2]			
Not married	473 (6.24)	676 (5.92)	<0.001
Married	6265 (82.63)	10659 (93.41)	
Cigarette smoking [2,4]			
Never	4320 (56.98)	7337 (64.30)	<0.001
Ever	3171 (41.82)	4012 (35.16)	
Alcohol drinking [2,4]			
Never	4130 (54.47)	6585 (57.71)	<0.001
Ever	3362 (44.34)	4697 (41.16)	
Benign thyroid disease [2,4]			
Never	2970 (39.17)	11108 (97.34)	<0.001
Ever	29 (0.38)	256 (2.24)	
Diabetes mellitus [2]			
Never	7089 (93.50)	9942 (87.13)	<0.001
Ever	263 (3.47)	551 (4.83)	
Hypertension [2]			
Never	6873 (90.65)	9746 (85.41)	<0.001
Ever	599 (7.89)	1601 (14.03)	
Family history of cancer [2,3]			
No	1880 (24.80)	6341 (55.57)	<0.001
Yes[2]	263 (3.47)	1683 (14.75)	
Pregnancy [5]			
Never	202 (5.06)	316 (4.47)	<0.001
Ever	1523 (38.12)	5346 (75.66)	
Menopausal status [4,5]			
Premenopausal	1296 (32.44)	2816 (39.85)	<0.001
Postmenopausal	2684 (67.18)	4233 (59.91)	

Abbreviation: N=number, SD=standard deviation; BMI=body mass index; UGI=upper gastrointestinal series; CT= computerized tomography.

1. The difference between the two groups (Unexposed to any type of diagnostic radiography vs. Exposed to any type of diagnostic radiography including x-ray, UGI, CT and mammography) was compared using t-test and Wilcoxon test for the continuous variables, and χ^2 test was used for the categorical variables.
2. Subjects with missing information were observed for BMI (6.80%), years of schooling (0.64%), marital status (4.84%), cigarette smoking (0.81%), alcohol drinking (1.15%), benign thyroid disease (24.38%), diabetes mellitus (6.04%), hypertension (0.92%), family history of cancer (46.47%), pregnancy (33.22%) and menopausal status (0.29%).
3. Among those who were with family history of cancer, 3 subjects reported their family history for thyroid cancer (2 for the unexposed group and 1 for the exposed group).
4. The factors were associated with both exposure status of diagnostic radiography and outcome, which were included as covariates in our regression analyses.
5. Only women were included.

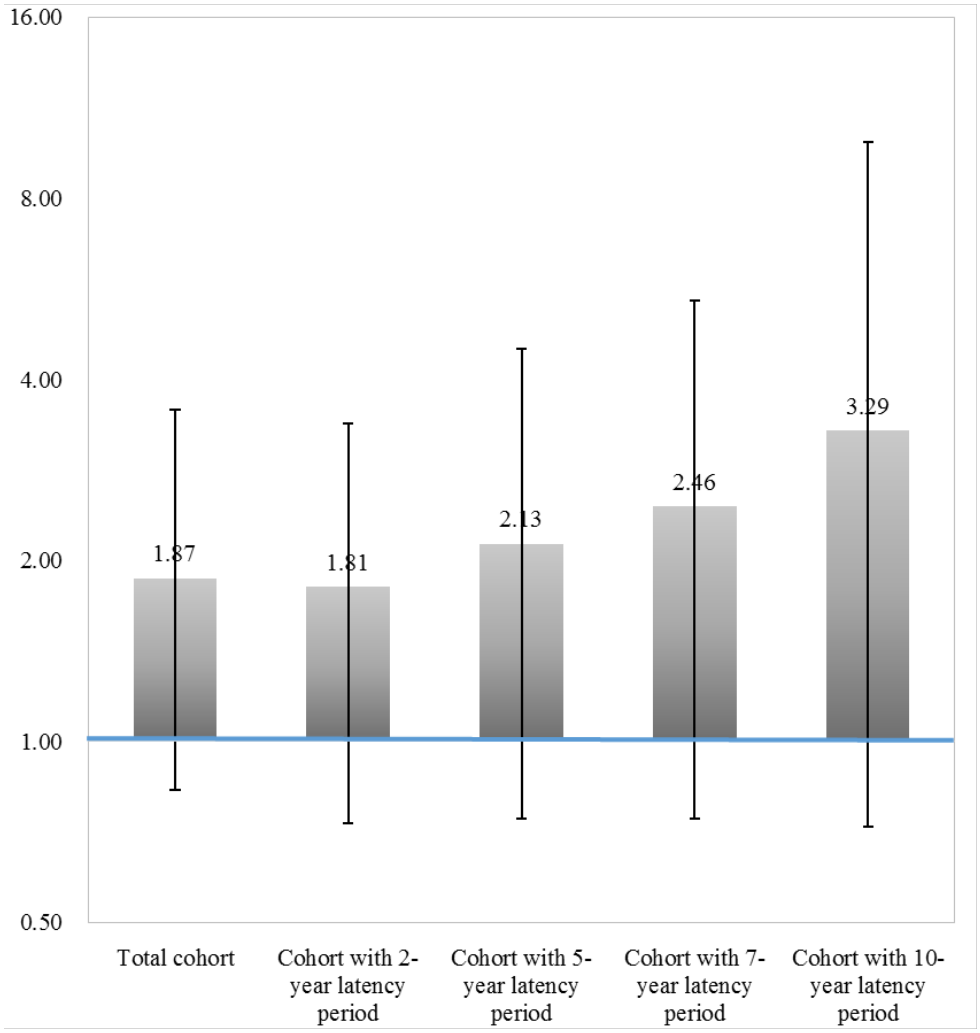

Figure 7 the exposure status of medical diagnostic radiography and thyroid cancer risk according to follow-up years considering a latency period, based on the Korean Multi-center Cancer Cohort study.

Table 25. Medical diagnostic radiography and thyroid cancer risk based on the KMCC study

	Person-years	N of cases	HR (95%CI)[1]
X-ray radiography			
Unexposed to any diagnostic radiography[2]	100403	32	1.00
1	19910	7	1.00 (0.36-2.79)
2-5	63530	39	1.85 (0.85-4.03)
6-10	22752	17	2.31 (0.97-5.49)
11+	17187	9	2.08 (0.78-5.54)
Unknown status for x-ray	4910	2	2.08 (0.78-5.55)
p-trend			*0.114*
UGI			
Unexposed to any diagnostic radiography[2]	100403	32	1.00
1	8692	5	1.18 (0.18-7.74)
2+	6989	6	1.73 (0.27-11.24)
Unknown status for UGI	112608	63	1.31 (0.26-6.57)
p-trend			*0.535*
CT			
Unexposed to any diagnostic radiography[2]	100403	32	1.00
1	9634	7	1.54 (0.26-9.36)
2+	4669	4	2.09 (0.31-4.15)
Unknown status for CT	113986	63	1.30 (0.26-6.51)
p-trend			*0.546*
Mammography[3]			
Unexposed to any diagnostic radiography[2]	56136	25	1.00
1	10598	11	3.09 (0.53-18.04)
2+	7152	8	3.21 (0.53-19.54)
Unknown status for mammography	64027	47	2.35 (0.41-13.28)
p-trend			*0.999*

Abbreviation: N=number, HR=hazard ratio; 95%CI=95% confidence interval; UGI=upper gastrointestinal series; CT=computerized tomography; BMI=body mass index; NA=not applicable.

1. Cox proportional hazards regression models were adjusted for age, enrollment year, sex, BMI, smoking, alcohol drinking, benign thyroid disease, menopausal status and the other procedures of medical diagnostic radiography.
2. Subjects unexposed to any diagnostic radiography including x-ray, UGI, CT and mammography were included.
3. Only women were included.

Table 26. Medical diagnostic radiography and thyroid cancer incidence considering a latency period in the KMCC study

Cohort with a latency period	2 years	5 years	7 years	10 years
	HR (95%CI)[1]	HR (95%CI)[1]	HR (95%CI)[1]	HR (95%CI)[1]
X-ray radiography				
Unexposed to any diagnostic radiography[2]	1.00	1.00	1.00	1.00
1	0.87 (0.30-2.51)	1.06 (0.32-3.54)	1.28 (0.36-4.51)	2.42 (0.55-10.55)
2-5	1.78 (0.82-3.90)	1.98 (0.79-4.95)	2.24 (0.83-6.08)	3.46 (0.97-12.31)
6-10	2.37 (0.99-5.64)	**3.07 (1.13-8.32)**	**3.54 (1.20-10.41)**	**4.49 (1.09-18.53)**
11+	1.89 (0.69-5.18)	2.60 (0.84-8.02)	2.82 (0.83-9.64)	3.46 (0.66-18.08)
Unknown status for x-ray	0.98 (0.20-4.75)	0.77 (0.09-6.66)	0.93 (0.10-8.28)	NA
p-trend	*0.096*	*0.051*	*0.073*	*0.440*
UGI				
Unexposed to any diagnostic radiography[2]	1.00	1.00	1.00	1.00
1	1.36 (0.21-9.06)	2.46 (0.21-28.95)	4.53 (0.36-56.47)	NA
2+	2.03 (0.31-13.29)	2.98 (0.24-36.48)	3.97 (0.28-55.65)	NA
Unknown status for UGI	1.32 (0.26-6.62)	1.13 (0.13-10.15)	1.36 (0.15-12.71)	3.29 (0.80-13.55)
p-trend	*0.535*	*0.808*	*0.837*	NA
CT				
Unexposed to any diagnostic radiography[2]	1.00	1.00	1.00	1.00
1	1.41 (0.23-8.85)	0.55 (0.04-7.77)	0.40 (0.02-8.43)	NA
2+	2.20 (0.32-15.04)	1.27 (0.09-18.19)	1.89 (0.12-28.76)	NA
Unknown status for CT	1.30 (0.26-6.56)	1.13 (0.13-10.09)	1.38 (0.15-12.86)	3.29 (0.80-13.55)
p-trend	*0.438*	*0.387*	*0.261*	NA
Mammography[3]				
Unexposed to any diagnostic radiography[2]	1.00	1.00	1.00	1.00
1	3.36 (0.57-19.61)	3.56 (0.35-36.60)	3.92 (0.38-40.84)	4.75 (0.72-31.54)
2+	3.07 (0.49-19.13)	2.33 (0.20-27.06)	2.61 (0.22-30.59)	5.93 (0.75-46.95)
Unknown status for mammography	2.35 (0.41-13.39)	2.05 (0.20-20.85)	2.06 (0.20-21.18)	**4.44 (1.01-19.48)**
p-trend	*0.786*	*0.453*	*0.453*	*0.877*

Abbreviation: N=number, HR=hazard ratio; 95%CI=95% confidence interval; UGI=upper gastrointestinal series; CT=computerized tomography; BMI=body mass index; NA=not applicable.

1. Cox proportional hazards regression models were adjusted for age, enrollment year, sex, BMI, smoking, alcohol drinking, benign thyroid disease, menopausal status and the other procedures of medical diagnostic radiography.
2. Subjects unexposed to any diagnostic radiography including x-ray, UGI, CT and mammography were included.
3. Only women were included.

We considered the possibility of correlations between procedures; therefore we examined the associations between various combinations of exposure types (Table 27). Exposure to X-ray only was not a significant risk factor (HR=1.80, 95%CI=0.85-3.81). The elevation in risk was observed in those who were exposed to four medical radiation types: X-ray, UGI, CT, and mammography (HR=4.47, 95%CI=1.04-19.19) as in Table 27. The magnitude of this associations were consistent considering a latency period for 2 years (HR=5.17, 95%CI=1.19-22.43); however, only one case was detected after 5 years (HR=4.15, 95%CI=0.42-40.82) and 7 years (HR=5.95, 95%CI=0.56-62.64) of follow-up duration. (Table 28). No clear evidence for potential effect modifier both individually and in combination as a multiplicative interaction were found in the analyses for baseline age (<50 vs. 50+ years old), sex (men vs. women) and menopausal status (premenopausal vs. postmenopausal women), which p-values were greater than 0.05 (data not shown).

Table 27. Combined exposures to medical diagnostic radiography and thyroid cancer risk considering a latency period in the KMCC study

	Total		
	Person-years	N of cases	HR (95% CI)[1]
Unexposed[2]	100403	32	1.00
Exposed to X-ray only	93307	47	1.80 (0.85-3.81)
Exposed to X-ray and mammography	10325	10	2.06 (0.78-5.40)
Exposed to X-ray and UGI	6034	2	1.45 (0.28-7.56)
Exposed to X-ray and CT	5236	3	2.51 (0.59-10.69)
Exposed to X-ray and additional 2 types of radiation	8467	7	2.82 (0.88-9.02)
Exposed to X-ray, UGI, CT and mammography	1796	3	4.47 (1.04-19.19)
Other combinations of radiation exposure	3123	2	1.79 (0.36-8.77)

Abbreviation: N=number, HR=hazard ratio; 95%CI=95% confidence interval; UGI=Upper gastrointestinal series; CT=computerized tomography; BMI=body mass index; NA=not applicable.

1. Cox proportional hazards regression models were adjusted for age, enrollment year, sex, BMI, smoking, alcohol drinking, benign thyroid disease, menopausal status and the other procedures of medical diagnostic radiography.

2. Subjects unexposed to any diagnostic radiography including x-ray, UGI series, CT and mammography were included.

Table 28. Combined exposures to medical diagnostic radiography and thyroid cancer risk considering a latency period in the KMCC study

Cohort with a latency period	2 years	5 years	7 years	10 years
	HR (95%CI)[1,2]	HR (95%CI)[1,2]	HR (95%CI)[1,2]	HR (95%CI)[1,2]
Unexposed to any diagnostic radiography	1.00	1.00	1.00	1.00
Exposed to X-ray only	1.71 (0.81-3.64)	1.97 (0.82-4.75)	2.26 (0.87-5.89)	3.28 (0.96-11.27)
Exposed to X-ray and mammography	2.16 (0.82-5.70)	2.95 (0.99-8.79)	3.59 (1.13-11.43)	3.75 (0.81-17.40)
Exposed to X-ray and UGI	1.59 (0.30-8.36)	4.36 (0.72-26.32)	3.76 (0.36-38.90)	NA
Exposed to X-ray and CT	2.74 (0.63-11.83)	2.44 (0.25-24.01)	NA	NA
Exposed to X-ray and additional 2 types of radiation	2.73 (0.81-9.18)	4.65 (1.03-21.05)	7.23 (1.45-36.19)	NA
Exposed to X-ray, UGI, CT and mammography	5.17 (1.19-22.43)	4.15 (0.42-40.82)	5.95 (0.56-62.64)	NA
Other combinations of radiation exposure	1.87 (0.38-9.21)	1.73 (0.20-15.08)	2.29 (0.25-20.78)	NA

Abbreviation: N=number, HR=hazard ratio; 95%CI=95% confidence interval; UGI=upper gastrointestinal series; CT=computerized tomography; BMI=body mass index; NA=not applicable.

1. Cox proportional hazards regression models were adjusted for age and enrollment year at baseline, sex, BMI, smoking, alcohol drinking, benign thyroid disease, menopausal status and the other procedures of medical diagnostic radiography.

2. Subjects unexposed to any diagnostic radiography including x-ray, UGI, CT and mammography were included.

3. The subset for DNA methylation study

The quality of the samples for the DNA methylation analyses was confirmed, as presented in Figs. 8 to 11. The hierarchical clustering was determined as shown in Figure 12. The correlations between the results were validated, as shown in Figure 13.

Among the 95 selected CpG sites, significant differences at 28 sites (p-value <0.05) were detected between the subjects and the controls (Table 29). Considering the FDR-corrected p-values for statistical significance, eleven sites (RAD51, ITGB3, RAD50, ERCC4, FANCG, PFC, PNMA5, MGC52057, PRSS22, SULT1A1 and DMPK) were found. These results were visualized in a heatmap (Figure 14) and in a PCA plot (Figure 15).

When we restricted the results to true CpG islands, a relatively low methylation level was observed in the three methylation sites of cg05293216 in the FANCG gene, cg12594641 in the MGC52057 gene and cg18345369 in the PRSS22 gene, as shown in Table 29. The mean differences in the FDR-corrected

p-values between the subjects and the controls were -0.038 (p-value <0.001), -0.010 (p-value=0.048) and -0.048 (p-value=0.030) for cg05293216, cg12594641 and cg18345369, respectably.

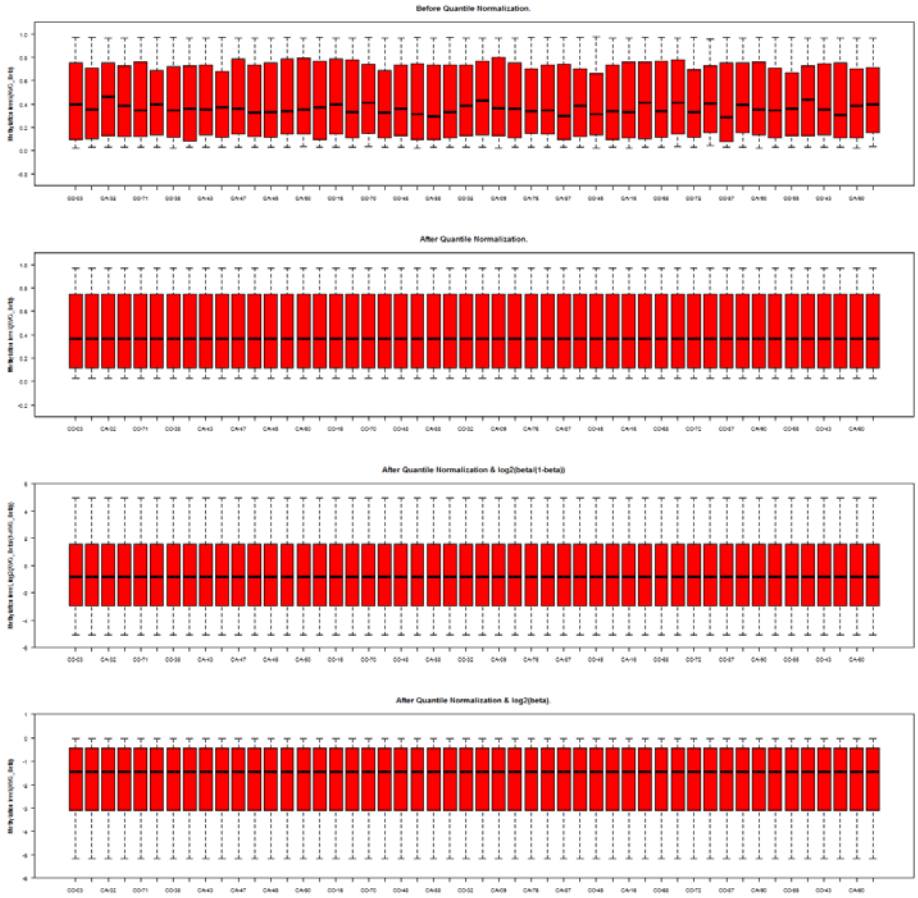

Figure 8 The box-plot before and after the quantile normalization of the samples.

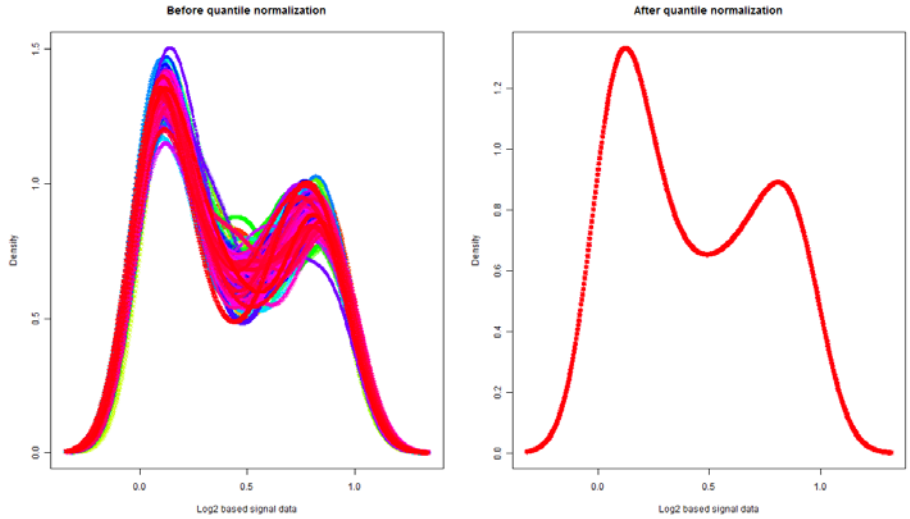

Figure 9 The density-plot before and after the quantile normalization of the samples.

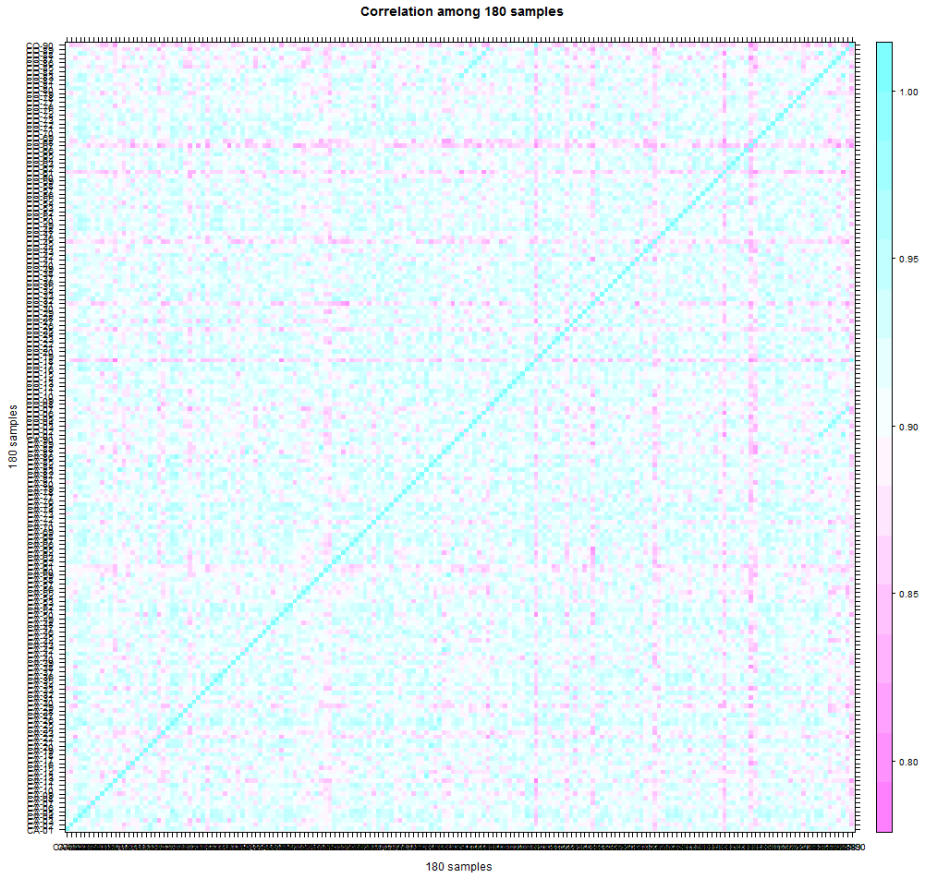

Figure 10 The level-plot of the total samples.

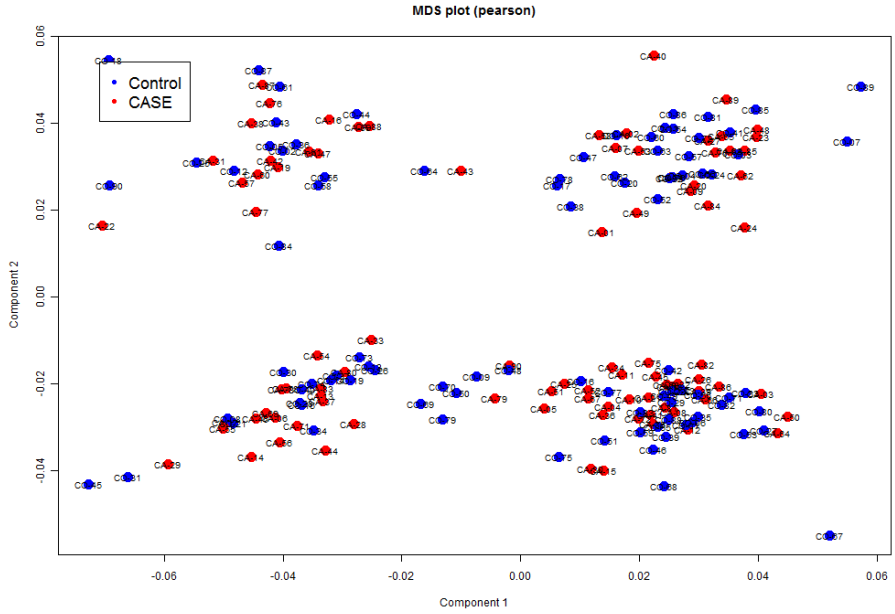

Figure 11 The MDS plot for the cases and the controls.

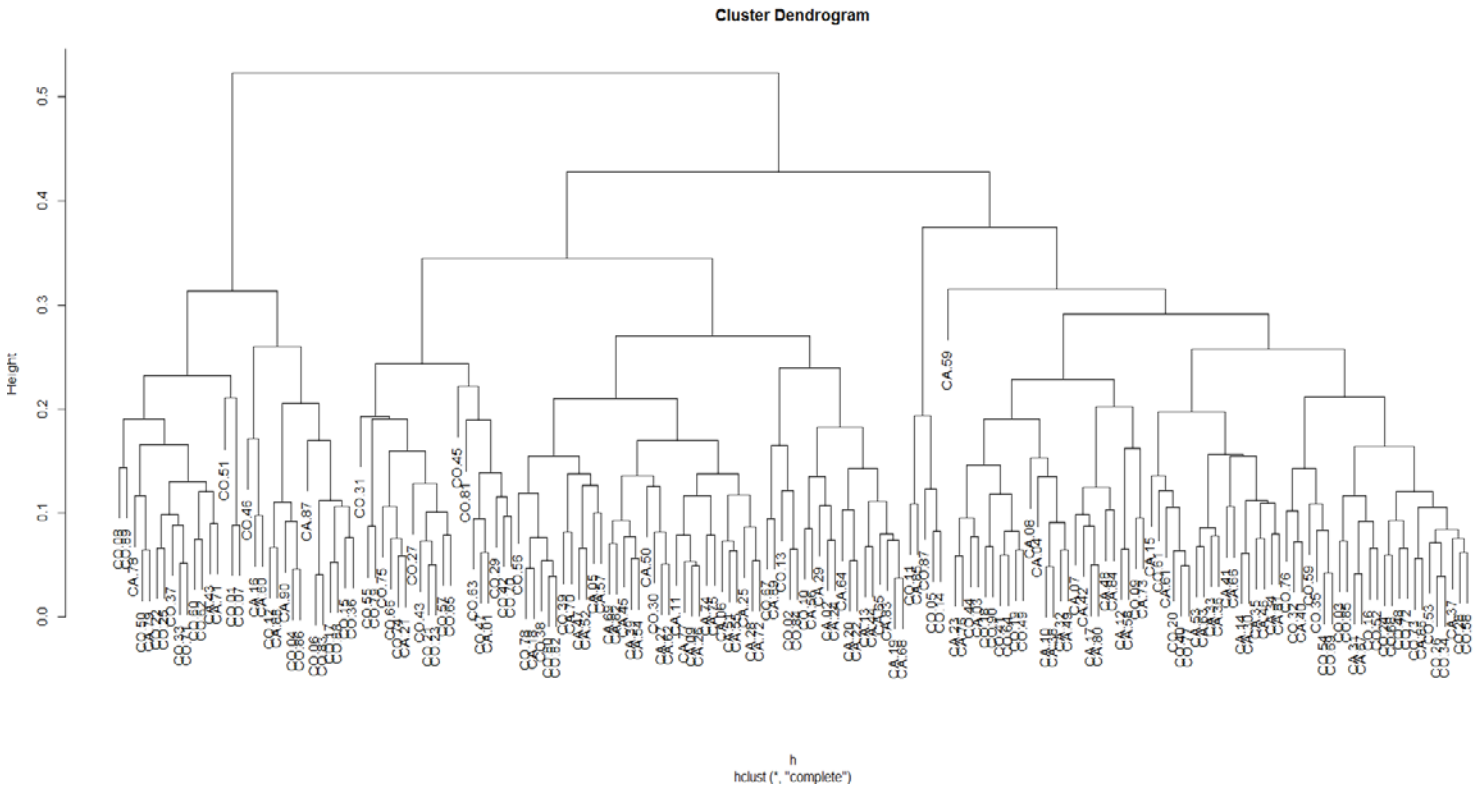

Figure 12 The hierarchical clustering of the DNA methylation level.

Figure 13 Correlations of the DNA methylation level for each sample.

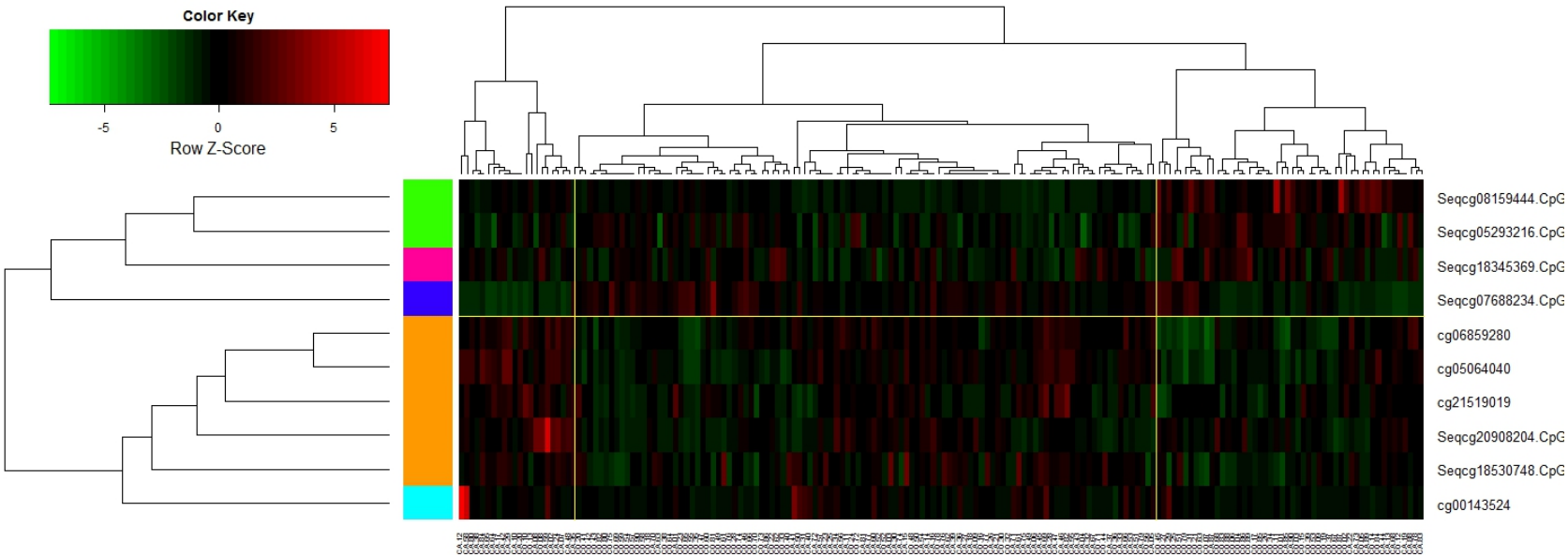

Figure 14 Heatmap for DNA methylation level for the selected CpG sites.

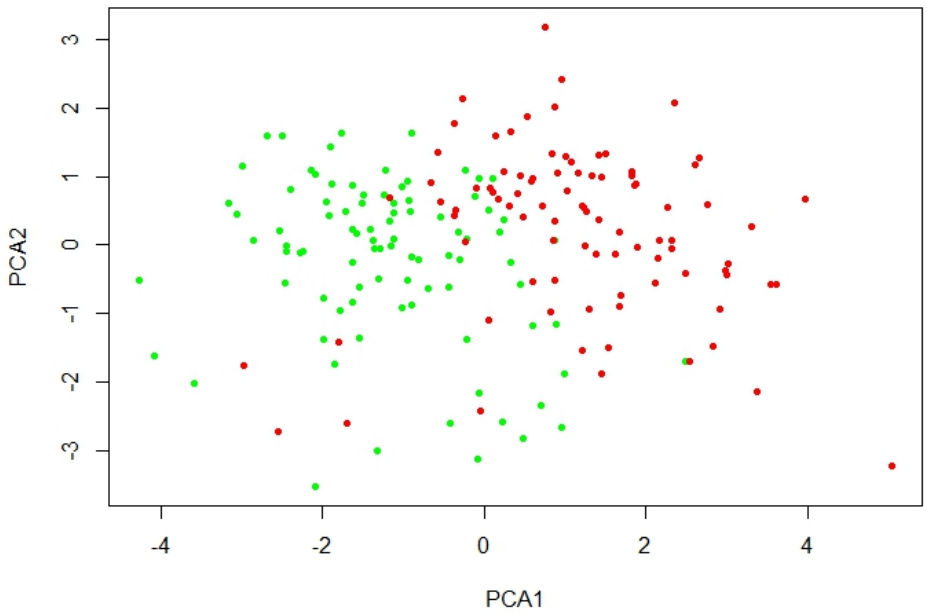

Figure 15 PCA plot for DNA methylation level for the selected CpG sites.

Table 29. Distribution of DNA methylation level compared between the cases and the controls

Locus	Chromosome	Gene	Cases	Controls	Mean difference	t	p.value	fdr.p.val	Cpg_Island
cg00143524	15	RAD51	0.0477	0.0398	0.0079	-4.3209	<0.001	0.0006	
cg05032573	3	SLC4A7	0.1693	0.1599	0.0094	-2.4498	0.0153	0.0771	
cg05064040	17	ITGB3	0.1044	0.0718	0.0327	-11.8826	<0.001	<0.001	
cg05359974	17	BRCA1	0.4858	0.4996	-0.0138	2.0835	0.0386	0.1427	
cg06859280	5	RAD50	0.1675	0.1308	0.0367	-8.2654	<0.001	<0.001	
cg21519019	16	ERCC4	0.0738	0.0666	0.0072	-3.3900	0.0009	0.0118	
cg01958189	20	DEFB125	0.8807	0.8689	0.0118	-2.0257	0.0445	0.1583	
cg03237153	19	PPAP2C	0.9641	0.9597	0.0044	-2.3393	0.0212	0.0969	TRUE
cg04008843	18	TTR	0.8050	0.8206	-0.0157	2.7638	0.0063	0.0505	FALSE
cg05293216	9	FANCG	0.6586	0.6972	-0.0386	6.4118	<0.001	<0.001	TRUE
cg07688234	X	PFC	0.1390	0.1634	-0.0244	3.0262	0.0029	0.0303	FALSE
cg08159444	X	PNMA5	0.0989	0.1258	-0.0270	3.9453	0.0001	0.0019	FALSE
cg08822227	4	SH3BP2	0.2158	0.2406	-0.0249	2.5403	0.0119	0.0637	TRUE
cg09553448	9	NUP214	0.2042	0.2377	-0.0335	2.7030	0.0075	0.0532	
cg10198932	21	C21orf129	0.7571	0.7780	-0.0209	2.6332	0.0092	0.0532	
cg11126134	13	FLJ14834	0.1144	0.1314	-0.0170	2.6619	0.0085	0.0532	TRUE
cg12164282	2	PXDN	0.1364	0.1266	0.0098	-2.6266	0.0094	0.0532	TRUE
cg12437481	16	MRPL28	0.8290	0.8674	-0.0384	2.0958	0.0375	0.1427	TRUE
cg12594641	2	MGC52057	0.1010	0.1116	-0.0106	2.8054	0.0056	0.0487	TRUE
cg15383120	6	DUSP22	0.2275	0.1927	0.0348	-2.1676	0.0315	0.1316	TRUE
cg16842214	3	KBTBD5	0.8067	0.8269	-0.0202	2.6317	0.0092	0.0532	
cg18345369	16	PRSS22	0.4706	0.5185	-0.0478	2.9942	0.0032	0.0303	TRUE
cg18530748	16	SULT1A1	0.2159	0.1812	0.0347	-6.2517	<0.001	<0.001	
cg19239342	5	REEP5	0.1609	0.1742	-0.0133	1.9973	0.0474	0.1624	TRUE
cg20727362	22	CYB5R3	0.8372	0.8094	0.0278	-2.1213	0.0353	0.1411	TRUE
cg20908204	19	DMPK	0.1070	0.0922	0.0148	-3.3269	0.0011	0.0131	FALSE
cg21835643	20	RBPSUHL	0.0508	0.0590	-0.0083	2.3715	0.0188	0.0904	TRUE
cg24655310	19	CYP4F11	0.4689	0.4464	0.0224	-2.2696	0.0244	0.1066	FALSE

IV. Discussions

1. Alcohol consumption and thyroid cancer

In the T-CALOS data, this study observed possible threshold effects of acute high-dose and chronic lifetime exposure to alcohol on increasing the DTC risk, which were more pronounced in women. Our findings were consistent even after considering clinicopathologic features.

Women showed more vulnerable to the duration of drinking compared that in the men and the differential effects of sex hormones on ethanol metabolism can be the one of the answer [152]. The androgens in men possibly increased alcohol dehydrogenase activity and enzymes responsible for the related pathways [153]. Women had a higher blood level of alcohol with a same amount of alcohol drinking [154], which may trigger development of alcohol-related organ damage [152]. In addition, the previous studies suggested a smaller organ size in women compared to that in men might related to differences in ethanol pharmacokinetics [152, 155].

We found consistent effects of drinking on DTC with a large tumor size or advanced TNM stage, suggesting the presence of

a potential link between drinking and DTC with a poor prognosis. One recent study proposed that alcohol consumption may increase thyroid cancer risk by increasing thyroid-stimulating hormone (TSH) levels, thereby stimulating related hormones and mitotic activity and altering tumor susceptibility [156]. The BRAF (V600E) mutation has been associated with unfavorable clinicopathologic characteristics in patients with PTC in previous studies [146, 157]. The possible effects of drinking and BRAF mutations on colon cancer risk have been examined [158, 159], although, no related studies of drinking and thyroid cancer risk have been performed. Therefore, further evidence is required for the integrating link between alcohol consumption, thyroid cancer, clinicopathologic features and DNA mutations.

Earlier studies presented an inverse relationship between alcohol consumption and thyroid cancer. Based on the NIH-AARP Diet and Health Study, consuming 2 or more drinks per day were related with decreased thyroid cancer risk in a combined sample of men and women (RR=0.57, 95%CI=0.36-0.89) [21]. In a recent pooled analysis that included 1,003 cases, consuming alcohol daily or more often was found to be associated with a decreased thyroid cancer risk (≥7 drinks per

week: hazard ratio (HR)=0.72, 95%CI=0.58-0.90) compared with never-drinkers [22]. On the other hands, Guignard et al. found that there was no association between alcohol consumption and thyroid cancer risk (men: >10 drinks per week: OR=0.92, 95%CI=0.24-3.45; women: >10 drinks per week: OR=0.32, 95%CI=0.05-1.95) [34]. Additionally, no significant results were reported among post-menopausal women in the Women's Health Initiative (≥7 drinks per week: HR=0.66, 95%CI=0.44-1.01; ≥4 g/day: HR=0.79, 95%CI=0.60-1.05) compared to non-drinkers [31]. As in the previous studies, we also found a small amount of alcohol consumption had preventive effects on thyroid cancer, but his had threshold effects beyond the cutoff points that have been used in the previous studies. In addition, the previous results were based on the subjects who had geological, ethnical and cultural differences to our study subjects in Korea.

2. Smoking and thyroid cancer

Although ever-smoking was not a risk factor for PTC, smoking initiation before 20 years of age was associated with PTC risk among those who reported early-age exposure to secondhand

smoking. Secondhand smoking was found to increase PTC risk, particularly for those who had been exposed to secondhand smoking in young ages. In addition, smoking at least 20 pack-years were more likely to have positive results for BRAF (V600E) mutation testing in the male PTC patients than those who were never smokers.

Previous literatures have reported inconsistent results about the association between smoking and thyroid cancer. A reduced thyroid cancer risk for current smokers was observed in pooled analyses of 5 cohort studies performed in the United States [22], pooled analyses of 14 case-control studies [25], and meta-analysis [49]. In particular, the most recent meta-analysis have demonstrated an inversed relationship of smoking with thyroid cancer (combined relative risk = 0.78; 95% CI = 0.70-0.88) [49]. Although we found negative association between current smoking and PTC, null or positive associations have also been reported in some previous studies [19, 30, 33]. However, in these studies, sample sizes of thyroid cancer patients were relatively small or Asians or men were not included as cases. For example, Kabat et al. reported null association (OR = 1.13, 95% CI = 0.88-1.45); however, the

study population included postmenopausal women only [31].

Several mechanisms could be suggested to explain the reductions in thyroid cancer risk. First, the low levels of thyroid stimulating hormone (TSH) that has been detected in the current smokers [48, 160, 161] might lead to a lower thyroid cancer risk because an elevated TSH level has been reported to be a predictor of thyroid cancer [162, 163]. Additionally, there is evidence that estrogen metabolism may stimulate the growth of thyroid tumor cells [164]. Smoking could have an anti-estrogenic effect, lowering the estrogen level and thereby decreasing the thyroid cancer risk in women [165-167].

Although smoking is an established carcinogen, the number of mechanistic studies that have explored the window of vulnerability is very limited. Previous studies have suggested that smoking have genotoxic effects and result in cancer development and that adolescents may be affected to a greater extent because of their rapid growth and dynamic hormonal changes [168]. The "critical period" hypothesis for cancer susceptibility was suggested that early-age smoking may cause accelerated DNA damage because of the high rate of organ development that occurs before or at approximately 20

years of age [169]. Early-life exposure to smoking can also contribute to adverse epigenetic effects related to cancer susceptibility during adulthood [170]. However, evidence for thyroid cancer has been rarely reported. Future studies should focus on the age-dependent effects of smoking exposure on thyroid cancer risk.

It is important to consider secondhand smoking in relation to PTC risk, because the majority of thyroid cancer patients are women and proportion of smokers in women is relatively lower than in men worldwide. However, published data on the effects of secondhand smoking on thyroid cancer development are very limited. Previous study reported that thyroid cancer patients are more likely to be exposed to maternal smoking of over 20 cigarettes per day (OR = 2.1, 95% CI = 0.2-21.2); however, due to small sample size which consisted of 151 cases and 139 controls, the confidence interval was relatively wide [171]. Our large-scale study assessing both active and secondhand smoking was important for clarifying the association with consideration of the timing of smoking exposure. Our results may be partly explained by the association of secondhand smoking with thyroid functioning or hormones. Secondhand

smoking has been reported to contribute to increases in the level of thyroid hormones, such as Tri-iodothyronine (T3) and free Thyroxine (T4), but not TSH [172]. Infants who have been exposed to secondhand smoking by both parents showed an increased thyroglobulin level and a decreased TSH level [173]. Further studies assessing the association of precisely quantified smoking doses or molecular indicators with thyroid cancer conducted in a prospective setting are required.

The paradoxical findings regarding stronger influences of secondhand smoking than that of subjects' smoking behaviors may be related to gender differences in smoking prevalence and cultural and sociological factors [174, 175]. A recent study has reported that the majority of thyroid cancer patients are female, identifying a relatively lower number of female smokers compared with male smokers [176]; thus, the effects of smoking may have been underestimated. In addition, females are more likely to report exposure to secondhand smoking than males (74.7% vs. 63.5%) [177]. Moreover, the attributable fraction of secondhand smoking is greater than that of ever smoking for lung cancer risks in the Korean female population [178]. However, we cannot exclude the possibilities of

information bias or recall bias, because the environmental exposure information was obtained from interview in the present study, and underreporting of smoking could be possible especially among women and cancer patients.

Cigarette smoking contains substances that are potentially involved in thyroid carcinogenesis, genetic or epigenetic alterations [179, 180]. For example, polycyclic aromatic hydrocarbons (PAHs) have been proposed to be a genotoxic carcinogens that promote reactive electrophilic estrogen metabolites, mutagenic oxygen radicals, estrogen-induced oxidants and DNA damage [181]. The genes that detected to be mutated in patients with smoking-induced cancer and thyroid cancer include BRAF, RAS, PI3K/AKT, PTEN and TP53 [164]. We hypothesize that the threshold effects of cumulative smoking doses (20 pack-years) might be the source of or an accelerating factor for mutagenesis for the somatic mutation BRAF (V600E) in male PTC patients; however the detailed mechanism has not yet been fully elucidated.

3. Obesity and thyroid cancer

In the previous studies, overweight and obesity were related to

PTC risk [69, 74, 182, 183]. Xu et al. recently confirmed weight (pooled OR=1.27, 95%CI=1.22−1.34) and BMI (pooled OR=1.77, 95%CI=1.64−1.91) as risk factors of thyroid cancer based on a pooled analysis of three case−control studies conducted in the United States, Italy and Germany [184]. In a prospective study of 484,326 American subjects that were followed up for eight years, a higher thyroid cancer incidence associated with increasing BMI was also found in men (higher thyroid cancer risk in men with a BMI above 30 kg/m2, HR=1.89, 95%CI=1.21−2.96) and not in women [185]. However, these results were based on data including non−Asian study subjects (the United States, Italy, and Germany) [184].

In the present study, we showed that excess weight was related to PTC risks. Our results are consistent with those of several previous studies [69, 74, 182, 183]. In the French E3N cohort, the highest quartile of weight (≥64 kg) presented a borderline significant association with thyroid cancer (HR=1.51, 1.02−2.22) compared with the lowest quartile of weight (<53 kg) [182]. Regarding the thyroid cancer risk related to obesity (BMI≥30), we observed a significant association in male

subjects but not in females. In a prospective study of 484,326 American subjects followed up for eight years, a higher thyroid cancer incidence associated with increasing BMI was also found in men (higher thyroid cancer risk in obese men with a BMI above 30 kg/m2, HR=1.89, 95%CI=1.21−2.96) and not in women [185].

Little is known about the relationship between current and past BSA and thyroid cancer. Clero et al. recently indicated that BSA was an important indicator of thyroid cancer among various anthropometric factors, based on a pooled analysis of two case−control studies that included 554 cases (men: OR=1.99, 95%CI=0.67−5.96; women: OR=3.39, 95%CI=2.28−5.04) [71].

The few studies evaluated the relation between anthropometric factors in early life and thyroid cancer risk. Relatively young−age obesity has been reported based on the Hospital−based Epidemiologic Research Program at Aichi Cancer Center (HERPACC) in Japan [72]. Excess weight and BMI at age 20 years old and thyroid cancer risk was positively related (the highest tertile vs. the lowest tertile, weight: OR=2.69, 95%CI=1.71−4.25; BMI: OR=1.54, 95%CI=0.97−2.34) [72],

which is consistent with our results. BMI in adolescence at age 13 years was significantly related to an increased PTC risk in adulthood (HR=1.28, 95%CI=1.06-1.53); however, the stratified results by sex were not significant due to the small number of cases (the number of total thyroid cancer cases: 158 women and 58 men) [186].

Little is known regarding the biological mechanism of obesity at age 18-20 and PTC risk. First, estrogens may play an important role in thyroid cancer, specifically 17 beta-estradiol, which is also related to the activation of mitogen-activated protein (MAP) kinase and growth factors in malignant thyroid tumors [187], as well as to a more aggressive presentation [188]. Second, increased concentrations of insulin-like growth factor (IGF-1) among the subjects with obesity may be associated with thyroid carcinogenesis [189] A correlation of obesity with high levels of leptin and thyroid stimulating hormone (TSH) has been indicated [190-192], which could substantially promote cell proliferation in the thyroid gland. An elevated level of thyroid function hormones or autoimmunity biomarkers [193], may also be predictors of thyroid cancer. Moreover, a role of chronic subclinical inflammation of the

adipose tissue in patients with obesity and local thyroid inflammation that may contribute to thyroid cancer development and its tumor progression has also been hypothesized [194]. Although BMI in adulthood has been reported to affect a more advanced stage and histopathological subtype of PTC [195] or developing a locoregional event [196], our results provide new evidence of the effects of weight and BMI at age 18–20 on consequent thyroid cancer risk.

Only a few studies have included results regarding adult weight change over long-term period and PTC development [30, 72, 197, 198]. While a recent meta-analysis found that each 5-kg increase in adult weight gain was related to various cancers including breast, ovarian, colon and kidney cancer[73], the impact of weight change during adulthood on elevated PTC risk has not been elucidated. Kitahara and colleagues assessed whether weight gains of more than 10 kg between the ages of 35 to 50 years could influence PTC development based on the NIH-AARP Diet and Health Study (NIH-AARP study) in the United States; however, the results were not statistically significant (men: hazard ratio (HR) 1.61, 95%CI, 0.91–2.43; women: HR, 0.83, 95%CI, 0.43–1.59) [197]. While Kabat el al.

also found an insignificant association between weight gains of 50 lbs or more after the age of 18 years and PTC risk (HR, 1.09, 95%CI, 0.53-2.22) based on the Women's Health Initiative (WHI), the results were based on a study that included only post-menopausal women [198]. One possible explanation for the insignificant associations in the previous studies is the different age distribution of the subjects. The more pronounced associations in our findings compared with previous results can be explained by our inclusion of younger study subjects (median age: 54 years for the men and 51 years for the women in our study; 63.8 years for men and 63.1 years for women in the NIH-AARP study [197] and 61.9 years for cases and 63.1 for non-cases in the WHI study [198]). Recruiting subjects who were more than 10 years older than the median age for thyroid cancer diagnosis (50 years old) at enrollment could lead to an underestimation of the actual effects of weight change on thyroid cancer risk because of information bias, such as memory decay regarding one's weight at age 35 years. Although the previous two studies had many strengths based on their prospective cohort study designs, it could be difficult to generalize the results to the general population (the

NIH-AARP Diet and Health Study recruited members of the American Association of Retired Persons [197], and the WHI data included postmenopausal women [198]). The insignificant difference may also indicate insufficient statistical power, as the samples included fewer than 200 PTC patients.[30, 72]

An increased risk of thyroid cancer was observed among subjects who reported a "large" body shape at 35-40 years, based on the "lean" body shape as the reference, in a cohort of 91,909 French women (HR, 1.48, 95%CI, 1.10-1.99) [182]. The results were assessed according to self-evaluated body shape over the lifetime [182], which may account for the influence of weight at middle age on PTC development.

In general, we found stronger associations between weight change and PTC risk in men than in women. The rising prevalence of overweight and obesity has been more striking in males than in females in Korea [199] and the male PTC patients in our study presented more aggressive tumor features compared with the female patients, which may be one of the reasons. We observed different associations in the subgroups based on menopausal status. Menopause was related to total and abdominal adiposity[200], and different profiles of

circulating estrogens and changed hormonal balances in sex steroids can promote cell differentiation and proliferation in thyroid tumors[201]. Although there are inconsistent results regarding physical activity as an independent determinant of PTC[185, 202-204], the reported inverse association [203, 204] at least partially support the assumption that regular exercise can reduce the effects of weight gain on male PTC development.

The first limitation of this study is the use of self-reported weight history information, which can introduce reporting errors and recall bias[205]. Second, there was the likelihood of increased PTC detection in people who were overweight or obese; who were diagnosed as a chronic disease and who were undergo relatively more frequent health check-ups at clinics. We cannot completely exclude the possibility of detection bias. Third, the PTC cases and controls came from the two different studies, even though the data were considerably comparable. Finally, the lack of more detailed information about benign thyroid conditions, radiation exposure and lifestyle variables could be a limitation, and may need to be considered as adjusting variables in multivariate models for the potential

confounders.

4. Medical radiation

Inconsistent results was found in the previous studies [77, 78, 89-92]. In support of that insignificant link in our findings, Neta et al., observed the lack of association between diagnostic x-rays and thyroid cancer (chest x-ray: HR=0.91, 95%CI=0.81-1.03; UGI: HR=1.01, 95%CI=0.90-1.12; mammography: HR=1.00, 95%CI=0.88-1.13) [90]. These HRs were estimated from the data including 75,494 subjects who had chronic occupational exposure to irradiation sources in the cohort of the US Radiologic Technologists Study, which was conducted between 1983 and 2006 [90]. Additionally, non-significant relation between a high calculated cumulative absorbed dose (2.9mGy or greater) of thyroid gland and elevated thyroid cancer risk was observed (odds ratio, OR=1.4, 95%CI=0.8-2.3), based on data from the Swedish Cancer Registry (132 cases and 251 controls) [78]. The life-time exposure frequency to diagnostic x-rays was not clearly associated in all various types of the relative radiation dose to thyroid gland (greater than 10 times exposure to chest,

shoulders, upper gastrointestinal tract: OR=0.99, 95%CI=0.47−2.08) [77]. None of the studies provided estimated associations between combined exposures to multiple procedures and thyroid cancer risk based on a community−based prospective cohort study.

In contrast to our results, clear evidence for the association between medical diagnostic radiation was supported (chest radiography: OR=1.79, 95%CI=1.06−3.02; UGI: OR=1.82, 95%CI=1.01−3.29; CT scanning of chest: OR = 2.63, 95%CI = 1.31–5.29) [89]. Moreover, there was a trend toward higher risk with higher exposure frequency [89]. Another study, which included Australian Medicare data composed of 11 million Australians, reported significantly higher thyroid cancer risk in those exposed to CT (incidence rate ratio, IRR=1.24, 95%CI=1.20–1.29) compared to those unexposed to CT [93]. Although it has a large−scale population−based data, the exposure variable was considered CT scans in childhood or adolescence (aged 0−19 years), and combined exposure with other types of medical diagnostic procedures was not investigated [93]. The observed inconsistencies among these previous study results might be related to ethnic, genetic, and

environmental factors affecting the radiation susceptibility of their study populations. In addition, thyroid-affecting dose levels from various radiographic procedures could vary considerably depending on site-specific examination types, medical devices, and hospital protocols [83].

Biologic explanations for the relationship between medical radiography and thyroid cancer risk have not been fully described. It has been reported that low-dose ionizing radiation can affect DNA damage both direct and indirect, such as cell death, unscheduled activation of the MAPK signaling pathway, abnormal chromosome rearrangement, an initiator role in mutagenic cell transformations, or carcinogenesis [206-210]. For thyroid cancer-specific effects related to radiation exposure, increased frequency of chromosomal rearrangements of the RET gene called RET/PTC [211-213]. Theses radiation-induced generic modifications possibly activate the MAPK and PI3K-AKT signaling pathway, which lead to develop thyroid cancer [214]. On the other hands, previous studies suggested that a high frequency of RET/PTC rearrangements or BRAF mutation was mostly observed with a high dose of radiation exposure [215, 216]. The estimated average radiation

doses from medical diagnostic procedures were expected very low considering estimated thyroid-specific radiation doses (chest X-ray: 0.01mGy; chest CT: 2.25mGy), and it was not significantly related to the cancer risk [217]. The estimated lifetime attributable risk from parathyroid imaging on thyroid cancer was conflicting [218, 219]. We thus cannot conclude that a low-dose radiation from medical diagnostic radiography is applicable to possible biological mechanisms explaining high-dose radiation effects. Therefore, further study is needed to explain the underlying mechanisms associated with radiation-induced carcinogenesis, particularly involving low-dose-rate radiation exposure.

5. DNA methylation and thyroid cancer

In our study, a low DNA methylation level at the three selected CpG sites, cg05293216 in FANCG gene, cg12594641 in MGC52057 gene and cg18345369 in PRSS22 gene, in a blood sample can be a biomarker for increased risk of thyroid cancer. FANCG gene is in the Fanconi anemia complementation group (FANC), and a possible link of Fanconi anemia and a genetically heterogeneous recessive disorder characterized by cytogenetic

instability, hypersensitivity to DNA crosslinking agents, increased chromosomal breakage, and defective DNA repair. The members of the Fanconi anemia complementation group do not share sequence similarity; they are related by their assembly into a common nuclear protein complex [220-224].

MGC52057 gene (LYPD6) is a member of LYPD6 family consisted of at least one 80-amino acid LU domain that contains 10 conserved cysteines with a defined disulfide-bonding pattern [225]. The functions of LYPD6 in relation to control Lrp6 activation specifically in membrane rafts, which is essential for downstream signaling [226] and Wnt/β-catenin signaling by promoting Lrp6 phosphorylation in raft plasma membrane domains [226] have been reported.

The PRSS22 gene, also known as BSSP-4, on chromosome 16 encodes a member of the trypsin family of serine proteases [227]. Chen et al., suggested that BSSP4 had an important role in the physiological changes and underlying mechanism of 5-triiodo-L-thyronine (T3)-mediated regulation in both the mRNA and protein levels [227]. In addition, T3 appears to regulate BSSP4 via the ERK1/2/C/EBPβ/VEGF cascade, enhancing progression of tumor cells [227].

The limitation of current study is that we used case-control study setting, which could introduce selection bias and recall bias. Using a total of 180 samples, the statistical power could be insufficient. However, we observed a difference of DNA methylation level between the cases and controls.

In conclusion, we provide new possibility that a simple blood testing for DNA methylation of specific loci can predict thyroid cancer. These finding could lead to further approaches for non-invasive diagnostic methods for thyroid cancer risk. Additional confirmation using a prospective study design and in-depth functional study explaining biological mechanism is warranted for the results.

For T-CALOS study, the strengths are as follows. First, a large sample size of thyroid cancer patients allowed us to analyses in various subgroups, particularly for men. Second, we accompanied data collection based on a standardized research protocol and a comprehensive epidemiologic questionnaire. Third, we used detailed clinicopathologic information on the analyses. Fourthly, using the KMCC study, a community-based cohort study, we could minimize possibility of recall biases,

because all subjects are asked about their radiation exposure status before their diagnosis of thyroid cancer. If there was a bias possibility, it would result in non-differential misclassification across groups of study subjects. Furthermore, we assessed the effects of alcohol consumption, both smoking behaviors and secondhand smoking and obesity on thyroid cancer after controlling for important potential confounders in the two data sources.

Several limitations of the present study should be noted. First, the self-reporting of exposure status is relatively unreliable compared with the use of an objective biomarker, such as the urinary cotinine levels [48, 228]. To minimize this bias and misclassification related to exposures, a structured questionnaire was administered by trained interviewers and by a standardized protocol. Second, the thyroid cancer patients were not recruited using multi-center based methods. Third, selection bias related to differential socioeconomic status could be an issue for evaluating impacts of lifestyle factors. Subjects who had ever been diagnosed with cancer or thyroid disorder were excluded in the process of the control selection, because we designed and restricted the control group to those with a

normal thyroid gland. Additionally, we cannot exclude the possibility that patients classified in the group with overweight or obesity at an early age are more likely to receive medical care, particularly thyroid screenings. Fourth, the possibility of a birth year effect also existed, although we found no significant variation in the overweight or obesity prevalence by enrollment year. For medical radiation study using the KMCC study data, the study questionnaire did not request detailed information about the type of radiography, the target organ, or the estimated radiation doses of each procedure. Among study participants who were exposed to medical radiation, some could not remember the frequency of undergoing each procedure. In the absence of this information, it is possible to underestimate the importance of radiation dose to the subsequent risk of thyroid cancer. Second, the thyroid glands of the young are more sensitive to radiation-induced carcinogenesis than those of older subjects [81]. In the absence of detailed information on the dates of radiation exposures, we could not assess the differential effects of exposures during childhood and adolescence. Through individuals' opportunities of getting more opportunities to be exposed to diagnostic procedures has

been increased in recent years, there also have been efforts to minimize absorbed radiation doses for each radiographic images[82, 90, 229, 230], such dose changes may have affected our results. Study data were further limited by the inability to trace radiation exposure status after subject enrollment. In contrary to the T-CALOS study, there was not sufficient number of cases especially for males in the statistical analyses.

V. Conclusions

Advanced imaging techniques in medical devices and increased screening rates were suggested as important factors to cause rapid increases in the thyroid cancer incidence.[231, 232] However, true increases of thyroid cancer were suggested, because the increases in the young thyroid cancer patients who usually not classified for cancer screening beneficiaries are also observed.[1, 233]. Potential contributing factors for thyroid cancer were suggested in recent studies[29, 234–236], as well as (1) alcohol consumption, (2) smoking, (3) obesity at age 18–20, (4) weight change in middle–aged adults, (5) body surface area (BSA), (6) medical diagnostic radiation and (7) epigenetic profiles suggested in this thesis, and further results are warranted.

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Abstract in Korean (국문초록)

한국인 갑상선암과 관련된 생활습관, 의료방사선 노출 및 후성유전적 요인 평가

황윤지

의과학과

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연구배경: 한국인 갑상선암 발생은 최근 전세계적으로 증가 추세이며, 한국에서 1999년부터 2012년을 기준으로 갑상선암 발생의 연평균백분률변화 (annual percentage change, APC)도 22.3%를 기록했다. 물론, 진단기술의 발전, 암검진 기회의 증가 및 상대적으로 저렴해진 갑상선암 초음파검사가 한국인의 작은 종양크기의 갑상선암 증가에 많은 부분 영향을 미친다는 의견도 많지만, 실제 갑상선암 발생의 증가 가능성에 대한 논의도 계속 되고 있다. 특히, 어린 나이의 방사능 노출력을 제외하고는, 갑상선암의 환경적 혹은 유전적 위험요인은 명확히 밝혀지지 않은 상태이다. 본 연구의 목표는, (1) 음주, (2)흡연, (3) 청소년비만, (4)

중년나이의 체중변화, (5) 체질량지수 (body mass index, BMI), (6) 의료용 진단방사선, 그리고 (7) 후생유전학 특성을 갑상선암 위험요인인지 평가한다.

연구방법: 본 연구는 두 개의 연구자료를 활용하였다. 첫 번째, the Thyroid Cancer Longitudinal Study (T-CALOS)는 2010년부터 2014년까지 서울대병원에서 갑상선암 수술 환자를 모집하였다. 20세 혹은 그 이상 연령을 대상으로 자발적으로 참여한 사람들에게 연구참여동의서를 받았고, 면접 설문을 통하여 환경적 요인, 유전적 요인 및 일반적인 건강상태에 대한 정보를 획득하였다. 갑상선암 환자의 경우, 의무기록 조사를 통하여 임상정보를 추가로 확보하고 각 환자의 암 종류를 병리학자가 확인 한 경우 포함 시켰다. 대조군으로, 지역사회 코호트 Health Examinees (HEXA) study 자료의 건강한 사람들을 환자 개인별로 짝짓기 하였다. 두번째 연구자료로, 한국인 다기관 암 코호트 (the Korean Multi-center Cancer Cohort, KMCC)는 1993년부터 암 발생의 원인과 연관 될 가능성이 있는 생활습관, 신체활동, 식이 및 생식력 관련 정보들을 수집했다. 모집된 연구대상자들은 1993년부터 매년 국가 암 등록 자료와 사망통계자료와 연계하여 추적관찰 하고 있다. 통계분석방법으로는, 일반적인 특성을 비교하기 위하여 t-test 와 Chi-square test 가 사용되었다. 후생유전학적인 차이를 보기 위해선 환자대조군 두 그룹의 메틸레이션 수치를 비교하였다. Odds

ratios (ORs), hazard ratios (HRs) 및 95% confidence intervals (95%CIs)이 각 잠재적 위험요인과 갑상선암 위험도의 연관성을 평가하기 위해 산출되었다.

연구결과: 가볍게 혹은 중간 정도 술을 마시는 음주행태는 갑상선암 위험을 감소시켰으나, 한번에 많은 양을 마시는 경우 (순수 알코올량 기준151g 혹은 그 이상) 남자 (OR=2.22, 95%CI=1.27−3.87)와 여자 (OR=3.61, 95%CI=1.52−8.58) 모두에서 술을 마시지 않는 사람에 비하여 높은 갑상선암 위험도를 보였다. 31년 이상 음주에 노출된 사람은 갑상선암의 중요한 위험인자로 남자와 (31−40 years: OR=1.58, 95%CI=1.10−2.28; 41+ years: OR=3.46, 95%CI=2.06−5.80) 여자 (31−40 years: OR=2.18, 95%CI=1.62−2.92; 41+ years: OR=2.71, 95%CI=1.36−5.05) 모두에서 확인 되었다. 특히, 한번에 많은 양의 알코올량을 섭취하는 것은 갑상선암 종양의 특징 (tumor size, lymph node metastasis, extrathyroidal extension and TNM stage)을 고려했을 때에도 의미 있는 요인이었다. 흡연의 경우, 실제 본인의 흡연 경험 여부 그 자체로는 갑상선암 위험 요인으로 보기 어려웠지만, 어릴 적 자라는 동안 집에서 간접흡연에 노출된 경우 혹은 20세 이전에 흡연을 일찍 시작했을 때에는, 흡연에 노출된 적이 없는 사람과 비교 했을 때, 갑상선암 위험이 증가했다 (OR = 1.64, 95% CI = 1.02−2.61). 청소년기 비만의 경우, 18세

체질량지수 (body mass index, BMI)가 갑상선암과 연관이 있었다 (BMI, 25.0−29.9: OR=4.0; 95%CI=3.2−5.0; BMI, 30.0+: OR=4.0, 95%CI=1.4−11.6). 18세 BMI 의 갑상선암 위험도에 미치는 영향은 남자에서 여자보다 조금 더 많이 나타났다 (men: OR=6.8, 95%CI=4.5−10.2; women: OR=3.2, 95%CI=2.4−4.2; p−heterogeneity=0.002). 중년비만의 경우, 35세 이후 10kg 이상 증가한 사람은 5kg 미만의 차이를 보이며 체중유지를 한 사람에 비해 갑상선암을 진단받을 확률이 높았다 (men, OR, 5.39, 95%CI, 3.88–7.49; women, OR, 3.36, 95%CI, 2.87–3.93). 체중변화를 1년 평균값 (annual average change of BMI $\geq$0.3 kg/m^2 /year)은 갑상선암 위험과 연관성 있는 지표였다. 그 중에서도 종양 크기가 1cm 이상인 경우 그 연관성의 정도가 더 강하게 나타났다 (<1 cm, OR, 2.34, 95%CI, 1.92–2.85; $\geq$1 cm, OR, 4.00, 95%CI, 2.91−5.49, p−heterogeneity=0.005). 청소년비만과 중년비만의 결과와는 반대로, T−CALOS 와 KMCC 두 자료를 모두 확인 한 결과, 연구에 참여 당시 측정된 현재 BMI 는 갑상선암과 유의한 연관을 보이지는 않았다. 추가로, 의학적 진단용 방사선 노출(X−ray radiography, upper gastrointestinal series (UGI), computerized tomography (CT), and mammography)은 각 종류별로 봤을 때 갑상선암 위험을 통계적으로 유의하게 높이지는 않았다. 하지만, 그 어떤 의학적 진단용 방사선 노출이 없는

사람들에 비하여, 네 가지 종류의 방사선 (X-ray, UGI, CT, and mammography)에 노출된 적이 있는 사람은 6배 이상 갑상선암 위험도가 높았다 (HR=5.91, 95%CI=1.45-24.07). 본 연구는 갑상선암 환자-대조군의 메틸레이션 수치를 비교했을 때 유의미한 차이를 확인했다.

연구결론: 음주, 간접흡연, 청소년기의 비만, 35세 이후 중년의 체중증가 및 후성유전학적 차이가 갑상선암 위험요인일 가능성이 있다. 더욱이, 메틸레이션수치는 갑상선암의 biomarker 로 활용 될 수 있다.

주요어: 갑상선암, 위험요인, 음주, 흡연, 비만, 체질량지수, 방사선노출, 메틸레이션.
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