

Dietary Iodine Intake, Fingernail Selenium, and Risk of Differentiated Thyroid Cancer: Analyses of Case-Control Studies from Epi-Thyr Consortium

Yan Ren

► To cite this version:

Yan Ren. Dietary Iodine Intake, Fingernail Selenium, and Risk of Differentiated Thyroid Cancer: Analyses of Case-Control Studies from Epi-Thyr Consortium. Food and Nutrition. Université Paris-Saclay, 2017. English. NNT: 2017SACLS120. tel-01816177

HAL Id: tel-01816177 https://theses.hal.science/tel-01816177

Submitted on 15 Jun2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.





THESE DE DOCTORAT DE L'UNIVERSITE PARIS-SACLAY PREPAREE A UNIVERSITE DE PARIS SUD

ECOLE DOCTORALE N° 570 EDSP Santé Publique

Spécialité de doctorat : Santé Publique - Épidémiologie

Par

Madame Yan Ren

L'apport alimentaire en iode, sélénium dans les ongles, et risque du cancer différencié de la thyroïde: Analyses d'études cas-témoins du consortium Epi-Thyr

Thèse présentée et soutenue à Villejuif, le 14 juin 2017

Composition du Jury :

Monsieur Bruno Falissard	Professeur des université – praticien hospitalier,	Président
	Inserm, Université Paris Sud, Université Paris Saclay	
Monsieur Marc Colonna	Directeur de recherche	Rapporteur
	CHU de Grenoble, Universitaire Grenoble Alpes	
Monsieur Marc Klein	Professeur des université – praticien hospitalier,	Rapporteur
	Chu Nancy, Universitaire de Nancy	
Madame Marie-Christine Boutron-Ruault	Directrice de recherche,	Examinatrice
	Inserm, Université Paris Sud, Université Paris Saclay	
Madame Mathilde Touvier	Chargé de recherche,	Examinatrice
	Inserm, Inra, Cnam, Universités Paris V, VII et XIII	
Monsieur Florent de Vathaire	Directeur de recherche	Directeur de thèse
	Inserm, Université Paris Sud, Université Paris Saclay	



Remerciements

Je remercie Florent de Vathaire pour m'avoir donné l'opportunité de réaliser cette thèse sur un sujet passionnant. Je le remercie de m'avoir ouverte un portail merveilleux d'un monde scientifique, pour m'avoir fait bénéficier de ses connaissances et de son expérience.

Je tiens à remercier Marc Colonna et Marc Klein d'avoir accepté d'être les rapporteurs de ce travail. Je remercie également Marie-Christine Boutron-Ruault, Mathilde Touvier et Bruno Falissard d'avoir accepté d'être membre de mon jury de thèse, ainsi que Jean Bouyer qui m'a suivie durant tout au long de cette thèse.

Mes remerciements vont ensuite à Rodrigue Allodji qui m'a soutenu tout au long de ma thèse, même commencé par mon stage de Master 1. Ses conseils scientifiques m'ont été d'une aide très précieuse. Malgré son emploi du temps chargé, il a toujours été disponible pour moi.

Merci à Constance Xhaard, pour son aide scientifique, ce fut un plaisir de partager le même bureau! Un grand merci à Nadia Haddy pour ses conseils et sa gentillesse, je la remercie pour son enthousiasme et son écoute qui m'ont permis de tenir le coup dans les moments difficiles. Merci à Carole Rubino, Ibrahima Diallo, Agnès Dumas, pour ses conseils scientifiques très précieux.

Je remercie Vincent Souchard pour m'aider nettoyer les bases de données avec soin et patience, et les discussions très intéressantes partagées avec lui. Je remercie Angéla Jackson pour son super support informatique et son enthousiasme qui m'a soutenue moralement.

Je remercie Françoise Terrier pour son aide administrative et sa gentillesse et ses soins qui m'ont portée.

Mes remerciements s'adressent aux Giao Vu-Bezin et Damien Llanas, pour ses souris et bon sens d'humeur, notamment le moment badminton partagé avec vous.

Merci à Isao Kobayashi pour son écoute, sa gentillesse, sa compréhension et ses soutiens silencieux. Merci à Fara Diop, Adel Kaddi, Cristina Veres, Imene Mansouri, pour ses compréhensions, aides et soutiens.

Je remercie tous les autres membres de l'équipe Cancer et Radiations de l'Unité 1018 Inserm, pour d'avoir partagé d'une expérience de vie merveilleuse professionnelle et personnelle avec moi.

Je remercie vivement à Catherine Hill, Serge Koscielny et Nils Ternès du Service de Biostatistique et d'Epidémiologie de l'IGR pour ses conseils précieux et ses aides tout au long de ma thèse. Mes remerciements s'adressent également à toutes les personnes ayant contribué à la réalisation des études du consortium Epi-Thyr, dont les collègues de France, de Polynésie française, de Nouvelle Calénodie et de Cuba: Thérèse Truong, Pascal Guénel, Françoise Doyon, Enora Cléro, Stéphane Maillard, Fabienne Lesueur, Catherine Tcheandjieu, Françoise Borson-Chazot, Geneviève Sassolas, Laurent Bailly, Eugènia Mariné Barjoan, Martin Schlumberger, Emilie Cordina-Duverger, Marie Sanchez, Elisabeth Adjadj, Axelle Leufroy, Thierry Guérin, Laurent Noël, Anne Boland, Jean-François Deleuze, Frédérique Rachedi, Jean-Louis Boissin, Joseph Sebbag, Larrys Shan, John Paoaafaite, Rosa M Ortiz, Juan J Lence-Anta, Celia M Pereda, Mae Chappe, Milagros Velasco, Idalmis Infante, Marlene Bustillo, Silvia Turcios, Regla Rodriguez.

J'aimerais adresser un remerciement particulier à mes collègues Australiens à l'occasion du programme d'échange pour les doctorants et post-doctorants entre l'Inserm et l'Université de Sydney: Bruce Robinson, Rory Clifton-Bligh, Martyn Bullock, Trisha Dwight, Diana Benn, Anthony Glover, Lauren Joo, Danielle Somers, Elizabeth Carter, et Joonsup Lee pour ses aides, soutiens précieuses, ses gentillesses et ses conseils. Merci tout particulièrement à Rory pour son encadrement, sa disponibilité et sa bienveillance qui a rendu mon séjour à Sydney riche et agréable. Merci aussi à Martyn pour m'avoir fait bénéficier de son savoir et de sa connaissance génétique. Ce fut un plaisir de travailler ensemble!

Un grand merci à mes moniteurs et amis plongeurs: Christian Aragon, Marie-Rose Aragon, Guillaume Aragon, Sébastien Aragon, Marco Delecluse, Pascal Liot, Pascal Passaquit... pour ses enthousiasmes, encouragements et amitiés qui m'ont soutenue moralement, avec qui j'ai gagné une famille sous-marine!

Je remercie fortement pour les recommandations fortes de la part de Josiane Schilling, de Jean-Marc Chantelot, et de Pascal Astagneau pour la demande de bourse, qui m'ont permis de réaliser cette thèse.

Je remercie la Fondation de France qui a financé ce travail de thèse. Je remercie la Ligue Nationale Contre le Cancer (LNCC), l'Association pour la Recherche contre le Cancer (ARC), l'Agence Nationale pour la Recherche (ANR), le comité de radioprotection d'Electricité De France (EDF), l'Agence Française de Sécurité Sanitaire de l'Environnement et du Travail (AFSSET) et la Direction Générale de la Santé (DGS) qui ont permis de réaliser les études en Polynésie française et en Nouvelle-Calédonie.

Et enfin, je tiens à remercier profondément mes parents sans qui rien de tout ça n'aurait été possible. Ils ont toujours été là pour moi et m'ont soutenue tout au long de mes études.

Résumé

Contexte : L'iode est un micronutriment provenant des aliments. Il est établi que, dans les régions d'endémie goitreuse caractérisées par un apport d'iode insuffisant, l'incidence des goitres et des cancers différenciés de la thyroïde (CDT) de type folliculaire est plus élevée qu'ailleurs. En revanche, l'influence de l'apport alimentaire en iode sur le risque de CDT de type papillaire, forme représentant actuellement plus de 80% des CDTs dans la plupart des pays, ne fait pas l'objet d'un consensus. Il semble cependant que cette incidence soit plus élevée dans les îles du pacifique qu'ailleurs. Par ailleurs, les propriétés de protection redox du sélénium pourraient protéger la glande thyroïdienne et aider à maintenir la production d'hormones thyroïdiennes, mais la relation entre l'apport en sélénium et le CDT n'avait été que très peu étudiée.

Objectifs : L'objectif général de ce travail de thèse était d'explorer la relation entre l'apport alimentaire en iode et le risque de CDT. Plus spécifiquement, il s'agissait 1) d'étudier la relation entre l'apport alimentaire en iode et le risque de CDT dans les cinq études cas-témoins du consortium Epi-Thyr, conduites en Nouvelle Calédonie, en Polynésie Française, en France métropolitaine (deux études) et à Cuba; 2) d'étudier l'interaction entre cet apport et quatre polymorphismes nucléotidiques (SNP) identifiés dans la plupart des études d'association pangénomiques (GWAS) comme associés au risque de CDT, ainsi qu'avec les facteurs de risque environnementaux connus; et 3) d'évaluer l'intérêt du sélénium présent dans les ongles comme un bio-marqueur potentiel du risque de CDT.

Matériel et Méthodes : La régression logistique conditionnelle a été utilisée pour analyser la relation entre l'apport alimentaire en iode et le risque de CDT d'abord dans l'étude Cuba incluant 203 cas et 212 témoins, puis dans l'ensemble des cinq études incluant au total 2162 cas et 2571 témoins. Les questionnaires alimentaires de ces cinq études étaient dérivés de ceux de l'étude de cohorte E3N. Les mesures de l'iode et du sélénium dans les aliments traditionnels polynésiens et cubains avaient été réalisées spécialement pour cette étude. Quatre SNPs avaient été génotypés, dont deux sur le gène *FOXE1*, un sur le gène *ATM* et un autre près du gène *NKX2-1*. Les mesures de l'iode, du sélénium et des autres oligoéléments métalliques avaient été réalisées dans les ongles des sujets de l'étude Polynésie Française.

Résultats : Il n'a pas été observée, une association significative entre l'apport alimentaire en iode et le risque de CDT dans l'étude réalisée à Cuba. Dans l'ensemble des cinq études, la majorité des cas et des témoins ont été considérée comme présentant une carence légère en iode selon la classification du Réseau Mondial d'Iode (IGN). Bien que le risque de CDT ne soit pas lié à l'apport alimentaire en iode, ce risque était significativement réduit avec l'augmentation de la consommation de poisson, ceci de manière plus importante dans l'étude réalisée à Cuba que dans les autres. En outre, une augmentation de l'apport alimentaire en iode a été significativement associée à une diminution du risque de CDT uniquement dans l'étude réalisée en Polynésie Française et chez les Polynésiens. Une augmentation significative du risque de CDT associée au nombre de l'allèle mineur (A) du SNP rs965513 près de *FOXE1* parmi les sujets qui ont consommé moins d'iode que la médiane dans l'étude réalisée à Cuba. Pour les femmes qui ont eu un nombre de grossesses élevé et qui étaient déficitaires en iode, l'augmentation de l'apport alimentaire en iode réduisait leur risque de CDT. Par ailleurs, il n'a pas été possible de mettre en évidence un intérêt du sélénium dans les ongles comme bio-marqueur prédictif du risque de CDT.

Mots clés : Cancer différencié de la thyroïde, apport alimentaire en iode, polymorphisme nucléotidique, sélénium dans les ongles, étude cas-témoins, Epi-Thyr

Context : Iodine is a trace element derived from food. In endemic goiter areas characterized by dietary iodine deficiency, it is established that the incidence of goiter and differentiated thyroid cancers (DTC) of follicular type is higher than elsewhere. On the other hand, the influence of dietary iodine intake in the risk of papillary thyroid cancer which currently representing more than 80% of DTCs in most countries, has not achieved a consensus. It appears, however, that this incidence is higher in the Pacific islands than elsewhere. In addition, the redox protection properties of selenium could protect the thyroid gland and help to maintain the production of thyroid hormones, but few studies evaluated the relationship between selenium intake and DTC.

Objectives : The general objective of this thesis was to explore the relationship between dietary iodine intake and DTC risk. More specifically, it was 1) to study the relationship between dietary iodine intake and the risk of DTC in five case-control studies of Epi-Thyr consortium, carried out in New Caledonia, French Polynesia, Metropolitan France (two studies) and Cuba; 2) to investigate the interaction between this intake and four single-nucleotide polymorphisms (SNP) identified in most genome-wide association studies (GWAS) as associated with DTC risk, as well as the interaction with the well-established environmental risk factors; and 3) to evaluate the benefit of selenium present in fingernails as a potential bio-marker of DTC risk.

Materials and Methods : Conditional logistic regression was used to analyze the relationship between dietary iodine intake and DTC risk first in the Cuba study including 203 cases and 212 controls, and then in the pooled analysis of the five studies including a total of 2162 cases and 2571 controls. The dietary questionnaires of these five studies were derived from those in the E3N cohort study. Measurements of iodine and selenium in traditional Polynesian and Cuban foods were specifically carried out for this pooled analysis. Four SNPs were genotyped, including two of *FOXE1* gene, one of *ATM* gene and another near *NKX2-1* gene. Measurements of iodine, selenium and other metal trace elements were made in the fingernails of the participants of the French Polynesia study.

Results : There was no significant association between dietary iodine intake and DTC risk in the Cuba study. In the pooled analysis of the five studies, the majority of the cases and the controls were considered as being in mild dietary iodine deficiency according to the IGN classification. Although DTC risk was not found to be linked to dietary iodine intake, this risk significantly decreased with high fish consumption, this reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than in the other studies. Additionally, higher dietary iodine intake significantly associated with lower DTC risk only in the French Polynesia study and in Polynesians. A strong increase in DTC risk associated with the number of the minor allele (A) of the SNP rs965513 near *FOXE1*, among participants who consumed less iodine than the median value in the Cuba study. For the women who had a high number of full term pregnancies and who were iodine deficient, increasing dietary iodine intake may reduce their risk of suffering from DTC. Moreover, it was not possible to demonstrate an interest in fingernail selenium as a biomarker that predicts DTC risk.

Keywords : Differentiated thyroid cancer, dietary iodine intake, single-nucleotide polymorphism, fingernail selenium, case-control study, Epi-Thyr

Scientific production

Publications

Ren Y, Kitahara CM, Berrington de Gonzalez A, Clero E, Brindel P, Maillard S, Cote S, Dewailly E, Rachedi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Xhaard C, Rubino C, De Vathaire F. Lack of association between fingernail selenium and thyroid cancer risk: a case-control study in French Polynesia. Asian Pac J Cancer Prev. 2014;15:5187-94.

Ren Y, Lence-Anta JJ, Pereda CM, Chappe M, Velasco M, Infante I, Bustillo M, Turcios S, Leufroy A, Guérin T, Noël L, Lesueur F, Maillard S, Cléro É, Xhaard C, Allodji RS, Rubino C, Rodriguez R, Ortiz RM, de Vathaire F. *FOXE1* Polymorphism Interacts with Dietary Iodine Intake in Differentiated Thyroid Cancer Risk in the Cuban Population. Thyroid. 2016 Dec;26(12):1752-1760.

Ren Y, Truong T, Xhaard C, Souchard V, Maillard S, Lence-Anta JJ, Pereda CM, Ortiz RM, Turcios S, Leufroy A, Guérin T, Noël L, Rachedi F, Boissin JL, Sebbag J, Shan L, Borson-Chazot F, Sassolas G, Bailly L, Barjoan EM, Schlumberger M, Tcheandjieu C, Cordina-Duverger E, Sanchez M, Adjadj E, Boland A, Deleuze JF, Allodji RS, Rubino C, Guénel P and de Vathaire F. Dietary Iodine Intake and Thyroid Cancer Risk - a Pooled analysis of 5 Case-Control Studies from Epi-Thyr Consortium. (manuscript done)

Conferences

Oral Presentation

Presentation in the annual conference of NACRe-Partenariat (The National Network of Food and Cancer Research) on «Fingernail selenium and thyroid cancer risk: a case-control study in French Polynesia», Paris, France, April 2014.

Presentation in the annual conference of NACRe-Partenariat on *«Foxe1* polymorphism interacts with dietary iodine intake in DTC risk in the Cuban population», Paris, France, March 2015.

Presentation in the Gustave Roussy's research days of the pooled analysis of my PhD study entitled «Dietary iodine intake and thyroid cancer risk: a pooled analysis of 5 case-control studies from Epi-Thyr Consortium», Tours, France, March 2016.

Poster Presentation

Presentation in the 86th Annual Meeting of the American Thyroid Association, a poster entitled «Dietary iodine uptake and thyroid cancer risk: a pooled analysis of 5 case-control studies from Epi-Thyr consortium », Denver, the USA, September 2016.

Exchange Program

Representative of Inserm's PhD students to take the doctoral and post-doctoral students exchanges program between Inserm and University of Sydney, Cancer Genetics Diagnostic Laboratory of Kolling Institute of Medical Research, Royal North Shore Hospital, 1) a clinical research project to study association between somatic mutations in the *TERT* gene promoter and outcomes of papillary thyroid cancer, the results successfully published through this exchange program (Bullock M and Ren Y et al., *TERT* promoter mutations are a major indicator of recurrence and death due to papillary thyroid carcinomas. Clin Endocrinol (Oxf). 2016 Aug;85(2):283-90. Epub 2016 Feb 8.); 2) Measuring iodine content in human thyroid tumor tissue samples using Raman spectroscopy, in the University of Sydney. Sydney, Australia, from June to September 2015.

List of abbreviations

ANSES: French Agency for Food, Environmental and Occupational Health & Safety ASR: Age-Standardized Rate ATC: Anaplastic Thyroid Cancer ATM: Ataxia-Telangiectasia Mutated BMI: Body Mass Index BSA: Body Surface Area CFCT 2014: Traditional Cuban Food Composition Table Made By the Collaboration with ANSES in 2014 **CI:** Confidence Interval Ciqual: Centre d'Information sur la Qualité des Aliments CT: Computed Tomography **CTN:** Calcitonin DTC: Differentiated Thyroid Cancer FAO: Food and Agriculture Organization of the United Nations FNAC: Fine-Needle Aspiration Cytology FOXE1: forkhead box E1, also called TTF2 for Thyroid Transcription Factor 2 FPFCT 2013: Traditional French Polynesian Food Composition Table Made By the Collaboration with ANSES in 2013 FTC: Follicular Thyroid Cancer GLOBOCAN: Estimated Cancer Incidence, Mortality and Prevalence Worldwide from International Agency for Research on Cancer of World Health Organization GWAS: Genome-Wide Association Study HR: Hazard Ratio IARC: International Agency for Research on Cancer ICP-MS: Inductively Coupled Plasma Mass Spectrometry **IGN:** Iodine Global Network INFOODS: International Network of Food Data Systems MAF: Minor Allele Frequency MHP: Mantel-Haenszel P

MTC: Medullary Thyroid Cancer

NKX2-1: NK2 homeobox 1, also called TTF1 for Thyroid Transcription Factor 1

OR: Odds Ratio

PDTC: Poorly Differentiated Thyroid Cancer

PTC: Papillary Thyroid Cancer

SNP: Single Nucleotide Polymorphism

TMAH: Tetramethylammonium hydroxide

UIC: Urinary Iodine Concentration

TABLE OF CONTENTS

1	P	ART	I: GE	NERAL INTRODUCTION	1
	1.1	THYR	ROID C	ANCER	2
	1.	1.1	HISTO	DLOGIC TYPES	2
		1.1.1.	.1	Papillary thyroid cancer (PTC)	2
		1.1.1.	.2	Follicular thyroid cancer (FTC)	2
		1.1.1.	.3	Poorly differentiated thyroid cancer (PDTC)	3
		1.1.1.	.4	Medullary thyroid cancer (MTC)	3
		1.1.1.	.5	Anaplastic thyroid cancer (ATC, undifferentiated thyroid cancer)	3
	1.	1.2	INCIE	DENCE AND MORTALITY	3
	1.	1.3	THYR	OID MICROCARCINOMAS	8
	1.	1.4	ETHN	IIC DIFFERENCES	9
	1.2	RISK	FACTC	ORS FOR DIFFERENTIATED THYROID CANCER	9
	1.	2.1	FAMI	LY HISTORY	9
	1.	2.2	EXPO	SURE TO IONIZING RADIATION DURING CHILDHOOD	. 10
	1.	2.3	PRE-E	EXISTING BENIGN THYROID DISEASES	. 10
	1.	2.4	HORI	MONAL AND REPRODUCTIVE FACTORS	. 12
	1.	2.5	DIFTA	ARY IODINF INTAKE	. 13
	1	26	ОТНЕ	ER DIFTARY FACTORS	14
		126	1	Fish	15
		1.2.6.	.2	Salt Water Fish	15
		1.2.6.	.3	Fresh Water Fish	16
		1.2.6.	.4	Shellfish	16
		1.2.6.	.5	Vegetables	16
		1.2.6.	.6	Cruciferous Vegetables	16
		1.2.6.	.7	Meat	16
	1.,	2.7	BODY	/ SIZE	. 17
	1.3	FOOI	р сом	IPOSITION TABLES AND IODINE CONTENT IN FOOD	. 17
	1.	3.1	THE I	MAIN FOOD COMPOSITION TABLES	. 17
		1.3.1.	.1	Food and Agriculture Organization of the United Nations (FAO) Databases (123)	17
		1.3.1.	.2	French Food Composition Table Centre d'Information sur la Qualité des Aliments Ciqual (version	
		2013)) (124)		18
		1.3.1.	.3	Traditional French Polynesian and Cuban Food Composition Tables Made By the Collaboration with	th
		ANSE	S in 20:	13 (FPFCT 2013) and in 2014 (CFCT 2014) Respectively	18
	1.	3.2	THE \	/ARIABILITY OF DIETARY IODINE DATA	. 19
	1	3.3	RESU	LTS OF THE DIETARY IODINE MEASUREMENT IN THE TRADITIONAL FRENCH POLYNESIAN	
	Al	ND CU	IBAN F	OOD SAMPLES	. 22
	1.4	OBJE	CTIVES	S OF THE THESIS	. 25

2	PA	RT I	EPI-THYR CONSORTIUM	26
	2.1 0	DBJEC	TIVE AND HISTORY	27
	2.2 N	латс	HING PROCESS	27
	2.2.	1	NDIVIDUAL MATCHING	28
	2.2.	2	FREQUENCY MATCHING	
	2.2.	3	WHY MATCHING?	
	2.3 0	CARAG	CTERISTICS OF THE 5 CASE-CONTROL STUDIES	29
	2.3.	1	THE NEW CALEDONIA STUDY	29
	2	.3.1.1	Background	30
	2	.3.1.2	Case Selection	
	2	.3.1.3	Control Selection	
	2	.3.1.4	Data Collection	32
	2	.3.1.5	Dietary Questionnaire	32
	2.3.	2	THE FRENCH POLYNESIA STUDY	
	2	.3.2.1	Background	35
	2	.3.2.2	Case Selection	36
	2	.3.2.3	Control Selection	
	2	.3.2.4	Data Collection	
	2	.3.2.5	Dietary Questionnaire	37
	2.3.	3	THE YOUNG-THYR STUDY	39
	2	.3.3.1	Background	40
	2	.3.3.2	Case Selection	
	2	.3.3.3	Control Selection	41
	2	.3.3.4	Data Collection	42
	2	.3.3.5	Dietary Questionnaire	43
	2.3.	4	THE CATHY STUDY	44
	2	.3.4.1	Case Selection	44
	2	.3.4.2	Control Selection	45
	2	.3.4.3	Data Collection	45
	2	.3.4.4	Dietary Questionnaire	46
	2.3.	5	THE CUBA STUDY	47
	2	.3.5.1	Background	48
	2	.3.5.2	Case Selection	49
	2	.3.5.3	Control Selection	50
	2	.3.5.4	Data Collection	50
	2	.3.5.5	Dietary Questionnaire	51
	2.3.	6	THE HOMOGENEITY AND THE HETEROGENEITY AMONG THE 5 STUDIES	52
	2.4	GENET	TIC ANALYSES	59
	2.4.	1	GENOTYPING	60
	2	.4.1.1	The French Polynesia Study	60
	2	.4.1.2	The Cuba Study	61

	2.4.1	1.3 The French Young-Thyr Study	62
	2.4.1	1.4 The New Caledonia and French Cathy Studies	62
3	PART	III: DIETARY IODINE INTAKE AND DIFFERENTIATED THYROID CAN	
RIS	K: THE	CASE-CONTROL STUDY IN CUBA	63
3.1	L MET	ſHOD	64
	3.1.1	ANALYZED PARAMETERS	64
	3.1.2	STATISTICAL ANALYSIS	65
3.2	2 RES	ULTS	65
	3.2.1	FOOD CONSUMPTIONS OF THE STUDY PARTICIPANTS	65
	3.2.2	DIETARY ENERGY INTAKE OF THE STUDY PARTICIPANTS	69
	3.2.3	DIETARY IODINE INTAKE OF THE STUDY PARTICIPANTS	70
	3.2.4	DIETARY SELENIUM INTAKE OF THE STUDY PARTICIPANTS	72
	3.2.5	FISH, SHELLFISH CONSUMPTION AND DTC RISK	74
	3.2.6	DIETARY IODINE INTAKE AND DTC RISK	75
	3.2.7	FOXE1 POLYMORPHISM INTERACTS WITH DIETARY IODINE INTAKE IN DTC RISK	80
3.3	B DISC	CUSSION	82
	3.3.1	SUMMARY OF THE RESULTS	82
	3.3.2	STRENGTHS AND LIMITATIONS OF THE STUDY	82
	3.3.3	COMPARISON RESULTS FROM THE LITERATURE	84
3.4		ICLUSION	85
4 RISP CON	SOR1	OOLED ANALYSIS OF THE 5 CASE-CONTROL STUDIES FROM EPI-1	чсек ГНҮR 86
4.1	l stu	DY PARTICIPANTS	87
4.2	2 MET	rhod	88
	4.2.1	ANALYZED PARAMETERS	88
	4.2.2	STATISTICAL ANALYSIS	93
	4.2.2	2.1 Analysis Strategy from the Matching Process	
	4.2.2	2.2 Mixed Models	
	4.2.2	2.3 Conditional Logistic Regression	
4.3	B POP	ULATION DESCRIPTION	
	4.3.1	CHARACTERISTICS OF THE STUDIED POPULATION	
	4.3.2	FOOD CONSUMPTIONS OF THE STUDY PARTICIPANTS	102
	4.3.3	DIETARY ENERGY INTAKE OF THE STUDY PARTICIPANTS	103
	4.3.4	DIETARY IODINE INTAKE OF THE STUDY PARTICIPANTS	105
	4.3.5	DIETARY SELENIUM INTAKE OF THE STUDY PARTICIPANTS	106
	4.3.6	DIETARY PATTERNS OF EACH STUDY IN THE POOLED ANALYSIS	108
4.4	4 RES	ULTS	111
	4.4.1	DIETARY IODINE INTAKE	111

	4.4.1.	1 Pooled Analysis of Epi-Thyr Consortium	111
4.4.1.2		2 Heterogeneity between Studies	112
	4.4.1.	3 Heterogeneity between Ethnicity	113
	4.4.1.	4 Interaction with the 4 Investigated SNPs	117
	4.4.1.	5 Interaction with the Number of Pregnancies	122
	4.4.2	FISH CONSUMPTION	123
4.5	DISC	JSSION	124
	4.5.1	SUMMARY OF THE RESULTS	124
	4.5.2	STRENGTHS AND LIMITATIONS OF THE STUDY	125
	4.5.3	COMPARISON RESULTS FROM THE LITERATURE	127
4.6	CON	CLUSION	131
5 CON	PART TROL	V: FINGERNAIL SELENIUM AND THYROID CANCER RISK: A CASE- STUDY IN FRENCH POLYNESIA	132
5.1	MAT	ERIALS AND METHODS	133
	5.1.1	STUDY PARTICIPANTS	133
	5.1.2	DATA COLLECTION	134
	5.1.3	FINGERNAIL SELENIUM AND IODINE MEASUREMENTS	134
	5.1.4	STATISTICAL ANALYSIS	134
5.2	RESU	LTS	135
5.3	DISC	JSSION	140
	5.3.1	SUMMARY OF THE RESULTS	140
	5.3.2	STRENGTHS AND LIMITATIONS OF THE STUDY	141
	5.3.3	COMPARISON RESULTS FROM THE LITERATURE	141
54	CON		144
5.4	con		144
CON	CLUSI	ON AND PERSPECTIVES	145
PER	SONA	L CONTRIBUTION	148
REF	ERENC	ES	152
APPI	ENDIX	ES	177
1	RESU	ME SUBSTANTIEL EN FRANÇAIS	177
1.1	CONT	EXTE	177
1.2	OBJEC	TIFS	179
1.3	1.3 Matériel et Méthodes		180
1.4	1.4 PROGRAMME DE TRAVAIL		181
1.5	Résul	TATS	182
	1.5.1	Apport alimentaire en iode et risque de cancer thyroïdien à Cuba	182
	1.5.2	Apport alimentaire en iode et risque de cancer thyroïdien dans les 5 études du consortium Epi-T	hyr. 184
	1.5.3	Sélénium présent dans les ongles	187
1.6	Discu	ssion et Conclusion	188

1.7	Perspectives
2 SI	JPPLEMENTAL TABLE 1 - DETAILS USED COMMON FOOD GROUPS AND ITEMS
FOR T	HE POOLED ANALYSIS190
3 SI	JPPLEMENTAL TABLE 2 - ADJUSTED ODDS RATIO OF DTC ASSOCIATED WITH
DIETA	RY IODINE INTAKE ACCORDING TO IGN CLASSIFICATION IN THE POOLED
ANALY	'SIS
4 SI	JPPLEMENTAL TABLE 3 - DIETARY IODINE INTAKE AND THYROID CANCER
RISK: \$	SUMMARY OF THE RESULTS FROM THE PUBLISHED CASE-CONTROL STUDIES
19	98
5 SI	JPPLEMENTAL TABLE 4 - THE ASSOCIATION BETWEEN FISH, FRUITS,
VEGET	TABLES, MEAT, DAIRY FOOD, AND THYROID CANCER RISK: SUMMARY OF THE
RESUL	TS FROM THE PUBLISHED OBSERVATIONAL STUDIES
6 SI	JPPLEMENTAL TABLE 5 - THE ASSOCIATION BETWEEN ALCOHOL
CONSI	UMPTION AND THYROID CANCER RISK: SUMMARY OF THE RESULTS FROM
THE P	UBLISHED OBSERVATIONAL STUDIES212
7 PI	JBLICATIONS WITHIN THE THESIS216

Table 1-1 - Top 10 Countries with the highest ASR of thyroid cancer incidence for both sexes
Table 1-2 - The Variation of Iodine Content in Different French Polynesian Food Samples . 20
Table 1-3 - Dietary Iodine and Selenium Intake from Typical Cuban Food in the Cases and in
the Controls based on the composition of 20 traditional Cuban food items measured by the
French ANSES (CFCT 2014)
Table 2-1 - Characteristics of the Studied 5 Case-Control Studies from Epi-Thyr Consortium
Table 2-2 - Description of the five studied polymorphisms in the French Polynesia Study
(Maillard et al. 2015)
Table 2-3 - Description of the five studied polymorphisms in the Cuba Study (Pereda et al.
2015)
Table 3-1 - Food consumptions of the study participants (By decreasing order of the 20 sub-
groups)
Table 3-2 - Dietary energy intake of the study participants 69
Table 3-3 - Dietary iodine intake of the study participants
Table 3-4 - Dietary selenium intake of the study participants 73
Table 3-5 - Odds Ratios of DTC Associated with Fish and Shellfish Consumption75
Table 3-6 - Odds Ratios of DTC Associated with Dietary Iodine Intake According to IGN
Classification in the Studied Cuban Population76
Table 3-7 - DTC Risk Associated with Dietary Iodine Intake by Various Confounding Factors
in the Studied Cuban Population77
Table 3-8 - Odds Ratios of DTC Risk Associated with Dietary Iodine Intake and the
Polymorphism of SNP rs965513 near FOXE181
Table 4-1 - Results of the generalized linear model
Table 4-2 - Description of the strata
Table 4-3 - Characteristics of the Studied 5 Case-Control Studies from Epi-Thyr Consortium
Table 4-4 - Food consumptions of the pooled analysis of the 5 studies' participants (By
decreasing order of the 20 sub-groups)

Table 4-5 - Dietary energy intake of the pooled analysis of the 5 studies' participants (By
decreasing order of the 20 sub-groups)
Table 4-6 - Dietary iodine intake of the pooled analysis of the 5 studies' participants (By
decreasing order of the 20 sub-groups)
Table 4-7 - Dietary selenium intake of the pooled analysis of the 5 studies' participants (By
decreasing order of the 20 sub-groups)
Table 4-8 - Dietary iodine intake and DTC risk, dietary iodine intake classified according to
the quartiles of the value distribution of the controls in each study of the pooled
analysis(µg/day)112
Table 4-9 - Heterogeneity between each study and the other 4 studies, and global
heterogeneity
Table 4-10 - Heterogeneity between each ethnical group and European group, and global
heterogeneity
Table 4-11 - DTC risk associated with dietary iodine intake adjusted by various confounding
factors
Table 4-12 - Odds-Ratio Associated with dietary iodine intake adjusted by Various
Confounding Factors
Table 4-13 - Odds-Ratio Associated with dietary iodine intake (all food items, but bread)
adjusted by Various Confounding Factors
Table 4-14 - Alleles Frequency of the Four Polymorphisms Which Associated with the Risk
of Developing DTC of the Genotyped Participants in each Study
Table 4-15 - Association between the Risk of Developing DTC and Investigated 4 SNPs in
the Pooled analysis
Table 4-16 - Association between the Risk of Developing DTC and the 4 Investigated SNPs,
as well as Their Interactions with Total Dietary Iodine Intake in the Pooled Analysis 121
Table 4-17 - DTC Risk Associated with Fish Consumption, Adjusted by Various
Confounding Factors in the Pooled Analysis
Table 5-1 - Predictor of selenium concentration in fingernails in thyroid cancer patients and
controls
Table 5-2 - Fingernail selenium and thyroid cancer by various confounding factors in French
Polynesia, 1981-2003
Table 5-3 - Fingernail selenium and thyroid cancer by the time between diagnosis and
interview in French Polynesia, 1981-2003

FIGURE LIST

Figure 1-1 - World wide Variation in Thyroid Cancer Incidence Age-Standardised Rates
(ASR) Worldwide
Figure 1-2 - Worldwide Age-Standardized Rate (ASR) of Incidence and Mortality of Thyroid
Cancer (GLOBOCAN 2012)
Figure 1-3 - Incidence and mortality of thyroid cancer chronological trend in France (10^5)
person-years) (Colonna et al. 2013)
Figure 1-4 - Forest plot for the association between thyroid cancer risk and fish consumption
stratified by location. 1 = iodine deficiency areas; 2 = iodine-rich areas. ^a Study in Sweden. ^b
Study in Norway
Figure 1-5 - Variation of iodine content in different food samples measured by different food
composition tables
Figure 1-6 - Iodine content in fish samples from the seven different French Polynesian areas
measured by FPFCT 2013
Figure 1-7 - Measurements of Iodine Content in Typical Cuban Food by the French ANSES
(CFCT 2014) (µg/g)
Figure 1-8 - Measurements of Selenium Content in Typical Cuban Food by the French
ANSES (CFCT 2014) (μg/g)
Figure 2-1 - Map of New Caledonia
Figure 2-2 - Photo booklet to help fill out the dietary questionnaire. Example of standard
portions of raw vegetables and standard beverages volumes
Figure 2-3 - Map of French Polynesia
Figure 2-4 - Map of constituent lands of the French Republic
Figure 2-5 - Map of Cuba
Figure 3-1 - Food consumptions of the study participants (By decreasing order of the 20 sub-
groups: g/day/person)
Figure 3-2 - Mean of dietary energy intake of the Cuba study participants
Figure 3-3 - Mean of dietary iodine intake of the Cuba study participants
Figure 3-4 - Mean of dietary selenium intake of the Cuba study participants
Figure 4-1 - Participant flow chart of inclusion criteria, the Pooled Analysis, 1979-2011 88
Figure 4-2 - Predicted values for bread consumption in the Cathy study
Figure 4-3 - Residuals in bread consumption prediction of the Cathy study

Figure 4-4 - Dietary iodine intake distributions of the cases and the controls of the pooled
analysis
Figure 4-5 - Mean of food consumptions of the pooled analysis of the 5 studies' participants
Figure 4-6 - Mean of dietary energy intake of the pooled analysis of the 5 studies' participants
(by decreasing order of the 20 sub-groups: kcal/day/person) 104
Figure 4-7 - Mean of dietary iodine intake of the pooled analysis of the 5 studies' participants
(by decreasing order of the 20 sub-groups: µg/day/person)
Figure 4-8 - Mean of dietary selenium intake of the pooled analysis of the 5 studies'
participants
Figure 4-9 - (1) Mean of the top 3 consumed foods of each study (g/day/person); (2) Mean of
dietary energy intake of each study (by order of the first 3 foods which brought the most
dietary energy intake: kcal/day/person); (3) Mean of dietary iodine intake of each study (by
order of the first 3 foods which brought the most dietary iodine intake: $\mu g/day/person$; (4)
Mean of dietary selenium intake of each study (by order of the first 3 foods which brought the
most dietary selenium intake: µg/day/person), by case, control and all study participants in the
pooled analysis
Figure 4-10 - Significant Interaction between Dietary Iodine Intake and The SNP rs965513
near FOXE1 for The French Polynesia and the Cuba Studies
Figure 4-11 - Significant Interaction between Dietary Iodine Intake and Number of
pregnancies in DTC risk (P-interaction=0.05)
Figure 5-1 - Fingernail selenium concentration in studied population of French Polynesia . 136

1 PART I: GENERAL INTRODUCTION

1.1 THYROID CANCER

The thyroid gland is a butterfly-shaped endocrine gland that is normally located in the lower front of the neck. The thyroid's job is to make thyroid hormones, which are secreted into the blood and then carried to every tissue in the body. Thyroid hormone helps the body use energy, stay warm and keep the brain, heart, muscles, and other organs working as they should (1). An abnormal growth of thyroid cells that forms a lump within the thyroid gland called thyroid nodule (2). Although the vast majority of thyroid nodules are benign (noncancerous), a small proportion (5%-10%) of thyroid nodules do contain thyroid cancer (3), which is the most frequent endocrine cancer and represents 1-2% of all solid human malignancies (4).

1.1.1 HISTOLOGIC TYPES

1.1.1.1 Papillary thyroid cancer (PTC)

PTC is referred to as follicular cell-derived differentiated thyroid cancer (DTC). PTC is the most common type, making up about 70% to 80% of all thyroid cancers in iodine sufficient areas. PTC can occur at any age and its incidence is maximal between 30 to 50 years of age (mean age: 45 years). The female to male ratio is 2:1. It tends to grow slowly and often spreads to lymph nodes in the neck. However, unlike many other cancers, papillary cancer has a generally excellent prognosis with 30 year disease specific survivals in excess of 95% (5, 6).

1.1.1.2 Follicular thyroid cancer (FTC)

FTC is also referred to as follicular cell-derived differentiated thyroid cancer (DTC), with capsular and/or vascular invasion or invasion of adjacent structures in the absence of nuclear features of papillary thyroid cancer (7). In epidemiological surveys, FTC tends to be more common in areas with iodine deficiency. Owing to a combination of changing diagnostic criteria and an increase in the incidence of PTC associated with dietary iodine supplementation, the diagnosis of FTC is less frequent. While usually a sporadic disease, FTC is more likely than PTC to spread to distant organs, particularly the lungs and bones (8).

1.1.1.3 Poorly differentiated thyroid cancer (PDTC)

PDTC is a concept proposed to include carcinomas of follicular thyroid epithelium that retain sufficient differentiation to produce scattered small follicular structures and some thyroglobulin, but generally lack the usual morphologic characteristics of papillary and follicular carcinoma (9, 10), it comprises a small subset of heterogeneous thyroid tumors, occupying an intermediate area between well differentiated follicular or papillary carcinoma and anaplastic carcinomas, from both a histopathogenetic and a clinical point of view, accounting for less than 5% of all thyroid cancer cases, includes the following 3 features: (11) a solid, trabecular or insular growth pattern, (11) lack of classic PTC nuclear features, and (12) tumor necrosis, 3 or more mitoses per 10 high power fields, or convoluted nuclei which is evidence for loss of differentiation in PTC (13). Using this definition, clinical outcomes of PDTCs are significantly worse than seen in PTC and FTC with 10 year survival rates of approximately 50% (13, 14).

1.1.1.4 Medullary thyroid cancer (MTC)

MTC arises from parafollicular or C-cells of the thyroid that produce calcitonin (CTN), and accounts for 3%-5% of all thyroid cancers. Approximately 25% of all MTC runs in families and is associated with other endocrine tumors. In family members of an affected person, a test for a genetic mutation in the RET proto-oncogene can lead to an early diagnosis of medullary thyroid cancer and, as a result, to curative surgery (8).

1.1.1.5 Anaplastic thyroid cancer (ATC, undifferentiated thyroid cancer)

ATC is a rare undifferentiated tumor derived from follicular cells. It is found in less than 2% of patients with thyroid cancer (8). ATC is the most advanced and aggressive thyroid cancer and the least likely to respond to treatment.

My PhD subject only deals with differentiated thyroid cancers (PTC and FTC).

1.1.2 INCIDENCE AND MORTALITY

The incidence of thyroid cancer is quite various worldwide. GLOBOCAN 2012 (15), from International Agency for Research on Cancer (IARC) of World Health Organization shows, thyroid cancer accounted for 2.1% of all cancers worldwide in 2012. Number of new thyroid cancers in 2012 was estimated at about 298,102: 229,923 for women and only 68,179 for men. In most parts of the world, the incidence of thyroid cancer was 2 to 6 cases per 10^5 person-years in women and 1 to 2 cases per 10^5 person-years in men. High-risk areas (areas whose incidence of men and women is more than 10 per 10^5 person-years), are mainly from the North America (Canada and the United States), Pacific Islands: Polynesia (French Polynesia and Samoa), Australia / New Zealand and Melanesia (Fiji, New Caledonia, Papua New Guinea, Solomon Islands and Vanuatu) (Figure 1-1, Figure 1-2).

Worldwide variations in thyroid cancer incidence may reflect not only true variations in incidence, but also variations of economic level, convenience of access to medical diagnosis, and health insurance system among countries. In general, people from high-income countries pay more attention to their health care, have easier access to the successive new imaging techniques of thyroid disease diagnosis, such as ultrasound guided fine needle aspiration. This may introduce overdiagnosis. Figure 1-1 and Figure 1-2 show that the "high-risk areas" are mainly from the more developed countries rather than the less developed countries. Vaccarella et al. provided a measure of thyroid-cancer overdiagnosis over the past two decades in 12 selected high-income countries. These authors estimated that between 2003 and 2007, overdiagnosis in women accounted for 90% of thyroid-cancer cases in South Korea; 70 to 80% in the United States, Italy, France, and Australia; and 50% in Japan, the Nordic countries, England and Scotland. In men, overdiagnosis was approximately 70% in France, Italy, and South Korea; 45% in the United States and Australia; and less than 25% in all other countries they examined (16).



Figure 1-1 - World wide Variation in Thyroid Cancer Incidence Age-Standardised Rates (ASR) Worldwide



Figure 1-2 - Worldwide Age-Standardized Rate (ASR) of Incidence and Mortality of Thyroid Cancer (GLOBOCAN 2012)

Among the 10 countries with the highest incidence rates of thyroid cancer (ASR) all around the world (Table 1-1), we can see that South Korea was on the top, followed by New Caledonia, Puerto Rico and French Polynesia. Incidence in these two French overseas territorial divisions $(22/10^5 \text{ person-years} \text{ in New Caledonia and } 15/10^5 \text{ person-years} \text{ in French Polynesia})$ were twice higher than it was in metropolitan France (8.4 / $10^5 \text{ person-years}$). If we compare the incidence (ASR) of these 3 areas in 2008 (14) with that in 2012, they all decreased $(24/10^5 \text{ person-years} \text{ in New Caledonia, } 21/10^5 \text{ person-years} \text{ in French Polynesia}$ and $10/10^5 \text{ person-years}$ in metropolitan France in 2008), especially for the one in French

Polynesia. This may be due to the current recommendation to avoid fine-needle aspiration cytology (FNAC) for tumors that are less than 10 mm in diameter when there is no evidence of extension beyond the thyroid capsule and of lymph node metastases (18).

In Northern America, especially the USA, which was also among the top 10 countries with the highest thyroid cancer incidence (ASR) for both sexes (Table 1-1), the annual incidence of thyroid cancer varies in different registries. The epidemiological data of Surveillance Epidemiology and End Results (SEER) reported an annual incidence of thyroid cancer of 5.3 and 17.3 cases per 10^5 person-years in men and women from 2009 through 2013, respectively (19). In South Korea, since 2000 when screening became frequently performed, the annual incidence increased from 10 up to 70 cases per 10^5 person-years (10).

	Worldwide ASR of Thyroid Cancer Incidence (10 ⁵ person-years)		
Countries	Woman	Man	Total
South Korea	88.6	17.3	52.8
New Caledonia	35.8	8.3	22.2
Puerto Rico	25.4	5.8	16.1
French Polynesia	24.0	6.8	15.2
The USA	20.0	6.4	13.2
Canada	20.6	4.8	12.7
Lithuania	19.3	3.7	12.2
Israel	17.2	5.4	11.5
Turkey	17.8	3.8	10.9
Italy	14.7	6.7	10.8

Table 1-1 - Top 10 Countries with the highest ASR of thyroid cancer incidence for both sexes

(GLOBOCAN 2012)

The global mortality from thyroid cancer did not decrease with screening, confirming overdiagnosis (12). Around 10% of patients with a clinical papillary or follicular thyroid cancer will die from thyroid cancer, but in countries like South Korea, where screening permits the discovery of multiple small thyroid cancers, less than 1% of patients with thyroid cancer will die from thyroid cancer.

The mortality rate from papillary thyroid cancer ranges from very low rates for small tumors detected by screening up to 20% of cancers among older patients who often have large, fixed

or invasive cervical lesions or distant metastases at the time of diagnosis, and the initial risk of dying from thyroid cancer is estimated by the tumor, node, metastases (TNM) staging system (19,20).

If we look at the chronological trend from 1980 to 2010, the observed incidence of thyroid cancer for both sex increased in France, but mortality decreased (21, Figure 1-3).



Figure 1-3 - Incidence and mortality of thyroid cancer chronological trend in France (10⁵ person-years) (Colonna et al. 2013)

1.1.3 THYROID MICROCARCINOMAS

Microcarcinomas are cancers that measure less than 10 mm in diameter. They often have the features of a classic papillary carcinoma even though aggressive variants have been described (diffuse sclerosing variant, tall cell variant) (22). The majority of these tumors would probably remain undiscovered without screening. This is confirmed by the high prevalence of thyroid microcarcinomas in autopsy series (ranging from 2.7% to 30% depending from the selected population) (22, 24, 25, 26, 27). In an observational study in Japan, it appeared that only 16% of non-operated microcarcinomas progressed after 10 years; the outcome of these tumors when operated at progression was similar to that of tumors that were initially operated (28). This is the basis for the current recommendation to avoid FNAC for tumors that are <1cm in diameter when there is no evidence of extension beyond the thyroid capsule and of

lymph node metastases. Also the name of papillary "microcarcinoma" may be changed to papillary "microtumor" (the Porto consensus) (29).

1.1.4 ETHNIC DIFFERENCES

The differences of incidence of thyroid cancer according to ethnic origins allow the study of the pathogenic role of genetic and environmental factors including dietary habits and their combination. In general, thyroid cancer is more frequent in males and females of Caucasian than that of African and Puerto Rican Hispanics (15,20).

1.2 RISK FACTORS FOR DIFFERENTIATED THYROID CANCER

Several risk factors for thyroid carcinoma have emerged from epidemiological studies (30,31), but the only clearly demonstrated etiologic factor is a history of radiation exposure during childhood. In most patients, and as for most other cancers, no risk factor is identified.

1.2.1 FAMILY HISTORY

Epidemiological studies have suggested that about 3 to 10% of PTC occur as familial cancers (32,33). DTC may have a greater familial component than other nonhereditary cancers, with relative risk estimates of 3-4 (or higher) for first-degree relatives of persons with DTC (34,35,36,37). PTC is the highest heritable cancer among all cancers and has been shown to be the only cancer for which the contribution of inherited genetic factors more than that of environmental factors (38). If three or more first degree relatives are diagnosed with DTC, there is a greater than 94% chance that this represents a hereditary familial syndrome (39).

Thyroid cancers are present in several familial syndromes. Patients with familial adenomatous polyposis coli (FAP) and its subtype the Gardner's syndrome caused by inherited mutations of APC (adenomatous polyposis coli) at chromosome 5q21, have a 5- to 10-fold increased risk of PTC compared to the general population, and its prevalence is about 2% of affected individuals (40,41). These PTCs are mainly found in females less than 35 years of age. Genome-wide association studies (GWAS) of SNPs with large cohorts of patients with DTC and controls have been performed. Associations between DTC and SNPs have been found at

9q22.33 located near *FOXE1* (*Forkhead factor E1, also called TTF2 for Thyroid Transcription Factor 2*) gene and 14q13.3 located near the *NKX2-1* (*NK2 homeobox 1, also called TTF1 for Thyroid Transcription Factor 1*) gene (42,43). Also, studies focusing on radiation induced PTC confirmed the association with the 9q22.33 SNPs (rs925489, rs7850258, rs965513 and rs10759944) (44). These associations were confirmed in several studies (45,46,47,48,49) and also associations with other loci were found including 2q35, 3q25, 7q21, 8p12, 9q34. The conclusion drawn from these studies is that predisposition to DTC is complex and is most likely due to low and moderate risk mutations (50,51).

1.2.2 EXPOSURE TO IONIZING RADIATION DURING CHILDHOOD

Exposure to external irradiation to the neck during childhood and adolescence increases the risk for PTC (30, 31, 52,53,54,55). The latency period between exposure and diagnosis is at least 5 years. In children exposed to a dose of 1 Gy (100 rad) to the thyroid, excess risk for thyroid cancer is 7.7-fold (55). The risk decreases with older age at exposure and is low or even not significant when radiation exposure occurs in adults. Similarly, diagnostic or therapeutic administration of I^{131} to adults does not seem to be associated with an increased risk for thyroid cancer (31, 55). However, the increased incidence of PTC in children in the Marshall Islands after atomic bomb testing and more recently in Belarus and Ukraine after the Chernobyl nuclear reactor accident indicates a direct carcinogenic effect of radioactive isotopes, both I^{131} and/or short-lived isotopes, on the thyroid gland during childhood (56,57,58,59,60).

1.2.3 PRE-EXISTING BENIGN THYROID DISEASES

Thyroid cancer is often preceded by other thyroid abnormalities, such as benign thyroid nodules or goiter (61). Franceschi et al. (62) did a pooled analysis of case-control studies of benign nodules and goiter and thyroid cancer, including 2519 cases (2008 were PTC) and 4176 controls from 11 studies of goiter and 8 studies of benign nodules/adenomas. The studies of goiter included populations from five regions with sufficient to excessive iodine intakes (four studies from the U.S. in the early 1980s, and one from Japan) and six populations with sufficient to mildly deficient intakes (one from coastal China before the introduction of iodized salt and five from Europe). For women, ORs for a goiter history for all thyroid cancer were 5.9 (95 % CI: 4.2-8.1), and for PTC and FTC were 5.5 (95 % CI: 3.9-7.8)

and 6.9 (95 % CI: 3.8-12.4), respectively. For men, the OR for all thyroid cancer was 38.3 (95 % CI: 5.0-291.2). In that review, with the exception of a Japanese study where the OR for thyroid cancer was very high for women (26.5), the individual ORs from the other ten studies did not vary widely. For women, OR for a benign nodules/adenomas history for all thyroid cancer were 29.9 (95 % CI: 14.5-62.0), and for PTC and FTC were 28.9 (95 % CI: 13.6-61.2) and 62.3 (95 % CI: 18.9-205.8), respectively. The excess risk for goiter and benign nodules/adenomas was greatest within 4 years prior to thyroid cancer diagnosis, but a significantly elevated OR was still present more than 10 years before diagnosis. Case-control and cohort studies published since this analysis (62) have supported a link between goiter and/or thyroid nodules and an increased risk of developing thyroid cancer (63,64,65). However, biases cannot be excluded, one thyroid disease paving the way for the discovery of another. Also nodules considered benign at FNAC and therefore not operated do not progress into a malignant tumor.

Higher risk for DTC has been noted in patients with Graves' disease and cold thyroid nodules, and increased aggressiveness of such Graves' disease-associated thyroid cancer has been proposed (66,67,68,69).

The global rate of malignancy is similar in case of single or multiple nodules, suggesting that the risk of malignancy for each individual nodule is lower in case of multiple nodules than that of a solitary nodule (70). A meta-analysis of 14 cross-sectional or retrospective cohort studies (71) found the risk of thyroid cancer was significantly lower in multi-nodular goiter than in single nodules (OR = 0.8; 95 % CI: 0.67-0.96). However, there was moderate inconsistency across studies ($I^2 = 35$ %): studies in iodine sufficient areas (U.S., Saudi Arabia, Nigeria, Croatia) found risk was lower in multi-nodular goiter than in single nodules (OR = 0.77; 95 % CI: 0.65-0.92), while studies in mildly iodine deficient areas (Italy, Turkey), where multi-nodular goiter would be expected to be more common, found no significant association (OR = 0.88; 95 % CI: 0.68-1.14).

Occasionally, thyroid cancer has been found in patients with congenital goiter, suggesting that prolonged and sustained stimulation by TSH may promote the clinical appearance of DTC. This association has been proposed by various authors (72,73), while some others did not (74,75).

1.2.4 HORMONAL AND REPRODUCTIVE FACTORS

The incidence of thyroid cancer is approximately the same for males and females before puberty and after the menopause; the female to male ratio is highest during puberty and declines thereafter. Various studies demonstrated that thyroid volume increases during puberty and pregnancy and fluctuates during the menstrual cycle (76,77,78). In in vitro studies, it has been demonstrated that estrogen receptors are present on thyroid cancer cell lines and can stimulate proliferation (79).

These findings suggest that hormonal or reproductive factors may play a role in the risk of thyroid cancer in females. Various epidemiological studies tried to explain the link between thyroid cancer and reproductive age of the woman, but the results were contrasting. A younger age at menarche was significantly associated with a higher risk of DTC in a casecontrol study performed among young women (35 years or younger) in eastern France, for girls' age of 11 years or younger vs. those 12-14 years old, OR = 1.3, 95% CI: 1.0-1.8) (80). However, this result was directly opposite of the findings of a pooled analysis (for girls age of 15 years or older vs. those younger than 13 years, OR = 1.2, 95% CI: 1.0-1.4) (81), and those from a cohort study conducted in California in which the analysis was restricted to women less than 45 years of age (for girls 14 years of age or older vs. those younger than 13 years, relative risk = 1.88, 95% CI: 1.13-3.13) (82). No such association with age at menarche was observed in several other studies, including one conducted in Serbia on subjects less than 20 years of age (83) and 3 in which the analysis was restricted to subjects less than 45 years of age in New Caledonia (84), French Polynesia (85), and California (86). These differences between age at menarche and DTC risk may be due to the age and ethnicity of subjects (84,86).

The only risk factor for which association has been established is a high parity rate. The casecontrol study performed among younger people in eastern France, showed that having high number of pregnancies was significantly associated with high DTC risk (80). A meta-analysis with 21 studies including 406,329 thyroid cancer cases suggested a strong association of parity (\geq 3 pregnancies) with the risk of thyroid cancer (87). Early population-based casecontrol study of thyroid cancer also demonstrated that among females, the radiogenic risk appeared to be potentiated by the number of subsequent live-births (88). Three case-control studies performed in the USA (89), in China (90) and in New Caledonia (84) respectively revealed evidence of high risk in women who had been pregnant and who had a high number of pregnancies. A possible explanation for the higher risk of DTC in women who have had a high number of pregnancies, could be the proliferation of thyroid cells, as maternal thyroid activity increases during pregnancy (91). Estrogens, which are elevated during pregnancy, are associated with elevated serum thyroxin and triiodothyronine levels, which might induce high cell turnover (92). The highest levels of serum TSH, even within the normal range, are associated with a subsequent diagnosis of thyroid cancer in individuals with thyroid abnormalities (93). Repeated pregnancies may increase the risk of DTC by revealing DNA damage due to carcinogens, such as ionizing radiation, that may have remained silent without pregnancies (94).

1.2.5 DIETARY IODINE INTAKE

It is established that in endemic goiter regions with iodine deficiency, incidences of goiter and follicular thyroid cancer are higher than elsewhere (95). However, the impact of iodine deficiency in the risk of papillary cancer, in most countries, has not achieved a consensus.

Some biologically experimental models revealed that under normal conditions, thyroid follicular cells only slowly proliferate, but in iodine deficient animals, serum thyroidstimulating hormone (TSH) increases and the proliferation rate of thyroid cells increases by 5 to 30-fold, leading to marked thyroid hyperplasia and hypertrophy. Rapidly proliferating thyroid cells are likely more vulnerable to mutagens such as radiation, chemical carcinogens and oxidative stress, and may accumulate a higher number of genetic alterations (96). Several authors have suggested that thyroid carcinomas caused by iodine deficiency are due to chronic TSH overstimulation (97,98,99,100), because serum TSH is increased in moderate to severe iodine deficiency in an effort to maintain euthyroidism (101) leading to the increasing of follicular thyroid cancer (FTC) and anaplastic thyroid cancer (ATC) in populations with severe endemic goiter. Excess iodide, by inhibiting thyroid hormone production (the Wolff-Chaikoff effect), can transiently increase TSH. However, in animal studies, chronic iodine excess does not increase serum TSH (96), and, in cell studies, moderate doses of iodide inhibit thyroid cell proliferation (102). Moreover, some population-based studies have reported, compared to the populations with mildly deficient iodine intake, populations with adequate or excess iodine intakes had slightly higher mean serum TSH, but this may be due to increased toxic adenomas in mild iodine deficiency (101). Thus, the mechanism by which iodine excess promotes thyroid tumorigenesis is uncertain.

The population-based case-control studies which had been carried out in high iodine intake areas (Hawaii and California), which means 2-3 fold higher than recommended intakes, didn't achieved the consensus about the association between dietary iodine intake and thyroid cancer risk. The case-control study based on 191 cases and 442 controls in Hawaiian adults revealed no association (103), however, another case-control study of 608 women with thyroid cancer and 558 controls in northern California showed that a significant reduction in the papillary thyroid cancer risk was seen in the highest iodine intake quintile (> 537 µg/day) compared with the lowest (< 273 μ g/day) (OR = 0.49, 95 % CI: 0.29-0.84). Iodine intake from food alone was not associated with risk, but iodine intake from supplements was (63). While, the other case-control studies done in Pacific Islands populations with mildly deficient intakes didn't have the same conclusion neither. The case-control study based on 293 cases and 354 controls in New Caledonia demonstrated no association (104), the study launched in French Polynesia with 229 cases and 371 controls evidenced the significant reduction of DTC risk with the increasing dietary iodine intake (105). The meta-analysis of publications of animal and human studies about iodine intake and thyroid cancer risk done by Zimmermann et al. (106) examined the four case-control studies described above, by adjusting OR for the highest iodine intake quartile compared to the lowest quartile, and revealed the odds for thyroid cancer risk are 23 % less in the highest quartile of iodine intake versus the lowest, although this effect was only borderline significant (OR = 0.77; 95 % CI: 0.58-1.02; p = 0.068).

1.2.6 OTHER DIETARY FACTORS

Several epidemiological studies were interested in the relationship between foods rich in iodine, such as fish, shellfish or meat (107,108,109,110), and some other dietary factors such as cruciferous vegetables, and thyroid cancer risk. However, studies showing the association between dietary factors and thyroid cancer risk have been inconsistent. A meta-analysis of 19 observational studies on dietary factors and thyroid cancer risk summarized the ORs for each dietary factor classified by sex, histologic type, located in an iodine deficiency or iodine rich region. Based on the highest level of total consumption versus the lowest level, results showed by the alimental group were as following (111):

1.2.6.1 Fish

Ten studies were included in the high vs. low analysis of fish consumption and thyroid cancer risk. The summary OR indicated a significant inverse association with a 21% decreased risk of thyroid cancer (OR = 0.79, 95 % CI: 0.66-0.94). When analysis was restricted to PTC or to women, no significant association between high vs. low fish intake and thyroid cancer risk was found. In the subgroup analysis according to location, the summary OR was 0.74 (95% CI, 0.59-0.92) in iodine deficiency areas while no significant association was found in iodine-rich areas (Figure 1-4, 111).



Figure 1-4 - Forest plot for the association between thyroid cancer risk and fish consumption stratified by location. 1 = iodine deficiency areas; 2 = iodine-rich areas. ^a Study in Sweden. ^b Study in Norway. (Liu ZT et al. 2014)

1.2.6.2 Salt Water Fish

Four studies provided information on the association of salt water fish intake with risk of thyroid cancer. The summary OR for the highest versus lowest intake was 0.95 (95% CI, 0.74-1.23). Subgroup analyses showed this non-significant association was not modified by sex, histological type of thyroid cancer, and location.

1.2.6.3 Fresh Water Fish

In 5 studies with information about fresh water fish intake, the summary estimate effect did not show association of it with thyroid cancer (OR = 0.86, 95% CI, 0.63-1.16). When adjusting this association by sex, histological type of thyroid cancer, and location (iodine deficiency areas or iodine-rich areas). The estimates OR was varied a little by them, showing that fresh water fish consumption had no effect on thyroid cancer risk.

1.2.6.4 Shellfish

A meta-analysis of 9 studies yielded a summary OR of 0.88 (95% CI, 0.72-1.08). When analysis was restricted to PTC or to women, this non-significant association reminded consistent. In the subgroup analysis according to location, a favorable effect was found in iodine deficiency areas (OR = 0.46, 95% CI, 0.27-0.75), whereas the estimate OR in iodine-rich areas was not statistically significant.

1.2.6.5 Vegetables

A total of 5 studies examined the association between vegetables intake and thyroid cancer risk. The pooled OR was 0.76 (95% CI, 0.58-1.00). In subgroup analysis, there was no significant association between vegetables consumption and thyroid cancer whatever adjusted for sex, histologic type or location.

1.2.6.6 Cruciferous Vegetables

Eight studies provided information on the association between cruciferous vegetable consumption and risk of thyroid cancer. The random effects summary OR was 0.93 (95% CI, 0.66-1.29). Subgroup analyses showed that sex, histological type, and location did not markedly influence the observed null association.

1.2.6.7 Meat

A meta-analysis of 5 studies for thyroid cancer risk with meat consumption yielded a summary OR of 0.96 (95% CI, 0.70-1.34). Subgroup analyses showed that histological type and location did not markedly influence this observed non-significant association.

1.2.7 BODY SIZE

Various epidemiological studies documented an increased risk in thyroid cancer in persons with tall height and large body size (measured as either body surface area or body mass index (BMI; weight in kilograms divided by height in meters squared)) (114,115,116,117,118,119). The mechanisms responsible for this association are still unknown, even if several explanations have been proposed (chronic inflammation (120), higher TSH levels in obese patients (121), cooperation between TSH and insulin-like growth factor 1 in activation of MAP kinase and PI3K pathways (122)).

1.3 FOOD COMPOSITION TABLES AND IODINE CONTENT IN FOOD

1.3.1 THE MAIN FOOD COMPOSITION TABLES

1.3.1.1 Food and Agriculture Organization of the United Nations (FAO) Databases (123)

The FAO/ International Network of Food Data Systems (INFOODS) Global Food Composition Databases provide nutrient values for pulses (a subgroup of legumes that includes dry edible seeds with low fat content), for fish and shellfish, for food supplements. The majority of data are analytical data complemented by data from other published sources covering data for foods in raw, cooked and processed forms on proximates, minerals, vitamins, phytate, amino acids and fatty acids fractions. The data compilation process followed standards and guidelines outlined by FAO/INFOODS, and the species were selected based on the importance of the food and the available data. Emphasize is put on nutrient variations among different species, varieties and origin. The data are presented in Excel format alongside with a comprehensive documentation in PDF format.

The FAO/INFOODS also provides the Density Database - a tool for researchers and professionals to convert volume into weight and vice-versa; the Biodiversity Database; the Analytical Food Composition Database - a global compendium of scrutinized analytical data (without any additional estimations, imputation or calculation of missing values) for commonly consumed foods; and The West African Food Composition Table contains 472 foods and 28 components and presents a compilation of existing compositional data from 9
countries (Benin, Burkina Faso, Gambia, Ghana, Guinea, Mali, Niger, Nigeria and Senegal) which were supplemented with data from other countries.

However, these food composition databases haven't included the data on iodine, selenium or other trace elements in foods.

1.3.1.2 French Food Composition Table Centre d'Information sur la Qualité des Aliments Ciqual (version 2013) (124)

Since 2012, Ciqual is part of the Observatory of Food Nutritional Quality (Ciqual-Oqali), unit of ANSES (French Agency for Food, Environmental and Occupational Health & Safety).

The first mission of Ciqual is to collect, evaluate and make available French food composition data.

The three main tasks consist in:

- Input and management of a reference database on food composition
- Contribution to risk assessment in nutrition, within the French agency for food, environmental and occupational health safety
- Communication and dissemination of food composition data to administrations, researchers, nutritionists, food companies and consumers

At present, the French food composition databank of ANSES is permanently enriched through CIQUAL's yearly analytical programs, research programs in collaboration with external partners, scientific literature, data from producers or suppliers, recipe calculation, or as a last resort borrowed data from foreign food composition tables.

1.3.1.3 Traditional French Polynesian and Cuban Food Composition Tables Made By the Collaboration with ANSES in 2013 (FPFCT 2013) and in 2014 (CFCT 2014) Respectively

In order to better estimate dietary iodine, selenium and other trace elements of Asia-Pacific Islands' populations and Cuban population (Explanation in details see 4) and understand the role of these dietary factors in the DTC risk, Cancer and Radiations team of Centre for research in epidemiology and population health (CESP)/Unit 1018 of the French National Institute of Health and Medical Research (Inserm) collaborated with the Laboratory for Food Safety of ANSES. The colleagues from French Polynesia and Cuba and me, we organized the

collection of a large number of traditional French Polynesian and Cuban food samples; including fruit, vegetables, seafood, and beverages in French Polynesia between 2011 and 2013 in seven areas: Tahiti and Maupiti (Society Islands), Hao and Rangiroa (Tuamotu Archipelago), Mangareva (Gambier Islands or Mangareva Islands), Tubuai (Austral Islands) and Hiva Oa (Marquesas Islands). Foods were selected so as to cover the typical diet of the French Polynesian population. Each of the composite samples from the seven areas of sampling was composed of up to five sub-samples from five different locations. We also selected some fruit, vegetables, beverages, grains, dairy and canned products, tuberous plant food samples in Havana and its suburb areas in 2013. These samples allowed ANSES to develop a new measurement method to quantify iodine (I), selenium (Se), vanadium (V), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd), tellurium (Te), mercury (Hg), lead (Pb), uranium (U) in these food samples. For iodine, it is the first table adapted to these populations. The composition of 124 traditional French Polynesian and 39 traditional Cuban food items were measured by this collaboration.

It is noteworthy that the ANSES used an innovative technology for determining total iodine (100). This technology is based on a European standard (125) that involves alkaline extraction using tetra tetramethylammonium hydroxide (TMAH), optimized with an additional enzymatic treatment for samples containing starch, and followed by inductively coupled plasma mass spectrometry (ICP-MS) analysis. A complete single-laboratory validation was performed according to the accuracy profile approach (126, 127) based on tolerance intervals to select the best calibration function and to determine the validated concentration ranges. This method was used to determine the iodine, selenium and other trace elements content in the main foods consumed by French Polynesians and Cubans (Supplementary Figs. S1 and S2).

1.3.2 THE VARIABILITY OF DIETARY IODINE DATA

Dietary iodine has high volatility and strong variability, for the same food item, dietary iodine data depend on soil, agricultural techniques, meal preparation, and food composition tables measured by different laboratories, for example, iodine content in cabbage was estimated to be 0.0106 μ g/g in France (CIQUAL 2013 table) (124), 0.0160 μ g/g in French Polynesia (FPFCT 2013), but 0.00427 μ g/g in Cuba (CFCT 2014) (Figure 1-5), between 0.0026 and 0.0037 μ g/g in Cameroon (128). Figure 1-5 shows us the variations of iodine content in

different food samples measured by the French CIQUAL 2013 (124), FPFCT 2013 and CFCT 2014, respectively. These variations exist for all foods, even those rich in iodine such as shellfish. The iodine content ranged from 0.56 μ g/g depending on the type of shellfish in France (CIQUAL 2013 table) (124) to 5.0 μ g/g in the Marshall Islands (129).

Table 1-2 also shows us the variation of iodine content in different French Polynesian food samples (100), and Figure 1-6 demonstrated iodine content in open-sea or lagoon fish samples from the seven different French Polynesian areas measured by ANSES.



Figure 1-5 - Variation of iodine content in different food samples measured by different food composition tables

Table 1-2 - The Variation o	f Iodine Content in	Different French	Polynesian	Food Samp	oles

Food group	N	LC	% Humidity	Iodine content (mg kg ⁻¹ dry weight [*])		
				Mean**	Min	Max
Fruits	34	31	75	0.014	<0.014	0.032
Pineapple	3	3	82	0.014	< 0.027	< 0.027
Banana	5	5	70	0.011	< 0.014	< 0.027
Copra	7	6	42	0.016	< 0.027	0.029
Mango	3	3	85	0.014	< 0.027	< 0.027
Melon	3	1	91	0.025	< 0.027	0.032
Grapefruit	5	5	84	0.010	< 0.014	< 0.027
Papaya	5	5	87	0.014	< 0.027	< 0.027
Watermelon	3	3	93	0.014	< 0.027	< 0.027
Starchy staples	19	17	63	0.018	< 0.014	0.081
Гагиа	4	4	66	0.012	< 0.014	< 0.027
Taro	3	2	53	0.034	< 0.014	0.081
Manioc	5	4	63	0.020	< 0.027	0.044
Sweet potato	3	3	69	0.014	< 0.027	< 0.027
Uru	4	4	64	0.012	< 0.014	< 0.027
Green vegetables	11	2	88	0.427	< 0.027	1.85
Cabbage leaf	4	1	90	0.162	< 0.027	0.316
Fafa	4	0	84	0.974	0.208	1.85
Green beans	3	1	91	0.052	< 0.027	0.073
Shellfish	9	0	78	46.2	6.51	85.6
Giant clam	9	0	78	46.2	6.51	85.6
Fish	41	0	75	1.57	0.222	5.19
Lagoon fish	16	0	76	2.68	0.520	5.19
Open-sea fish	25	0	72	0.851	0.222	2.54
Beverages	10	8	C 1/	0.219	< 0.004	1.39
Drinking water	4	2	-	0.544	< 0.004	1.39
Coconut water	6	6	-	0.002	< 0.004	< 0.008

20

N, number of samples; LC, number of left-censored data.

* Except for beverages.

Mean calculated using the middle bound approach (WHO, 1995).



Figure 1-6 - Iodine content in fish samples from the seven different French Polynesian areas measured by FPFCT 2013

1.3.3 RESULTS OF THE DIETARY IODINE MEASUREMENT IN THE TRADITIONAL FRENCH POLYNESIAN AND CUBAN FOOD SAMPLES

Table 1-3 shows us the results of dietary iodine and selenium intake from typical Cuban food in the cases and in the controls of the Cuba study (see 2.3.5), based on the composition of 20 traditional Cuban food items in the dietary questionnaire of this study, and measured by the French ANSES (CFCT 2014, see 1.3.1.3). Within these food items, the results confirmed that iodine is rich in meats, cheese and egg (Figure 1-7).

Food Items	Iodine Content (μg/100 g fresh weight)	Туре	Mean (Min, Max) (µg/day) ⁵	Selenium Content (µg/100 g fresh weight)	Туре	Mean (Min, Max) (µg/day) ⁵
	(IC 95%)			(IC 95%)		
Tomato	0.26	Cases	0.13 (0, 1.16)	$< LOD^1$	Cases	0.14 (0, 1.31)
	(0.21-0.31)	Controls	0.11 (0, 0.92)	(-)	Controls	0.12 (0, 1.05)
Lettuce	4.78	Cases	1.17 (0, 15.34)	3.2	Cases	0.78 (0, 10.27)
	(4.00-5.56)	Controls	0.81 (0, 4.38)	(1.92 - 4.48)	Controls	0.54 (0, 2.93)
Cabbage	0.43	Cases	0.21 (0, 2.69)	2.4	Cases	1.18 (0, 15.12)
U	(0.37 - 0.49)	Controls	0.16 (0, 1.54)	(1.44 - 3.36)	Controls	0.92 (0, 8.64)
Watercress	3.99	Cases	0.24 (0, 3.99)	3.5	Cases	0.21 (0, 3.50)
	(3.33-4.65)	Controls	0.09 (0, 3.99)	(2.10 - 4.90)	Controls	0.08 (0, 3.50)
Mango	0.74	Cases	1.00 (0, 9.77)	1.4	Cases	1.89 (0, 18.38)
U	(0.63 - 0.85)	Controls	0.78 (0, 6.51)	(0.84 - 1.96)	Controls	1.47 (0, 12.25)
Guava	2.03	Cases	2.60 (0, 16.40)	4.2	Cases	5.40 (0, 34.02)
	(1.71 - 2.35)	Controls	1.50 (0, 14.58)	(2.94 - 5.46)	Controls	3.12 (0, 30.24)
Banana	<lod (plantain)<="" td=""><td>Cases</td><td>0.39 (0, 2.82)</td><td><lod (plantain)<="" td=""><td>Cases</td><td>0.75 (0, 5.40)</td></lod></td></lod>	Cases	0.39 (0, 2.82)	<lod (plantain)<="" td=""><td>Cases</td><td>0.75 (0, 5.40)</td></lod>	Cases	0.75 (0, 5.40)
	\geq LOD and $<$ LOQ ^{1, 2}	Controls	0.50 (0, 4.70)	\geq LOD and $<$	Controls	0.96 (0, 9.00)
	(fruit banana)			LOO ^{1, 2}		
	(-)			(fruit banana)		
				(-)		
Papaya	<LOD ¹	Cases	0.04(0, 0.83)	\geq LOD and <	Cases	0.59 (0, 11.88)
.1	(-)	Controls	0.05(0, 0.32)	LOO ^{1, 2}	Controls	0.77 (0, 4.50)
				(-)		
Pasta	<LOD ¹	Cases	0.32 (0, 3.78)	\geq LOD and <	Cases	0.68 (0, 8.10)
	(-)	Controls	0.31 (0, 3.78)	_ LOQ ^{1, 2}	Controls	0.66 (0, 8.10)
				(-)		
Rice	\geq LOD and $<$ LOQ ^{1, 2}	Cases	7.04 (0, 12.30)	3.5	Cases	12.02 (0, 21.00)
	(-)	Controls	6.00 (0, 12.30)	(2.10 - 4.90)	Controls	10.25 (0, 21.00)
Potato	\geq LOD and $<$ LOQ ^{1, 2}	Cases	0.14 (0, 3.28)	<LOD ¹	Cases	0.13 (0, 3.00)
	(-)	Controls	0.11 (0, 1.31)	(-)	Controls	0.10 (0, 1.20)
Delicatessen	27.7	Cases	0.46 (0, 11.08)	7.5	Cases	0.12 (0, 3.00)
	(23.32-32.08)	Controls	0.83 (0, 22.16)	(5.25-9.75)	Controls	0.22 (0, 6.00)
Ham	57.89	Cases	4.45 (0, 43.00)	13.7	Cases	1.05 (0, 10.18)
	(50.06-65.72)	Controls	7.31 (0, 75.26)	(9.55-17.85)	Controls	1.73 (0, 17.81)
Sausage	24.71	Cases	2.43 (0, 29.65)	10.2	Cases	1.00 (0, 12.24)
-	(20.87-28.55)	Controls	2.35 (0, 39.54)	(7.15–13.25)	Controls	0.97 (0, 16.32)
Poultry	1.19	Cases	0.80 (0.04, 7.84)	35.5	Cases	23.97 (1.05, 234.30)
	(1.00 - 1.38)	Controls	0.75 (0, 7.13)	(24.85-46.15)	Controls	22.39 (0, 213.00)
Pork	<LOD ¹	Cases	0.09 (0, 1.22)	27.4	Cases	11.67 (0, 164.40)
	(-)	Controls	0.11 (0, 1.62)	(19.18-35.62)	Controls	14.50 (0, 219.20)
Egg	22.2	Cases	6.67 (0, 35.52)	28.8	Cases	8.66 (0, 46.08)
	(18.77-25.63)	Controls	8.39 (0, 53.28)	(20.16-37.44)	Controls	10.89 (0, 69.12)
Melted cheese	24.98	Cases	1.81 (0, 29.97)	16.8	Cases	1.22 (0, 20.16)
	(21.10-28.86)	Controls	1.96 (0, 14.99)	(11.76-21.84)	Controls	1.32 (0, 10.08)
Beer	<LOD ³	Cases	0.04 (0, 1.01)	\geq LOD and $<$	Cases	0.10 (0, 2.28)
	(-)	Controls	0.12 (0, 4.26)	LOQ ^{3, 4}	Controls	0.27 (0, 9.59)
				(-)		
Iodized salt	1024.6	Cases	102.46^{*}	2.2	Cases	0.22^*
(dried)	(865.2–1184.0)	Controls	102.46^{*}	(1.30 - 3.10)	Controls	0.22^{*}

Table 1-3 - Dietary Iodine and Selenium Intake from Typical Cuban Food in the Cases and in the Controls based on the composition of 20 traditional Cuban food items measured by the French ANSES (CFCT 2014)

¹ LOD = Limit Of Detection: $1.4 \ \mu g/100 g$ (dry weight) for iodine and $1.0 \ \mu g/100 g$ (dry weight) for selenium;

² LOQ = Limit Of Quantification: 2.7 μ g/100g (dry weight) for iodine and 2.0 μ g/100g (dry weight) for selenium;

 3 LOD = Limit Of Detection: 0.4 µg/100g (liquid weight) for iodine and 0.3 µg/100g (liquid weight) for selenium; 4 LOQ = Limit Of Quantification: 0.8 µg/100g (liquid weight) for iodine and 0.6 µg/100g (liquid weight) for selenium;

⁵ In these calculations, "< LOD" has been arbitrarily replaced by half of the LOD; and " \geq LOD and < LOQ" have been replaced by the mean of LOD and LOQ;

^{*} Dietary consumption of iodized salt was not measured in the questionnaire and assumed mean to be 10g per person and per day.



Figure 1-7 - Measurements of Iodine Content in Typical Cuban Food by the French ANSES (CFCT 2014) (µg/g)



Figure 1-8 - Measurements of Selenium Content in Typical Cuban Food by the French ANSES (CFCT 2014) $(\mu g/g)$

1.4 OBJECTIVES OF THE THESIS

The overall objectives of this thesis is to explore the relation between dietary iodine intake and differentiated thyroid cancer risk in a multiethnic set of case-control studies, to identify environmental and genetic risk factors which interact with this relation, and to investigate the possibility to use selenium present in nails as a potential biomarkers in thyroid cancer casecontrol studies.

The specific objectives are:

- To explore the role of specific food groups or items, especially in regard to iodine intake, in the risk of developing DTC, in case-control study performed in Cuba. It was hypothesized, fish consumption may reduce DTC risk.
- To establish or reestablish dietary iodine, selenium, energy intake for the participants in Epi-Thyr consortium of 5 cases-control studies performed in New Caledonia, French Polynesia, metropolitan France (2 studies), and Cuba, according to different food composition tables.
- To investigate the relationship between dietary iodine status and the risk of DTC in the pooled analysis of Epi-Thyr consortium of the 5 cases-control-studies.
- To assess the possibility of interaction between iodine status and number of pregnancies in DTC risk, in the 5 Epi-Thyr case-control studies. It was hypothesized, iodine deficiency may interact with high number of pregnancies that leads to a high DTC risk.
- To study, in the 5 Epi-Thyr case-control studies, the possible interaction between dietary iodine intake and 4 common variants of the SNPs previously identified in most of GWAS as associated with DTC risk. Two of these variants are on *FOXE1*, which is a thyroid hormones transcription factor.
- To investigate in a study performed in French Polynesia the possibility to use selenium present in nails as a potential biomarker in thyroid cancer case-control studies.

2 PART II: EPI-THYR CONSORTIUM

2.1 OBJECTIVE AND HISTORY

In order to identify the genetic and environmental risk factors of thyroid cancer and their interactions, Cancer and Radiations group and Cancer and Environment group, Center for research in Epidemiology and Population Health (CESP) - Unit 1018, the French National Institute of Health and Medical Research (Inserm) established a set of 5 large case-control studies which have been carried out in French Polynesia, New Caledonia, metropolitan France (2 studies) and Cuba, conducted homogeneously in contrasting populations, both genetically and in terms of the environment and life style, using similar questionnaire and DNA collection, to date, about 2212 cases and 2581 controls were interviewed face-to-face by trained professionals (nursing and medical staff), using the standardized dietary questionnaire and the photo booklet from Epic cohort. Buccal cell taken by cytobrush or saliva taken by DNA Genotek Oragene collection brush/kit (Ottawa, Canada) given to each study participant for their DNA collection. Since 2013, Cancer and Radiations group and Cancer and Environment group have established a consortium and synchronized the data of these 5 Case-Control studies together called Epi-Thyr consortium (Detailed characters of these 5 studies see Table 2-1). To the best of our knowledge, this consortium is the largest case-control consortium about thyroid cancer going across different population and ethnicity samples all over the world, and this consortium still expands.

2.2 MATCHING PROCESS

Confounding implies that the confounding factor (which is one of the exposures) is not evenly distributed between cases and controls. Therefore in order to prevent confounding the simplest solution would be to design a study in which cases and controls would have an equal distribution of the confounding factor. This process is called matching. Matching is most often applied into case-control studies.

We usually identify two types of matching process, individual matching and frequency matching. Both individual and frequency matching have the same consequence: matching will have to be taken into account during the analysis (130).

2.2.1 INDIVIDUAL MATCHING

In this first method, matching is performed subject by subject. This is called individual matching. For example, if age is a confounding factor, for each case age 30 years, one control of the same age will be selected, and so far and so on for all cases included in the study. The results are pairs of individuals belonging to the same study population and sharing one common characteristic (in this example, a specific age).

In individual matching, we may also consider to select more than one control per case. Then two or more controls have then the same characteristic of the case. We have then constituted triplets (one case and 2 controls), quadruplets (one case and 3 controls), etc (130).

2.2.2 FREQUENCY MATCHING

In a second type of matching process, matching is no longer done individually but for groups of subjects. In such instance a group of controls is matched to a group of cases with respect to a particular characteristic (the confounding factor). For example if in a case-control study with 50 cases there are 20 men and 30 women, we would select a control group having the same gender distribution. We would first select 20 men from the male study population and then 30 women from the female study population (130).

2.2.3 WHY MATCHING?

Matching controls to cases is nothing more than stratifying in advance of analysis. Instead of constituting strata at the time of the study analysis we prepare them before the study is done, at the time of controls selection. When we select one control per case, each stratum will include one case and one control, when we select two controls per case, each stratum will include one case and two controls, and so on. We will therefore have as many strata as pairs in the study. The objective of matching is to prepare the analysis. Matching optimizes the number of cases and controls per stratum. It avoids having no case or no control in a stratum, as could happen when doing a stratified analysis afterwards (The biggest inefficiency in a stratified analysis done afterward would occur when in a stratum there is either no case or no control). This is why matching is frequently mentioned as a way to improve the efficiency of an analysis by better distributing cases and controls between strata (130).

2.3 CARACTERISTICS OF THE 5 CASE-CONTROL STUDIES

2.3.1 THE NEW CALEDONIA STUDY

New Caledonia had a very high thyroid cancer incidence during the last decade, but the risk factors in this particular area were still little known, to investigate the remarkably high incidence of thyroid cancer and to clarify the role of certain risk factors for thyroid cancer in general, a countrywide population-based case-control study was conducted in general population of New Caledonia in 1998.

Previously, this study reported that, thyroid cancer was associated with goiter, age at menarche, irregular menstruation, hysterectomy and miscarriage as an outcome of the first pregnancy. There was a dose-response trend with number of full-term pregnancies (84). The sharp increase of thyroid cancer incidence in 1985-1999 in New Caledonia was partly related to enhanced detection of small size carcinomas (131). Thyroid cancer was negatively associated with tobacco smoking and alcohol drinking. Strong positive associations with weight and body mass index were observed in Melanesian women aged 50 years or more (132). Later, in order to show the dominant role of body surface area (BSA) in DTC risk, this study had been pooled with another case-control study carried out in French Polynesia, a high proportion of cases (73%) were overweight or obese before diagnosis of thyroid cancer was found in this pooled Analysis. An increased risk of thyroid cancer was observed with greater height, weight, BMI, BF%, and BSA. The association of thyroid cancer risk with height, weight, BMI, and BF% did not remain when adjustment was made for BSA (114). Brackish water fish consumption was inversely related to DTC risk in Melanesian women; but high consumption of cruciferous vegetables was associated with thyroid cancer risk among women with low iodine intake (104). Thyroid cancer was associated with a history of thyroid cancer in first-degree relatives and with a family history of multinodular goiter; however, thyroid cancer was not associated with a family history of thyroid diseases in Melanesians from the Loyalty Islands, the area with the highest incidence rates for thyroid cancer, possibly indicating a high frequency of genetic susceptibility variants and lack of genetic variation in this population subgroup (133). In the recent publication about fine-mapping of two DTC susceptibility loci at 9q22.33 and 14q13.3 in Europeans from metropolitan France and Melanesians from New Caledonia, the people auto-declaring as having European ancestry

and Melanesian ancestry from the New Caledonia study was pooled with the people autodeclaring as having European ancestry of the French metropolitan Cathy study (see 2.4.1.4), the principal results were that the association with the four SNPs previously identified in GWAS (rs965513, rs1867277 at loci 9q22, and rs944289, rs116909734 at loci 14q13) was replicated in Europeans while only rs944289 was replicated in Melanesians. Among Europeans, the two SNPs previously reported at 9q22 were not independently associated to DTC and that rs965513 was the predominant signal; at 14q13, the haplotype rs944289[C]rs116909374[C]-rs999460[T] was significantly associated with DTC risk. Among Melanesians, a new independent signal was observed at 14q13 for rs1755774 which is strongly correlated to rs2787423; this latter is potentially a functional variant. Significant interactions with parity and body mass index were observed for rs1755774 and rs2787423 (134).

2.3.1.1 Background

New Caledonia is a special collectivity of France located in the southwest Pacific Ocean, 1,210 km east of Australia and 16,136 km east of Metropolitan France (135). The archipelago, part of the Melanesia sub-region, includes the main island of Grande Terre, the Loyalty Islands, the Chesterfield Islands, the Belep archipelago, the Isle of Pines, and a few remote islets (136). The Chesterfield Islands are in the Coral Sea (*Figure 2-1*).



Figure 2-1 - Map of New Caledonia

New Caledonia has a land area of 18,576 km². Its population of 268,767 (Aug. 2014 census) (137) consists of a mix of Kanak people (the original inhabitants of New Caledonia), people

of European descent (Caldoches and Metropolitan French), Polynesian people (mostly Wallisians), and Southeast Asian people, as well as a few people of Pied-Noir and Maghreban descent. The capital of the territory is Nouméa (135).

2.3.1.2 Case Selection

All patients with papillary or follicular thyroid cancer diagnosed between January 1, 1993, and December 31, 1999, and having resided in New Caledonia for at least 5 years at the time of cancer diagnosis were eligible for the study (84).

The cases were identified from the pathology records kept by the two histopathology laboratories, one public and one private, operating in New Caledonia. All pathology reports including a diagnosis of thyroid cancer were retrieved by manual or electronic searches. The cancer diagnoses were made by the local pathologists. Most of the histologic slides were also reviewed by the Pathology Department of the Royal Prince Alfred Hospital in Sydney, Australia. The case list was cross-checked with the New Caledonia cancer registry, which collects information on cancer diagnoses from medical facilities throughout the country. However, no additional case of thyroid cancer was detected. All of the pathology reports were retrieved in order to code the histology, number, and size of the cancerous nodules. Mixed papillary-follicular cases were included in the papillary group (138). If insufficient information had been included in the missing information. The thyroid cancer cases were classified according to histology and size of the largest cancerous nodule (84).

All cases diagnosed during the 5-year period before the start of data collection in 1998 and all cases newly diagnosed up to the end of the study period in 1999 were recruited for the study. Good prognosis of differentiated thyroid tumors, residential stability, and enabled subject enrollment were achieved several years after the initial diagnosis (84).

Among the 369 eligible differentiated thyroid cancer cases, 37 (10%) were not interviewed because they refused to participate (n=10), had died (n=21), could not be contacted (n=5), or was too sick to reply the questionnaire (n=1). The study participants thus consisted of 332 cases.

2.3.1.3 Control Selection

Controls were randomly selected from updated electoral rolls that included the name, address, and date of birth of all New Caledonia residents aged 18 years or older. The controls were frequency matched to the cases by sex and 5-year age group in order to control the distribution of these two variables in controls was the same as it in cases. The distribution by sex and 5-year age group of the cases diagnosed during the period 1990-1995 was used as a first approximation from the cancer registry data. This distribution was used to constitute a pool of randomly selected controls from the electoral rolls after stratification on sex and age group. From the pool of controls thus constituted, three lists of controls were created at the beginning, in the middle and at the end of the data collection period, by random selection. Controls were allocated a year of reference equal to the year of diagnosis of the case group for which they were selected. Only those events or exposures that occurred before the reference date were considered in the analyses. Controls were excluded if they had not lived in New Caledonia for at least 5 years before the reference date or if they had had thyroid cancer before that date. For practical reasons, prior to study initiation, the decision was made to restrict the total number of controls to 500 (84).

Among the 473 eligible controls, 61 (13%) were not interviewed because they refused to participate (n=21), had died (n=13), could not be contacted (n=24), were too sick to reply the questionnaire (n=2), or did not respond with for another reason (n=1). The remaining 412 controls were included in the study.

2.3.1.4 Data Collection

Trained interviewers conducted a face-to-face interview with the cases and controls at their home, using a structured questionnaire, after having obtained informed consent. The interviewers elicited information on socio-demographic characteristics, diet, alcohol drinking, tobacco smoking, anthropometric factors, gynecologic and reproductive history, medical conditions and medical x-ray exposure, occupational and residential history, personal history of thyroid disease and familial history of thyroid cancer (84).

2.3.1.5 Dietary Questionnaire

The dietary questionnaire included two parts on the consumption (frequency and quantity) of 11 sub-groups and 5 questions on dietary habit before or at the time of the interview. Overall,

the questionnaire provided estimation for daily consumption of 122 food items, 17 of which were fish or shellfish including fresh open-sea fish, fresh water fish, brackish water fish, shellfish, oysters, mussels, octopus, lobster, shrimp, coconut crab, mangrove crab, smoked, dried or salted fish, canned sardines, canned tuna, and canned pilchards.

In the dietary questionnaire, for each food item, the study participants were asked to indicate the frequency of consumption (never or almost never, number of times per day, per week or per month) and the quantity of consumption (in portion or in a standard unit). The consumption of foods indicated by a portion (mainly marked A, B or C) was estimated with the help of the photo booklet from the E3N cohort validated by the EPIC (European Prospective Investigation into Cancer) study (139, 140, Figure 2-2 shows one page of this photo booklet), which indicating the weight corresponding to each portion according to the different food sub-groups, in order to facilitate the estimation of portion sizes. Information on the seasonality and weight of local fruits and vegetables was reviewed with a nutritionist from New Caledonia. Then, the daily consumption (in g/day) per person was calculated for each food item on multiplying the frequency by the quantity of the portion. Season factor was taken into account for certain seasonal foods as well, in converting the food daily consumption for the whole year into the food consumption only when it is in season (the food daily consumption for the whole year \times the proportion of the numbers of available consumption days in 365 days).

We used the FPFCT 2013 (presented above, see 1.3.1.3) to convert the traditional New Caledonian foods into daily dietary iodine intake because these local foods are closed to those of French Polynesian. For the other general food in the study, the French CIQUAL 2013 food composition table (124) was a necessary complement. For each food item in the diet questionnaire, iodine intake was estimated by multiplying the daily consumption of each food by the iodine content in this food based on these composition tables (if iodine content were measured from several similar foods in these food composition tables, the mean of the iodine content in this sub-group iodine content by summing the iodine content in this sub-group. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium for the standardization of the next pooled analysis (details see in the supplemental Table1).

We used the FAO Databases (presented above, see 1.3.1.1) to convert the daily food consumption into daily dietary energy intake (kcal/day). For each study participant, we

calculated the total energy intake corresponding to the energy sum of all foods consumed per day.



Figure 2-2 - Photo booklet to help fill out the dietary questionnaire. Example of standard portions of raw vegetables and standard beverages volumes

2.3.2 THE FRENCH POLYNESIA STUDY

As I demonstrated above, thyroid cancer incidence was high in French Polynesia, in particular among natives of this set of archipelago (141). However, up to date, there isn't enough data or qualified information to reveal whether this area is iodine deficiency. To investigate the reasons for this level of incidence and the potential role of atmospheric fallout from nuclear tests performed by France between 1966 and 1974, Cancer and Radiations group conducted between 2002 and 2005 a population-based case-control study among natives of French Polynesia in Tahiti Islands, Windward Islands, Leeward Islands, Marquesas Islands, Australes Islands, Tuamotu-Gambier Archipelago, including most of the thyroid cancer patients diagnosed between 1985 and 2003, who were aged 55 years or less and still alive. The study was approved by the French Polynesian Ethics Committee. Written informed consent was obtained from all participants so that we could contact their physician.

Previously, this study reported that a low level of education (85), tallness and a high body mass index (142), a family history of thyroid cancer (34), and, in women, a large number of pregnancies (85) were associated with an increased risk of thyroid cancer in this population. It also estimated the radiation doses received by the study subjects during the atmospheric

nuclear tests (143) and concluded that nuclear radiation fallout played a small but significant role in the high incidence of thyroid cancer in French Polynesia (94). Moreover, it showed that traditional Polynesian dietary pattern was associated with a lower thyroid cancer incidence than western pattern (144). French Polynesia is a mild iodine deficiency area in which a higher consumption of food from the sea and a higher dietary iodine intake were significantly associated with a decreased risk of thyroid cancer (105, 145). Lastly, among French Polynesian individuals with sufficient levels of fingernail selenium, there was lack of association between greater long-term exposure to selenium and thyroid cancer risk (112).

2.3.2.1 Background

French Polynesia is an overseas collectivity of the French Republic, which is located in the South Pacific Ocean, about 5,000 km from east of New Caledonia. It is administratively divided into 5 archipelagos: The Society Islands archipelago composed of the Windward Islands (Tahiti and Moorea) and the Leeward Islands (including Bora Bora), the Tuamotu Archipelago, the Gambier Islands, the Marquesas Islands and the Austral Islands. It consists of 118 geographically dispersed islands and atolls (67 are inhabited) stretching over an expanse of more than 2,000 kilometers. Its total land area is 4,167 square kilometres equivalent to that of Europe (4,032 km² of land scattered over a maritime area of 5 million km²) (Figure 2-3).



Figure 2-3 - Map of French Polynesia

The Polynesian islands are volcanic origin: the activity of hot spots situated under the oceanic plate gave birth to submarine volcanoes which grew up progressively and emerged to form tall islands that next evolved into Motus (islets) then into atolls (coral rings surrounding a lagoon).

Total population at the August 2012 census was 268,270 inhabitants (146). At the 2012 census, 68.5% of the population of French Polynesia lived on the island of Tahiti (146), which is located within the Society Islands, is the largest and most populous island and the seat of the capital of the collectivity, the urban area of Papeete, the capital city, has 133,627 inhabitants (2012 census). In 2016, the population was estimated at more than 285,000, with a steady population growth rate of 1.1% (147, 148). In 2015, the ethnic makeup of the population was estimated to be 78% native Polynesian, 12% Chinese, 6% French Polynesian (referred to in the source as "local French") and 4% French (referred to in the source as "metropolitan French").

2.3.2.2 Case Selection

All patients diagnosed with a differentiated thyroid cancer before the age of 56, born and living in French Polynesia, were eligible for the study. This cutoff age was chosen in order to include mainly subjects who were younger than 15 at the time of the atmospheric nuclear tests (between 1966 and 1974). Four patients aged from 56 to 62 years at the time of the diagnosis, one case diagnosed in 1979, and two cases diagnosed in 2004 were included erroneously and were kept in the analysis because their characteristics were close to the inclusion criteria and results were unchanged when these cases and their controls were excluded. The cases were identified from the cancer registry of French Polynesia and medical insurance files and by the four endocrinologists in Tahiti. Histological information was obtained from the two histopathology laboratories (one public and one private) in Tahiti and from the medical files of endocrinologists. The histological slides of 68 patients who had received I^{131} treatment at the Institute of Gustave Roussy were reviewed in the institute's pathology department, and the diagnoses were in agreement with those established in French Polynesia for 64 patients (94%). Of the 255 eligible differentiated thyroid cancer cases, 26 (10%) individuals were not interviewed because they had died (n=14), could not be located (n=6), refused to participate (n=5), or the person was too ill to be interviewed (n=1). Finally, the study population consisted of 229 cases (105).

2.3.2.3 Control Selection

For each eligible case, two potential controls closest in terms of the date of birth and matched on sex were randomly selected from the registry of births, which records all inhabitants born in French Polynesia. An SAS random number generator (SAS Institute, Inc., Cary, NC) was used for random selection. At the end of the interviews, a few cases had no interviewed controls. Each of those cases was matched with a control initially selected for another case of the same sex and with the same date of birth. Matching on the date of birth was then extended if necessary. Finally, of the 229 interviewed cases, 87 were matched with one control (38%) and 142 with two controls (62%). Of the 458 randomly selected controls, 85 (19%) were not interviewed because the subjects had died (n=9), could not be located (n=29), refused to participate (n=29), were too ill to be interviewed (n=2), or had left French Polynesia (n=16). Among interviewed controls, we had no information on dietary consumption for two controls: overall, 371 controls were considered in the present analysis (105).

2.3.2.4 Data Collection

The addresses of cases and controls were obtained from the territorial medical insurance plan, which covers all inhabitants, whatever their professional status. Interviews were conducted face-to-face by trained Polynesian interviewers and medical staff using a structured questionnaire. Cases diagnosed between 1979 and 2004 and matched controls answered the questionnaire between 2002 and 2004. Data collection included their ethnicity, education, smoking habits, lifetime weight changes, personal and family history of thyroid disease and cancer, places of residence, gynecologic and reproductive factors, medical X-ray exposure, and diet. For each subject, the total thyroid dose was calculated by summing the thyroid doses reconstructed for each nuclear test, taking into account age, source of drinking water, and data collected in the diet questionnaire (143).

2.3.2.5 Dietary Questionnaire

The dietary questionnaire was composed of two parts with a booklet of photographs from the E3N cohort validated by the EPIC study, in order to facilitate the estimation of portion sizes. The two parts included questions on the consumption (frequency and quantity) of seven food groups and one question on dietary habit: the first part concerned consumption at the time of the interview and the second part concerned consumption during childhood. Overall, the questionnaire provided estimation for daily consumption of 66 food items, 5 of which were

food from the sea including pelagic fish, lagoon fish, fafaru, and shellfish (giant clams, other shellfish) (105).

In the dietary questionnaire, for each food item, the study participants were asked to indicate the frequency of consumption (never or almost never, number of times per day, per week or per month) and the quantity of consumption (in portion or in a standard unit). The consumption of foods indicated by a portion (mainly marked A, B or C) was estimated with the help of the photo booklet from the E3N cohort validated by the EPIC study (139), which indicating the weight corresponding to each portion according to the different food subgroups, in order to facilitate the estimation of portion sizes. Food consumption in a standard unit (for example, number of yoghurt), was estimated from the data of a dietary survey (149), conducted by the French Polynesian Health Directorate in 1995, indicating the average weight of local foods such as fe'i (cooking banana, typical in French Polynesia) and uru (fruit of the breadfruit tree). Then, the daily consumption (in g/day) per person was calculated for each food item on multiplying the frequency by the quantity of the portion. Season factor was taken into account for certain seasonal foods as well, in converting the food daily consumption for the whole year into the food consumption only when it is in season (the food daily consumption for the whole year \times the proportion of the numbers of available consumption days in 365 days).

We used the FPFCT 2013 (presented above, see 1.3.1.3) to convert the traditional French Polynesian foods into dietary iodine. For the other general food in the study, the French CIQUAL 2013 food composition table (124) was a necessary complement. The overall iodine intake took into account all the foods of questionnaire, except iodized salt, because we had no precise information on the amount of iodized salt consumption. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium for the standardization of the next pooled analysis (details see in the supplemental Table1).

We used the FAO Databases (presented above, see 1.3.1.1) to convert the daily food consumption into daily dietary energy intake (kcal/day). For each study participant, we calculated the total energy intake corresponding to the energy sum of all foods consumed per day.

2.3.3 THE YOUNG-THYR STUDY

After the Chernobyl nuclear power plant accident in April 1986, an increased incidence of DTC was observed in children living in contaminated areas of Belarus, Ukraine, and the Russian Federation (150, 151). Although the radioactive fallout was much less severe and the thyroid radiation dose was much lower in France than in the previously mentioned contaminated areas, a case-control study was performed to investigate the role of the fallout and of other potential risk factors in the incidence of DTC in young people who lived through that period in eastern France.

The study was approved by the French Data Protection Authority Commission Nationale de l'Informatique et des Libertés (agreement 05-1120; April 5, 2005). Written consent forms were obtained from all participants.

Each eligible subject who agreed to participate in the study received an information notice about the study's aims and a consent form to be signed and returned. Once consent forms were received, a database of eligible subjects was compiled so that controls could be selected randomly according to the selection criteria, with at least one control per case.

Previously, this study reported that a higher number of pregnancies, used a lactation suppressant, or had early menarche were associated with a higher DTC risk; conversely, breastfeeding, oral contraceptive use, and late age at first pregnancy were associated with a lower DTC risk. No association was observed between thyroid cancer and having irregular menstrual cycle, undergoing treatment for menstrual cycle regularity shortly after menarche, having a cessation of menstruation, use of another contraceptive, history of miscarriage or abortion for the first pregnancy, or having had gestational diabetes. The results support the fact that exposure to estrogens increases thyroid cancer risk (80). The risk of PTC was higher for participants with a high body surface area (BSA), great height, or excess weight and for women with a high body fat percentage. Conversely, no significant association was found between body mass index and the PTC risk. These results provide further evidence of the role of BSA and excess weight in the risk of thyroid cancer (152). In the pooled analysis of two case-control studies including the present Young-Thyr study focusing on the relationship between recreational physical activity and DTC risk, results showed DTC risk was slightly reduced among subjects who reported recreational physical activity. The weekly frequency (i.e. h/week) seems to be more relevant than the duration (years) (153). Furthermore, a recent paper based on this case-control study discussed about whether dietary reports are biased by risk perception of Chernobyl fallouts, the results shows us that leafy vegetable consumption during the two months after the Chernobyl accident was associated with belief among cases, but not among controls or among all subjects, leafy vegetable consumption had an association with subject status (case or control) but not with beliefs; conversely, fresh dairy products consumption was not correlated to the status or beliefs of subjects (154).

2.3.3.1 Background

France is a country with territory in western Europe and several overseas regions and territories (French Guiana is located in South America; Guadeloupe and Martinique are in the Caribbean; and Réunion and Mayotte are in the Indian Ocean, off the coast of Africa. All five are considered integral parts of the republic. France also comprises Saint Pierre and Miquelon in North America; Saint Barthélemy and Saint Martin in the Caribbean; French Polynesia, New Caledonia, Wallis and Futuna and Clipperton Island in the Pacific Ocean; and finally the French Southern and Antarctic Lands.). The metropolitan area of France extends from the Mediterranean Sea to the English Channel and the North Sea, and from the Rhine to the Atlantic Ocean. Overseas France include French Guiana on the South American continent and several island territories in the Atlantic, Pacific and Indian oceans (Figure 2-4). France spans 643,801 square kilometres (155) and had a total population of almost 67 million people as of January 2017.



Figure 2-4 - Map of constituent lands of the French Republic

2.3.3.2 Case Selection

All patients who were diagnosed with DTC between January 1, 2002, and December 31, 2006, were born after January 1, 1971, were less than 35 years of age, and had their main residence in one of the regions of eastern France (Alsace, Champagne-Ardennes, Corse, Franche-Comté, Lorraine, Rhône-Alpes, or Provence-Alpes-Côte d'Azur) were eligible for this study. Incident cases were identified by thoroughly investigating 3 main sources according to the region: 1) the General Cancer Incidence Registry in Champagne-Ardennes, Alsace, and Rhône-Alpes; 2) the National Childhood Cancer Registry (which contains information on children <15 years of age) in all regions; and 3) private and public hospitals in Lorraine, Franche-Comté, Corse, and Provence-Alpes-Côte d'Azur. In these last 4 regions, which are not covered by the General Cancer Incidence Registry, the identification of cancer cases was performed in collaboration with local public health structures. In this way, collaboration was set up with pathologist networks, specifically with one in the Provence-Alpes-Côte d'Azur region, for which the exhaustiveness and the quality of database has been assessed in previous works (156, 157).

All cases of DTC were histologically confirmed. Information on histopathological diagnosis, the size of the largest cancerous nodule, tumor extension beyond the thyroid capsule, and multifocality were obtained from either the registries or histopathology laboratories. All DTC cases were eligible, regardless of tumor size. Mixed papillary follicular cancers were considered as papillary lesions.

Of the 1,049 eligible persons with DTC, 223 (21%) were not interviewed, because they refused to participate (n=120), could not be contacted (n=95), were excluded for another raison (n=6), or had died (n=2). Of the 826 cases who were interviewed, 70 were matched with 2 controls; the others were matched to 1 control. One case who did not live in the region in which he was interviewed and 20 cases who were not matched to a control at the end of this process were excluded. In all, the study consisted of 805 cases, 633 of whom were female.

2.3.3.3 Control Selection

Controls were selected from the general population and individually matched to a single case who was the same sex and had the same date of birth (plus or minus 1 year) and region of residence in the year that the case was diagnosed with cancer. Potentially eligible controls were randomly selected in each region from the landline telephone subscriber directory. This was achieved using an SAS random number generator (SAS Institute, Inc., Cary, North Carolina) and a file from France Telecom containing all landline phone numbers (approximately 100 million) in France. Because a large proportion of the population in the targeted age group (10-40 years old at time of interview) only has mobile phones and because a number of people are not included in the directory, controls included not just those selected from the directory but also relatives of those people. Each established contact was asked whether they knew people who fit the selection criteria (year of birth, sex, and region of residence) and whether they could communicate the phone contact details of these potentially eligible controls. This step was done parallel to but independently of the case selection and interview process. This was possible because we used national incidence data to anticipate the age distribution of the cases and therefore the age distribution of the controls required in each region. Only people without DTC were eligible to serve as controls. At the time of the first phone contact, controls were asked about their lack of thyroid cancer, but they did not undergo a medical examination (80).

Of the 1,045 contacted controls, 164 (16%) were refused to be interviewed. Of the 881 controls who were interviewed, 5 were not matched to a case at the end of this process and were excluded. In all, the study consisted of 876 controls, 679 of whom were female. The median difference in the date of birth between each case and his or her control was 103 (standard deviation, 282) days.

2.3.3.4 Data Collection

Between July 2005 and October 2010, cases and controls were interviewed face to face by a trained interviewer using a structured questionnaire that was sent to the subjects at least one week before the interview so that they could gather documents (e.g., medical examination and radiography results) and ask their parents questions relating to their youth. Interviews were conducted mostly at each subject's place of residence. The same interviewer systematically interviewed all subjects in the same strata (same sex, same age at the reference year: the year that the case was diagnosed with cancer and same residence region).

The questionnaire included items on ethnicity, lifetime height-weight changes, specific questions during the period (26/04/1986-30/06/1986) when the Chernobyl accident took place, smoking habit, physical activities, personal history of thyroid disease, family history of thyroid disease and cancer, places of residence, education and occupation, gynecologic and

reproductive factors, medical x-ray exposure, diet, both at the time of the interview and during childhood, and perception of the risk due to the Chernobyl accident.

2.3.3.5 Dietary Questionnaire

The dietary questionnaire was composed of two parts. The first part included questions on the consumption (frequency and quantity) of 12 food sub-groups and 6 questions on dietary habit concerned the food consumption one year before the time of the interview, the questionnaire provided estimation for daily consumption of 73 food items, 8 of which were fish or shellfish including open-sea fish, river or lake fish, canned sardines, canned tuna, frozen fish, oysters or mussels, lobster or crab, and shrimp; and the second part included questions on the consumption (frequency and quantity) of 5 food sub-groups and 2 questions on dietary habit concerned the food consumption during childhood of the study participant when the Chernobyl accident took place (26/04/1986-30/06/1986).

In the dietary questionnaire, for each food item, the study participants were asked to indicate the frequency of consumption (never or almost never, number of times per day, per week or per month) and the quantity of consumption (in portion or in a standard unit). The consumption of foods indicated by a portion (mainly marked A, B or C) was estimated with the help of the photo booklet from the E3N cohort validated by the EPIC study (139), which indicating the weight corresponding to each portion according to the different food subgroups, in order to facilitate the estimation of portion sizes. For food consumption in a standard unit (for example, number of yoghurt), the estimation of the unit was from the average weight of the local food in several food markets or super markets. Then, the daily consumption (in g/day) per person was calculated for each food item on multiplying the frequency by the quantity of the portion. Season factor was taken into account for certain seasonal foods as well, in converting the food daily consumption for the whole year × the proportion of the numbers of available consumption days in 365 days).

We used the French CIQUAL 2013 food composition table (124) to convert the daily foods consumption into daily dietary iodine intake. For each food item in the diet questionnaire, iodine intake was estimated by multiplying the daily consumption of each food by the iodine content in this food based on the French CIQUAL 2013 food composition table (if iodine content were measured from several similar foods in the composition table, the mean of the

iodine content in these similar foods was taken), sub-group iodine content by summing the iodine content in each food item in this sub-group. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium for the standardization of the next pooled analysis (details see in the supplemental Table1).

We also used the French CIQUAL 2013 food composition table (124) to convert the daily food consumption into daily dietary energy intake (kcal/day) for this French study. For each study participant, we calculated the total energy intake corresponding to the energy sum of all foods consumed per day.

2.3.4 THE CATHY STUDY

The Cathy study is a population-based case–control study conducted in metropolitan France, including three French administrative areas (départements): Marne, Ardennes and Calvados, covered by a cancer registry. All participants signed written consent. The study has focused special interest on the role of some environmental factors such as pesticides, dioxin, xeno-estrogens, in DTC risk.

Up to date, Tcheandjieu et al. (134) selected Europeans ethnicity of this study and pooled them with the people auto-declaring as having European ancestry from the New Caledonia study in order to detect novel candidate functional SNPs in Europeans from metropolitan France and Melanesians from New Caledonia by Fine-mapping of two differentiated thyroid carcinoma susceptibility loci at 9q22.33 and 14q13.3, the principal results were that the association with the four SNPs previously identified in GWAS (rs965513, rs1867277 at loci 9q22, and rs944289, rs116909734 at loci 14q13) was replicated in Europeans. Among Europeans, the two SNPs previously reported at 9q22 were not independently associated to DTC and that rs965513 was the predominant signal; at 14q13, the haplotype rs944289[C]-rs116909374[C]-rs999460[T] was significantly associated with DTC risk and that the association with rs116909374 differed by smoking status.

2.3.4.1 Case Selection

Eligible cases were all patients diagnosed with DTC between 01/01/2002 and 31/12/2007 residing in one of the three areas mentioned above, aged 25 years and over at the time of diagnosis.

The cases were identified by the general cancers register of Calvados and the specialized thyroid cancers register of Marne-Ardennes. Topography and morphology were coded according to the third version of the International Classification of Diseases for Oncology (ICD-O-3). Thyroid cancers were classified according to the characteristics (size, histology) of the largest cancer nodule.

Since the study had begun in 2005, the cases diagnosed between 2002 to 2004 were identified retrospectively. The cases diagnosed from 2005 to 2007 were identified prospectively.

Among 853 eligible cases, 232 (27%) could not be interviewed: 148 refused to participate, 29 died at the time of the interview, 52 could not be contacted, and 3 were too sick to respond. Finally, the study involved 621 cases.

2.3.4.2 Control Selection

Controls were randomly selected by the survey institute CSA (Conseil-Sondage-Analyse) and who were at least 25 years old, residing in the same area (Calvados, Marne or Ardennes) at the time of cases' diagnoses. The random draw was made from telephone directories, based on telephone numbers incremented by one unit in order to be able to contact the people on the red list as well. A first telephone contact was used to determine whether one of the members of the household met the inclusion criteria of the study. If applicable, the person was proposed to participate in the study by receiving an interviewer at home.

Controls were frequency-matched to cases by sex and 5-year age groups. The randomly selected controls were stratified on the socio-professional category so that the distribution of socio-professional categories among the controls was representative of that of their residence departments.

Therewith, a reference year between 2002 and 2007 was also allocated to the controls. Only exposures that occurred before the reference year were included in the analyses.

Among 943 eligible controls, 236 (25%) could not be interviewed: 232 refused to participate and 4 could not be contacted. Finally, the study involved 707 controls.

2.3.4.3 Data Collection

Data from the Cathy study were collected using standardized questionnaires by face-to-face interviews at home, conducted by interviewers specially trained for the study. The collected

data included the information about socio-demographic, medical history (personal and family medical history), characteristics of hormonal and reproductive life, anthropometric characteristics, lifestyle (physical activity, tobacco and alcohol consumptions), recreation, occupation, and residential history.

2.3.4.4 Dietary Questionnaire

The dietary questionnaire included two questions on the consumption (frequency and quantity) of 15 sub-groups and 5 questions on dietary habit before or at the time of the interview. Overall, the questionnaire provided estimation for daily consumption of 161 food items, 15 of which were fish or shellfish including open-sea fish, river or lake fish, breaded fish, natural or oiled canned tuna, natural or oiled canned sardines or anchovies, natural or oiled canned mackerel, mussels, oysters, other shells (whelks, periwinkles, clams...), shrimp, crab, and lobster.

In the dietary questionnaire, for each food item, the study participants were asked to indicate the frequency of consumption (never or almost never, number of times per day, per week or per month) and the quantity of consumption (in portion or in a standard unit). The consumption of foods indicated by a portion (mainly marked A, B or C) was estimated with the help of the photo booklet from the E3N cohort validated by the EPIC study (139), which indicating the weight corresponding to each portion according to the different food subgroups, in order to facilitate the estimation of portion sizes. For food consumption in a standard unit (for example, number of yoghurt), the estimation of the unit was from the average weight of the local food in several food markets or super markets. Then, the daily consumption (in g/day) per person was calculated for each food item on multiplying the frequency by the quantity of the portion. Season factor was taken into account for certain seasonal foods as well, in converting the food daily consumption for the whole year × the proportion only when it is in season (the food daily consumption for the whole year × the proportion of the numbers of available consumption days in 365 days).

We used the French CIQUAL 2013 food composition table (124) to convert the daily foods consumption into daily dietary iodine intake. For each food item in the diet questionnaire, iodine intake was estimated by multiplying the daily consumption of each food by the iodine content in this food based on the French CIQUAL 2013 food composition table (if iodine content were measured from several similar foods in the composition table, the mean of the

iodine content in these similar foods was taken), sub-group iodine content by summing the iodine content in each food item in this sub-group. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium for the standardization of the next pooled analysis (details see in the supplemental Table1).

We also used the French CIQUAL 2013 food composition table (124) to convert the daily food consumption into daily dietary energy intake (kcal/day) for this French study. For each study participant, we calculated the total energy intake corresponding to the energy sum of all foods consumed per day.

2.3.5 THE CUBA STUDY

Cuba is an island of 100,000 km² where 35% of the population belongs to the African ethnic group (158). Although thyroid cancer is the sixth most common female cancer in Cuba, its incidence is quite low. Indeed, while in North America the world age-standardized rate (ASR) of DTC incidence was 10.0/100,000 in white females during the period 1998–2002 (159), the ASR of DTC incidence in Cuba was around 4.1/100,000 for females and was even six-fold less frequent in males in 2009 (160). For other Latin American countries such as Colombia, the ASR of DTC incidence was 9.4/100,000 in females during the period 1998–2002 (159). Why is DTC incidence low in Cuba? Is this situation related to dietary iodine intake or other dietary factors?

Traditional Cuban food includes staple food such as potato, taro, yam, vegetable banana, rice, and spaghetti; meat such as chicken, pork, beef, and ham; eggs; vegetables such as watercress, lettuce, tomato, red bean, and cabbage; fruits such as guava, mango, papaya, and banana; and fish or seafood such as sardine, tuna (canned or fresh), lobster, crab, shrimp, and shellfish. However, it was not clear whether dietary factors contribute to thyroid cancer risk in the Cuban population. Moreover, no clear explanation for the low incidence of DTC in the Cuban population has been established.

In order to establish the role of environmental and lifestyle factors and to relate some common SNPs previously associated with the risk of developing DTC in Cuba, between 2009 and 2011, we established a case-control study in Havana and its surrounding area. This study was revised and approved by the Clinical Research Ethics Committee of the National Institute

of Oncology and Radiobiology (INOR), Havana, Cuba. Informed written consent was obtained from all of the participants in the study.

Previously, this study reported that non-African ethnicity, non-smokers, parity, and high body mass index, being rhesus factor-positive, having a personal history of benign thyroid disorder, agricultural occupation, and consumption of artesian well water significantly associated with an increased risk of developing DTC (161). It also found that the thyroid volume was higher in current or former smokers and in persons with AB or B blood type (162). Significant association with DTC risk was found for rs1867277 in the promoter region and the polyalanine tract expansion polymorphism rs71369530 of the FOXE1 gene; Women who had 2 or more pregnancies had a 3.5-fold increase in risk of DTC if they carried the A allele of the SNP rs18015616 in the ATM gene, while 0.8 in those who had fewer than 2 (48). This study was involved in a pooled analysis of two case-control studies focusing on the relationship between recreational physical activity and DTC risk, results showed DTC risk was slightly reduced among subjects who reported recreational physical activity. The weekly frequency (i.e. h/week) seems to be more relevant than the duration (years) (153). Furthermore, it observed DTC risk was significantly reduced with increasing fish consumption, but no association with total dietary iodine intake. Nevertheless, DTC risk was significantly associated with the number of copies in the minor allele (A) for SNP rs965513 near FOXE1 among people who consumed less iodine than the median value (113).

2.3.5.1 Background

Cuba is a country comprising the island of Cuba as well as Isla de la Juventud and several minor archipelagos. It is located in the northern Caribbean where the Caribbean Sea, the Gulf of Mexico, and the Atlantic Ocean meet. Cuba is the largest island in the Caribbean, with an area of 109,884 square kilometres, and the second-most populous after Hispaniola, with over 11 million inhabitants (163, Figure 2-5).

Prior to Spanish colonization in the late 15th century, Cuba was inhabited by Amerindian tribes. It remained a colony of Spain until the Spanish-American War of 1898, which led to nominal independence as a de facto United States protectorate in 1902. Then it was under the dictatorship of Fulgencio Batista in 1952. Further unrest and instability led to Batista's ousting in January 1959 by the July 26 Movement, which afterwards established a government under the leadership of Fidel Castro. A point of contention during the Cold War between the Soviet

Union and the United States, a nuclear war nearly broke out during the Cuban Missile Crisis of 1962 (164).

Culturally, Cuba is considered part of Latin America (165). It is a multiethnic country whose people, culture and customs derive from diverse origins, including the aboriginal Taíno and Ciboney peoples, the long period of Spanish colonialism, the introduction of African slaves, and a close relationship with the Soviet Union in the Cold War (164).



Figure 2-5 - Map of Cuba

Cuba is one of the world's last planned economies and its economy is dominated by the exports of sugar, tobacco, coffee and skilled labor. According to the Human Development Index, Cuba is described as a country with high human development and is ranked the eighth highest in North America (166). It also ranks highly in some metrics of national performance, including health care and education (167, 168).

2.3.5.2 Case Selection

Patients aged between 17 and 60 years who were living in Havana and its surrounding municipality of Jaruco (30 km from Havana), and were treated for DTC between 2000 and

2011 at Cuban National Institute of Oncology and Radiobiology (INOR) and at the Institute of Endocrinology, were eligible for the study. Potential cases were selected from the National Cancer Registry databases (C73 ICD-10) and were cross-referenced with the INOR Pathology Register. On average, around 25–30 patients who fit these criteria were identified for each year of the study period. In order to achieve the estimated sample size, the selection was extended to include incident cases (161).

Of the 240 eligible DTC cases, 37 (15%) individuals were not interviewed because they could not be located (n=32) or refused to participate (n=5). The final study population consisted of 203 cases. 37% of the cases were interviewed just after their cancers diagnoses (either 0 or 1 year of the time lag between diagnosis and interview), while 19% of the cases had a lag time >5 year.

2.3.5.3 Control Selection

Controls (229) were randomly selected from the general population living in Havana city and its surrounding areas municipality of Jaruco using consultation files from primary care units (family doctors). They were frequency-matched with cases by sex and age at cancer diagnoses of the cases (± 5 years). Of the 229 potential controls, 17 refused and 212 agreed to be interviewed.

2.3.5.4 Data Collection

Both cases and controls were interviewed face-to-face by trained professionals (nursing and medical staff) using a structured questionnaire between January 2009 and December 2011. A standardized questionnaire was used to collect data on demographic characteristics (age, sex, place of residence, and occupation), blood group, rhesus factor status, anthropometric parameters, reproductive and hormonal history, lifestyle (smoking habits, dietary habits), exposure to radiation or chemicals, personal medical history and family medical history in first-degree relatives. Size, blood group and rhesus factor collected by interviewers were compared to those in the national identity card and in medical records for cases.

Interviewers were trained to collect information related to one year before diagnosis for cases and one year before interview for controls. The majority of interviews were conducted in the presence of a parent or relative. Some were conducted at the workplace in the presence of a general practitioner.

2.3.5.5 Dietary Questionnaire

The dietary questionnaire was composed of two parts which included questions on the consumption (frequency and quantity): the first part concerned general food consumption of 10 sub-groups and 5 questions on dietary habit, and the second part concerned the traditional Cuban food consumption including 2 sub-groups, one year before the time of the interview. Overall, the questionnaire provided estimation for daily consumption of 87 food items, 8 of which were fish or shellfish including open-sea fish, river or lake fish, canned sardines, canned tuna, frozen fish, oysters or mussels, lobster or crab, and shrimp.

In the dietary questionnaire, for each food item, the study participants were asked to indicate the frequency of consumption (never or almost never, number of times per day, per week or per month) and the quantity of consumption (in portion or in a standard unit). The consumption of foods indicated by a portion (mainly marked A, B or C) was estimated with the help of the photo booklet from the E3N cohort validated by the EPIC study (139), which indicating the weight corresponding to each portion according to the different food subgroups, in order to facilitate the estimation of portion sizes. For food consumption in a standard unit (for example, number of yoghurt), the estimation of the unit was from the average weight of the local food in several food markets or super markets. Then, the daily consumption (in g/day) per person was calculated for each food item on multiplying the frequency by the quantity of the portion. Season factor was taken into account for certain seasonal foods as well, in converting the food daily consumption for the whole year × the proportion of the numbers of available consumption days in 365 days).

We used the CFCT 2014 (presented above, see1.3.1.3) to convert the 20 traditional Cuban foods into dietary iodine. For the other general food in the study, the French CIQUAL 2013 food composition table (124) was a necessary complement. For each food item in the diet questionnaire, iodine intake was estimated by multiplying the daily consumption of each food by the iodine content in this food based on the two food composition tables mentioned above (if iodine content were measured from several similar foods in the composition tables, the mean of the iodine content in these similar foods was taken), sub-group iodine content by summing the iodine content in each food item in this sub-group.

An item for quantifying iodized salt intake in the questionnaire was only included in this study. In fact, since 2005, Cuba has established compulsory iodization of salt for human

consumption for the whole Cuban population, because a global strategy at the national level is the best solution for a cost-effective prevention and control of iodine deficiency (169). A fixed value of 10 g of iodized salt per person per day was included. This value was estimated to be the average consumption of iodized salt per inhabitant per day by the Center for Nutrition and Food Hygiene, the National Institute of Hygiene, Epidemiology, and Microbiology (Inhem) of Cuba. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium for the standardization of the next pooled analysis (details see in the supplemental Table1).

We used the FAO Databases (presented above, see 1.3.1.1) to convert the daily food consumption into daily dietary energy intake (kcal/day). For each study participant, we calculated the total energy intake corresponding to the energy sum of all foods consumed per day.

2.3.6 THE HOMOGENEITY AND THE HETEROGENEITY AMONG THE 5 STUDIES

The 5 studies conducted homogeneously in contrasting populations, both genetically and in terms of the environment and life style, using similar questionnaire and DNA collection, study participants were interviewed face-to-face by trained professionals, using the standardized dietary questionnaire and the photo booklet from Epic cohort.

The five different studies of Epi-Thyr consortium are heterogeneous in terms of their size, of the period of diagnosis, of age at diagnosis and of proportion of papillary thyroid cancer. The proportion of papillary thyroid cancer in the French Polynesia study was lower than in the other studies, probably because this study included cases diagnosed much earlier (1979-2004) than the other ones. It has also to be noted that the difference in age at diagnosis between studies not only reflect difference in age structure of studied populations and in thyroid cancer incidence, but also restriction in age at diagnosis imposed in the design of studies: no more than 15 years at time of the first atmospheric test, in French Polynesia, i.e. born in 1950 or after, and no more than 15 years at time of Chernobyl accident in the Young-Thyr study, i.e. born in 1971 or after (Table 2-1).

Table 2-1 - Characteristics of the Studied 5 Case-Control Studies from Epi-Thyr Consortium

	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
	(744)	(600)	(1681)	(1293)	(415)
Cases / Controls, N (%)	332 (44.6)/412 (55.4)	229 (38.2)/371 (61.8)	805 (47.9)/876 (52.1)	593 (45.9)/700 (54.1)	203 (48.9)/212 (51.1)
Women, cases / controls N (%)	293 (88.3)/354 (85.9)	203 (88.7)/324 (87.3)	633 (78.6)/679 (77.5)	463 (78.1)/501 (71.6)	179 (88.2)/173 (81.6)
Incidence (10^5 person-years): W / M	35.8 / 8.3	24.0 / 6.8	12.6 /	4.1	2.4 / 1.0
Globocan 2012					
Year of diagnosis (Min, Max)	1993-1999	1979-2004	2002-2006	2002-2007	2000-2011
Period of study enrollment	1998-2002	2002-2004	2005-2010	2005-2007	2009-2012
Age at diagnosis (Year),	49 (17, 80)	38 (10, 62)	27 (9, 35)	51 (24, 83)	38 (17, 60)
Mean (Min, Max)					
Identification of cases	The two histopathology	The cancer registry of	Three main sources	The general cancers	The National Cancer
	laboratories, one public	French Polynesia and	according to the region: 1)	register of Calvados and	Registry databases (C73
	and one private, operating	medical insurance files	the General Cancer	the specialized thyroid	ICD-10) and were cross-
	in New Caledonia.	and by the four	Incidence Registry in	cancers register of Marne-	referenced with the INOR
		endocrinologists in	Champagne-Ardennes,	Ardennes	Pathology Register
		Tahiti. Histological	Alsace, and Rhône-Alpes;		
		information was obtained	2) the National Childhood		
		from the two	Cancer Registry (which		
		histopathology	contains information on		
		laboratories (one public	children <15 years of age)		
		and one private) in Tahiti	in all regions; and 3) private		
		and from the medical	and public hospitals in		
		files of endocrinologists	Lorraine, Franche-Comté,		
			Corse, and Provence-Alpes-		53
			Côte d'Azur		
	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
-------------------	-----------------------------	---------------------------	-------------------------------	--------------------------	---------------------------
	(744)	(600)	(1681)	(1293)	(415)
Case eligibility	All patients with papillary	All patients diagnosed	All patients who were	All patients diagnosed	Patients aged between 17
	or follicular thyroid	with a differentiated	diagnosed with DTC and	with DTC and were	and 60 years who were
	cancer and having resided	thyroid cancer and born	who were born after	residing in one of the	living in Havana and its
	in New Caledonia for at	after January 1, 1950,	January 1, 1971, were less	three areas of	surrounding municipality
	least 5 years at the time	born and living in French	than 35 years of age, and	metropolitan France	of Jaruco (30 km from
	of cancer diagnosis	Polynesia, were eligible	had their main residence in	(Marne, Ardennes and	Havana), and were treated
		for the study	one of the regions of eastern	Calvados), aged 25 years	for DTC at Cuban
			France (Alsace,	and over at the time of	National Institute of
			Champagne-Ardennes,	diagnosis	Oncology and
			Corse, Franche-Comté,		Radiobiology (INOR) and
			Lorraine, Rhône-Alpes, or		at the Institute of
			Provence-Alpes-Côte		Endocrinology
			d'Azur)		
Eligible cases, N	369	255	1049	853	240
Refusals	10	5	120	148	5
Deceased	21	14	2	29	
Too sick to	1	1		3	
response					
Impossible to	5	6	95	52	32
reach					
Excluded			6		
Not matched with			21		
Controls					
No information on				28	

	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
	(744)	(600)	(1681)	(1293)	(415)
dietary					
consumption					
Participants	332	229	805	593	203
Papillary histology, N (%)	288 (86.8)	177 (77.3)	720 (89.4)	526 (88.7)	189 (93.1)
Type of control		General po	pulation without any personal I	DTC history	
Control selection	Randomly selected from	Randomly selected from	Randomly selected in each	Randomly selected from	Randomly selected using
	updated electoral rolls of	the registry of births of	region from the landline	telephone directories by	consultation files from
	New Caledonia	French Polynesia	telephone subscriber	the survey institute CSA	primary care units (family
			directory and a file from	(Conseil-Sondage-	doctors)
			France Telecom containing	Analyse) and who were at	
			all landline phone numbers	least 25 years old at the	
			(approximately 100 million)	time of cases' diagnosis	
			in France		
Matching criteria	Sex and 5-year age group	Sex and date of birth	Sex, date of birth (plus or	Sex, 5-year age groups	Sex and age at cancer
			minus 1 year), and region of	and residing area at the	diagnoses of the cases (± 5
			residence in the year that	time of cases' diagnoses	years)
			the case was diagnosed with		
			cancer		
Type of matching	Frequency	Individual	Individual	Frequency	Frequency
Eligible controls, N	473	458	1045	943	229
Refusals	21	29	164	232	17
Deceased	13	9			
Too sick to	2	2			
response					

	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
	(744)	(600)	(1681)	(1293)	(415)
Impossible to	24	29		4	
Reach					
No information on		2		7	
Dietary					
Consumption					
Left study		16			
launched area					
Not matched with			5		
cases					
Participants	412	371	876	700	212
Type of interviews			Face-to-face		
Data collection	Socio-demographic	Ethnicity, education,	Ethnicity, lifetime height-	Socio-demographic	Demographic
	characteristics, diet,	smoking habits, lifetime	weight changes, specific	characteristics, medical	characteristics (age, sex,
	alcohol drinking, tobacco	weight changes, personal	questions during the period	history (personal and	place of residence, and
	smoking, anthropometric	and family history of	(26/04/1986-30/06/1986)	family medical history),	occupation), blood group,
	factors, gynecologic and	thyroid disease and	when the Chernobyl	characteristics of	rhesus factor status,
	reproductive history,	cancer, places of	accident took place,	hormonal and	anthropometric
	medical conditions and	residence, gynecologic	smoking habit, physical	reproductive life,	parameters, reproductive
	medical x-ray exposure,	and reproductive factors,	activities, personal history	anthropometric	and hormonal history,
	occupational and	medical X-ray exposure,	of thyroid disease, family	characteristics, lifestyle	lifestyle (smoking habits,
	residential history,	diet, and the total	history of thyroid disease	(physical activity, tobacco	dietary habits), exposure
	personal history of thyroid	thyroid irradiation doses	and cancer, places of	and alcohol	to radiation or chemicals,
	disease and familial		residence, education and	consumptions),	personal medical history
	history of thyroid cancer		occupation, gynecologic	recreation, occupation,	and family medical history

	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
	(744)	(600)	(1681)	(1293)	(415)
			and reproductive factors,	and residential history	in first-degree relatives
			medical x-ray exposure,		
			diet, both at the time of the		
			interview and during		
			childhood, and perception		
			of the risk due to the		
			Chernobyl accident		
Dietary questionnaire	- 11 food sub-groups and	- 7 food sub-groups and	- 12 food sub-groups and 6	- 15 food sub-groups and	- 10 general food sub-
	5 questions on dietary	1 question on dietary	questions on dietary habit	5 questions on dietary	groups, 5 questions on
	habit, including 122 food	habit concerned both	concerned the consumption	habit, including 161 food	dietary habit and 2 sub-
	items, 17 of which were	consumption at the time	one year before the time of	items, 15 of which were	groups of the traditional
	fish or shellfish.	of the interview and	the interview, including 73	fish or shellfish.	Cuban food consumption
	- Portion sizes estimation:	during childhood,	food items, 8 of which were	- Portion sizes estimation:	one year before the time
	the photo booklet from the	including 66 food items,	fish or shellfish; and 5 food	the photo booklet from	of the interview, including
	E3N cohort.	5 of which were fish or	sub-groups and 2 questions	the E3N cohort.	87 food items, 8 of which
	- Used food composition	shellfish.	on dietary habit concerned	- Used food composition	were fish or shellfish.
	tables:	- Portion sizes	the consumption during	table:	- Portion sizes estimation:
	the FPFCT 2013 (daily	estimation: the photo	childhood of the study	the French CIQUAL 2013	the photo booklet from the
	dietary iodine intake), the	booklet from the E3N	participant when the	(daily dietary iodine and	E3N cohort.
	French CIQUAL 2013	cohort.	Chernobyl accident took	energy intake).	- Used food composition
	(daily dietary iodine	- Used food composition	place (26/04/1986-		tables:
	intake); the FAO	tables:	30/06/1986).		the CFCT 2014 for the 20
	Databases (daily dietary	the FPFCT 2013 (daily	- Portion sizes estimation:		traditional Cuban foods
	energy intake).	dietary iodine intake),	the photo booklet from the		(daily dietary iodine

New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba
(744)	(600)	(1681)	(1293)	(415)
	the French CIQUAL	E3N cohort.		intake), the French
	2013 (daily dietary	- Used food composition		CIQUAL 2013 for the
	iodine intake); the FAO	table:		other general food in the
	Databases (daily dietary	the French CIQUAL 2013		study (daily dietary iodine
	energy intake).	(daily dietary iodine and		intake); the FAO
		energy intake).		Databases (daily dietary
				energy intake).

2.4 GENETIC ANALYSES

Both NKX2-1 (NK2 homeobox 1, also called TTF1 for Thyroid Transcription Factor 1) and FOXE1 (Forkhead factor E1, also called TTF2 for Thyroid Transcription Factor 2) encode thyroid-specific transcription factors. They play a crucial role in the development of the thyroid gland and their expression is modified in thyroid tumors (170, 171, 172, 173). In 2009, a GWAS reported the contribution of two SNPs nearby NKX2-1 and FOXE1 to the risk of developing DTC in Icelandic and European populations (42). The first SNP, rs944289, is located 337-kb upstream of the NKX2-1 gene and was described to increase the risk of DTC (42, 43). The second SNP, rs965513, is located in an intergenic region, 57-kb upstream of the FOXE1 gene. It has been related to many endocrine and metabolic disorders (174), to changes in the concentration of thyroid hormones (42), and to an increased risk of DTC (42, 43, 45). Rs965513 has been reported as a strong genetic factor for both sporadic and radioinduced PTC (44). Located within the 5' untranslated region (UTR) of FOXE1, the SNP rs1867277 has been reported as a risk factor for DTC and has been suggested as the causal SNP (45, 46). It has been also proposed that the minor allele A could modulate a transcriptional regulation pathway of the FOXE1 gene, by recruiting USF1/USF2 transcription factors (47). The ATM (Ataxia-Telangiectasia Mutated) gene is involved in the repair of DNA double-strand breaks. Mutations in this gene have been related to ataxia telangiectasia and other disorders, especially characterized by radiation sensitivity and cancer predisposition (175). Variations in this gene were reported to play a role in hormone cancers (176, 177). An association between the missense substitution D1853N (rs1801516) and a reduced risk of PTC was also reported for both sporadic and radiation-induced thyroid cancer (178). Recently, we also showed that this coding SNP in ATM contributes to the risk of PTC in Belarusian children exposed to ionizing radiation from the Chernobyl nuclear power plant accident (179).

We thus sought to investigate the contribution of genetic variations at the *NKX2-1*, *FOXE1* and *ATM* loci to the risk of DTC in the 5 studies of Epi-Thyr Consortium, especially for the minor allele of the 4 common SNPs of Epi-Thyr Consortium: rs965513, rs1867277, rs1801516 and rs944289.

2.4.1 GENOTYPING

2.4.1.1 The French Polynesia Study

For SNP rs965516 and rs1867277, 10 ng gDNA were analysed using a specific TaqMan Predesigned SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). Fluorescence readings and data analyses were performed with the ABI PRISM 7900HT Sequence detection system. For rs944289 and rs1801516, 25ng gDNA were analysed using High-Resolution Melting curve (HRM), with a specific probe. Some representative samples were re-sequenced by dye-terminator to confirm the genotype (180). Fluorescence readings and data analyses were done with the Idaho Technology LightScanner Inc. Hi-Res Melting System.

The proportion of successfully genotyped DNA samples was 95.1% for rs944289, 90.3% for rs965513, 79.4% for rs1867277 and 98.5% for rs1801516. Table 2-2 gives us a clear description of the five studied polymorphisms in the French Polynesia Study.

						Participan	ts (N = 4	52)	
			Polymo	Polymorphism N		Minor Allele Ha Frequency E		lardy-Weinberg Equilibrium χ ² p-value	
Reference	Location	Chromosome	Allele change	Residue change	Cases	Controls	Cases	Controls	
rs944289	Intergenic, 337 kb telomeric of NKX2-1	14q13.3	C>T	÷	0.32	0.27	0.01	0.001	
rs965513	Intergenic, 57 kb upstream to FOXE1	9q22.33	G>A	-	0.27	0.21	0.27	0.97	
rs1867277	5'UTR of FOXE1	9q22.33	G>A		0.20	0.19	0.43	0.64	
rs71369530	FOXE1	9q22.33	Poly-alanine tract expansion		0.17	0.15	0.49	0.47	
rs1801516	Missense substitution at codon 1853 of ATM	11q22-23	G>A	D[Asp]>N [Asn]	0.03	0.02	0.67	0.80	

Table 2-2 - Description of the five studied polymorphisms in the French Polynesia Study (Maillard et al. 2015)

In 2015, Maillard S et al. (49) published a paper about common variants at 9q22.33, 14q13.3, and ATM Loci, in the risk of DTC within the French Polynesia study, he found an association between genotypes G/A and A/A of the GWAS SNP rs965513 near *FOXE1*, and the risk of DTC. A multiplicative effect of allele A was even noted. However, no association was observed with rs1867277 falling in the promoter region of the gene. In contrast, the GWAS SNP rs944289 (*NKX2-1*) did not show any significant association. Although the missense substitution D1853N (rs1801516) in *ATM* was rare in the population, carriers of the minor allele (A) also showed an excess risk. The relationships between these four polymorphisms and the risk of DTC were not contingent on the body surface area, body mass index, ethnicity

or dietary iodine intake. However, an interaction was evidenced between the thyroid radiation dose and rs944289.

2.4.1.2 The Cuba Study

For SNPs rs944289, rs965513, rs1867277, and rs1801516, 25 ng of gDNA were analysed using High-Resolution Melting curve (HRM) with a specific probe. Some representative samples were re-sequenced by dye-terminator to confirm the genotype (180). Fluorescence readings and data analyses were done with the Idaho Technology LightScanner Inc. Hi-Res Melting System (Idaho Technology, Salt Lake City, UT).

The proportion of successfully genotyped DNA samples was 99.0% for rs944289, 96.9% for rs965513, 99.0% for rs1867277, and 97.1% for rs1801516. Quality control analysis showed a concordance rate >99% between duplicate samples. Table 2-3 gives us a clear description of the five studied polymorphisms in the Cuba Study.

						Participan	ts (N = 45	52)
			Polymorphism		Minor Allele Frequency		Hardy-Weinberg Equilibrium χ^2 p-value	
Reference L	Location	Chromosome	Allele change	Residue change	Cases	Controls	Cases	Controls
rs944289	Intergenic, 337 kb telomeric of NKX2-1	14q13.3	C>T	5	0.32	0.27	0.01	0.001
rs965513	Intergenic, 57 kb upstream to FOXE1	9q22.33	G>A	÷	0.27	0.21	0.27	0.97
rs1867277	5'UTR of FOXE1	9q22.33	G>A		0.20	0.19	0.43	0.64
rs71369530	FOXE1	9q22.33	Poly-alanine tract expansion		0.17	0.15	0.49	0.47
rs1801516	Missense substitution at codon 1853 of ATM	11q22-23	G>A	D[Asp]>N [Asn]	0.03	0.02	0.67	0.80

Table 2-3 - Description of the five studied polymorphisms in the Cuba Study (Pereda et al. 2015)

In 2015, Pereda CM et al (48). replicated the same genetic variants as these in the French Polynesia Study in DTC risk, but within the Cuban population samples. Significant association with DTC risk was found for rs944289 near *NKX2-1* (OR per A allele = 1.6, 95% CI: 1.2-2.1), and two polymorphisms near or within *FOXE1*, rs965513 (OR per A allele = 1.7, 95% CI: 1.2-2.3), rs1867277 (OR per A allele = 1.5, 95% CI: 1.1-1.9), only the 1 latter remaining significant when correcting for multiple tests. Overall, no association between DTC and the coding SNP D1853N (rs1801516) in *ATM* (OR per A Allele = 1.1, 95% CI: 0.7-1.7) was seen. Nevertheless women who had 2 or more pregnancies had a 3.5-fold increase in risk of DTC if they carried the A allele (OR = 3.5, 95% CI: 3.2-9.8) as compared to 0.8 (OR = 0.8, 95% CI: 0.4-1.6) in those who had fewer than 2 pregnancies.

2.4.1.3 The French Young-Thyr Study

For the 1417 Young-Thyr samples, TaqMan genotyping was performed at the National Centre of Genotyping (CEA, Evry, France), on a 7900HT Sequence detection system (Applied Biosystem).

All probes were purchased from ThermoFisher-Applied Biosystems. Probes for rs1801516 (C_11736668_10) and rs965513 (C_1444137_10) were purchased as TaqMan SMP genotyping assays (Ref : 4351376), and probes rs1867277 and rs944289 as custom Taqman genotyping assays (Ref : 4332072). Genotyping was performed on 5 ng of gDNA, using standard protocols. All samples were treated in a 384-well plate format, and each plate included several positive and negative control samples in order to validate the process. Fluorescence reading and data analyses were performed with the ABI7900HT sequence detection systems version 2.4.1 (Applied Biosystem), in duplicate by two different people.

The proportion of successfully genotyped DNA samples was 99.29% for rs1801516, 99.22% for rs965513, 98.58% for rs1867277, and 98.44% for rs944289. Quality control analysis showed a concordance rate >99% between duplicate samples.

2.4.1.4 The New Caledonia and French Cathy Studies

The four common SNPs of Epi-Thyr Consortium mentioned above were genotyped in International Agency of Cancer Research (IRAC, Lyon, France) of the World Health Organization (WHO) using the Fluidigm Dynamic array chip (société Intégragen). Oligonucleotide probes and primer were validated in 90 CEPH DNA sample. All the genotyped SNPs had a call rate>95% and did not deviated from hardy Weinberg equilibrium (p>0.001). We exclude subjects with low DNA concentration (64 cases/65 controls in the French Cathy study) and with genotype completions less than 90% (19 cases/11 controls in both the New Caledonia and French Cathy studies).

The proportion of successfully genotyped DNA samples was 97.74% and 99.14% for rs1801516, 97.74% and 98.66% for rs965513, 96.71% and 98.19% for rs1867277, and 97.53% and 98.76% for rs944289, in the New Caledonia study and French Cathy study, respectively.

3 PART III: DIETARY IODINE INTAKE AND DIFFERENTIATED THYROID CANCER RISK: THE CASE-CONTROL STUDY IN CUBA

Basing on the scientific background, interesting and the materials that I previously presented in 2.3.5, 2.4.1.2 of the Cuba study, in this part, I will explore the role of specific food groups or items, especially in regard to iodine intake, in the risk of developing DTC, and to assess the possibility of interaction between environmental factors and some common SNPs previously associated with DTC in the Cuban population that we haven't known before.

It was hypothesized, first, that fish consumption may reduce the risk of DTC; second, that dietary iodine deficiency and excess dietary iodine could be associated with an increasing risk of DTC; and third, that dietary iodine deficiency may interact with *FOXE1* due to its role in thyroid hormone pathways.

3.1 METHOD

3.1.1 ANALYZED PARAMETERS

For quantitative factors, such as fish, shellfish, and total seafood consumption, the study participants were categorized into tertiles according to the consumption value distribution in controls.

Overall, the study participants were classified into three categories according to the Iodine Global Network (IGN) (181): mild iodine deficiency (75–149 μ g/day), optimal iodine intake (150–299 μ g/day), and more than adequate or excess iodine intake (\geq 300 μ g/day). Furthermore, the study participants were regrouped into quartiles according to the dietary iodine intake in controls.

The possible interaction between dietary iodine intake and the following polymorphisms was investigated: rs944289 (near *NKX2-1*), rs965513 (near *FOXE1*), rs1867277 (5' UTR of *FOXE1*), and rs71369530 (polyalanine length polymorphism in *FOXE1*), which were significantly associated with the risk of developing DTC in the same Cuban case-control study (48). For this purpose, the values of dietary iodine intake were classified into two categories: either less or more than the median (186 μ g/day) value observed in controls.

3.1.2 STATISTICAL ANALYSIS

Nineteen strata were defined on the basis of age and sex: seven for men and twelve for women. For each parameter studied, the OR stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, and body surface area (BSA), was estimated using conditional logistic regression with SAS v9.3 (SAS Institute, Inc., Cary, NC). Linear trend tests were performed by creating a variable that assigned the median value for each category of the studied variable consumption, and this variable was then modeled as a continuous variable. Likelihood ratio tests comparing models with and without multiplicative interaction terms were performed to assess the significance of interactions. Sensitivity analyses were performed by excluding individuals diagnosed more than five years before the interviews.

3.2 RESULTS

3.2.1 FOOD CONSUMPTIONS OF THE STUDY PARTICIPANTS

Table 3-1 shows us the average of food consumptions (g/day) in the 20 sub-groups as well as their included detailed food items of the cases, the controls and all the study participants, respectively. Overall, fruits (600 g/day), rice (318 g/day) and other vegetables (261 g/day) were the first three most consumed foods among all the study participants comparing with cheese (17 g/day), fish (15 g/day) and shellfish (6 g/day) which were the least consumed foods in the studied Cuban population (Figure 3-1).

Food item or sub-group	Details	Case Mean (g/day)	Control Mean (g/day)	All the study participants Mean (g/day)	
	Orange, grapefruit, mandarin, lemon		530		
	Mango			600	
Emite	Guava	(72			
Fruits	Banana	673			
	Pineapple				
	Papaya				
	Melon				
Rice	Rice	343	293	318	

Table 3-1 - Food consumptions of the study participants (By decreasing order of the 20 sub-groups)

Food item or sub-group	Details	Case Mean (g/day)	Control Mean (g/day)	All the study participants Mean (g/day)	
	Raw leffuce				
	Raw sov				
	Raw mushroom				
	Cooked carrot				
	Cooked pumpkin				
	Cooked broceli				
	Cooked blocoli				
Other vegetables		267	255	261	
	Cooked leek				
	Cooked celery				
	Cooked spinach				
	Cooked green pea, peas,				
	Cooked exemient memory				
	Dried pulse (lentil navy				
Pulses	bean split pea cassoulet	147	143	145	
1 01000	Fresh cow's milk		1.0		
Milk	Fresh goat's or sheep's milk	109	73	91	
	Raw tomato				
Tomatoes	Cooked tomato	84	93	89	
	Yogourt, fromage blancs.				
Yoghurt	petit suisse	97	77	87	
	Deer				
	Beef		81		
	Calf	76		70	
Meats	Lamb	/6		/8	
	Pork				
	Liver (calf, pork, poultry)				
	Poultry (chicken, turkey,				
Poultry	duck, rabbit)	68	63	65	
	Ice cream				
Dessert	Cream dessert (chocolate or	63	51	57	
	caramel cream)				
	Wine				
	Beer				
Alcohol	Whisky	29	73	52	
	Other aperitif (fortified	_>	15		
	wine, pastis)				
	Alcohol, liqueur				
	Raw cabbage				
~	Cooked cabbage		43		
Cabbages	Cooked brussels sprout	55		49	
	Cooked cauliflower				
		4.7		4.7	
Pasta	Pasta, samolina	45	44	45	

Food item or sub-group	Details	Case Mean (g/day)	Control Mean (g/day)	All the study participants Mean (g/day)
Potato	Potato	42	34	38
Chips	Fry, chips	36	38	37
Egg	Hard-boiled egg, soft- boiled, omelet	30	38	34
	Cured or cooked ham,			
Sausage or charcuterie	Sausage (pâté, saucisson, rillettes)	19	25	22
	Hot sausage (blood sausage, andouillette)			
	Cow's cheese	18	16	17
Chassa	Goat's cheese			
Cheese	Sheep's cheese			
	Soft cheese			
	Fresh sea fish			
	Fresh game fish			
Eich	Canned sardine	12	16	15
ГISII	Canned tuna	15	10	15
	Other canned fish			
	Frozen fish			
	Shellfish (oyster, mussel,)			
Shellfish	Lobster, crab	5	7	6
	Shrimp			
Total foods consumption		2220	1992	2103



Figure 3-1 - Food consumptions of the study participants (By decreasing order of the 20 sub-groups: g/day/person)

3.2.2 DIETARY ENERGY INTAKE OF THE STUDY PARTICIPANTS

Table 3-2 shows us the average of dietary energy intake (kcal/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, rice (447 kcal/day), fruits (370 kcal/day) and meats (171 kcal/day) were the first three food sub-groups which had the most dietary energy intake among all the study participants comparing with fish (23 kcal/day), cabbages (14 kcal/day) and shellfish (7 kcal/day) which had the least dietary energy intake in the studied Cuban population (Figure 3-2).

Food item or sub- group	Case Mean (kcal/day)	Control Mean (kcal/day)	All study participants Mean (kcal/day)
Rice	484	413	447
Fruits	411	331	370
Meats	164	177	171
Pulses	154	149	152
Poultry	131	122	127
Dessert	142	108	124
Chips	94	98	96
Other			
vegetables	95	96	95
Yoghurt	91	71	81
Pasta	61	60	60
Egg	49	62	56
Sausage or			
charcuterie	48	60	54
Potato	59	48	53
Cheese	53	45	49
Milk	53	36	44
Tomatoes	37	47	42
Alcohol	18	42	30
Fish	21	25	23
Cabbages	16	13	14
Shellfish	6	7	7
Total	2185	2009	2095

Table 3-2 - Dietary energy intake of the study participants
(By decreasing order of the 20 sub-groups)



Figure 3-2 - Mean of dietary energy intake of the Cuba study participants (by decreasing order of the 20 sub-groups: kcal/day/person)

3.2.3 DIETARY IODINE INTAKE OF THE STUDY PARTICIPANTS

Table 3-3 shows us the average of dietary iodine intake (μ g/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, yoghurt (12.88 μ g/day), chips (10.95 μ g/day) and milk (9.08 μ g/day) were the first three food sub-groups which had the most dietary iodine intake among all the study participants comparing with alcohol (0.30 μ g/day), cabbages (0.26 μ g/day) and potato (0.12 μ g/day) which had the least dietary iodine intake in the studied Cuban population (Figure 3-3).

Food item or sub- group	Case Mean (µg/day)	Control Mean (µg/day)	All study participants Mean (µg/day)
Yoghurt	14.44	11.39	12.88
Chips	10.68	11.20	10.95
Milk	11.02	7.23	9.08
Sausage or			
charcuterie	7.34	10.48	8.95
Dessert	8.64	6.69	7.64
Egg	6.67	8.39	7.55
Rice	7.04	6.00	6.51
Fish	6.09	6.60	6.35
Pulses	5.98	5.82	5.90
Fruits	6.38	4.28	5.31
Cheese	5.08	4.36	4.71
Other			
vegetables	4.86	4.40	4.63
Shellfish	3.31	3.90	3.61
Tomatoes	2.33	3.35	2.85
Meats	2.50	2.09	2.29
Poultry	0.80	0.75	0.78
Pasta	0.32	0.31	0.31
Alcohol	0.17	0.42	0.30
Cabbages	0.28	0.25	0.26
Potato	0.14	0.11	0.12
Total	104.08	98.02	100.99

Table 3-3 - Dietary iodine intake of the study participants(By decreasing order of the 20 sub-groups)



Figure 3-3 - Mean of dietary iodine intake of the Cuba study participants (by decreasing order of the 20 sub-groups: µg/day/person)

3.2.4 DIETARY SELENIUM INTAKE OF THE STUDY PARTICIPANTS

Table 3-4 shows us the average of dietary selenium intake (μ g/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, poultry (23.17 μ g/day), meats (15.57 μ g/day) and pulses (11.96 μ g/day) were the first three food sub-groups which had the most dietary selenium intake among all the study participants comparing with chips (0.37 μ g/day), alcohol (0.27 μ g/day) and potato (0.11 μ g/day) which had the least dietary selenium intake in the studied Cuban population (Figure 3-4).

Food item or sub- group	Case Mean (µg/day)	Control Mean (µg/day)	All study participants Mean (µg/day)
Poultry	23.97	22.39	23.17
Meats	14.34	16.75	15.57
Pulses	12.13	11.79	11.96
Fruits	13.71	9.48	11.55
Rice	12.02	10.25	11.11
Egg	8.66	10.89	9.80
Other			
vegetables	7.43	7.29	7.36
Fish	5.52	6.42	5.98
Sausage or	7 19	2.02	256
Vachurt	2.18	2.92	2.50
i ognurt	2.40	1.90	2.14
Shellfish	1.70	1.99	1.85
Cheese	1.86	1.78	1.82
Cabbages	1.44	1.23	1.33
Milk	0.98	0.67	0.82
Dessert	0.81	0.72	0.77
Pasta	0.68	0.66	0.67
Tomatoes	0.42	0.53	0.47
Chips	0.36	0.37	0.37
Alcohol	0.14	0.39	0.27
Potato	0.13	0.10	0.11
Total	110.88	108.52	109.68

Table 3-4 - Dietary selenium intake of the study participants(By decreasing order of the 20 sub-groups)



Figure 3-4 - Mean of dietary selenium intake of the Cuba study participants (by decreasing order of the 20 sub-groups: µg/day/person)

3.2.5 FISH, SHELLFISH CONSUMPTION AND DTC RISK

The risk of DTC reduced significantly with increasing consumption of fish whatever via univariate analysis (p-trend=0.005) or via multivariate analysis (p-trend=0.04; Table 3-5).

	Cases/Controls	Age and sex	P for	Multivariate OR ²	P for
	(203/212)	stratified OR	trend ¹	(95% CI)	trend ²
		(95% CI)			
Fish consumpti	ion (g/day)				
< 4	103/77	1.00*	0.005	1.00*	0.04
4–14	50/65	0.58 (0.36-0.95)		0.60 (0.36–1.00)	
≥15	50/70	0.52 (0.33-0.84)		0.55 (0.34–0.92)	
Shellfish consu	mption (g/day)				
0	128/102	1.00*	0.04	1.00*	0.2
1–4	21/44	0.38 (0.21–0.69)		0.31 (0.17–0.58)	
≥ 5	54/66	0.68 (0.43-1.06)		0.70 (0.43–1.13)	
Total consumpt	tion of seafood (g/da	ay)			
< 5	89/64	1.00*	0.02	1.00*	0.1
5–19	60/81	0.54 (0.34–0.87)		0.58 (0.36-0.96)	
≥20	54/67	0.57 (0.35–0.92)		0.58 (0.35-0.98)	

Table 3-5 - Odds Ratios of DTC Associated with Fish and Shellfish Consumption

¹ Mantel-Haenszel Chi-2 trend test stratified on age group and sex;

² Multivariate logistic regression stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, BSA (body surface area), and, for trend test, calculated by assigning the median value to each category and treating this variable as a continuous variable; * Reference category.

3.2.6 DIETARY IODINE INTAKE AND DTC RISK

We observed that only 15% of the studied population samples were estimated as having mild iodine deficiency, and the majority (78%) of our studied population had an optimal dietary iodine intake according to the IGN classification. We did not identify a significant association between dietary iodine intake, when classified according to the IGN classification, and DTC risk in the entire Cuban sample (adjusted p-trend=0.5; Table 3-6). This association was still not significant (p-trend=0.7) when we regrouped the population sample by quartiles of dietary

iodine intake in controls (Table 3-7). Furthermore, this non-significant association was not modified by age, smoking status, dietary selenium intake, or ethnicity (all p-interactions > 0.05) (Table 3-7).

An interaction that was close to significance was identified between dietary iodine intake and BMI (adjusted p-interaction=0.08). Among people whose BMI was equal to or more than 25 kg/m², the risk of DTC was 2.47 times higher (multivariate OR=2.47, 95% CI=0.89–6.85) in people who were in the lowest quartile of dietary iodine intake compared with those who were in the highest quartile, although this difference was not significant (adjusted p-value for trend=0.1). This increase of DTC risk when decreasing iodine intake was not observed in people with a BMI lower than 25 kg/m² (Table 3-7).

 Table 3-6 - Odds Ratios of DTC Associated with Dietary Iodine Intake According to IGN Classification in the Studied Cuban Population

	Dietary iodine intake, IGN classification (µg/day)					
	Mild deficiency Optimal		More than adequate or excess	p-trend ²		
	(75–149)	(150–299)	(≥300)			
Cases/controls	34/28	147/175	22/9			
Age and sex stratified OR (95% CI)	1.44 (0.83–2.51)	1.00*	2.78 (1.24–6.25)	0.6 ^a		
Multivariate OR ¹ (95% CI)	1.27 (0.70–2.30)	1.00*	2.32 (0.98-5.48)	0.5 ^b		

¹ Stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, BSA (body surface area);

² Calculated using the Wald test for dietary iodine intake modeled as a continuous variable (per classification); * Reference category;

^a Crude p-trend;

^b Adjusted p-trend.

Dietary iodine intake in quartile of the value distribution in controls ($\mu g/day$)						
	< 164.9	(164.9–186.5)	(186.5–237.5)	(≥237.5)	p- trend ²	p- interaction ³
Overall						
Cases/controls	62/53	27/53	57/53	57/53		
Age and sex stratified	1.07 (0.62–1.83)	0.46 (0.26–0.84)	0.96 (0.56-1.66)	1.00*	0.7^{a}	
OR (95% CI)						
Multivariate	1.12 (0.62–2.03)	0.56 (0.30-1.05)	1.23 (0.69–2.22)	1.00*	0.7 ^b	
OR ¹ (95% CI)						
By age						
Age at the diagnosis ≤ 40						
Cases/controls	30/27	18/30	31/27	39/31		
Age and sex stratified	0.93 (0.46–1.90)	0.47 (0.22–1.00)	0.83 (0.40-1.70)	1.00*	0.5^{a}	
OR (95% CI)						
Multivariate	0.81 (0.35-1.86)	0.48 (0.20-1.14)	1.08 (0.47–2.48)	1.00*	0.3 ^b	
OR ¹ (95% CI)						
Age at the diagnosis > 40						
Cases/controls	32/26	9/23	26/26	18/22		
Age and sex stratified	1.37 (0.59–3.16)	0.45 (0.17–1.21)	1.20 (0.51–2.79)	1.00*	0.8^{a}	0.5 ^c
OR (95% CI)						
Multivariate	1.59 (0.62–4.04)	0.48 (0.16–1.38)	1.35 (0.55–3.33)	1.00*	0.7^{b}	0.4^d
OR ¹ (95% CI)						
BMI (kg/m ²)						
<25						
Cases/controls	31/35	11/36	26/31	30/24		
Age and sex stratified	0.74 (0.36–1.53)	0.28 (0.12-0.66)	0.70 (0.33-1.50)	1.00*	0.2^{a}	
OR (95% CI)						
Multivariate	0.77 (0.34–1.74)	0.32 (0.13-0.80)	0.85 (0.37-1.92)	1.00*	0.2^{b}	
OR ¹ (95% CI)						
≥25						
Cases/controls	31/18	16/17	31/22	27/29		

Table 3-7 - DTC Risk Associated with Diete	ry Iodine Intake by	v Various Confounding	Factors in the Studied Cuban Population

Dietary iodine intake in quartile of the value distribution in controls ($\mu g/day$)						
	< 164.9	(164.9–186.5)	(186.5–237.5)	(≥237.5)	p- trend ²	p- interaction ³
Age and sex stratified	1.79 (0.78–4.12)	0.94 (0.39–2.29)	1.20 (0.55–2.66)	1.00*	0.3 ^a	0.1 ^c
OR (95% CI)						
Multivariate	2.47 (0.89-6.85)	1.41 (0.53–3.78)	1.39 (0.56–3.45)	1.00*	0.1 ^b	0.08 ^d
OR ¹ (95% CI)						
Smoking status						
Nonsmoker						
Cases/controls	41/27	19/29	38/37	46/32		
Age and sex stratified	1.20 (0.60–2.39)	0.46 (0.22-0.95)	0.70 (0.37-1.35)	1.00*	0.97 ^a	
OR (95% CI)						
Multivariate	1.17 (0.54–2.56)	0.62 (0.29–1.35)	0.90 (0.45-1.81)	1.00*	0.98 ^b	
OR ¹ (95% CI)						
Current smoker						
Cases/controls	21/26	8/24	19/16	11/21		
Age and sex stratified	1.19 (0.41–3.42)	0.49 (0.15–1.61)	1.85 (0.61–5.61)	1.00*	0.8^{a}	0.9 ^c
OR (95% CI)						
Multivariate	1.09 (0.34–3.55)	0.48 (0.13–1.76)	2.41 (0.65–9.02)	1.00*	0.5^{b}	0.8^{d}
OR ¹ (95% CI)						
Dietary selenium intake (µg	/day)					
<95						
Cases/controls	59/52	14/32	16/16	4/7		
Age and sex stratified	1.02 (0.46–2.28)	0.44 (0.17–1.15)	1.00*	0.47 (0.11-2.00)	0.2 ^a	
OR (95% CI)						
Multivariate	0.90 (0.37-2.22)	0.44 (0.15–1.28)	1.00*	0.37 (0.08–1.72)	0.3 ^b	
OR ¹ (95% CI)						
≥95						
Cases/controls	3/1	13/21	41/37	53/46		
Age and sex stratified	$?^{\dagger}$	0.58 (0.25–1.34)	1.00*	1.02 (0.55–1.91)	0.5 ^a	0.09 ^c
OR (95% CI)						
Multivariate	$?^{\dagger}$	0.50 (0.19–1.26)	1.00*	0.71 (0.35–1.47)	0.99 ^b	0.3 ^d

	Dietary iodine intake in quartile of the value distribution in controls ($\mu g/day$)					
	< 164.9	(164.9–186.5)	(186.5–237.5)	(≥237.5)	p- trend ²	p- interaction ³
OR ¹ (95% CI)						
Ethnic group						
European						
Cases/controls	30/14	7/8	12/6	8/10		
Age and sex stratified	2.50 (0.74-8.44)	1.22 (0.26–5.74)	2.61 (0.58–11.74)	1.00*	0.3 ^a	
OR (95% CI)						
Multivariate	2.56 (0.57–11.58)	0.93 (0.11-7.90)	3.24 (0.51–20.56)	1.00*	0.4 ^b	
OR ¹ (95% CI)						
African						
Cases/controls	13/15	7/17	15/28	14/15		
Age and sex stratified	1.06 (0.34–3.30)	0.54 (0.17–1.72)	0.90 (0.31-2.64)	1.00*	0.8^{a}	0.3 ^c
OR (95% CI)						
Multivariate	1.00 (0.23-4.40)	0.52 (0.13-2.12)	0.81 (0.22–2.95)	1.00*	0.8^{b}	0.3 ^d
OR ¹ (95% CI)						
Other and mixed						
Cases/controls	19/24	13/28	30/19	35/28		
Age and sex stratified	0.53 (0.23–1.21)	0.39 (0.17-0.90)	1.20 (0.53–2.70)	1.00*	0.03 ^a	
OR (95% CI)						
Multivariate	0.61 (0.25–1.48)	0.44 (0.18–1.07)	1.35 (0.57–3.21)	1.00*	0.07 ^b	
OR ¹ (95% CI)						

¹ Stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of ² Calculated using the likelihood ratio test comparing a model with an interaction term to one without;

* Reference category;

?[†] Not estimable;

^{*a*} Crude *p*-trend;

^b Adjusted p-trend;

^c Crude p-interaction;

^d Adjusted p-interaction.

3.2.7 *FOXE1* POLYMORPHISM INTERACTS WITH DIETARY IODINE INTAKE IN DTC RISK

We did not find any interaction between dietary iodine intake and the following polymorphisms, rs944289, rs1867277 and rs71369530 in the risk of developing DTC. Conversely, a strong interaction (multivariate p-interaction=0.005) was identified between dietary iodine intake and rs965513, near the *FOXE1* gene on chromosome 9q22.33 (Table 3-8). A strong increased DTC risk was observed with the number of copies in the minor allele (A) of rs965513 among people who consumed less iodine than the median (< 186 µg/day). The risk of DTC was 3.4 times higher (adjusted p-trend=0.0005, 95% CI=1.7–6.5) for subjects carrying one copy of the A allele, and 6.7 times higher (adjusted p-trend=0.0005, 95% CI=1.6–27.3) for subjects carrying two copies of the A allele when compared to the reference group of subjects with no copies in the minor allele (A). This dose effect with regard to the DTC risk with the increasing number of copies in the A allele was not observed in subjects consuming more iodine than the median (> 186 µg/day); independent of the number of copies in the minor allele (A), the risk of DTC was around three times higher than that of the reference group whose subjects were not carrying any A allele and had a dietary iodine intake below the median (Table 3-8).

Dietary Iodine Intake		Polymorphis	P-value		
		G/G	G/A	A/A	for trend
< median (186 μ g/day)	Cases/Controls	31/68	45/31	11/3	
	OR (95% CI)	1.0*	3,2 (1.7-5.9)	8.0 (2.1-30.9)	< 0.0001 ¹
	Age and sex stratified OR (95% CI)	1.0*	3.2 (1.7–6.2)	8.0 (1.6-40.3)	< 0.0001 ²
	Multivariate OR ³ (95% CI)	1.0*	3.4 (1.7–6.5)	6.7 (1.6–27.3)	0.0005 ³
> median	Cases/Controls	50/48	44/46	16/9	
	OR (95% CI)	2.3 (1.3-4.1)	2.1 (1.2-3.8)	3.9 (1.6-9.8)	0.5^{1}
	Age and sex stratified OR (95% CI)	2.6 (1.4-4.8)	2.3 (1.2–4.3)	4.8 (1.7–13.8)	0.3 ²
	Multivariate OR ³ (95% CI)	2.4 (1.3–4.4)	2.4 (1.3–4.5)	3.3 (1.2–8.8)	0.4 ³

Table 3-8 - Odds Ratios of DTC Risk Associated with Dietary Iodine Intake and the Polymorphism of SNP rs965513 near FOXE1

1 Mantel-Haenszel Chi-2 trend test;

2 Mantel-Haenszel Chi-2 trend test stratified on age group and sex;

3 Multivariate logistic regression stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, BSA (body surface area), and for trend test, calculated by assigning the number of A allele in FOXE1;

* *Reference category*.

Missing data: 2 cases and 4 controls with dietary iodine intake < median and 4 cases and 3 controls with dietary iodine intake > median due to low quality of samples for genotyping;

P-interaction between the number of A allele in FOXE1 and dietary iodine intake (+ or - median), crude = 0.007, stratified = 0.003, multivariate = 0.005.

3.3 DISCUSSION

3.3.1 SUMMARY OF THE RESULTS

We have observed that the risk of DTC significantly decreased with increasing consumption of fish in the Cuban population. In addition, we found a strong increase in DTC risk with the number of copies in the minor allele (A) of rs965513 near *FOXE1* on chromosome 9q22.33 among people who consumed less than the median value of iodine in controls. Furthermore, the risk of DTC tended to be higher among people whose BMI was equal to or more than 25 kg/m² and who were in the lowest quartile of dietary iodine intake.

3.3.2 STRENGTHS AND LIMITATIONS OF THE STUDY

The present study is the first case-control study to focus on the risk factors of DTC in the Cuban population. We used the food photo booklet from the E3N cohort (140) in this study in order to estimate the portions of food and drink daily consumption by the study cohort, which complemented the quantificational questionnaire and gave us normative, accurate and qualitative information on which to build the database. Another noteworthy point is that we have constructed a special food composition table to measure the quantity of iodine in typical Cuban food for this study. However, only 28% of the food items in the questionnaire of this study were measured using this special and innovative table, knowing that until now, no such measurement of iodine has been made by other organizations for typical Cuban or Latino food.

This case-control study has for the first time identified a strong increase in DTC risk associated with the number of copies in the minor allele (A) of SNP rs965513, near *FOXE1* at 9q22.33, among people who consumed less iodine than the median, but no variation among people who consumed more (multivariate P-interaction=0.005). To the best of my knowledge, gene-environment interactions in DTC have only been investigated in relation to radiation exposure in a candidate gene approach, but no convincing association has been reported so far. In particular, no gene-environment interaction study has investigated an association with *FOXE1* (at 9q22.33) and dietary iodine intake.

As with any case-control study, this study has some limitations. Self-reported food consumption is associated with imprecise measures and recall bias. Another limitation is that

for a large part of the Cuban diet, we estimated iodine content from the CIQUAL 2013 food composition table, which had been established for French food, and may not be applicable to Cuban food. The French CIQUAL 2013 food composition table was used in this study in order to evaluate the iodine content in fish and shellfish. This could introduce some uncertainties. Nevertheless, the iodine content in fish is highly variable even in one country (182). It depends strongly on the species and the places of capture, and most of the sea fish consumed in Cuba are from areas far away from Cuban coasts. Given the low number of fish captured by Cuban boats and low fish consumption in Cuba, the difficulty in measuring the iodine content in fish, and the cost-effectiveness of this study, we did not measure the iodine content in either Cuban fish or shellfish.

Moreover, because cases usually take more care of their health and are more motivated to participate in research studies than controls, the cases may have reported more precise information than the controls did, and this could have introduced a differential bias in the final results and overestimated the risk of DTC. Nevertheless, this potential bias cannot explain the significant relation between the number of copies in the minor allele (A) of rs965513 and the risk of DTC among people who consumed less iodine than the median. Indeed, it is very unlikely that our finding regarding an interaction between SNP rs965513 and iodine intake was due to chance because it remained present when correcting the p-value for the number of SNPs tested, which was four SNPs in the analyses (183). Additionally, this result was almost exactly identical when adjusting or not for other DTC risk factors and stratifying or not for age groups and sex. Lastly, recall bias was probably reduced by the fact that food import and export limitations exist in Cuba, which keeps the typical Cuban food more stable and less diversified than Western food.

According to the IGN classification, an optimal iodine intake corresponds to 150–299 µg/day (181). The quartiles of the dietary iodine intake in the controls in this study were: < 164.9, 164.9–186.5, 186.5–237.5 and ≥ 237.5 µg/day. The last three quartiles cover the optimal iodine intake, as well as more than adequate or excess iodine intake. However, a case-control study using a food frequency questionnaire is not an adequate way to estimate iodine status (because a case-control study without iodine measurement in urine is not designed to estimate the iodine status of a population, but to perform comparisons between the cases and the controls).

3.3.3 COMPARISON RESULTS FROM THE LITERATURE

This study shows that in the Cuban population, the risk of DTC significantly decreased with the increasing consumption of fish at the time of the interview (Table 3-5). This important finding is in line with another case-control study, which was carried out in French Polynesia (105). A meta-analysis of 19 observational studies on dietary factors and thyroid cancer risk summarized the ORs for each risk factor. Based on the highest level of total consumption versus the lowest level, the aggregate OR (95% CI) of thyroid cancer was 0.79 (0.66, 0.94) for fish consumption. Subgroup analysis showed that fish (OR=0.74, 95% CI=0.59–0.92) and shellfish (OR=0.46, 95% CI=0.27-0.75) consumption has a protective effect in iodinedeficient areas (111). Another related review showed that iodine-rich food such as fish and shellfish may provide a protective role in populations with insufficient daily iodine intake (184). In contrast, a Japanese cohort where subjects were followed up for a mean of 14.5 years showed that a high intake of seaweed was positively associated with thyroid cancer risk, although this result was not statistically significant. In addition, a high intake of seaweed was positively associated with papillary thyroid cancer risk in postmenopausal women (especially for papillary carcinoma, the hazard ratio (HR) for almost daily consumption compared with 2 days/week or less was 3.81, 95% CI: 1.67–8.68; p-trend<0.01) (185).

First-degree relatives of DTC patients have an increased risk for developing thyroid cancer, and a positive family history is reported in approximately 5% of cases (38, 186, 187, 188). These observations strongly suggest the contribution of genetic factors to the susceptibility to the disease. Six potential regions for harboring a high-risk DTC gene have been identified through family-based linkage studies in the early 2000s: *MNG1* (14q32) (189), *TCO* (19p13.2) (190,191), *fPTC/PRN* (1q21) (192), *NMTC1* (2q21) (193), *FTEN* (8p23.1-p22) (194), and the telomere-telomerase complex (195). More recently, population-based studies, particularly GWASs, have identified and replicated susceptibility loci at 9q22, 14q13, 2q35 and 8p12 (196). In a previous study, in the same sample of the Cuban population, we validated the association between DTC risk and variants of genes localized at 9q22.33 (*FOXE1*) and 14q13.3 (*NKX2-1*) (48). The *FOXE1* locus is the most widely replicated susceptibility locus associated with DTC risk in a number of population-based studies of various ethnic origins, especially associated with sporadic and radiation-related papillary thyroid cancer and also with familial nonmedullary thyroid carcinoma (197, 198).

The majority of the studied population had an optimal dietary iodine intake. If this result was confirmed by other studies, it would emphasize the effectiveness of the compulsory public health policy by using iodized salt in urban, rural and mountainous areas of Cuba. A cross-sectional study to assess the effectiveness of this program through determination of UIC and goiter prevalence has been previously completed in Cuban schoolchildren aged 6 to 11 years in 2011–2012. The results of this study by Terry-Berro et al. showed that the median UIC was 176.3 μ g/L (199). Only 7.6% of the children showed iodine deficiency (2.2% had severe deficiency <50 μ g/L) and 15.3% had an UIC above 300 μ g/L. The prevalence of optimal iodine nutrition was 43.5% (52.5% in mountain areas). The overall goiter prevalence was 17.6%, which reflects mild endemic goiter. However, in mountain areas, the prevalence of goiter was 32.6% (severe endemic goiter). The authors concluded that iodine deficiency is no longer a public health problem in urban and rural areas of Cuba, which is attributable to successful salt iodization. Nevertheless, high goiter rates are still observed in mountain areas.

In a comprehensive synthesis of animal and human studies (106), Zimmermann et al. concluded that iodine deficiency increases DTC risk. However, the present study does not have enough participants in the moderate iodine deficiency group to verify this conclusion with sufficient statistical power.

3.4 CONCLUSION

In conclusion, the majority of the studied population had an optimal dietary iodine intake. Although DTC risk was not found to be linked to dietary iodine intake in this study, DTC risk significantly decreased with high fish consumption. Additionally, DTC risk significantly increased with an increasing number of copies in the minor allele (A) of rs965513 near *FOXE1* among people who consumed less iodine than the median. Because these findings are based on post-diagnostic measures, studies with pre-diagnostic dietary iodine are needed for confirmation.

4 PART IV: DIETARY IODINE INTAKE AND DIFFERENTIATED THYROID CANCER RISK: A POOLED ANALYSIS OF THE 5 CASE-CONTROL STUDIES FROM EPI-THYR CONSORTIUM

Basing on the 5 case-control studies which carried out in New Caledonia, French Polynesia, metropolitan France (2 studies) and Cuba from Epi-Thyr Consortium that I presented previously (see 2 PART II), I did a pooled analysis of the individual data on pooling these 5 studies together, to the best of my knowledge, this is the first and the largest pooled analysis of case-control studies to investigate iodine status and thyroid cancer risk in heterogenetic populations. My aim was to explore the role of specific food groups or items, especially in regard to iodine intake, in the risk of developing DTC, to study the specificity for each case-control study, and to assess the possibility of interaction between iodine status and number of pregnancies in DTC risk. It was hypothesized, first, fish consumption may reduce DTC risk; second, dietary iodine deficiency and dietary iodine excess could be associated with an increasing risk of DTC; third, iodine deficiency may interact with high number of pregnancies that leads to a high DTC risk; and fourth, dietary iodine intake may interact with the 4 investigated SNPs in the oncotargeted genes that presented previously (see 2.4), thereby accelerating the risk of developing DTC.

4.1 STUDY PARTICIPANTS

Among the 5,914 identified individuals in the study populations, 2,766 eligible subjects with DTC, among them, 555 (20%) were not interviewed, among them, 287 refused to participate the studies, 66 died at the moment of interview, 12 were too sick to response the questionnaire, 190 couldn't be reached. Of the 2,211 cases who were interviewed, one case who did not live in the region where he was interviewed and 20 cases who were not matched with a control at the end of this process were excluded. Of the 2,190 included cases, 28 hadn't had valid dietary information and were excluded from the statistical analyses of this article. Finally, the present study consisted of 2,162 cases, 1,771 of whom were women; Of the 3,148 contacted controls, 563 (18%) were not interviewed, among them, 466 refused to participate the studies, 22 died at the moment of interview, 2 were too sick to response the questionnaire, 73 couldn't be reached. Of the 2,585 controls who were interviewed, 5 were not matched with a case at the end of this process and were excluded. Of the 2,580 included controls, 9 hadn't had valid dietary information and were excluded. Of the 2,580 included controls, 9 hadn't had valid dietary information and were excluded. Of the 2,580 included controls, 9 hadn't had valid dietary information and were excluded from the statistical analyses of this article. Finally, the present study consisted of 2,571 controls, 2,031 of whom were women (Figure 4-1).



Figure 4-1 - Participant flow chart of inclusion criteria, the Pooled Analysis, 1979-2011

4.2 METHOD

4.2.1 ANALYZED PARAMETERS

Overall, the study participants were classified according to the classification of dietary iodine intake, OR and 95% CI were calculated corresponding to each scenario: 1) the cases and the controls were classified into four categories according to the Iodine Global Network (IGN) (181): severe or moderate iodine deficiency ($\leq 74 \ \mu g/day$), mild iodine deficiency (75–149 $\mu g/day$), optimal iodine intake (150–299 $\mu g/day$), and more than adequate or excess iodine intake ($\geq 300 \ \mu g/day$). 2) Dietary iodine intake as a continuous variable ($\mu g/day$). First, total dietary iodine intake was calculated as the sum of iodine from 66 food items consumption, in which, iodine content in bread presented in the New Caledonia, French Polynesia and Cathy studies, but totally missing in the Young-Thyr and Cuba studies, because bread was the food item that its consumption was not reported homogeneously among the 5 studies, but it was involved in the statistical analysis, both in logit linear model and linear model; second, total dietary iodine intake was calculated as the sum of iodine from 65 food items consumption, in which, iodine intake from bread consumption was excluded for all the 5 studies (virtually, iodine intake from bread consumption was excluded in the New Caledonia, French Polynesia and Cathy studies), in order to make dietary iodine intake homogeneously reported among the 5 studies in the statistical analysis, both in logit linear model and linear model. 3) The cases and the controls were categorized into quartiles according to dietary iodine intake value distribution of the controls in each study. First, total dietary iodine intake was calculated as the sum of iodine from 66 food items consumption, including iodine content in bread presented in the New Caledonia, French Polynesia and Cathy studies, but totally missing in the Young-Thyr and Cuba studies, both in logit linear model and linear model; second, total dietary iodine intake was calculated as the sum of iodine from 65 food items consumption, excluding iodine intake from bread consumption for all the 5 studies, both in logit linear model and linear model. These 3 scenarios were applied to the analyses of heterogeneity between studies, between ethnicity; and the last 2 scenarios were applied to the detailed analyses of interaction between dietary iodine intake and Polynesian ethnicity, in all the cases and their eligible controls; in the cases whose cancer longer diameter was more than 10 millimeters.

For quantitative food group or item factors, such as fish, the study participants were categorized into quartiles according to the food group or item consumption value distribution in controls for each study. The possible interactions between dietary iodine intake and the 4 SNPs cited above (see 2.4), which were significantly associated with the risk of developing DTC in the previous GWAS or functional studies (42, 44, 46), for estimating these interactions, the values of dietary iodine intake were classified into two categories: either less or more than the median value observed in controls for each study. Furthermore, the possible interaction between dietary iodine intake and number of full term pregnancies was investigated. For this purpose, the values of dietary iodine intake were classified into two categories: either iodine deficiency (< 150 μ g/day) or adequate or more iodine intake ($\geq 150 \mu$ g/day) according to the IGN classification.

We did not impute bread consumption of the participants of the Young-Thyr and the Cuba studies. The main reason was that we evidenced that bread consumption was poorly correlated to other food consumptions. Indeed, within the Cathy study, which was the only study susceptible to be used to predict bread consumption in the Young-Thyr study, and less
convincing to be used to predict bread consumption in the Cuba study, age at the diagnosis, sex and all the other food items which were significant in correlation tests together permitted to predict only 44% of the variability of bread consumption. Results of the generalized linear model are in Table 4-1. Additionally, plotting the predicted values for bread (Figure 4-2) and the residuals (Figure 4-3) showed a strong bias in prediction, by overestimation of the low reported values and underestimation of the high reported values. That's why it was not possible to impute bread consumption of the participants of the Young-Thyr and the Cuba studies. In fact, such imputed values would decrease the power of the study and skews the predictions.

Source of variability	Sum of square	Degree of	F	P > F
		freedom		
Total	122376952.5	1292		
Model	23567410.9	11	27.78	<.0001
Age of diagnosis (year)	458160.781	1	50.59	0.0149
Sex (W/M)	3799672.168	1	50.59	<.0001
Meats	4464219.165	1	57.39	<.0001
Body Surface Area	216730.527	1	2.85	0.0939
Pasta	926599.026	1	9.40	0.0005
Sausage and charcuterie	7777.630	1	0.13	0.7509
Potato	1162063.312	1	17.30	0.0001
Cheese	1286621.581	1	15.31	<.0001
Poultry	3234.876	1	0.00	0.8378
Rice	53102.978	1	1.02	0.4068
Yoghurt	612965.346	1	8.67	0.0049
Error	98809541.6	1281	77134.7	

Table 4-1 - Results of the generalized linear model







Figure 4-3 - Residuals in bread consumption prediction of the Cathy study

Sensitivity analyses were performed by excluding the individuals diagnosed as microcarcinomas (cancers measured less than 10 mm in longer diameter, see 1.1.3). Sensitivity analysis was also done in order to find the thresholds of iodine consumption that

the risk of developing DTC increased, which can give me a suggestion of iodine consumption classification in the pooled analysis. Nevertheless, from the Figure 4-4, we cannot see differences between iodine consumption of the cases and of the controls, so it is difficult to find the thresholds of iodine consumption which increasing DTC risk in these data.



Figure 4-4 - Dietary iodine intake distributions of the cases and the controls of the pooled analysis

4.2.2 STATISTICAL ANALYSIS

4.2.2.1 Analysis Strategy from the Matching Process

In the two case-control studies (Young-Thyr and French Polynesia), each case was individually matched with one or two controls of the same age and sex, whereas in the other three (New Caledonia, Cathy and Cuba), the cases and the controls were frequency matched, in the present pooled analysis, frequency Matching was used for all the studies. Twenty-six strata were defined on the basis of age class and sex: thirteen for women and thirteen for men. Table 4-2 shows the number of the cases and the controls in each stratum by each study, as well as it in each stratum in the pooled analysis of the 5 studies; moreover, mean age at diagnosis (year) in each stratum by each study, as well as it in each stratum in the pooled analysis of the cases and the control among the strata for each study is not homogeneous neither, this situation leads me to use conditional logistic regression for the pooled analysis of the 5 studies.

	New	French	French	French Cathy	Cuba	Pooled
	Caledonia	Polynesia	Young-Thyr			Study
Characteristics	(744)	(600)	(1681)	(1293)	(415)	(4733)
	Case /	Case /	Case /	Case /	Case /	Case /
	Control	Control	Control	Control	Control	Control
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Over all	332	229	805	593	203	2162
	(44.6)/412	(38.2)/371	(47.9)/876	(45.9)/700	(48.9)/21	(45.7)/2571
	(55.4)	(61.8)	(52.1)	(54.1)	2 (51.1)	(54.3)
Strata						
F & < 15	0/0	4/6	14/14	0/0	0/0	18/20
F & 15-19	1/1	4/5	46/50	0/0	7/5	58/61
F & 20-24	9/12	12/22	136/142	0/2	13/17	170/195
F & 25-29	24/24	21/36	213/235	13/34	14/17	285/346
F & 30-34	34/42	29/45	209/221	28/35	21/19	321/362
F & 35-39	32/38	40/63	15/17	43/49	32/30	162/197
F & 40-44	29/37	27/45	0/0	42/67	43/42	141/191

Table 4-2 - Description of the strata

	New	French	French	French Cathy	Cuba	Pooled
	Caledonia	Polynesia	Young-Thyr			Study
Characteristics	(744)	(600)	(1681)	(1293)	(415)	(4733)
	Case /	Case /	Case /	Case /	Case /	Case /
	Control	Control	Control	Control	Control	Control
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
F & 45-49	36/32	29/49	0/0	59/59	32/29	156/169
F & 50-54	30/41	32/47	0/0	92/69	11/10	165/167
F & 55-59	40/37	4/5	0/0	68/58	4/2	116/102
F & 60-64	24/44	1/1	0/0	46/42	2/2	73/89
F & 65-69	18/19	0/0	0/0	31/43	0/0	49/62
$F \& \ge 70$	16/27	0/0	0/0	41/43	0/0	57/70
M & <15	0/0	0/0	4/4	0/0	0/0	4/4
M & 15-19	0/0	1/1	11/14	0/0	1/0	12/14
M & 20-24	0/0	3/5	34/41	0/2	2/2	39/48
M & 25-29	0/0	3/6	53/55	3/11	0/6	59/78
M & 30-34	1/1	1/1	66/81	7/22	5/5	80/110
M & 35-39	1/4	6/12	4/2	12/15	6/8	29/41
M & 40-44	6/7	5/9	0/0	17/22	6/7	34/45
M & 45-49	5/6	2/4	0/0	17/23	2/7	26/40
M & 50-54	9/14	4/7	0/0	23/30	1/3	37/54
M & 55-59	2/4	2/3	0/0	19/32	1/1	24/40
M & 60-64	9/13	0/0	0/0	9/9	0/0	18/22
M & 65-69	1/5	0/0	0/0	13/12	0/0	14/17
M & ≥ 70	5/4	0/0	0/0	10/23	0/0	15/27
Mean age at diag	gnosis (year)					
F & < 15	-	13	12	-	-	12
F & 15-19	18	17	18	-	18	18
F & 20-24	23	23	22	24	22	22
F & 25-29	27	27	27	27	27	27
F & 30-34	32	32	32	32	33	32
F & 35-39	37	37	35	38	37	37
F & 40-44	43	42	-	43	42	42
F & 45-49	47	47	-	47	47	47
F & 50-54	52	52	-	52	51	52
F & 55-59	57	55	-	57	58	57

	New	French	French	French Cathy	Cuba	Pooled
	Caledonia	Polynesia	Young-Thyr			Study
Characteristics	(744)	(600)	(1681)	(1293)	(415)	(4733)
	Case /	Case /	Case /	Case /	Case /	Case /
	Control	Control	Control	Control	Control	Control
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
F & 60-64	62	62	-	62	60	62
F & 65-69	68	-	-	67	-	68
F & ≥ 70	74	-	-	75	-	75
M & <15	-	-	12	-	-	12
M & 15-19	-	-	17	-	18	17
M & 20-24	-	21	22	-	23	22
M & 25-29	-	26	28	28	26	27
M & 30-34	34	31	32	33	32	32
M & 35-39	38	38	35	37	37	37
M & 40-44	43	43	-	42	42	42
M & 45-49	47	48	-	47	46	47
M & 50-54	52	53	-	53	52	52
M & 55-59	58	57	-	57	57	57
M & 60-64	63	-	-	62	-	62
M & 65-69	69	-	-	68	-	68
M & ≥ 70	73	-	-	75	-	74

F: Female; M: Male.

4.2.2.2 Mixed Models

Although I did consider using linear generalized mixed model (using PROC GLIMMIX procedure of SAS® v9.4), in order to take into account possible random effect for study and for dietary iodine intake, I did not use the obtained results with this method and the results presented in this thesis were obtained with conditional logistic regression without random effect.

Indeed, the number of studies in this present pooled analysis is small, only five, and linear generalized mixed model to estimate random effect for dietary iodine intake between studies is not well adapted to such a small number, in particular when using logit link (200).

In our case, the GLIMMIX procedure of SAS® was unable to estimate both a random study effect (intercept in dose-response for iodine) and a random effect for iodine (slope of the dose-response for iodine), this inability whatever the number of qpoints for the quadrature we specified in the GLIMMIX procedure in order to fit the integration which approximates the likelihood (201).

4.2.2.3 Conditional Logistic Regression

The data were analyzed by conditional logistic regression (202) with the software SAS® v9.4 (Statistical Analysis System, SAS Institute, Inc., Cary, North Carolina), applying the PROC LOGISTIC procedure.

Logistic regression (203) is a model used to estimate the probability of a binary response Y based on one or more independent variables X_i which can be quantitative or qualitative. It allows one to say that the presence of a risk factor X_i increases the probability of a given disease Y (here, differentiated thyroid cancer) by a specific percentage: $P(D^+|X_1, X_2, ..., X_p)$.

In the case of a single variable X, the logistic regression model is written: $P(D^+|X) = f(X) = \frac{1}{1 + e^{-(\alpha + \beta X)}}$, here f is the logistic function.

For example, the association between the disease D and an exposure X (exposed X_1 / unexposed X_0) is measured with the following logistic model:

- for the exposed subjects:
$$P(D^+|X_1) = P_1 = \frac{1}{1 + e^{-(\alpha + \beta)}}$$
 and $1 - P_1 = \frac{e^{-(\alpha + \beta)}}{1 + e^{-(\alpha + \beta)}}$

96

$$Logit(P_1) = Ln \frac{P_1}{1+P_1} = \alpha + \beta X_1$$

- for the unexposed subjects: $P(D^+|X_0) = P_0 = \frac{1}{1+e^{-\alpha}}$ et $1-P_0 = \frac{e^{-\alpha}}{1+e^{-\alpha}}$

$$Logit(P_0) = Ln \frac{P_0}{1+P_0} = \alpha + \beta X_0$$

We thus obtain the odds ratio (OR) defined by:

$$Ln(OR) = Logit(P_1) - Logit(P_0) = \beta(X_1 - X_0) \text{ or } OR = \frac{P_1/(1 - P_1)}{P_0/(1 - P_0)} = e^{\beta(X_1 - X_0)}.$$

 β is estimated by maximum likelihood estimation (MLE) method, which estimating the parameters of a statistical model given observations, by finding the parameter values that maximize the likelihood of making the observations given the parameters.

In case-control studies with matched data, the conditional logistic regression model is used. A matched population sample can be considered as a stratified sample at the extreme, each stratum consisting of the subjects of the same pair. Then, the conditional logistic regression model is written: $P(D^+|X) = \frac{1}{1 + e^{-(\alpha_k + \beta X)}}$, here k is the number of strata and α_k varies from one stratum to another.

To estimate the parameters, the likelihood V_k in each stratum was calculated. After simplification, the conditional likelihood for the stratum k is written: $V_k = \frac{e^{-\beta x_{0k}}}{e^{-\beta x_{0k}} + e^{-\beta x_{1k}}}$, V_k no longer depends on α_k . Then, the product of V_k is made to obtain the total likelihood.

The value $\hat{\beta}$ that maximizes this total likelihood makes it possible to calculate $\exp(\hat{\beta})$ which is an unbiased estimate of the OR.

For potential risk factors, ORs (95% CI) for the risk of DTC were computed by conditional logistic regression models, stratified by age class, sex and study, and they were later adjusting for smoking status, ethnicity, education level, number of full term pregnancies, body surface area (BSA), radiotherapy for previous cancer, first-degree relative thyroid cancer history, and energy intake. 3428 individuals have completed data for all these variables. In addition, to

look for a dose- response relationship, intake of selected nutrients and food items was divided into quartiles of consumption, and adjusted odds ratios were obtained for each consumption level. To test for linear trend in the logit of risk, a trend variable for consumption was entered into the logistic model, this variable was assigned the median value of each quartile, then modeled as a continuous variable. Likelihood ratio tests comparing models with and without multiplicative interaction terms were performed to assess the significance of interactions, by entering into the model the main effects and an interactive term.

Assuming a prevalence of exposure close to 30%, the size of this study (about 2,200 cases and 2,500 controls) was large enough to allow us to reach a power of 80% for identifying the role of a risk factor associated with an odds ratio (OR) of 1.8 when accepting a type I error of 5%.

4.3 POPULATION DESCRIPTION

4.3.1 CHARACTERISTICS OF THE STUDIED POPULATION

Average age of the study participants in this pooled analysis was 39, the youngest was 9 and oldest was 83. Table 4-3 shows us the distribution of the cases and the controls in each age class. Study participants were older in the Cathy study and younger in the Young-Thyr study, comparing with the other studies. The majority of the population in this pooled analysis was females (80%), nonsmoker (53%) and Europeans (64%, ethnicity was declared by the study participants regarding to the ethnicity of his or her parents). The French Polynesia and the Cuba studies had the more mixed races participants compared to the other studies; In the French Polynesia study, Polynesian - European mixed race had the highest rate among all the mixed races and in the Cuba study, Afro-Cubans accounted for 30% of the total study participants in accord with the 2012 Cuban Census, Afro-Cubans accounted for 35% of the population (204). Their ancestors originated predominantly from the Congo in Central Africa. The average of body surface area was a little bit higher in the French Polynesia study comparing with the other studies. The 2 metropolitan France studies had more participants whose education level was Baccalaureate or higher, and in the pooled analysis, more controls than cases whose education level was higher than BEPC, CAP, BEP. More than 40% of the female cases in the New Caledonia and the French Polynesia studies declared having 4 or more full term pregnancies. In this pooled analysis, the majority of the DTC was papillary thyroid cancer (88%), only 3% of the cases declared that they had had radiotherapy treatment to a previous cancer (excluding thyroid cancer), and only 5.5% of the cases declared that they had had a first-degree relative thyroid cancer history (Table 4-3).

	New Caledonia	French Polynesia	French Young-Thyr	French Cathy	Cuba	Pooled Study
Characteristics	(744)	(000) Casa/Control	(1001) Casa/Control	(1293)	(413)	(4/33)
Characteristics	N (%)	N (%)	N(%)	N (%)	N (%)	N (%)
Over all	332 (44.6)/412 (55.4)	229 (38.2)/371 (61.8)	805 (47.9)/876 (52.1)	593 (45.9)/700 (54.1)	203 (48.9)/212 (51.1)	2162 (45.7)/2571 (54.3)
Age at diagnosis						
< 15	0 (0.0)/0 (0.0)	4 (1.8)/6 (1.6)	18 (2.2)/18 (2.1)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	22 (1.0)/24 (0.9)
15-19	1 (0.3)/1(0.2)	4 (1.8)/5 (1.4)	57 (7.1)/64 (7.3)	0 (0.0)/0 (0.0)	8 (3.9)/5 (2.4)	70 (3.2)/75 (2.9)
20-24	9 (2.7)/12 (2.9)	15 (6.6)/27 (7.3)	170 (21.1)/183 (20.9)	0 (0.0)/2 (0.3)	15 (7.4)/19 (9.0)	209 (9.7)/243 (9.5)
25-29	24 (7.2)/24 (5.8)	24 (10.5)/42 (11.3)	266 (33.0)/290 (33.1)	16 (2.7)/45 (6.4)	14 (6.9)/23 (10.9)	344 (15.9)/424 (16.5)
30-34	35 (10.5)/43 (10.4)	30 (13.1)/45 (12.1)	275 (34.2)/302 (34.5)	35 (5.9)/57 (8.1)	26 (12.8)/24 (11.3)	401 (18.6)/471 (18.3)
35-39	33 (9.9)/42 (10.2)	46 (20.1)/76 (20.5)	19 (2.4)/19 (2.2)	55 (9.3)/64 (9.1)	38 (18.7)/38 (17.9)	191 (8.8)/238 (9.3)
40-44	35 (10.5)/44 (10.7)	32 (14.0)/53 (14.3)	0 (0.0)/0 (0.0)	59 (10.0)/89 (12.7)	49 (24.1)/49 (23.1)	175 (8.1)/235 (9.1)
45-49	41 (12.4)/38 (9.2)	31 (13.5)/53 (14.3)	0 (0.0)/0 (0.0)	76 (12.8)/82 (11.7)	34 (16.8)/36 (17.0)	182 (8.4)/209 (8.1)
50-54	39 (11.8)/55 (13.4)	36 (15.7)/54 (14.6)	0 (0.0)/0 (0.0)	115 (19.4)/99 (14.1)	12 (5.9)/13 (6.1)	202 (9.3)/221 (8.6)
55-59	42 (12.7)/41 (10.0)	6 (2.6)/9 (2.4)	0 (0.0)/0 (0.0)	87 (14.7)/90 (12.9)	5 (2.5)/3 (1.4)	140 (6.5)/142 (5.5)
60-64	33 (9.9)/57 (13.8)	1 (0.4)/1 (0.3)	0 (0.0)/0 (0.0)	55 (9.3)/51 (7.3)	2 (1.0)/2 (0.9)	91 (4.2)/111 (4.3)
65-69	19 (5.7)/24 (5.8)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	44 (7.4)/55 (7.9)	0 (0.0)/0 (0.0)	63 (2.9)/79 (3.1)
≥ 70	21 (6.3)/31 (7.5)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	51 (8.6)/66 (9.4)	0 (0.0)/0 (0.0)	72 (3.3)/97 (3.8)
Sex						
Female	293 (88.3)/354 (85.9)	203 (88.7)/324 (87.3)	633 (78.6)/679 (77.5)	463 (78.1)/501 (71.6)	179 (88.2)/173 (81.6)	1771 (81.9)/2031 (79.0)
Male	39 (11.8)/58 (14.1)	26 (11.4)/47 (12.7)	172 (21.4)/197 (22.5)	130 (21.9)/199 (28.4)	24 (11.8)/39 (18.4)	391 (18.1)/540 (21.0)
Smoking						
Nonsmoker	196 (59.0)/236 (57.3)	101 (44.1)/196 (52.8)	420 (52.2)/412 (47.0)	323 (54.5)/355 (50.7)	144 (70.9)/125 (59.0)	1184 (54.8)/1324 (51.5)
Formal smoker	51 (15.4)/64 (15.5)	34 (14.9)/32 (8.6)	126 (15.7)/112 (12.8)	175 (29.5)/191 (27.3)	18 (8.9)/24 (11.3)	404 (18.7)/423 (16.5)
Current smoker	85 (25.6)/122 (27.2)	94 (41.1)/143 (38.5)	259 (32.2)/352 (40.2)	95 (16.0)/154 (22.0)	41 (20.2)/63 (29.7)	574 (26.6)/824 (32.1)
Ethnicity						
European	42 (12.7)/133 (32.3)	0 (0.0)/1 (0.3)	684 (85.0)/811 (92.6)	568 (95.8)/683 (97.6)	57 (28.1)/38 (17.9)	1351 (62.5)/1666 (64.8)
Polynesian	10 (3.0)/9 (2.2)	123 (53.7)/205 (55.3)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	133 (6.2)/214 (8.3)
Melanesian	244 (73.5)/189 (45.9)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	244 (11.3)/189 (7.4)
Asian (Vietnamese, Chinese,)	15 (4.5)/31 (7.5)	4 (1.8)/20 (5.4)	0 (0.0)/0 (0.0)	2 (0.3)/0 (0.0)	0 (0.0)/0 (0.0)	21 (1.0)/51 (2.0)
African or Caribbean	0 (0.0)/0 (0.0)	0 (0.0)/0 (0.0)	68 (8.5)/24 (2.7)	5 (0.8)/2 (0.3)	49 (24.1)/75 (35.4)	122 (5.6)/101 (3.9)
Other (Maghreb, Wallisian)	21 (6.3)/50 (12.1)	102 (44.5)/145 (39.1)	53 (6.6)/41 (4.7)	18 (3.0)/15 (2.1)	97 (47.8)/99 (46.7)	291 (13.5)/350 (13.6)

Table 4-3 - Characteristics of the Studied 5 Case-Control Studies from Epi-Thyr Consortium

	New Caledonia (744)	French Polynesia (600)	French Young-Thyr (1681)	French Cathy (1293)	Cuba (415)	Pooled Study (4733)
Characteristics	Case/Control N (%)	Case/Control N (%)	Case/Control N (%)	Case/Control N (%)	Case/Control N (%)	Case/Control N (%)
BSA						
Mean, Median (Min - Max)	1.8, 1.8 (1.1-2.6)	1.9, 1.9 (1.4-2.9)	1.7, 1.7 (0.8-2.6)	1.8, 1.8 (1.3-2.6)	1.8, 1.7 (1.3-2.7)	1.8, 1.8 (0.8-2.9)
Education						
Never go to school / Primary school	170 (51.2)/161 (39.1)	101 (44.1)/136 (36.7)	51 (6.3)/27 (3.1)	46 (7.8)/37 (5.3)	23 (11.3)/10 (4.7)	391 (18.1)/371 (14.4)
BEPC, CAP, BEP	135 (40.7)/198 (48.1)	119 (52.0)/182 (49.1)	190 (23.6)/163 (18.6)	340 (57.3)/367 (52.4)	113 (55.7)/125 (59.0)	897 (41.5)/1035 (40.3)
Baccalaureate or higher	27 (8.1)/53 (12.9)	9 (3.9)/53 (14.3)	564 (70.1)/686 (78.3)	207 (34.9)/296 (42.3)	67 (33.0)/77 (36.3)	874 (40.4)/1165 (45.3)
Number of full term pregnancies						
0	74 (22.3)/107 (26.0)	53 (23.1)/106 (28.6)	486 (60.4)/549 (62.7)	168 (28.3)/250 (35.7)	59 (29.1)/100 (47.2)	840 (38.9)/1112 (43.3)
1	36 (10.8)/55 (13.4)	18 (7.9)/46 (12.4)	124 (15.4)/150 (17.1)	86 (14.5)/90 (12.9)	50 (24.6)/54 (25.5)	314 (14.5)/395 (15.4)
2	31 (9.3)/77 (18.7)	27 (11.8)/58 (15.6)	120 (14.9)/130 (14.8)	166 (28.0)/186 (26.6)	66 (32.5)/40 (18.9)	410 (19.0)/491 (19.1)
3	42 (12.7)/47 (11.4)	35 (15.3)/48 (12.9)	42 (5.2)/32 (3.7)	105 (17.7)/120 (17.1)	18 (8.9)/15 (7.1)	242 (11.2)/262 (10.2)
≥4	149 (44.9)/126 (30.6)	96 (41.9)/113 (30.5)	33 (4.1)/15 (1.7)	68 (11.5)/54 (7.7)	10 (4.9)/3 (1.4)	356 (16.5)/311 (12.1)
Histology						
Papillary	288 (86.8)/0 (0.0)	177 (77.3)/0 (0.0)	720 (89.4)/0 (0.0)	526 (88.7)/0 (0.0)	189 (93.1)/0 (0.0)	1900 (87.9)/0 (0.0)
Follicular	44 (13.3)/0 (0.0)	52 (22.7)/0 (0.0)	75 (9.3)/0 (0.0)	67 (11.3)/0 (0.0)	14 (6.9)/0 (0.0)	252 (11.7)/0 (0.0)
Missing data	0 (0)/412 (100.0)	0 (0)/371 (100.0)	10 (1.2)/ 876 (100.0)	0 (0.0)/700 (100.0)	0 (0.0)/212 (100.0)	10 (0.4)/ 2571 (100.0)
Radiotherapy treatment to cancer						
Yes	13 (3.9)/9 (2.2)	8 (3.5)/2 (0.5)	15 (1.9)/4 (0.5)	25 (4.2)/16 (2.3)	6 (3.0)/3 (1.4)	67 (3.1)/34 (1.3)
No	319 (96.1)/403 (97.8)	221 (96.5)/369 (99.5)	790 (98.1)/872 (99.5)	568 (95.8)/684 (97.7)	197 (97.0)/209 (98.6)	2095 (96.9)/2537 (98.7)
First-degree relative thyroid cancer hist	tory					
Yes	28 (8.4)/14 (3.4)	12 (5.2)/7 (1.9)	46 (5.7)/28 (3.2)	25 (4.2)/10 (1.4)	7 (3.5)/3 (1.4)	118 (5.5)/62 (2.4)
No	304 (91.6)/398 (96.6)	217 (94.8)/364 (98.1)	759 (94.3)/848 (96.8)	568 (95.8)/690 (98.6)	196 (96.6)/209 (98.6)	2044 (94.5)/2509 (97.6)

4.3.2 FOOD CONSUMPTIONS OF THE STUDY PARTICIPANTS

Table 4-4 shows us the average of food consumptions (g/day) in the 20 sub-groups by the case, the control, and all the study participants, the detailed food items of the sub-groups were the same as those in the Cuba study that I presented before (see 3.2.1 Table 3-1). Overall, fruits (456 g/day), other vegetables (239 g/day) and yoghurt (148 g/day) were the first three most consumed foods among all the study participants comparing with chips (16 g/day), egg (16 g/day) and shellfish (9 g/day) which were the least consumed foods in the studied pooled populations (Figure 4-5).

Food item or sub-group	Case Mean (g/day)	Control Mean (g/day)	All study participants Mean (g/day)
Fruits	444	466	456
Other vegetables	238	241	239
Yoghurt	153	143	148
Rice	98	91	94
Milk	68	69	69
Meats	68	67	68
Alcohol	46	68	58
Tomatoes	56	56	56
Pasta	52	53	53
Dessert	55	45	50
Potato	49	47	48
Cheese	41	39	40
Poultry	39	37	38
Fish	29	31	30
Pulses	29	27	28
Cabbages	29	26	27
Sausage or charcuterie	20	21	20
Chips	17	16	16
Egg	15	16	16
Shellfish	9	9	9
Total foods consumption	1554	1569	1562

Table 4-4 - Food consumptions of the pooled analysis of the 5 studies' participants(By decreasing order of the 20 sub-groups)



Figure 4-5 - Mean of food consumptions of the pooled analysis of the 5 studies' participants (by decreasing order of the 20 sub-groups: g/day/person)

4.3.3 DIETARY ENERGY INTAKE OF THE STUDY PARTICIPANTS

Table 4-5 shows us the average of dietary energy intake (kcal/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, fruits (260 kcal/day), rice (139 kcal/day) and dessert (134 kcal/day) were the first three food sub-groups which had the most dietary energy intake among all the study participants comparing with tomatoes (23 kcal/day), shellfish (10 kcal/day) and cabbages (9 kcal/day) which had the least dietary energy intake in the studied populations (Figure 4-6).

Food item or sub-group	Case (kcal/day)	Control (kcal/day)	All study participants (kcal/day)
Fruits	248	270	260
Rice	145	134	139
Dessert	145	125	134
Meats	133	134	134
Yoghurt	135	124	129
Cheese	125	121	123
Other vegetables	86	87	87
Pasta	75	78	77
Poultry	76	73	74
Potato	64	62	63
Milk	52	56	54
Sausage or charcuterie	49	52	50
Alcohol	37	53	46
Fish	43	46	45
Chips	44	41	42
Pulses	29	27	28
Egg	26	28	27
Tomatoes	23	23	23
Shellfish	10	10	10
Cabbages	9	9	9
Total	1556	1551	1553
0	50 100	150 20	0 250 30
Rice Rice Dessert Meats Yoghurt Cheese Other vegetables Pasta Poultry Potato Milk Sausage or charcuterie Alcohol Fish Chips Pulses Egg		All stud	ly participants
Egg Tomatoes Shellfish Cabbages		Case Control	

Table 4-5 - Dietary energy intake of the pooled analysis of the 5 studies' participants (By decreasing order of the 20 sub-groups)

Figure 4-6 - Mean of dietary energy intake of the pooled analysis of the 5 studies' participants (by decreasing order of the 20 sub-groups: kcal/day/person)

4.3.4 DIETARY IODINE INTAKE OF THE STUDY PARTICIPANTS

Table 4-6 shows us the average of dietary iodine intake (μ g/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, yoghurt (23.43 μ g/day), fish (13.82 μ g/day) and cheese (11.13 μ g/day) were the first three food sub-groups which had the most dietary iodine intake among all the study participants comparing with pulses (1.11 μ g/day), alcohol (0.86 μ g/day) and cabbages (0.30 μ g/day) which had the least dietary iodine intake in the studied populations (Figure 4-7).

Food item or sub- group	Case (µg/day)	Control (µg/day)	All study participants (µg/day)
Yoghurt	24.24	22.74	23.43
Fish	13.71	13.92	13.82
Cheese	11.33	10.97	11.13
Shellfish	8.67	9.73	9.25
Milk	8.77	8.45	8.60
Dessert	8.23	6.97	7.55
Egg	6.89	7.23	7.07
Other vegetables	5.85	6.73	6.33
Meats	4.32	4.42	4.38
Chips	4.61	4.08	4.32
Fruits	4.49	3.97	4.21
Sausage or			
charcuterie	3.21	3.44	3.33
Rice	3.32	3.31	3.31
Tomatoes	2.22	2.24	2.23
Poultry	2.20	2.11	2.15
Pasta	1.75	1.70	1.72
Potato	1.46	1.40	1.43
Pulses	1.15	1.07	1.11
Alcohol	0.69	1.01	0.86
Cabbages	0.33	0.27	0.30
Total	117.45	115.77	116.54

Table 4-6 - Dietary iodine intake of the pooled analysis of the 5 studies' participants(By decreasing order of the 20 sub-groups)



Figure 4-7 - Mean of dietary iodine intake of the pooled analysis of the 5 studies' participants (by decreasing order of the 20 sub-groups: µg/day/person)

4.3.5 DIETARY SELENIUM INTAKE OF THE STUDY PARTICIPANTS

Table 4-7 shows us the average of dietary selenium intake (μ g/day) in the 20 sub-groups of the cases, the controls and all the study participants, respectively. Overall, fish (17.18 μ g/day), meats (7.25 μ g/day) and poultry (6.50 μ g/day) were the first three food sub-groups which had the most dietary selenium intake among all the study participants comparing with tomatoes (0.35 μ g/day), alcohol (0.29 μ g/day) and chips (0.14 μ g/day) which had the least dietary selenium intake in the studied pooled populations (Figure 4-8).

Food item or sub-group	Case (µg/day)	Control (µg/day)	All study participants (µg/day)
Fish	17.00	17.33	17.18
Meats	7.29	7.22	7.25
Poultry	6.76	6.29	6.50
Fruits	5.98	5.43	5.68
Egg	5.35	5.37	5.36
Other vegetables	5.05	5.21	5.14
Yoghurt	3.63	3.40	3.51
Pasta	3.03	3.41	3.24
Shellfish	3.29	3.08	3.18
Rice	3.16	2.66	2.89
Cheese	2.88	2.74	2.81
Pulses	2.39	2.16	2.27
Sausage or charcuterie	2.16	2.30	2.23
Dessert	1.38	1.18	1.27
Cabbages	1.33	1.11	1.21
Milk	0.98	0.97	0.98
Potato	0.84	0.84	0.84
Tomatoes	0.35	0.35	0.35
Alcohol	0.23	0.34	0.29
Chips	0.15	0.13	0.14
Total	73.22	71.53	72.30
0.00	5.00	10.00 15	.00 20.00
Fish			
Meats			
Poultry			
Fruits			
Egg Other vegetables			
Yoghurt			
Pasta			
Shellfish			
Rice			
Pulses			
Sausage or charcuterie			
Dessert			
Cabbages	•		
Milk Poteto	•	All study parti	cipants
Tomatoes		Case	
Alcohol			

Table 4-7 - Dietary selenium intake of the pooled analysis of the 5 studies' participants(By decreasing order of the 20 sub-groups)

Figure 4-8 - Mean of dietary selenium intake of the pooled analysis of the 5 studies' participants (by decreasing order of the 20 sub-groups: µg/day/person)

Chips

Control

4.3.6 DIETARY PATTERNS OF EACH STUDY IN THE POOLED ANALYSIS

Figure 4-9 is a brief summary of the mean of the top 3 consumed foods (g/day/person) as well as the mean of dietary energy (kcal/day/person), iodine and selenium intake (µg/day/person) by the cases, the controls and all the study participants of each study in the pooled analysis, by order of the first 3 foods which had most dietary elements intake; In general, fruits, other vegetables and rice were the most consumed foods for all studies, particularly, there was a more fruits consumption in the insular populations, especially in the French Polynesian population (1108 g/day/person), and there was a more yoghurt consumption in the metropolitan French population. The main dietary energy intake was from fruits, rice, and meats consumptions in the New Caledonia study and the Cuba study. In the French Polynesia study, fruits, milk and rice consumptions brought principal parts of dietary energy (687, 177 and 171 kcal/day/person respectively). In Young-Thyr study, the most dietary energy intake was from yoghurt, meats and cheese consumptions, almost the same case in Cathy study, excepting that the most dietary energy intake was from dessert consumption (200 kcal/day/person). When we look at the foods which brought the most dietary iodine intake, except the Cuba study, in the other 4 studies, fish consumption brought the more dietary iodine intake. Except the French Polynesia study, in the other 4 studies, dairy products (milk, yoghurt, cheese) consumptions brought the more dietary iodine intake. In the metropolitan French population on using the same food composition table (the French CIQUAL 2013 food composition table 124), the Young-Thyr study and the Cathy study had the same foods (yoghurt, cheese and fish) which brought the most dietary iodine intake, regarding the study participants in the Young-Thyr study had about twice yoghurt consumption than the study participants in the Cathy study, mean of dietary iodine intake from yoghurt consumption among the study participants in the Young-Thyr study was also about twice as it among the study participants in the Cathy study. When we look at the foods which brought the most dietary selenium intake, except the Cuba study, in the other 4 studies, fish was the top food which brought the most dietary selenium intake, confirmed that iodine or selenium content is rich in fish. In the Cuba study, poultry, meats and pulses consumptions brought the most dietary selenium intake (23.17, 15.57 and 11.96 µg/day/person respectively). Dietary selenium intake from fish consumption was higher in the French Polynesia study (62.24 $\mu g/day/person$) than in the other studies. Dietary selenium intake from poultry consumption was higher in the Cuba study (23.17 $\mu g/day/person$) than in the other studies.



Figure 4-9 - (1) Mean of the top 3 consumed foods of each study (g/day/person); (2) Mean of dietary energy intake of each study (by order of the first 3 foods which brought the most dietary energy intake: kcal/day/person); (3) Mean of dietary iodine intake of each study (by order of the first 3 foods which brought the most dietary iodine intake: μg/day/person); (4) Mean of dietary selenium intake of each study (by order of the first 3 foods which brought the most dietary selenium intake: μg/day/person), by case, control and all study participants in the pooled analysis

4.4 RESULTS

4.4.1 DIETARY IODINE INTAKE

4.4.1.1 Pooled Analysis of Epi-Thyr Consortium

Overall, the majority of the cases (n=966, 45%) and the controls (n=1,164, 45%) were considered as being in mild iodine deficiency according to the IGN classification (APPENDIXES 3Supplement Table 2). When considering the pooled analysis of the 5 case-control studies from Epi-Thyr consortium, we did not evidence a significant association between dietary iodine intake and DTC risk, either when considering the quartiles of dietary iodine intake of the controls in each study (with or without iodine in bread, Table 4-8, Table 4-12, Table 4-13), or when considering estimated dietary iodine intake (with or without iodine in bread) as a continuous variable in a logit linear or a linear model (Table 4-11), or when classifying by the IGN classification (Crude p-trend = 0.1, APPENDIXES 3Supplement Table 2).

This no significant association was confirmed when excluding the microcarcinoma cases (Table 4-11, Table 4-12, Table 4-13).

Dietary iodine intake, in quartiles of the value distribution of the controls in each study						
	Q1	Q2	Q3	Q4	p-trend	
Pooled Analysis						
OR ¹ (95% CI)	0.98 (0.78–1.2)	0.99 (0.81–1.2)	0.89 (0.73–1.1)	1.0^{*}	0.5	
The New Caledonia study						
OR ¹ (95% CI)	0.63 (0.35–1.1)	0.61 (0.33-0.95)	0.54 (0.32–0.93)	1.0^{*}	0.1	
The French Polynesia study						
OR ¹ (95% CI)	1.7 (0.86–3.5)	1.6 (0.81–3.0)	1.2 (0.64–2.2)	1.0^{*}	0.05	
The Young-Thyr study						
OR ¹ (95% CI)	1.0 (0.68–1.6)	1.2 (0.81–1.7)	0.96 (0.69–1.3)	1.0^{*}	0.7	
The Cathy study						
OR ¹ (95% CI)	0.94 (0.56–1.6)	1.1 (0.72–1.7)	0.87 (0.60–1.3)	1.0^{*}	0.4	
The Cuba study						
OR ¹ (95% CI)	1.1 (0.62–2.0)	0.56 (0.30-1.1)	1.2 (0.69–2.2)	1.0^{*}	0.7	
Pooled Analysis restricted to non-microcarcinoma						
OR ¹ (95% CI)	1.0 (0.79–1.3)	0.98 (0.78–1.2)	0.87 (0.70–1.1)	1.0*	0.5	

Table 4-8 - Dietary iodine intake and DTC risk, dietary iodine intake classified according to the quartiles of thevalue distribution of the controls in each study of the pooled analysis($\mu g/day$)

¹ Stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, dietary energy intake; *Reference category.

4.4.1.2 Heterogeneity between Studies

The global heterogeneity between studies in dose-response for estimated dietary iodine intake was significant, when classifying iodine intake by the IGN classification (p-heterogeneity = 0.02), or when dietary iodine intake as a continuous variable in μ g/day in a log linear model (p-heterogeneity = 0.02), (Table 4-9).

Because of this significant global heterogeneity between studies, we investigated heterogeneity between each study and the four others. Dose-response in the French Polynesia study was significantly different from the other 4 studies, for all the 3 dietary iodine intake variable classifications. For other studies, interaction reaches significance only for 1 or none of these variable classifications (Table 4-9).

Overall, these results were confirmed when excluding iodine present in bread, the microcarcinoma cases.

Dietary iodine intake	The New	The French	The Young-	The Cathy	The Cuba	Global
classification	Caledonia	Polynesia	Thyr study	study	study	P-
(µg/day)	study	study		•	·	heterogeneity ^{1, 2}
IGN	0.9	0.003	0.1	0.1	0.5	0.02
Continuous variable	0.9	0.001	0.04	0.2	0.8	0.02
Quartiles of the controls in each study	0.6	0.02	0.2	0.6	0.2	0.09

Table 4-9 - Heterogeneity between each study and the other 4 studies, and global heterogeneity

¹Calculated using the likelihood ratio test comparing a model with an interaction term to one without; ² Stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, and dietary energy intake.

4.4.1.3 Heterogeneity between Ethnicity

Because of the heterogeneity observed between studies, and the studies were multiethnic, we thus investigated the interaction between ethnicity and iodine dose-response.

A significant global heterogeneity between ethnicity in dose-response for estimated dietary iodine intake was evidenced when classifying iodine intake by the IGN classification (p-heterogeneity = 0.02), or when dietary iodine intake as a continuous variable (μ g/day) in a log linear model (p-heterogeneity = 0.02), but onrly near from signification when considering the quartiles of the dietary iodine intake of the controls in each study (p-heterogeneity = 0.08), (Table 4-10).

Overall, these results were confirmed when excluding iodine present in bread, the microcarcinoma cases.

European ethnicity counted for about 64% (3017/4733) of the participants in the pooled analysis, that's why this ethnicity was considered as the reference for ethnicity by ethnicity comparison. When considering dietary iodine intake dose-response in European as the reference, iodine dose-response in Polynesian was significantly different for all the 3 of the dietary iodine intake variable classifications (Table 4-10).

Dietary iodine intake classification (µg/day)	Melanesian	Polynesian	African	Others	Global P-heterogeneity ^{1, 2}
IGN	0.5	0.002	0.1	0.2	0.02
Continuous variable	0.6	0.002	0.2	0.3	0.02
Quartiles of the controls in each study	0.6	0.005	0.9	0.04	0.08

Table 4-10 - Heterogeneity between each ethnical group and European group, and global heterogeneity

¹*Calculated using the likelihood ratio test comparing a model with an interaction term to one without;*

² Stratified by age class, sex and study, and adjusted for smoking status, ethnicity (Polynesian versus others), education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, dietary energy intake.

Given the significant heterogeneity in dietary iodine intake dose-response by ethnicity was mostly due to the difference between Polynesian ethnicity and the others, we chose to regroup these other ethnicities in one group to investigate in details this dose-response (Table 4-11, Table 4-12, Table 4-13).

When considering the dose-response for dietary iodine intake in other ethnicities as the reference, the dose-response in Polynesians was significantly different, when dietary iodine intake was considered as a continuous variable in a logit linear model or in a linear model (Table 4-11), or when considering the quartiles of the dietary iodine intake of the controls in each study (Table 4-12, Table 4-13). These results were confirmed when excluding iodine present in bread, near from signification when excluding the microcarcinoma cases (Table 4-11, Table 4-12, Table 4-13).

	Logit linear Model					Linear Model						
Coefficients for iodine (Continuous	All food items Coeffecient (95% CI) ¹		All food items, but bread Coeffcient (95% CI) ¹		All food items Coefficient (95% CI) ¹		All food items, but bread Coefficient (95% CI) ¹					
variable) in µg/day * 1000	Polynesia n	Other ethnici ties	P-value	Polynesia n	Other ethniciti es	P-value	Polynesia n	Other ethnici ties	P-value	Polynesia n	Other ethniciti es	P-value
All cases (4733:	All cases (4733: 2162 cases vs.2571 controls)											
Without interaction	0.64 (-0.5	6, 1.84)	0.3ª	0.32 (-0.7	78, 1.42)	0.6 ^a	0.73 (-0.7	7, 2.25)	0.2 ^a	0.66 (-1.0)2, 2.34)	0.4 ^a
With interaction	-1.83 (-3.89, 0.23)	1.41 (0.04, 2.79)	0.002 ^b	-2.15 (-4.18, - 0.12)	1.13 (-0.16, 2.42)	0.003 ^b	- 0.89 (-1.67, - 0.11)	1.55 (-0.45, 3.55)	0.004 ^b	-0.87 (- 1.73, - 0.02)	1.84 (- 0.54, 4.22)	0.004 ^b
Cases whose can	cer longer di	ameter >	10 mm (39	71: 1400 cas	ses vs.2571	controls)						
Without interaction	0.78 (-0.5	4, 2.11)	0.2 ^a	0.73 (-0.5	51, 1.96)	0.2^{a}	0.76 (-1.04	4, 2.57)	0.3 ^a	0.92 (-1.1	7, 3.01)	0.3 ^a
With interaction	-0.97 (-3.35, 1.53)	1.17 (-0.31, 2.64)	0.09 ^b	-1.13 (-3.61, 1.35)	1.16 (-0.22, 2.55)	0.08 ^b	-0.52 (-2.22, 1.17)	1.33 (-0.91, 3.56)	0.1 ^b	-0.48 (- 2.29, 1.33)	1.68 (- 0.98, 4.34)	0.09 ^b

Table 4-11 - DTC risk associated with dietary iodine intake adjusted by various confounding factors

¹ Stratified by age class, sex and study, and adjusted for smoking status, education level, number of full term pregnancies, body surface area, radiotherapy for *previous cancer, first-degree relative thyroid cancer history, dietary energy intake, and ethnicity (Polynesian versus others);* ^a Adjusted p-trend: calculated using the Wald test for dietary iodine intake modeled as a continuous variable; ^b Adjusted p-interaction: calculated using the likelihood ratio test comparing a model with an interaction term to one without.

	Dieta	ary iodine intake	in quartile o	f the value distril	oution of controls	in each study	
	Logit linea	ur Model ¹	P-value	Linear	Linear Model ¹		
	Coefficient	(95% CI)		Coefficier	nt (95% CI)		
	Polynesian	Other		Polynesian	Other		
		Ethnicities			Ethnicities		
All cases (4733: 21	62 cases vs.2571 co	ntrols)					
Without	-0.012 (-0.084, 0.059)		0.7^{a}	0.0049 (-0.035, 0.045)		0.8^{a}	
interaction							
With	-0.31 (-0.53, -	-0.0014 (-	0.005 ^b	-0.089 (-0.15,	0.0093 (-	0.02 ^b	
interaction	0.09)	0.073, 0.071)		-0.033)	0.032, 0.050)		
Cases whose cancer	longer diameter >	10 mm (3971: 140	0 cases vs.2	571 controls)			
Without	-0.012 (-0.093, 0.070)		0.8^{a}	-0.00092 (-0.045, 0.043)		0.9 ^a	
interaction							
With	-0.27 (-0.56, -	-0.0066 (-	0.06 ^b	-0.084 (-0.16,	0.0025 (-	0.09^{b}	
interaction	0.013)	0.089, 0.075)		-0.0052)	0.043, 0.048)		

Table 4-12 - Odds-Ratio Associated with dietary iodine intake adjusted by Various Confounding Factors

 Table 4-13 - Odds-Ratio Associated with dietary iodine intake (all food items, but bread) adjusted by Various

 Confounding Factors

Dietary	v iodine intake in qua	rtile of the value	distribution o	of controls in each s	study, without iodin	e in bread.			
	Logit linear	Model ¹	P-value	Linear	Model ¹	P-value			
	Coefficient ((95% CI)		Coefficien	t (95% CI)				
	Polynesian	Other		Polynesian	Other				
		Ethnicities			Ethnicities				
All cases (4733: 21	All cases (4733: 2162 cases vs.2571 controls)								
Without	0.00041 (-0.0	64, 0.065)	0.9 ^a	0.0075 (-0.	0.9 ^a				
interaction									
With	-0.28 (-0.50, -	0.013 (-0.052,	0.007 ^b	-0.19 (-0.29, -	0.014 (-0.059, -	0.006^{b}			
interaction	0.068)	0.078)		0.10)	0.086)				
Cases whose cancer	longer diameter > 1() mm (3971· 1400	cases vs 257	71 controls)					
With a d		7(0.072)			001 0 070)	0.08			
Without	-0.0016 (-0.076, 0.073)		0.9	-0.0014 (-0.	.081, 0.078)	0.9			
interaction									
With	-0.25 (-0.53,	0.0055 (-	0.07 ^b	-0.17 (-0.31, -	0.0035 (-0.078,	0.08^{b}			
interaction	0.029)	0.070, 0.081)		0.029)	0.085)				

¹ Stratified by age class, sex and study, and adjusted for smoking status, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, dietary energy intake, and ethnicity (Polynesian versus others);

^{*a*} Adjusted *p*-trend: calculated using the Wald test for dietary iodine intake modeled as a continuous variable (per quartile);

^b Adjusted p-interaction: calculated using the likelihood ratio test comparing a model with an interaction term to one without.

4.4.1.4 Interaction with the 4 Investigated SNPs

For the 4 investigated polymorphisms which had been identified as associated with the risk of developing DTC, the 2 French metropolitan studies have the most Minor Allele Frequencies (MAF) of the SNP rs965513 in both cases and controls comparing with the other studies; For the SNP rs1867277, at the opposite of the other studies, the GG genotype in the cases (22%) of the Young-Thyr study was less frequent than the AA genotype (27%); In general, the A/A allele comparing with the other alleles of the SNP rs1801516 was very less present in both cases and controls of all these 5 studies, which could be considered as a minor allele of *ATM* in general population; However, the T/T allele comparing with the other alleles which was less frequent in both cases and controls in the southern Asia-Pacific populations samples, while it was more frequent in the French population samples, whereas it was more frequent in the cases (35%) but less frequent in the controls (19%) of the Cuban population sample (Table 4-14).

	New Ca	aledonia	French	Polynesia	France (Yo	ung-Thyr)	France	(Cathy)	Cu	ıba
Genotypes	Cases	Controls								
	N (%)									
rs965513 (near FOXE1)	N = 183	N = 194	N = 177	N = 273	N = 697	N = 686	N = 468	N = 540	N = 203	N = 212
G/G	69 (37.7)	94 (48.4)	89 (50.3)	155 (56.8)	186 (26.7)	278 (40.5)	159 (34.0)	242 (44.8)	81 (39.9)	116 (54.7)
G/A	85 (46.5)	84 (43.3)	57 (32.2)	81 (29.7)	334 (47.9)	319 (46.5)	211 (45.1)	231 (42.8)	89 (43.8)	77 (36.3)
A/A	29 (15.9)	16 (8.3)	14 (7.9)	11 (4.0)	170 (24.4)	85 (12.4)	98 (20.9)	66 (12.2)	27 (13.3)	12 (5.7)
Missing data [†]	0 (0.0)	0 (0.0)	17 (9.6)	26 (9.5)	7 (1.0)	4 (0.6)	0 (0.0)	1 (0.2)	6 (3.0)	7 (3.3)
rs1867277 (5'UTR of FOXE1)	N = 183	N = 194	N = 177	N = 273	N = 697	N = 686	N = 468	N = 540	N = 203	N = 212
G/G	72 (39.3)	88 (45.4)	89 (50.3)	147 (53.9)	152 (21.8)	231 (33.7)	139 (29.7)	190 (35.2)	56 (27.6)	87 (41.1)
G/A	86 (47.0)	87 (44.9)	41 (23.2)	65 (23.8)	338 (48.5)	320 (46.6)	223 (47.7)	280 (51.8)	104 (51.2)	91 (42.9)
A/A	24 (13.1)	16 (8.3)	7 (4.0)	9 (3.3)	186 (26.7)	124 (18.1)	102 (21.8)	69 (12.8)	41 (20.2)	32 (15.1)
Missing data [†]	1 (0.6)	3 (1.6)	40 (22.6)	52 (19.1)	21 (3.0)	11 (1.6)	4 (0.9)	1 (0.2)	2 (1.0)	2 (0.9)
rs1801516 (ATM)	N = 183	N = 194	N = 177	N = 273	N = 697	N = 686	N = 468	N = 540	N = 203	N = 212
G/G	175 (95.6)	176 (90.7)	164 (92.7)	260 (95.3)	526 (75.5)	510 (74.4)	356 (76.1)	413 (76.5)	153 (75.4)	162 (76.4)
G/A	6 (3.3)	15 (7.7)	11 (6.2)	8 (2.9)	151 (21.7)	156 (22.7)	99 (21.2)	116 (21.5)	44 (21.7)	42 (19.8)
A/A	2(1.1)	3 (1.6)	0 (0.0)	0 (0.0)	15 (2.2)	15 (2.2)	13 (2.8)	11 (2.0)	0 (0.0)	2 (1.0)
Missing data [†]	0 (0.0)	0 (0.0)	2 (1.1)	5 (1.8)	5 (0.7)	5 (0.7)	0 (0.0)	0 (0.0)	6 (3.0)	6 (2.8)
rs944289 (near NKX2-1)	N = 183	N = 194	N = 177	N = 273	N = 697	N = 686	N = 468	N = 540	N = 203	N = 212
T/T	29 (15.9)	37 (19.1)	25 (14.1)	30 (11.0)	287 (41.2)	207 (30.2)	202 (43.2)	183 (33.9)	70 (34.5)	41 (19.4)
C/T	94 (51.4)	82 (42.3)	59 (33.3)	82 (30.0)	315 (45.2)	346 (50.4)	218 (46.6)	266 (49.3)	85 (41.9)	98 (46.2)
C/C	60 (32.8)	75 (38.6)	84 (47.5)	149 (54.6)	84 (12.1)	122 (17.8)	47 (10.0)	91 (16.8)	47 (23.2)	70 (33.0)
Missing data [†]	0 (0.0)	0 (0.0)	9 (5.1)	12 (4.4)	11 (1.6)	11 (1.6)	1 (0.2)	0 (0.0)	1 (0.5)	3 (1.4)

Table 4-14 - Alleles Frequency of the Four Polymorphisms Which Associated with the Risk of Developing DTC of the Genotyped Participants in each Study

† Missing data were due to low quality of samples for genotyping.

We would like to reproduce the association between these 4 investigated polymorphisms and the risk of DTC in the pooled analysis. In fact, according to our statistical analyses, the SNP rs965513 (near *FOXE1*) and the SNP rs944289 (near *NKX2-1*) significantly associated with DTC risk both in univariate and multivariate analyses (p-trend ≤ 0.0001 , in the multivariate analysis, adjusted variable including dietary iodine intake); However, the SNP rs1867277 (*FOXE1*) significantly associated with DTC risk only in univariate analysis (p-trend < 0.0001); Nevertheless, we did not observe a significant association between the SNP rs1801516 (*ATM*) and DTC risk, neither in univariate nor in multivariate analyses (p-trend = 0.8) (Table 4-15).

	Genotyped par	ticipants	Odds-Ratios			
Genotypes	Cases	Controls	Stratified OR ¹ (95%	Multivariate OR ²		
	N (%)	N (%)	CI)	(95% CI)		
rs965513 (near FOXE1)	N = 1728	N = 1905				
G/G	584 (33.8)	885 (46.5)	1.00*	1.00*		
G/A	776 (44.9)	792 (41.6)	1.46 (1.26–1.69)	1.52 (1.27-1.82)		
A/A	338 (19.6)	190 (9.9)	2.64 (2.14-3.26)	2.70 (2.05-3.55)		
p-trend			<0.0001 ^a	< 0.0001		
Missing data ^{\dagger}	30 (1.7)	38 (2.0)				
rs1867277 (5'UTR	of N = 1728	N = 1905				
FOXE1)						
G/G	508 (29.4)	743 (39.0)	1.00*	1.00*		
G/A	792 (45.8)	843 (44.3)	1.35 (1.16–1.57)	1.07 (0.89-1.29)		
A/A	360 (20.8)	250 (13.1)	2.03 (1.65-2.49)	1.16 (0.89–1.51)		
p-trend			<0.0001 ^a	0.3		
Missing data [†]	68 (3.9)	69 (3.6)				
rs1801516 (ATM)	N = 1728	N = 1905				
G/G	1374 (79.5)	1521 (79.8)	1.00*	1.00*		
G/A	311 (18.0)	337 (17.7)	0.98 (0.82-1.16)	1.00 (0.83-1.21)		
A/A	30 (1.7)	31 (1.6)	0.99 (0.60-1.66)	1.18 (0.67-2.08)		
p-trend			0.8^{a}	0.8		
Missing data [†]	13 (0.8)	16 (0.8)				
rs944289 (near NKX2-1)	N = 1728	N = 1905				
T/T	613 (35.5)	498 (26.1)	1.00*	1.00*		
C/T	771 (44.6)	874 (45.9)	0.72 (0.61-0.84)	0.67 (0.57-0.79)		
C/C	322 (18.6)	507 (26.6)	0.52 (0.43-0.63)	0.45 (0.36-0.55)		
p-trend			0.0001^{a}	< 0.0001		
Missing data [†]	22 (1.3)	26 (1.4)				

Table 4-15 - Association between the Risk of Developing DTC and Investigated 4 SNPs in the Pooled analysis

¹ Stratified by age class, sex and study;

² Stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, dietary energy intake, dietary iodine intake, and number of minor alleles of SNP rs965513, rs1867277, rs1801516, rs944289; 3428 individuals have completed data for all these variables;

* *Reference category;*

^a Crude p-trend, calculated using the conditional maximum likelihood estimation for SNP rs965513; rs1867277; rs1801516; rs944289 (per minor allele); dietary iodine intake modeled as a continuous variable (per quartile);
 [†] Missing data were due to low quality of samples for genotyping.

In the French Polynesia study, a reduction of DTC risk (p-trend = 0.009) was observed among people who consumed more iodine than the median value (p-interaction = 0.02) and does not carry minor allele (A) of the SNP rs965513 near *FOXE1*, this reduction of risk when increasing dietary iodine intake was not observed in the rare allele carriers (Figure 4-10). At the opposite, in the Cuba study, I identified a strong increase of DTC risk associated with the number of copies in the minor allele (A) of this SNP (p-trend = 0.0003), among people who consumed less iodine than the median value, this association did not exist among those who consumed more iodine than the median value (p-interaction=0.01) (Figure 4-10). However, other from the French Polynesia study, the Cuba study didn't have independent effect of iodine intake (Adjusted p-trend = 0.7, Table 4-8). No significant interaction was observed in other studies or in ethnicity, and other SNPs (Table 4-16).

All the above results remained very similar when reanalyzing this pooled analysis on excluding iodine in bread. Moreover, all these results remained very similar when restricting only for papillary thyroid cancer (PTC) patients (88% in this pooled analysis).

Genotypes	Multivariate Odd	ls-Ratios (95%) ^{1, 2}	p-interaction between SNP and dietary iodine intake ²					
	\leq Median Iodine	> Median Iodine		By study		By Ethnicity		
			Pooled					
			study					
rs965513 (near <i>FOXE1</i>)				New Caledonia	0.8	Melanesian	0.3	
G/G	1.00*	1.00*		French Polynesia	0.02	Polynesian	0.4	
G/A	1.43 (1.10–1.86)	1.56 (1.20–2.01)	0.8	French Young- Thyr	0.4	European	0.7	
A/A	2.87 (1.91-4.33)	2.42 (1.65-3.55)		French Cathy	0.3	Others	0.3	
p-trend Missing data [†]	<0.0001	< 0.0001		Cuba	0.01	African	0.07	
rs1867277				New Caledonia	0.4	Melanesian	0.2	
(5'UTR of <i>FOXE1</i>)			0.9					
G/G	1.00*	1.00*		French Polynesia	0.7	Polynesian	0.3	
G/A	1.00 (0.77–1.31)	1.12 (0.85–1.47)		French Young- Thyr	0.7	European	0.5	
A/A	1.21 (0.82–1.79)	1.12 (0.77-1.63)		French Cathy	0.6	Others	0.06	
p-trend Missing	0.4	0.5		Cuba	0.5	African	0.4	
$\frac{\text{data}}{\text{re}^{1901516}(ATM)}$				New Caladonia	0.06	Malanasian	9	
G/G	1.00*	1.00*		French Polynesia	0.90	Polynesian	0.6	
G/A	0.95 (0.72–1.26)	1.01 (0.78–1.32)	0.5	French Young- Thyr	0.9	European	0.8	
A/A	0.91 (0.35-2.32)	1 40 (0 67–2 91)	0.5	French Cathy	0.9	Others	0.5	
p-trend Missing data [†]	0.8	0.6		Cuba	0.1	African	0.1	
rs944289 (near <i>NKX2-1</i>)				New Caledonia	0.4	Melanesian	0.2	
C/C	1.00*	1.00*		French Polynesia	0.4	Polynesian	0.6	
C/T	0.73 (0.57–0.93)	0.61 (0.48–0.77)	0.08	French Young- Thyr	0.4	European	0.7	
T/T	0.50 (0.37-0.69)	0.41 (0.30-0.56)		French Cathy	0.3	Others	0.7	
p-trend Missing data [†]	<0.0001	<0.0001		Cuba	0.4	African	0.2	

Table 4-16 - Association between the	Risk of Developing DTC and the 4 Investigated SNPs, as well as Their
Interactions with	Total Dietary Iodine Intake in the Pooled Analysis

¹ Stratified by age class, sex and study; ² Adjusted p-trend, stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, dietary energy intake and number of minor alleles of SNP rs965513, rs1867277, rs1801516, rs944289; 3428 individuals have completed data for all these variables;

* Reference category; [†] Missing data were due to low quality of samples for genotyping.

[?]Not convertible



Figure 4-10 - Significant Interaction between Dietary Iodine Intake and The SNP rs965513 near FOXE1 for The French Polynesia and the Cuba Studies

4.4.1.5 Interaction with the Number of Pregnancies

We found a significant interaction (p-interaction = 0.05) between number of full term pregnancies and dietary iodine intake classified by IGN classification: DTC risk significantly increased with increasing number of full term pregnancies among the iodine deficiency women (p-trend < 0.0001); less strong association was observed among women who had optimal or more than adequate iodine intake (p-trend =0.02) (Figure 4-11).



Figure 4-11 - Significant Interaction between Dietary Iodine Intake and Number of pregnancies in DTC risk (P-interaction=0.05)

4.4.2 FISH CONSUMPTION

In the pooled analysis, fish consumption provided about 11% of total dietary iodine intake, ranging from 6% in the Cuba study, to 24% in the French Polynesia study. Overall, DTC risk significantly decreased with high fish consumption (Adjusted p-trend = 0.003), the lowest quartile being associated to a DTC risk of 1.33 (95% CI: 1.08-1.64), as compared to the highest quartile (Table 4-17), this result was confirmed when excluding the microcarcinoma cases (Adjusted p-trend = 0.0002).

A significant interaction was observed between studies, the reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than it in the other studies. This significant interaction was observed when considering all the DTC cases (Adjusted p-interaction = 0.04), but was not kept when excluding the microcarcinoma cases (Adjusted p-interaction = 0.1), the OR of DTC risk decreases per quartile of fish consumption being 4.1 in this former analysis.

A significant interaction was also observed with the SNP rs944289 near *NKX2-1*, the reduction in DTC risk per quartile of fish consumption was more important in carrier of at least one minor allele of this SNP. This interaction was almost significant when considering all the DTC cases (Adjusted p-interaction = 0.1), and when excluding the microcarcinoma cases (Adjusted p-interaction = 0.07), the ratio of risk decreases per quartile of fish

consumption between carriers of at least 1 minor allele and the others being also 3.9 in this former analysis.

Table 4-17 - DTC Risk Associated with Fish Consumption, Adjusted by Various Confounding Factors in the Pooled Analysis

	Fish Consumption in quartile of the value distribution of the controls in each study								
	1 st quartile	2 nd quartile	3 rd quartile	4 th quartile	p-trend ³				
Fish									
Cases/controls	618/646	511/634	506/660	527/631					
Stratified OR ¹ (95% CI)	1.14 (0.97-1.33)	0.95 (0.81-1.12)	0.93 (0.79-1.10)	1.00^{*}	0.1^{a}				
Multivariate OR ² (95% CI)	1.33 (1.08–1.64)	1.20 (0.97–1.48)	1.03 (0.84–1.26)	1.00^{*}	0.003 ^b				

¹ Stratified by age class, sex and study;

² Stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, energy intake;

³ Calculated using the Wald test for fish consumption modeled as a continuous variable (per quartile);

* Reference category;

^{*a*} *Crude p*-*trend*;

^b Adjusted p-trend.

4.5 DISCUSSION

4.5.1 SUMMARY OF THE RESULTS

Overall, the majority of the cases (n=966, 45%) and the controls (n=1,164, 45%) were considered as being in mild iodine deficiency according to the IGN classification. There was non-significant association between dietary iodine intake and DTC risk except in the French Polynesia study, a higher dietary iodine intake was significantly associated with a lower DTC risk. This heterogeneity between studies was due to heterogeneity between ethnic groups, mainly due to a difference in dose-effect relationship between Polynesians and other ethnic groups. Among the Polynesians, a higher dietary iodine intake was significantly associated with a lower DTC risk, whereas this association did not present in other ethnic groups.

A reduction of DTC risk was observed among people who consumed more iodine than the median value and does not carry minor allele (A) of the SNP rs965513 near *FOXE1*, this reduction of risk when increasing iodine intake was not observed in the minor allele carriers, this effect has been identified only in the French Polynesia study; however, a strong increase of DTC risk associated with the number of minor allele (A) of this SNP among people who consumed less iodine than the median value, this effect has been identified only in the Cuba study.

DTC risk also significantly increased with increasing number of full term pregnancies among the iodine deficient women.

In the pooled analysis, DTC risk significantly decreased with increasing fish consumption, a significant interaction was observed between studies, the reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than it in the other studies.

4.5.2 STRENGTHS AND LIMITATIONS OF THE STUDY

To the best of the authors' knowledge, this present pool study is the first and the largest casecontrol study to investigate iodine status and thyroid cancer risk in heterogenetic ethnicities across different geographic zone of the world. Another point worth mentioning is that we have elaborated two special food composition tables (FPFCT2013, CFCT 2014, see 1.3.1.3) to measure the quantity of iodine in typically French Polynesian and Cuban food samples for this study, which gave us normative, accurate dietary iodine measurements for the French Polynesian and Cuban populations. Although the two tables have not been applied to all of the food items in the two studies, knowing that until now, no such measurement of iodine has been performed by other organizations for typically French Polynesian, Cuban or Latino food.

As with any case-control study, this pooled analysis of the 5 case-control studies has some limitations. Self-reported food consumptions led to imprecise measures and recall bias. The dietary questionnaires were heterogenetic between each individual study, each study did not have the equal and same food items, because these 5 studies were carried out in 5 different places and the dietary patterns could be different between one and others, in order to solve this problem, we stratified all the statistical analyses by study. Besides, in the daily life, each food item is a dependent variable but in the questionnaire, we inquire the study participant in an independent way, it may over-estimate the daily food consumption, thereby, over-estimate the daily dietary iodine intake. Nevertheless, if this over-estimation exists, it will be systematically applied to both cases and controls in this pooled analysis, so the overall estimation of the DTC risk OR will not be deflective. Additionally, this independent effect was compensated by the frequency of the food consumption (by day, week or month). We agree that urine or blood iodine concentration is regarded as the worldwide standardization to estimate the iodine status of general population. Nevertheless, our case-control studies used food frequency questionnaire rather than urine or blood iodine concentration to measure the dietary iodine intake is not seems like a good way, because they were not designed to estimate
the iodine status of general population, but to perform comparisons of certain potential characteristics (in this present study is the dietary iodine intake) between the cases and the controls. In addition, even if we had performed urine iodine measurement, it would have been confused by thyroid hormone replacement therapy received by the DTC cases of our studies due to their previous thyroidectomy. Therefore, iodine measured in urine of the cases would not have any clinical meaning. Another limitation could come from the difficulties to quantify iodine in diet, as iodine is very volatile, and the use of food composition tables in which the different values for different food items have not been assessed using the same iodine quantification methodology, which led to extremely variable value, in particular for fishes, it strongly depends on the species, the places of capture and the laboratory validation method of iodine measurement in this food, for example, in the French CIQUAL 2013 food composition table (124), for fish group, iodine content in natural albacore tuna was 1 μ g/100g, but in raw tuna was 36.7 μ g/100g. With such a large distribution echelon of iodine measurement among places of capture, laboratories, it did not seem pertinent to propose an average value. This problem only concerns the CIQUAL food composition table, but not the two tables performed by ANSES for our studies.

Another point worth discussing is that, we didn't include iodized salt in the statistical analyses, although we didn't have quantified but qualified information (always; sometimes; no) about iodized salt in our questionnaire, nevertheless, we did have the iodine (10.25 μ g/g, 95% CI = 8.65-11.84) and selenium (0.022 μ g/g, 95% CI = 0.013-0.031) content measurement in the iodized salt in CFCT 2014 (see 1.3.1.3) and in the French CIQUAL 2013 food composition table (124). Indeed, it is difficult to take iodized salt into account because firstly, we didn't know exactly when was the systematic iodized salt policy carried out in the countries or regions included in this pooled analysis. If it was carried out prior to the reference year (cancer diagnostic year for the cases and the same year for their eligible matched controls) of the study participants, normally, 100% of the participants had to use iodized salt in their daily life; if it was carried out during interval of their reference years, it is difficult to know how many percent of the study participants using iodized salt in their daily life, in particular, some participants were even not able to make difference between ordinary salt and iodized salt; if it was carried out after the upper bound of their reference years interval, especially for the elder participants in the French Polynesia and the New Caledonia studies, normally, 0% of the participants used iodized salt in their daily life because it hadn't existed at that time. Moreover, even if we had known the time when launching the iodized salt policy, we would

not have had the accurate information about the frequency and the quantity of using iodized salt in their daily life because salt is not an independent variable, it dependents on the cooking patterns and the dietary habits, for example, table salt supplementation is a dietary habit rather for the French than for the French Polynesian, the New Caledonian and the Cuban. Due to these variabilities and uncertainties, the "iodized salt" issue is quite difficulty to interpret because of the possible confusion.

4.5.3 COMPARISON RESULTS FROM THE LITERATURE

In this pooled analysis, dietary iodine intake didn't significantly associated with DTC risk whatever classified by the IGN classification or by the distribution value of the quartiles in the controls of each study, or considering iodine intake as a continuous variable, or considering the ratio between dietary iodine intake and dietary energy intake. A case-control study with the similar method of ours reported the same result as ours based on 191 cases and 442 controls in Hawaiian adults (103). Another case-control study of 608 women with thyroid cancer and 558 controls in northern California showed that a significant reduction in the papillary thyroid cancer risk was seen in the highest iodine intake quintile (> 537 μ g/day) compared with the lowest (< 273 μ g/day) (OR = 0.49, 95 % CI: 0.29, 0.84). Iodine intake from food alone was not associated with risk, but iodine intake from supplements was (63). The previous paper of the New Caledonia study about iodine intake and thyroid cancer risk found the same result as the present pooled analysis, but at that time, iodine intake was computed only using the French food composition table CIQUAL 1995 (205), which did not have data for some local seafood, so iodine intakes may have been underestimated (104); However, even the present pooled analysis took this previous study limitation into account and used the new measurements made by French ANSES (FPFCT 2013 and CFCT 2014, see 1.3.1.3) for the local food, and for the other general food, the French CIQUAL 2013 food composition table (124) was as a necessary complement, this non-significant association has not been changed. Nevertheless, the present pooled analysis confirmed with the previous paper of the French Polynesia study about the significant reduction of DTC risk with the increasing dietary iodine intake (105). The meta-analysis of publications of animal and human studies about iodine intake and thyroid cancer risk done by Zimmermann et al. (106) examined the four case-control studies described above, by adjusting OR for the highest iodine intake quartile compared to the lowest quartile, and revealed the odds for thyroid cancer risk were 23 % less in the highest quartile of iodine intake versus the lowest, although this effect was only borderline significant (OR = 0.77; 95 % CI: 0.58-1.02; p = 0.068). Moderate evidence for between-study heterogeneity ($I^2 = 42.6$ %; p = 0.058) was reported.

Except the SNP rs944289 near *NKX2-1*, minor alleles of the other three investigated SNPs were not more frequent in the controls of the French Polynesia, New Caledonia and Cuba studies than in the controls of the two French studies (Table 4-14). That suggests the risk allele of the two SNPs in *FOXE1* gene could be more frequent in European populations rather than in Polynesian, Melanesian, Caribbean or other island populations. However, this situation was just reverse for minor allele of the SNP rs944289 near *NKX2-1*. These results were concordant with the previous findings (134).

The result that dietary iodine intake interacts with number of minor allele A of the SNP rs965513 near FOXE1 in the risk of DTC, was valid only for the French Polynesia and Cuba studies, and dietary iodine intake only interacts with this SNP, it may be due to the genetic specificity in these populations. Furthermore, the French Polynesia and the Cuba studies had the more mixed races people compared to the other studies, we assume that being mixed race present less number of minor allele (A) of the SNP rs965513, and less risk of DTC, especially for the half-breeds in French Polynesia who consumed more iodine and for the half-breeds in Cuba who consumed less. To the best of the authors' knowledge, except my previous publication of the Cuba study (113), gene-environment interactions in DTC risk have only been investigated in relation to radiation exposure in two candidate regions approach, in fact, a Chernobyl accident post consequence investigation conducted a genome-wide association study, employing Belarusian patients with PTC aged 0-18 years at the time of accident and age-matched Belarusian control subjects. They identified 4 SNPs (rs925489, rs7850258, rs965513 and rs10759944) at chromosome 9q22.33 showing significant associations with radiation-related PTC (Mantel-Haenszel P: mhp = 1.7×10^{-9} to 4.9×10^{-9}). The association was further reinforced by a validation analysis using one of these SNP markers, rs965513 near *FOXE1*, with a new set of samples (overall mhp = 4.8×10^{-12} , OR = 1.65, 95% CI: 1.43-1.91). They also tested rs944289 near NKX2-1 at 14p13.3, no association with radiation-related PTC was obtained (mhp = 0.17) (44). However, until now, except the studies of Epi-Thyr consortium, no gene-environment interaction study has investigated an association with FOXE1 and dietary iodine intake.

In this pooled analysis, we found that DTC risk significantly increased with increasing number of full term pregnancies among the iodine deficient women. Moreover, the infants born of the mothers with iodine deficiency have high cretinism risk, characterized by severe delays in physical and mental growth, language disorders, deafness and dwarfism. According to the meta-analysis of Bleichrodt and Born, iodine deficiency is the leading cause of brain damage in infants, and it can lose 13.5 IQ points on average (206). A more recent metaanalysis conducted on studies in China produced a very similar result (207). During the pregnancy, hormone synthesis is increased to maintain both mother and fetus metabolism, and iodine requirements are increased by 150 µg/day to 220 µg/day during pregnancy, and 270 µg/day during breastfeeding (208). In the previous publication of the Young-Thyr study about reproductive factors in DTC risk, Xhaard et al. found that having high number of pregnancies was significantly associated with high DTC risk in women with 35 years old or below who lived in eastern France (80). A meta-analysis with 21 studies including 406,329 study participants suggested a strong association of parity (≥ 3 pregnancies) with the risk of thyroid cancer (87). Early population-based case-control study of thyroid cancer also demonstrated that among females, the radiogenic risk appeared to be potentiated by the number of subsequent live-births (88). Three case-control studies performed in the USA (89), in China (90) and in New Caledonia (84) respectively revealed evidence of high risk in women who had been pregnant and who had a high number of pregnancies. However, the results above simply based on the association between number of pregnancies and thyroid cancer risk, in this present pool analysis, we are the first to investigate the interaction between number of pregnancies and iodine status in thyroid cancer risk. A possible explanation for the higher risk of DTC in women who have had a high number of pregnancies and were in iodine deficiency status could be the acceleration on proliferation of thyroid cells, among iodine deficiency people, maybe among pregnant women who were iodine deficiency, the effect of thyroid cells proliferation is accumulate, as maternal thyroid activity increases during pregnancy (91). Estrogens, which are elevated during pregnancy, are associated with elevated serum thyroxin and triiodothyronine levels, which might induce high cell turnover (92). The highest levels of serum TSH, even within the normal range, are associated with a subsequent diagnosis of thyroid cancer in individuals with thyroid abnormalities (93). Repeated pregnancies may increase the risk of DTC by revealing DNA damage due to carcinogens, such as ionizing radiation, that may have remained silent without pregnancies (94).

DTC risk significantly decreased with increasing fish consumption but there was no association with dietary iodine intake within the pooled analysis, however, in general, iodine is rich in fish. This result could be explained by the presence of protection factors in fish,

maybe selenium, maybe omega-3 polyunsaturated fatty acids or others. Several publications showed current evidence for an interaction between genetic polymorphisms and dietary LCn-3 PUFA in the risk of breast, prostate and colorectal cancers, in regards to inflammation and eicosanoid synthesis: Lower activity genotypes of the GSTM1, GSTT1, GSTP1 genes associated with higher breast cancer protection with high intake of marine n-3 FA (209); High intake of n-3 FA associated with low risk of prostate cancer (210); C allele at locus rs5275 correlated with low risk of prostate cancer with high intake of fatty fish (211); Carriers of T allele of rs7873087 of PGES gene with high fish intake have low colorectal cancer risk; Contrarily, carriers of Ile variant of EP4 gene showed correlation between high fish intake but high colorectal cancer risk (212). However, a meta-analysis with systematic review showed that omega-3 polyunsaturated fatty acids has been found to be beneficial for brain development, furthermore, higher fish intake significantly related with lower risk of brain cancer based on nine observational studies, dose-response analysis showed that the RR per 100 g/day increase in fish intake was 0.95 (95% CI: 0.91-0.98) (213). In a recent study by Haslam et al. (214), the effects of fish consumption on the risk of thyroid cancer in humans were examined. Prior to this study, there was no report on any association between ingestion of PCBs (Polychlorinated biphenyls from fish consumption) and thyroid cancer, PCBs are known as endocrine disruptors and have potential effects on thyroid functions. The authors recruited participants (135 sports fishermen and their spouses) from the New York State Angler Cohort Study (215). The controls were matched with the same sex ratio of the thyroid cancer cases. The scientists asked the participants about their fish consumption patterns at the beginning of the study in 1991, and then followed the participants for 17 years (from 1991 to 2008), to observe incidences of thyroid cancer and to determine any differences between the fish consumers and non-fish consumers. Using this information, the authors estimated exposure to PCBs and the intake of omega-3 fatty acids from fish consumption. In total, 27 thyroid cancer cases were reported with 108 control participants. Results showed that there was no significant association between total fish consumption or the estimated PCBs intake from Great Lakes fish and the thyroid cancer incidence. Previously reported findings indicate that fish consumption was associated with a higher thyroid cancer risk in areas with an iodinerich diet, but high fish consumption had a protective effect of thyroid cancer in areas with endemic goiters (216). In the analyses of Haslam et al. (214), there were no differences in previous diagnosis of a thyroid goiter between cases and controls, but fish asked about in these analyses did not come from iodine-rich waters. Taken together, this may partially explain why there was no association seen between Great Lake fish consumption and thyroid cancer. Nevertheless, those who had higher long-term estimated omega-3 fatty acids consumption from Great Lakes fish had lower incidence of thyroid cancer than those with lower omega-3 fatty acids consumption. These results suggest that long-term intake of omega-3 fatty acids from Great Lakes fish may protect against the development of thyroid cancer.

Other explanation maybe the inaccuracy in the estimation of dietary iodine intake in this pooled analysis, it did not only depend on the auto-declaration of our dietary questionnaire, but also depended on all the food composition tables we used for dietary iodine intake calculation. Different food species items, different gastronomies and especially, different criterions of laboratory measuring validation (for example, for the same food item which reached the limit of detection or the limit of quantification of iodine, one laboratory chose the average iodine content of the limit of detection value and the limit of quantification value of several different measurements, another laboratory preferred the least restrictive value which could mediate the limit of detection value or the limit of quantification value), all these variations introduce the inaccuracy, that may be more important than the inaccuracy in the estimation of fish consumption which only depended on the auto-declaration of our dietary questionnaire.

4.6 CONCLUSION

Overall, in this pooled analysis, the majority of the studied population was considered as being in mild dietary iodine deficiency according to the IGN classification. Although DTC risk was not found to be linked to dietary iodine intake, this risk significantly decreased with high fish consumption, this reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than it in the other studies. Additionally, higher dietary iodine intake may reduce DTC risk only in French Polynesia and in Polynesians. A strong increase in DTC risk associated with the number of the minor allele (A) of the SNP rs965513 near *FOXE1*, among people who consumed more iodine than the median value in the French Polynesia study, but among people who consumed less iodine than the median value in the Cuba study. For the women who had a high number of full term pregnancies and who were iodine deficient, increasing dietary iodine intake may reduce their risk of suffering from DTC, thus reduce the hereditary risk to their infants.

5 PART V: FINGERNAIL SELENIUM AND THYROID CANCER RISK: A CASE-CONTROL STUDY IN FRENCH POLYNESIA

Selenium is an essential trace element present mainly in grains, meat, fish, eggs, and dairy products, as well as multivitamin supplements (217, 218). Like iodine, the selenium content of a given food strongly depends upon the geographic location where it is produced, as plant uptake is largely influenced by the availability and chemical species of selenium in the soil (217). The redox-protective properties of selenium are important during oxidative thyroid hormone production, in which thyroid cells produce excess H_2O_2 and reactive oxygen species. Due to its ability to protect the thyroid gland and maintain thyroid hormone production, there is a high concentration of selenium in the thyroid (217), and supply of selenium to the thyroid is prioritized even during periods of selenium deficiency (219).

Although numerous studies have shown that selenium deficiency may be associated with an increased risk for several types of cancer (220, 221, 222, 223), the relationship between selenium and thyroid cancer has been examined in few studies. One in vitro study showed that selenium causes growth inhibition of thyroid cancer cells accompanied by cell-cycle arrest in the S and G2/M phases (224). In addition, a small case-control study of 43 thyroid cancer cases showed a significant increased risk for low compared to high prediagnostic serum selenium concentrations (225).

In the present study, we examined the association between fingernail selenium concentrations with thyroid cancer in a case-control study of men and women in French Polynesia, a country who has high incidence of thyroid cancer in females (226) also highly exposed to selenium mainly through fish consumption (227). In contrast to other biomarker measures of selenium, such as blood or urine, nail concentrations represent longer-term exposure, typically the previous three to six months.

5.1 MATERIALS AND METHODS

5.1.1 STUDY PARTICIPANTS

The process for selection and interviews of thyroid cancer patients and controls has been extensively described in 2.3.2. In total, the study population included 229 interviewed cases and 373 interviewed controls, with 85 cases (37%) matched to one control and 144 cases (63%) matched to two controls. We further excluded 6 cases and 9 controls for missing data on fingernail selenium, and a batch of 8 cases and 33 controls for which all measures of any

elements in the fingernails were returned as null. The final analytic population was 215 cases and 331 controls.

5.1.2 DATA COLLECTION

Details in data collection through the structured questionnaire also have been described in 2.3.2. The slight difference applied to this present study was at the time of the analysis process, the FPFCT 2013 (see 1.3.1.3) was in the laboratory validation process and was not ready for this application, so we used French CIQUAL-REGAL food composition table (205) for the calculation of iodine and selenium intake. The overall iodine intake took into account all foods of the questionnaire, except iodized salt because we had no information on the amount of iodized salt consumption.

All participants provided fingernail clippings on the date of the interview. Clippings were stored in paper envelopes at room temperature before being transferred to the toxicology centre of the Institut National de Santé Publique du Quebec (INSPQ).

5.1.3 FINGERNAIL SELENIUM AND IODINE MEASUREMENTS

Nail samples were digested under basic conditions using trimethylammonium hydroxide for iodine and under acidic conditions with nitric acid for selenium. The digested material was directly analysed by ICP-MS (inductively coupled plasma mass spectrometry, Perkin Elmer Sciex, Elan DRCII with autosampler ESI SC-4 and work station Elan version 3.0) (228). Certified reference materials used were: Human hair powder GBW 07601 and GBW 09101b.

5.1.4 STATISTICAL ANALYSIS

Descriptive statistics and trend tests in univariate analysis were performed using nonparametric Jonckheere-Terpstra test for association between two quantitative variables; nonparametric Wilcoxon rank test for association between a qualitative variable with two classes and a quantitative variable; and non-parametric Kruskal-Wallis rank tests for association between a qualitative variable with more than two classes and a quantitative variable.

The association between selenium in nails and other parameters has been investigated using generalized linear models.

We used conditional logistic regression to estimate OR and 95%CI for thyroid cancer by fingernail selenium concentration. Selenium was categorized into quartiles based on the distribution among the controls. All models were conditioned on year of birth (< 1945, 1945-1955, 1955-1965, \geq 1965) and sex to reduce the potential bias due to the matching process and adjusted for year of birth (continuous, per year) to control for residual confounding by age. Multivariable models were additionally adjusted for ethnicity (Polynesian, mixed, other), education (primary school or lower vs. middle school or higher), BMI at the moment of cancer (< 25, 25-29.9, \geq 30 kg/m², missing), current smoking (yes vs. no), and radiotherapy treatment to head or neck (ever vs. never). These associations were also evaluated by sex, smoking status, BMI, thyroid cancer histology, tumor size, fingernail iodine and dietary iodine. Effect modification of selenium levels by sex, smoking status, BMI, thyroid cancer histology, tumor size, fingernail iodine and dietary iodine test comparing a model with an interaction term to one without. We also investigated for potential bias due to the delay between thyroid cancer diagnosis and fingernails sampling, by performing separate analysis for various categories of delay.

All statistical analyses were conducted using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).

5.2 RESULTS

The cases and controls were generally comparable in terms of ethnicity and level of education. Thirty percent of the cancers' longer diameters were less than 10 mm. About 70% of cases had papillary thyroid cancer, and 30% had a follicular type.

Among controls, higher fingernail selenium levels were observed in older people (p < 0.001), and people who have higher BMI (p = 0.001) and in non-smokers (p < 0.0001), but in a multivariate analysis, only age (p = 0.01) and smoking (p < 0.0001) remained significant (Table 5-1). The highest fingernail selenium levels were in inhabitants of the Marquises Islands ($M = 0.87 \mu g/g$) and of the Tuamotu-Gambier Archipelago ($M = 0.86 \mu g/g$) (Figure 5-1). Adjusting by other factors, including estimated selenium and iodine in diet, selenium in fingernails of the controls from the Tuamotu-Gambier Archipelago remained significantly higher than in the rest of French Polynesia, but not from the Marquises Islands (Table 5-1). On the contrary, among cases, only selenium in fingernails of the Marquises Islands

inhabitants remained higher than in the other inhabitants of French Polynesia (p = 0.05) (Table 5-1).

Table 5-1	- Predictor of	selenium	concentration	in fin	pernails	in thyroid	cancer n	atients i	and	controls
Tuble J-1	- 1 160101 0j	selenium	concentration	ın jınz	gernans i	n myroiu	cuncer p	unemis	unu	connois

	Cases		Controls		
	RegressionCoefficient (SD ^{\dagger})		Regression Coefficient (SD [†])	<i>p</i> -value [*]	
Dietary selenium (mg/1,000 kcal)	<0.001 (<0.001)	0.60	-0.0002 (<0.001)	0.06	
Dietary iodine (mg/1,000 kcal)	-0.0002 (<0.001)	0.30	0.0001 (<0.001)	0.20	
Gender (women/men)	-0.01 (0.03)	0.80	0.04 (0.02)	0.06	
Age (years)	0.001 (0.001)	0.20	0.002 (<0.001)	0.01	
BMI (kg/m ²)	0.003 (0.002)	0.03	0.002 (0.001)	0.07	
Currently smoking (yes/no)	-0.05 (0.02)	0.01	-0.06 (0.01)	0.0001	
Marquises Islands (yes/no)	0.14 (0.07)	0.05	0.04 (0.06)	0.40	
Tuamotu-Gambier Archipelago (yes/no)	0.06 (0.04)	0.10	0.12 (0.03)	0.0002	

[†] Standard deviation; ^{*} Regression coefficient t-test



Figure 5-1 - Fingernail selenium concentration in studied population of French Polynesia

We did not evidence a significant association between fingernail selenium levels and thyroid cancer risk in models that were conditioned on year of birth and gender and further adjusted for year of birth (highest versus lowest quartile: OR = 1.12, 95% CI: 0.66-1.90; p-trend = 0.30) (Table 5-2). After additional adjustment for age at the moment of the diagnosis of cancer, ethnicity, education, BMI, smoking status and radiotherapy treatment to the head or neck, this

association with the risk of thyroid cancer comparing the highest to the lowest quartile was also non-significant (OR = 1.02, 95%CI: 0.56-1.84; p-trend = 0.60) (Table 5-2).

Among the studied population, average fingernail selenium concentration was 0.75 μ g/g in smokers and 0.80 μ g/g in non-smokers (p < 0.0001). We did not observe evidence for a significant effect modification by sex, smoking status, BMI, histological type, fingernail iodine and dietary iodine (all p-interaction > 0.05; Table 5-2). A significant interaction was evidenced between selenium and tumor size (p = 0.04): when restricting the analysis to differentiated thyroid cancer's longer diameter of 10 mm or more, selenium in nails was nearly significantly positively (p = 0.09) linked to thyroid cancer risk. This relationship was enhanced when restricting the analysis to papillary thyroid cancer's longer diameter of 10 mm or more (p = 0.05). Although no significant interaction was evidenced between iodine in nails and selenium in nails effect (p = 0.70), a nearly significant positive association (p = 0.10) between fingernail selenium and thyroid cancer risk was seen in patients with less than 3 ppm of iodine in nails. A similar result also has been found in which a nearly significant positive association (p = 0.10) between fingernail selenium and thyroid cancer risk was seen in patients with less than 117 mg/1,000 kcal of dietary iodine intake (Table 5-2).

Quartiles of selenium ($\mu g/g$)						
	Q1 Q2 Q3 Q4				p- trend [*]	p- interaction ^{**}
	(0.00-0.68)	(0.68-0.76)	(0.76-0.86)	(0.86-1.30)		
Overall						
Cases/controls	51/93	56/81	50/79	58/78		
OR (95% CI) ¹	1.00	1.16 (0.70-1.94)	1.06 (0.64-1.77)	1.12 (0.66-1.90)	0.30	
OR (95% CI) ^{2,3}	1.00	1.08 (0.62-1.90)	1.10 (0.62-1.96)	1.02 (0.56-1.84)	0.60	
Men						
Cases/controls	2/15	8/9	6/5	7/11		
OR (95% CI) ^{2,3}	1.00	$?^{\dagger}$	$?^{\dagger}$	752.6(<0.001->? [†])	0.99	
Women						
Cases/controls	49/78	48/72	44/74	51/67		
OR (95% CI) ^{2, 3}	1.00	0.95 (0.53-1.71)	0.95 (0.51-1.74)	0.90 (0.48-1.70)	0.90	0.20

Table 5-2 - Fingernail selenium and thyroid cancer by various confounding factors in French Polynesia, 1981-2003

Quartiles of selenium ($\mu g/g$)							
	Q1	Q2	Q3	Q4	<i>p</i> -	p-	
	(0.00-0.68)	(0.68-0.76)	(0.76-0.86)	(0.86-1.30)	irena	interaction	
Non-smoker							
Cases/controls	15/33	20/40	27/47	32/49			
OR (95% CI) ^{2,3}	1.00	0.91 (0.38-2.18)	1.02 (0.45-2.32)	1.07 (0.47-2.46)	0.50		
Current smoker							
Cases/controls	36/60	36/41	23/32	26/29			
OR (95% CI) ^{2,3}	1.00	1.29 (0.68-2.43)	1.08 (0.53-2.19)	1.19 (0.59-2.41)	0.40	0.70	
$BMI < 30 \text{ kg/m}^2$							
Cases/controls	35/68	27/52	23/53	24/48			
OR (95% CI) ^{2,3}	1.00	0.95 (0.49-1.84)	0.81 (0.41-1.62)	0.76 (0.37-1.55)	0.80		
$BMI \geq 30 kg/m^2$							
Cases/controls	16/25	28/28	27/26	34/30			
OR (95% CI) ^{2,3}	1.00	1.65 (0.70-3.90)	1.74 (0.73-4.18)	1.97 (0.83-4.66)	0.15	0.30	
Papillary							
Cases/controls	40/58	39/56	37/66	49/56			
OR (95% CI) ^{2,3}	1.00	0.95 (0.52-1.76)	0.81 (0.44-1.49)	1.18 (0.64-2.18)	0.30		
Follicular							
Cases/controls	11/25	17/19	13/10	9/20			
OR (95% CI) ^{2,3}	1.00	1.74 (0.59-5.08)	3.79 (1.12-12.8)	0.98 (0.29-3.29)	0.80	0.60	
Tumours < 10 mm							
Cases/controls	9/25	15/20	17/25	18/24			
OR (95% CI) ^{2,3}	1.00	1.84 (0.59-5.75)	1.79 (0.61-5.29)	1.84 (0.59-5.75)	0.30		
Tumours $\geq 10 \text{ mm}$							
Cases/controls	32/59	30/49	28/43	33/39			
OR (95% CI) ^{2,3}	1.00	1.04 (0.53-2.02)	1.19 (0.60-2.36)	1.37 (0.69-2.72)	0.09	0.04	
Fingernail iodine < 3 ppm							
Cases/controls	24/49	23/43	25/45	27/39			
OR (95% CI) ^{2,3}	1.00	0.98 (0.46-2.09)	1.18 (0.56-2.49)	1.44 (0.66-3.11)	0.10		
Fingernail iodine \geq 3 ppm							
Cases/controls	27/44	33/38	25/34	31/39			
OR (95% CI) ^{2,3}	1.00	1.15 (0.56-2.37)	1.13 (0.52-2.47)	0.99 (0.47-2.06)	0.90	0.70	
Dietary iodine < 117							
(mg/1,000 kcal)	(mg/1,000 kcal)						
Cases/controls	29/38	29/49	23/35	35/36			
OR (95% CI) ^{2,3}	1.00	0.83 (0.41-1.70)	0.95 (0.44-2.06)	1.40 (0.67-2.95)	0.10		

Quartiles of selenium ($\mu g/g$)							
	Q1 Q2 Q3 Q4			p- trend [*]	p- interaction ^{**}		
	(0.00-0.68)	(0.68-0.76)	(0.76-0.86)	(0.86-1.30)			
Dietary iodine ≥ 117							
(mg/1,000 kcal)							
Cases/controls	22/55	27/32	27/44	23/42			
OR (95% CI) ^{2,3}	1.00	1.71 (0.80-3.64)	1.31 (0.63-2.72)	0.94 (0.43-2.06)	0.90	0.50	
Papillary and Tumours							
$\geq 10 \text{ mm}$							
Cases/controls	25/36	19/32	20/35	27/25			
OR (95% CI) ^{2,3}	1.00	0.93 (0.40-2.17)	0.98 (0.43-2.23)	1.76 (0.75-4.12)	0.05	-	

Calculated using the Wald test for selenium modeled as a continuous variable (per quartile);

**Calculated using the likelihood ratio test comparing a model with an interaction term to one without;

[†]Not estimable;

¹Adjusted for year of birth (continuous);

²Adjusted for year of birth (continuous), age at the moment of the diagnosis of cancer (continuous), gender, ethnicity,

education, BMI, smoking status, radiotherapy treatment to head or neck;

³One case and one control were excluded from the analysis due to missing data on BMI.

There was no clear association of fingernail selenium for cases who were interviewed and provided fingernail samples less than 3 years, 3 to 7, 7 to 11 or more than 11 years after diagnosis (all p-trend > 0.05; Table 5-3).

	Т			
	Q1	Q2	Q3	
	(0.00-0.71)	(0.71-0.82)	(0.82-1.30)	p-trena
<3 years				
Cases/controls	17/34	13/24	16/23	
OR (95% CI) ¹	1.00	1.03 (0.38-2.80)	1.24 (0.48-3.23)	0.20
3-7 years				
Cases/controls	16/33	18/24	28/38	
OR (95% CI) ¹	1.00	1.15 (0.43-3.04)	1.19 (0.49-2.90)	0.80
7-11 years				
Cases/controls	21/37	20/23	18/31	
OR (95% CI) ¹	1.00	1.82 (0.72-4.60)	1.03 (0.41-2.61)	0.50
≥ 11 years				
Cases/controls	13/18	17/27	18/19	
OR (95% CI) ¹	1.00	0.93 (0.30-2.88)	1.75 (0.53-5.77)	0.40

Table 5-3 - Fingernail selenium and thyroid cancer by the time between diagnosis and interview in FrenchPolynesia, 1981-2003

^{*}Calculated using the Wald test for selenium modeled as a continuous variable (per tertile); ¹Adjusted for year of birth (continuous), age at the moment of the diagnosis of cancer (continuous), gender, ethnicity, education, BMI, smoking status, radiotherapy treatment to head or neck

5.3 **DISCUSSION**

5.3.1 SUMMARY OF THE RESULTS

Our objective was to examine the association between fingernail selenium concentration and thyroid cancer in French Polynesia, a country highly exposed to selenium mainly through fish consumption and characterized by mild iodine deficiency (105).

Overall, we did not evidence a significant association between fingernail selenium levels and thyroid cancer risk. Fingernail selenium levels were higher in subjects with high BMI and in non-smokers, and among controls only, in older subjects. Furthermore, fingernail selenium levels were higher in inhabitants of the Marquises Islands and the Tuamotu-Gambier Archipelago. Selenium in nails was nearly significantly positively linked to the risk of thyroid cancer's longer diameter of 10 mm or more (p = 0.09). Lastly, a nearly significant positive association (p = 0.10) between fingernail selenium and thyroid cancer risk was seen in patients with less than 3 ppm of iodine in nails.

5.3.2 STRENGTHS AND LIMITATIONS OF THE STUDY

This analysis has important strengths including, the population-based design with virtually exhaustive identification of thyroid cancer cases in isolated Pacific Islands and a high participation rate among cases and population control subjects, which were from the population register of French Polynesia (142). Another important strength is that French Polynesia ranks as one of world's leading consumers of fish (229), a major dietary source of selenium (227), which increased the power of our analysis. Furthermore, despite recent changes in lifestyle and nutrition, an important proportion of inhabitants (at least the ones living outside Tahiti), still have a traditional diet of fish, starchy roots and fruits, and consumed at the time of interview few imported foods that are high in fat and low in fibre (230), and thus are more likely to accurately report their dietary intake.

This analysis also has some limitations. In regard to the statistical method, when we conditioned on age and sex using the original matching criteria (year of birth \pm 6 months and sex), we observed similar results but reduced statistical power since matched groups that were missing either a case or control were dropped from the analysis.

Because of the transition in diet habits from a traditional Polynesian diet to a more Westernized one, the results of this case-control study about diet in adulthood could be confounded by diet in previous periods.

We did not find a correlation between selenium measured in fingernails and dietary selenium intake, as estimated using dietary questionnaire data and a French food composition. This lack of correlation was not surprising because the concentration of selenium in foods is largely variable and its quantification from food composition table data is uncertain. Indeed, its concentration in vegetables is dependent on the soil content in a particular geographical location (217), and its concentration in fishes is dependent on the type of cooking and variable between species (205). Therefore, our estimation of selenium in diet is probably uncertain. Thus, biomarker measures are essential in investigating the relationship between selenium exposure and chronic disease risk.

5.3.3 COMPARISON RESULTS FROM THE LITERATURE

Selenium concentration in nails is a highly reproducible value. In a cohort on the investigation of dietary supplement use and cancer risk in Western Washington state, the concentration of

toenail selenium without selenium supplement was about six times higher than the concentration of plasma selenium without selenium supplement, mean toenail and plasma concentrations were $1.02 \pm 0.21 \ \mu\text{g/g}$ and $161 \pm 29 \ \mu\text{g/L}$, respectively (231). There are advantages to measure selenium concentration in fingernails rather than blood: the quantity of selenium in nails is more stable, cumulative, and integrates exposure occurring from 12 to 18 months before sampling (232). Additionally, collecting, transporting and storing fingernails are easier than blood.

A previous cross-sectional study, including 195 adults aged 18 years old and over from Tahiti and the Moorea islands, found that serum selenium levels in French Polynesia in 2008 were similar to those observed in Inuit adults in 1992; however, these are four times lower than France (foods imported to Polynesia come from France), even considering the whole blood/plasma ratio (229).

We found that the highest fingernail selenium concentration of French Polynesia were in the Marquises Islands and in the Tuamotu-Gambier Archipelago, which is similar to observations performed on cord blood selenium concentration in French Polynesian new-borns in 2008 (229). These highest values are probably mostly attributable to higher seafood consumption, but the results shown in Table 5-1, in which the higher fingernail selenium values in controls living in the Tuamotu-Gambier Archipelago remained when adjusting for estimated dietary selenium intake, showing that other factors could interact, or that selenium level could be higher in the Tuamotu-Gambier Archipelago fishes. Because most of the fishes eaten in the atolls of the Tuamotu-Gambier Archipelago come from the lagoon, rather than the open sea like other archipelagos of French Polynesia, this result could be due to an under-estimation of selenium contents for lagoon fishes in food composition tables.

Among the studied population, fingernail selenium concentration averages in French Polynesian, 0.75 ppm in smokers and 0.80 ppm in non-smokers (p < 0.0001), are very similar to those measured in other studies, and in particular in a large cohort study of US women, which have shown that toenail selenium was significantly reduced among cigarette smokers (M = 0.75, SD = 0.12, among 146 current smokers; and M = 0.82, SD = 0.16, among 311 never smokers; p < 0.001) (233).

We found a positive relationship between BMI and selenium in fingernails, both in cases and controls (Table 5-1). Our result is in opposition with most European surveys (234,235,236) or US populations (237), in which obese patients have lower serum selenium concentration, but

in agreement with Dewailly's survey of a French Polynesian sampling in which selenium concentration in blood was positively linked to BMI (229), the regression coefficient (β) being 0.02, i.e. ten times higher than in our study, among the controls ($\beta = 0.002$). A possible explication is that in the general population, BMI has a trend to rise with age, if we do not consider extreme ages. Furthermore, older people have more possibilities to eat traditional food, which is rich in selenium.

There may be a range of selenium that offers optimal protection against cancer development in general, as a deficiency in selenium has been associated with an increased risk of cancer, this association having been evidenced for selenium in hair and nails and prostate cancer in Malaysia (238), and selenium in serum and, respectively bladder carcinoma in China (223), and breast cancer in India (239). In the latter case-control study, it has been estimated that each increase of one μ mol/l of selenium was associated to decrease in 7% of breast cancer risk (239). Nevertheless, selenium supplementation may reduce cancer risk only in seleniumdeficient individuals.

Few studies have examined the association between selenium and thyroid cancer risk, however, we haven't found association between fingernail selenium and thyroid cancer risk in our results. In a case-control study of 43 cases and 129 matched controls in Norway, prediagnostic serum selenium levels were inversely associated with thyroid cancer; levels \leq 1.25 and 1.26-1.64 µmol/l were associated with a 7.70- and 6.10-fold increased risk of thyroid cancer compared to levels \geq 1.65 µmol/l (225). This case-control study also showed, when considering the delay from blood sampling to diagnosis of the case, that the protective effect of high serum selenium concentration was restricted to the seven-year period prior to the diagnosis of thyroid cancer, because the serum selenium concentration of cases tended to decrease, relatively to controls, when the delay from blood sampling to the diagnosis was shorter. Similarly, in an Austrian survey the average selenium blood concentration was 1.14 µmol/l in 554 healthy controls, 0.97 µmol/l in 42 patients with follicular thyroid carcinoma and 1.02 µmol/l in 73 patients with papillary thyroid carcinoma (240).

Our study did not evidence a significant association between fingernail selenium levels and thyroid cancer, even when considering only patients for which the delay between cancer diagnosis and fingernail sample was less than seven or less than three years (Table 5-3). It has to be noted that over a period of six years, selenium in toenails was one of the most reproducible trace elements (241). Therefore, our non-significant results are probably not due

to the fact that we investigated post-diagnosis selenium levels rather than prediagnosis ones, except if thyroid cancer, by itself, modifies selenium level in nails. A possible explication for the lack of association could be the iodine status of French Polynesians, who could be less frequently iodine deficient than Norwegians because selenium acts synergistically with iodine. These results could nevertheless also be due to the fact that fingernail samplings among cases were performed after thyroid cancer diagnosis and therefore during thyroid hormone substitution treatment, such as levothyrox, which could interact with selenium. Indeed, all three mono-deiodinase enzymes are selenium-dependent and are involved in thyroid hormone regulation. In this way, selenium status may affect both thyroid hormone homeostasis and iodine availability (242, 243, 244). Lastly, we evidenced a nearly significant positive association (p = 0.15) between fingernail selenium and thyroid cancer risk in patients with more than 30 kg/m² BMI (Table 5-2). This result has to be considered with caution because it is nearly significant, but it could be due to some specificity of obese patients.

Because cigarette smoking has been shown to be inversely associated with both selenium status (245) and thyroid cancer risk (246), it was important to adequately control for these exposures in our analysis. Despite having evidenced a lower selenium status in smokers than in non-smokers, we failed to show an interaction between fingernail selenium levels, smoking habit and thyroid cancer risk: both in smokers and nonsmokers, selenium status did not influence thyroid cancer risk. Nevertheless, it should be noted that such interaction tests are not very powerful in our study, given the number of patients.

5.4 CONCLUSION

In summary, our results do not support, among individuals with sufficient levels of selenium, that greater long-term exposure to selenium may reduce thyroid cancer risk. Because these findings are based on post-diagnostic measures, studies with prediagnostic selenium are needed for corroboration.

CONCLUSION AND PERSPECTIVES

There was no significant association between dietary iodine intake and DTC risk in the Cuba study. In the pooled analysis of the five studies, the majority of the cases and the controls was considered as being in mild dietary iodine deficiency according to the IGN classification. Although DTC risk was not found to be linked to dietary iodine intake, this risk significantly decreased with high fish consumption, this reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than in the other studies. Additionally, higher dietary iodine intake significantly associated with lower DTC risk only in the French Polynesia study and in Polynesians. A strong increase in DTC risk associated with the number of the minor allele (A) of the SNP rs965513 near *FOXE1*, among participants who consumed less iodine than the median value in the Cuba study. For the women who had a high number of full term pregnancies and who were iodine deficient, increasing dietary iodine intake may reduce their risk of suffering from DTC, thus reduce the hereditary risk to their infants. Moreover, it was not possible to demonstrate an interest in fingernail selenium as a biomarker that predicts DTC risk.

Dietary iodine intake declared by auto-questionnaire and fingernail selenium seem like impossible to predict DTC risk, the next step, maybe we should focalize on urine iodine concentration or thyroid-stimulating hormone (TSH). My publication on *FOXE1* interacts with dietary iodine intake in DTC risk within the Cuba study, and this significant interaction has been replicated in the pooled analysis for the French Polynesia and the Cuba studies, which could lead to the future biological investigation on evolution of the interaction between germline mutation and dietary iodine, thus from the dietary source, prevent thyroid cancer risk. This effect could bring big scientific interest in clinical nutrition for the prognosis of thyroid cancer patients if dietary iodine interacts with some somatic mutations as well, thus giving thyroid cancer patients high iodine content diet, may be helpful for their recovery. Composition of high iodine content diet, as well as the quantity of optimal iodine intake, need further investigations.

Since the early 90s, iodine supplement intake from iodized salt for people in endemic-goiter areas has been compulsively carried out in order to reduce goiter incidence, however, several studies demonstrated that excess iodine intake, especially from iodine supplement may increase the incidence of papillary thyroid cancer, especially in endemic-goiter areas, if future study confirms this result, we should reconsider iodized salt compulsive policy and conditionally apply it according to different iodine status areas. As we don't have enough cases and controls classified in the excess iodine intake class according to the IGN

classification, in future study, we'd better to include more study participants regarding the Epi-Thyr consortium still extends, and we will be able to include the Chernobyl post effect case-control study and the case-control study niched in the E3N cohort.

Classification of the colors of food label which indicate high iodine content food will bring some interesting scientific issues for public health.

The main perspective of my work concerns deepening my results about the specificity of the Polynesian population, which can be achieved thanks to an extension of the French Polynesia study on DTC risk associated with high number of pregnancies in women with iodine deficiency. Validation of this result will require specific studies.

Our group is organizing a GWAS on the 7 case-controls studies of the Epi-Thyr consortium in collaboration with Cancer and Environment group of Unit 1018 Inserm, and the colleagues from Curie Institute, IARC, and CNG. We use OncoArray chips (Illumina ®) designed for common cancers (lung cancer, breast cancer, prostate cancer, colon cancer and ovary cancer), composing 570,000 SNPs (including 250,000 tag-SNPs), enriched with 10,000 SNPs specially chosen for thyroid cancer, including thyroid pathway genes (cancer, signaling, synthesis), fine-Mapping at 9q22, 14q13, 2q35 and 8p12, candidate SNPs (linked with thyroid cancer, or tumor size or obesity), and tag-SNPs from candidate genes (*HTR1B*, *LOC727677*, *MGMT*, *Mir146a*, *TSHR*, *WDR11*), in order to replicate and identify common variants that can be related to DTC pathogenesis and potential interaction with environmental risk factors in multiethnic population samples.

PERSONAL CONTRIBUTION

My personal contribution first of all, consisted in the literature review on dietary factors, especially on dietary iodine intake and the risk of thyroid cancer.

The analyses of these factors actualized on the data of Epi-Thyr consortium of the 5 casescontrol studies performed in New Caledonia, French Polynesia, metropolitan France (2 studies), and Cuba. The first objective of my thesis was to explore the role of dietary iodine intake, in the risk of developing DTC, in the case-control study performed in Cuba. For this purpose, I participated in the organization of the traditional Cuban food samples' collection and storage, the Cuban colleague - Celia Pereda brought them from Cuba to France, then I transported them to the laboratory for food safety of ANSES (French Agency for Food, Environmental and Occupational Health & Safety, Maisons-Alfort) for the CFCT 2014 establishment. In 2014, I spent one month in Cuba in order to do quality & control of the data in the dietary questionnaire auto-declared by the study participants, working with the help of Dr. Rosa M. Ortiz and the biostatistician - Dr. Juan J. Lence-Anta, Celia Pereda, Mae Chappe from Department of Clinical Research, Institute of Oncology and Radiobiology, Havana, Cuba, I reinterviewed several study participants for the dietary questionnaire face to face or by telephone to correct their previous aberrant declarations. Afterwards, I filed the dietary questionnaire and updated the data. Then I reestablished the unit and the seasonal adjustment of each food item in the Cuba study from the investigations of several local fruits and vegetables markets in Havana and from the review of documents from the institute of investigation in tropical fruit under the direction of Ministry of Agriculture of Cuba, with the help of Dr. C. Blanca Terry-Berro - director of the Center for Nutrition and Food Hygiene, the National Institute of Hygiene, Epidemiology and Microbiology (Inhem) under the direction of Ministry of Public Health of Cuba, we fixed an average value of 10 g of iodized salt consumption per inhabitant per day, in order to better fit the local situation and have better estimation of local foods consumptions, thereby have better estimation of dietary iodine intake in the Cuba study. Later, I searched in the CFCT 2014 and the FAO Databases, the food items corresponding to those of the dietary questionnaire of the Cuba study, and established dietary iodine, selenium, energy intake for the participants in this study. Finally, I published a scientific paper to analyze these work (APPENDIXES7).

Besides, I also reestablished dietary iodine, selenium, energy intake for the participants in the French Polynesia study, according to the FPFCT 2013 and the FAO Databases (see 1.3.1.3 and 1.3.1.1), and added the seasonality of certain fruits and vegetables from the investigations of several local fruits and vegetables markets in French Polynesia, in order to better fit the

local situation and have better estimation of these trace elements intakes in this study. And I established fingernail selenium dosage for the study participants in order to test whether fingernail selenium could be a good biomarker for thyroid cancer risk, then I published a scientific paper to analyze these work (APPENDIXES7).

The New Caledonia study and the Cathy study have been administrated by Cancer and Environment group, Center for research in Epidemiology and Population Health (CESP) - Unit 1018 - Inserm, Dr. Thérèse Truong sent me the dataset of the adjustment variables of the New Caledonia study and the dietary variables of the New Caledonia and the Cathy studies, Dr. Emilie Cordina-Duverger sent me the dataset of the adjustment variables of the Cathy study, Dr. Catherine Tcheandjieu sent me the dataset of the 4 investigated SNPs of the New Caledonia and the Cathy studies, I did quality & control, checked and corrected these datasets, then, I combined the adjustment variables datasets together with the ones of the French Polynesia, the Young-Thyr and the Cuba studies administrated by our group, and standardized all these variables' coding for the next pooled analysis.

For the dietary variables, with the help of the data manager of our group - Vincent Souchard, we did the quality & control, checked and corrected the aberrant values and the missing data of these variables in these datasets by statistical imputation. We searched in the different food composition tables presented above, the food items corresponding to those of the dietary questionnaire of the Young-Thyr, Cathy and New Caledonia studies, I established and reestablished dietary iodine, selenium, energy intake for the participants in these studies, and I added the seasonality of certain fruits and vegetables for the Young-Thyr study according to the Cathy study. Later, we recreated 21 sub food groups and selected 66 common food items of the 5 case-control studies from Epi-Thyr consortium and standardized all these dietary variables' coding for the standardization of the next pooled analysis.

I participated in the organization of TaqMan genotyping of the 4 investigated SNPs for the 1417 Young-Thyr study participants' samples, with the collaboration of the National Centre of Genotyping (CEA, Evry, France), when I received the genotyping data, I did quality & control in order to check whether both allele and genotype frequencies in this population samples following the Hardy-Weinberg Equilibrium Proportions. Then I combined these SNPs variables' dataset together with the ones of the other 4 studies, and standardized all these variables' coding for the next pooled analysis.

I merged the datasets of the adjustment variables, the dietary variables, and 4 investigated SNPs variables of the 5 case-controls studies from Epi-Thyr consortium into a unique database and did the pooled analysis, I wrote the manuscript of this pooled analysis.

Moreover, I studied different statistic models and methods, such as mixed model, logit linear model, linear model, analysis of genetic association studies, multiple imputation etc., in order to know which model or method is the best to adapt to the pooled analysis.

During my PhD study, I did several scientific communications by oral presentation, poster presentation or exchange program: I presented the study of « Fingernail selenium and thyroid cancer risk: a case-control study in French Polynesia », in the annual conference of NACRe-Partenariat (The French National Network of Food and Cancer Research), in Paris, in 2014; I presented the study entitled « Foxel Polymorphism Interacts with Dietary Iodine Intake in DTC Risk in the Cuban Population », in the annual conference of NACRe-Partenariat, in Paris, in 2015; I presented the pooled analysis of my PhD study « Dietary iodine intake and thyroid cancer risk: a pooled analysis of 5 case-control studies » in the Gustave Roussy's research days, in Tours, in 2016; I presented a poster entitled « Dietary iodine uptake and thyroid cancer risk - a pooled analysis of 5 case-control studies of Epi-Thyr consortium » in the 86th Annual Meeting of the American Thyroid Association, in Denver, in 2016; I was selected as representative of Inserm's PhD students to take the doctoral and post-doctoral students exchanges program between Inserm and University of Sydney, in 2015, I spent three months in Cancer Genetics Diagnostic Laboratory of Kolling Institute of Medical Research, Royal North Shore Hospital, in Sydney, under the direction of Pr. Roderick Clifton-Bligh for a clinical research project to study association between somatic mutations in the TERT gene promoter and outcomes of papillary thyroid cancer, whose results successfully published through this exchange program which I was the first co-auteur (Bullock, Ren et al., TERT promoter mutations are a major indicator of recurrence and death due to papillary thyroid carcinomas. Clin Endocrinol (Oxf). 2016 Aug;85(2):283-90. Epub 2016 Feb 8.). I also learnt and launched an experiment in measuring iodine content in human thyroid tumor tissue samples using Raman spectroscopy, in the University of Sydney. In addition, I participated in several doctoral seminars organized by our Doctoral School of Public Health (EDSP: Ecole Doctorale de Santé Publique) and the Doctoral College of the University Paris-Saclay, the scientific seminars or conferences organized by NACRe, which assembling the experts in the analysis of nutritional factors, by French Endocrine Society, which assembling the

endocrinologists in the treatment of thyroid disease, by the Department of Biostatistics of the University of Liverpool, specialises in statistical analysis of genetic association studies.

In summary, within my PhD study, I wrote 3 scientific articles, 2 of them were published (see APPENDIXES 7).

REFERENCES

- American Thyroid Association, the USA 2017: Thyroid Cancer (Papillary and Follicular)\ Thyroid Cancer FAQs\WHAT IS THE THYROID GLAND? Available at: <u>http://www.thyroid.org/thyroid-cancer/</u> (Accessed February 15, 2017).
- American Thyroid Association, the USA 2017: Thyroid Nodules. Available at: <u>http://www.thyroid.org/thyroid-nodules/</u> (Accessed February 15, 2017).
- 3. Choi KU, Kim JY, Park DY, Lee CH, Sol MY, Han KT, Kim YG. Recommendations for the management of cystic thyroid nodules. ANZ J Surg. 2005 Jul;75(7):537-41.
- 4. Agate L, Lorusso L, Elisei R. New and old knowledge on differentiated thyroid cancer epidemiology and risk factors. J Endocrinol Invest. 2012;35(6 Suppl):3-9.
- 5. Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, Maurer MS, McIver B, Mullan BP, Oberg AL, Powell CC, van Heerden JA, Goellner JR. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. World J Surg. 2002 Aug;26(8):879-85. Epub 2002 May 21.
- 6. Mazzaferri EL, Kloos RT. Clinical review 128: Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab. 2001 Apr;86(4):1447-63.
- Dionigi G, Kraimps JL, Schmid KW, Hermann M, Sheu-Grabellus SY, De Wailly P, Beaulieu A, Tanda ML, Sessa F. Minimally invasive follicular thyroid cancer (MIFTC)--a consensus report of the European Society of Endocrine Surgeons (ESES). Langenbecks Arch Surg. 2014 Feb;399(2):165-84.
- American Thyroid Association, the USA 2017: Thyroid Cancer (Papillary and Follicular)\ Thyroid Cancer FAQs\WHAT ARE THE TYPES OF THYROID CANCER? Available at: <u>http://www.thyroid.org/thyroid-cancer/</u> (Accessed February 15, 2017).
- Burman KD, Ringel MD, Wartofsky L, et al. Unusual types of thyroid neoplasms. Endocrinol Metab Clin North Am. 1996;25:49-68.

- 10. Livolsi VA. Surgical Pathology of the Thyroid. Philadelphia, Pa: Saunders; 1990.
- Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, Biddinger PW, Nikiforov YE. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol. 2006 Feb;30(2):216-22.
- 12. Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer "epidemic"--screening and overdiagnosis. N Engl J Med. 2014 Nov 6;371(19):1765-1767.
- 13. Volante M, Landolfi S, Chiusa L, Palestini N, Motta M, Codegone A, Torchio B, Papotti MG. Poorly differentiated carcinomas of the thyroid with trabecular, insular, and solid patterns: a clinicopathologic study of 183 patients. Cancer. 2004 Mar 1;100(5):950-7.
- Carcangiu ML, Zampi G, Rosai J. Poorly differentiated ("insular") thyroid carcinoma. A reinterpretation of Langhans' "wuchernde Struma". Am J Surg Pathol. 1984 Sep;8(9):655-68.
- GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012, International Agency for Research on Cancer, World Health Organization 2012. Available at: <u>http://globocan.iarc.fr/Pages/Map.aspx</u> (Accessed December 20, 2016).
- 16. Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide Thyroid-Cancer Epidemic? The Increasing Impact of Overdiagnosis. N Engl J Med. 2016;375:614-7.
- 17. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
- 18. Schlumberger M, Pacini F, Tuttle RM. THYROID TUMORS. FOURTH EDITION. tcgraphite. ISBN 978-2-7466-7881-1.
- Howlader N, Noone AM, Krapcho M, Miller D, Bishop K, Altekruse SF, Kosary CL, Yu M, Ruhl J, Tatalovich Z, Mariotto A, Lewis DR, Chen HS, Feuer EJ, Cronin KA (eds). SEER Cancer Statistics Review, 1975-2013, National Cancer Institute. Bethesda, MD, Available at: http://seer.cancer.gov/csr/1975_2013/, based on November 2015 SEER data submission, posted to the SEER web site, April 2016 (Accessed February 15, 2017).
- 20. Bosetti C, Bertuccio P, Levi F, Lucchini F, Negri E, La Vecchia C. Cancer mortality in the European Union, 1970-2003, with a joinpoint analysis. Ann Oncol. 2008 Apr;19(4):631-40.
- Colonna M, Guizard A-V, Dabakuyo S, Schvartz C. Thyroïde. Estimation nationale de l'incidence et de la mortalité par cancer en France entre 1980 et 2012. Partie 1 – Tumeurs solides. 2013 July;p.96-99.

- 22. Kuo EJ, Goffredo P, Sosa JA, Roman SA. Aggressive variants of papillary thyroid microcarcinoma are associated with extrathyroidal spread and lymph-node metastases: a population-level analysis. Thyroid. 2013 Oct;23(10):1305-11.
- 23. Sampson RJ, Woolner LB, Bahn RC, Kurland LT. Occult thyroid carcinoma in Olmsted County, Minnesota: prevalence at autopsy compared with that in Hiroshima and Nagasaki, Japan. Cancer. 1974 Dec;34(6):2072-6.
- 24. Fukunaga FH, Yatani R. Geographic pathology of occult thyroid carcinomas. Cancer. 1975 Sep;36(3):1095-9.
- 25. Silverberg SG, Vidone RA. Carcinoma of the thyroid in surgical and postmortem material. Analysis of 300 cases at autopsy and literature review. Ann Surg. 1966 Aug;164(2):291-9.
- 26. Bisi H, Fernandes VS, de Camargo RY, Koch L, Abdo AH, de Brito T. The prevalence of unsuspected thyroid pathology in 300 sequential autopsies, with special reference to the incidental carcinoma. Cancer. 1989 Nov 1;64(9):1888-93.
- 27. Harach HR, Franssila KO, Wasenius VM. Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. Cancer. 1985 Aug 1;56(3):531-8.
- 28. Ito Y, Miyauchi A, Inoue H, Fukushima M, Kihara M, Higashiyama T, Tomoda C, Takamura Y, Kobayashi K, Miya A. An observational trial for papillary thyroid microcarcinoma in Japanese patients. World J Surg. 2010 Jan;34(1):28-35.
- 29. Rosai J, LiVolsi VA, Sobrinho-Simoes M, Williams ED. Renaming papillary microcarcinoma of the thyroid gland: the Porto proposal. Int J Surg Pathol. 2003 Oct;11(4):249-51.
- 30. Franceschi S, Boyle P, Maisonneuve P, La Vecchia C, Burt AD, Kerr DJ, MacFarlane GJ. The epidemiology of thyroid carcinoma. Crit Rev Oncog. 1993;4(1):25-52.
- 31. Schneider AB, Ron E. Carcinoma of follicular epithelium: pathogenesis in Werner & Ingbar's The Thyroid 2000, 8th ed, Lippincott, Williams, Wilkins (Philadelphia)
- 32. Loh KC. Familial nonmedullary thyroid carcinoma: a meta-review of case series. Thyroid. 1997 Feb;7(1):107-13.
- 33. Uchino S, Noguchi S, Kawamoto H, Yamashita H, Watanabe S, Yamashita H, Shuto S. Familial nonmedullary thyroid carcinoma characterized by multifocality and a high recurrence rate in a large study population. World J Surg. 2002 Aug;26(8):897-902.
- 34. Brindel P, Doyon F, Bourgain C, Rachédi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Paoaafaite J, Teuri J, de Vathaire F. Family history of thyroid cancer and the risk of differentiated thyroid cancer in French Polynesia. Thyroid. 2010 Apr;20(4):393-400.

- 35. Frich L, Glattre E, Akslen LA. Familial occurrence of nonmedullary thyroid cancer: a population-based study of 5673 first-degree relatives of thyroid cancer patients from Norway. Cancer Epidemiol Biomarkers Prev. 2001 Feb;10(2):113-7.
- 36. Hemminki K, Eng C, Chen B. Familial risks for nonmedullary thyroid cancer. J Clin Endocrinol Metab. 2005 Oct;90(10):5747-53.
- 37. Xu L, Li G, Wei Q, El-Naggar AK, Sturgis EM. Family history of cancer and risk of sporadic differentiated thyroid carcinoma. Cancer. 2012 Mar 1;118(5):1228-35.
- Hemminki K, Li X. Familial risk of cancer by site and histopathology. Int J Cancer. 2003 Jan 1;103(1):105-9.
- Kebebew E. Hereditary non-medullary thyroid cancer. World J Surg. 2008 May;32(5):678-82.
- 40. Bell B, Mazzaferri EL. Familial adenomatous polyposis (Gardner's syndrome) and thyroid carcinoma. A case report and review of the literature. Dig Dis Sci. 1993 Jan;38(1):185-90.
- 41. Cetta F, Montalto G, Gori M, Curia MC, Cama A, Olschwang S. Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. J Clin Endocrinol Metab. 2000 Jan;85(1):286-92.
- 42. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, et al. Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet. 2009;41: 460–464.
- 43. Matsuse M, Takahashi M, Mitsutake N, Nishihara E, Hirokawa M, Kawaguchi T, Rogounovitch T, Saenko V, Bychkov A, Suzuki K, Matsuo K, Tajima K, Miyauchi A, Yamada R, Matsuda F, Yamashita S. The FOXE1 and NKX2-1 loci are associated with susceptibility to papillary thyroid carcinoma in the Japanese population. J Med Genet.2011;48: 645–648.
- 44. Takahashi M, Saenko VA, Rogounovitch TI, Kawaguchi T, Drozd VM, Takigawa-Imamura H, Akulevich NM, Ratanajaraya C, Mitsutake N, Takamura N, Danilova LI, Lushchik ML, Demidchik YE, Heath S, Yamada R, Lathrop M, Matsuda F, Yamashita S. The FOXE1 locus is a major genetic determinant for radiation-related thyroid carcinoma in Chernobyl. Hum Mol Genet 2010;19(12):2516–2523.
- 45. Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, et al. Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 8q24 locus and failure to replicate a locus on 5q24. J Med Genet. 2012;49: 158–163.

- 46. Landa I, Ruiz-Llorente S, Montero-Conde C, Inglada-Pérez L, Schiavi F, Leskelä S, Pita G, Milne R, Maravall J, Ramos I, Andía V, Rodríguez-Poyo P, Jara-Albarrán A, Meoro A, del Peso C, Arribas L, Iglesias P, Caballero J, Serrano J, Picó A, Pomares F, Giménez G, López-Mondéjar P, Castello R, Merante-Boschin I, Pelizzo MR, Mauricio D, Opocher G, Rodríguez-Antona C, González-Neira A, Matías-Guiu X, Santisteban P, Robledo M. The variant rs1867277 in *FOXE1* gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors. PLoS Genet. 2009;5: e1000637.
- 47. Carré A, Castanet M, Sura-Trueba S, Szinnai G, Van Vliet G, Trochet D, Amiel J, Léger J, Czernichow P, Scotet V, Polak M. Polymorphic length of FOXE1 alanine stretch: evidence for genetic susceptibility to thyroid dysgenesis. Hum Genet. 2007;122: 467–476.
- 48. Pereda CM, Lesueur F, Pertesi M, Robinot N, Lence-Anta JJ, Turcios S, Velasco M, Chappe M, Infante I, Bustillo M, García A, Cléro É, Xhaard C, Ren Y, Maillard S, Damiola F, Rubino C, Salazar S, Rodriguez R, Ortiz RM, de Vathaire F. Common variants at the 9q22.33, 14q13.3 and ATM loci, and risk of differentiated thyroid cancer in the Cuban population. BMC Genet. 2015 Mar 1;16:22.
- 49. Maillard S, Damiola F, Cléro É, Pertesi M, Robinot N, Rachédi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Doyon F, Xhaard C, Rubino C, Blanché H, Drozdovitch V, Lesueur F, de Vathaire F. Common variants at 9q22.33, 14q13.3, and ATM loci, and risk of differentiated thyroid cancer in the French Polynesian population. PLoS One. 2015 Apr 7;10(4):e0123700.
- Malchoff CD, Malchoff DM. The genetics of hereditary nonmedullary thyroid carcinoma. J Clin Endocrinol Metab. 2002 Jun;87(6):2455-9.
- 51. Nagy R, Ringel MD. Genetic predisposition for nonmedullary thyroid cancer. Horm Cancer. 2015 Feb;6(1):13-20.
- 52. DeGroot L, Paloyan E. Thyroid carcinoma and radiation. A Chicago endemic. JAMA. 1973 Jul 30;225(5):487-91.
- 53. Favus MJ, Schneider AB, Stachura ME, Arnold JE, Ryo UY, Pinsky SM, Colman M, Arnold MJ, Frohman LA. Thyroid cancer occurring as a late consequence of head-andneck irradiation. Evaluation of 1056 patients. N Engl J Med. 1976 May 6;294(19):1019-25.
- 54. Refetoff S, Harrison J, Karanfilski BT, Kaplan EL, De Groot LJ, Bekerman C. Continuing occurrence of thyroid carcinoma after irradiation to the neck in infancy and childhood. N Engl J Med. 1975 Jan 23;292(4):171-5.

- 55. Ron E, Lubin JH, Shore RE, Mabuchi K, Modan B, Pottern LM, Schneider AB, Tucker MA, Boice JD Jr. Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. Radiat Res. 1995 Mar;141(3):259-77.
- 56. Baverstock K, Egloff B, Pinchera A, Ruchti C, Williams D. Thyroid cancer after Chernobyl. Nature. 1992 Sep 3;359(6390):21-2.
- 57. Dobyns BM, Hyrmer BA. The surgical management of benign and malignant thyroid neoplasms in Marshall Islanders exposed to hydrogen bomb fallout. World J Surg. 1992 Jan-Feb;16(1):126-39; discussion 139-40.
- Kazakov VS, Demidchik EP, Astakhova LN. Thyroid cancer after Chernobyl. Nature. 1992 Sep 3;359(6390):21.
- 59. Pacini F, Vorontsova T, Demidchik EP, Molinaro E, Agate L, Romei C, Shavrova E, Cherstvoy ED, Ivashkevitch Y, Kuchinskaya E, Schlumberger M, Ronga G, Filesi M, Pinchera A. Post-Chernobyl thyroid carcinoma in Belarus children and adolescents: comparison with naturally occurring thyroid carcinoma in Italy and France. J Clin Endocrinol Metab. 1997 Nov;82(11):3563-9.
- 60. Tronko N, Bogdanova T, Kommisarenko I. Thyroid cancer in children and adolescents in Ukraine after the Chernobyl accident (1986-1995) in The Radiological Consequences of the Chernoby Accident 1996 (Luxemburg)
- 61. Belfiore A, La Rosa GL, La Porta GA, Giuffrida D, Milazzo G, Lupo L, Regalbuto C, Vigneri R. Cancer risk in patients with cold thyroid nodules: relevance of iodine intake, sex, age, and multinodularity. Am J Med. 1992 Oct;93(4):363-9.
- 62. Franceschi S, Preston-Martin S, Dal Maso L, Negri E, La Vecchia C, Mack WJ, et al. A pooled analysis of case–control studies of thyroid cancer. IV. Benign thyroid diseases. Cancer Causes Control. 1999;10:583–95.
- 63. Horn-Ross PL, Morris JS, Lee M, West DW, Whittemore AS, McDougall IR, et al. Iodine and thyroid cancer risk among women in a multiethnic population: the Bay Area Thyroid Cancer Study. Cancer Epidemiol Biomarkers Prev. 2001;10:979–85.
- 64. Memon A, Varghese A, Suresh A. Benign thyroid disease and dietary factors in thyroid cancer: a case–control study in Kuwait. Br J Cancer. 2002;86:1745–50.
- 65. Iribarren C, Haselkorn T, Tekawa IS, Friedman GD. Cohort study of thyroid cancer in a San Francisco Bay area population. Int J Cancer. 2001;93:745–50.
- 66. Farbota LM, Calandra DB, Lawrence AM, Paloyan E. Thyroid carcinoma in Graves' disease. Surgery. 1985 Dec;98(6):1148-53.

- 67. Pacini F, Elisei R, Di Coscio GC, Anelli S, Macchia E, Concetti R, Miccoli P, Arganini M, Pinchera A. Thyroid carcinoma in thyrotoxic patients treated by surgery. J Endocrinol Invest. 1988 Feb;11(2):107-12.
- 68. Shapiro SJ, Friedman NB, Perzik SL, Catz B. Incidence of thyroid carcinoma in Graves' disease. Cancer. 1970 Dec;26(6):1261-70.
- 69. Pellegriti G, Mannarino C, Russo M, Terranova R, Marturano I, Vigneri R, Belfiore A. Increased mortality in patients with differentiated thyroid cancer associated with Graves' disease. J Clin Endocrinol Metab. 2013 Mar;98(3):1014-21.
- 70. Frates MC, Benson CB, Doubilet PM, Kunreuther E, Contreras M, Cibas ES, Orcutt J, Moore FD Jr, Larsen PR, Marqusee E, Alexander EK. Prevalence and distribution of carcinoma in patients with solitary and multiple thyroid nodules on sonography. J Clin Endocrinol Metab. 2006 Sep;91(9):3411-7.
- 71. Brito JP, Yarur AJ, Prokop LJ, McIver B, Murad MH, Montori VM. Prevalence of thyroid cancer in multinodular goiter versus single nodule: a systematic review and meta-analysis. Thyroid. 2013;23:449–55.
- 72. Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC, Franklyn JA. Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J Clin Endocrinol Metab. 2006 Nov;91(11):4295-301.
- 73. Fiore E, Rago T, Provenzale MA, Scutari M, Ugolini C, Basolo F, Di Coscio G, Berti P, Grasso L, Elisei R, Pinchera A, Vitti P. Lower levels of TSH are associated with a lower risk of papillary thyroid cancer in patients with thyroid nodular disease: thyroid autonomy may play a protective role. Endocr Relat Cancer. 2009 Dec;16(4):1251-60.
- 74. Castro MR, Espiritu RP, Bahn RS, Henry MR, Gharib H, Caraballo PJ, Morris JC. Predictors of malignancy in patients with cytologically suspicious thyroid nodules. Thyroid. 2011 Nov;21(11):1191-8.
- 75. Kim KW, Park YJ, Kim EH, Park SY, Park do J, Ahn SH, Park do J, Jang HC, Cho BY. Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. Head Neck. 2011 May;33(5):691-5.
- 76. Fleury Y, Van Melle G, Woringer V, Gaillard RC, Portmann L. Sex-dependent variations and timing of thyroid growth during puberty. J Clin Endocrinol Metab. 2001 Feb;86(2):750-4.
- 77. Berghout A, Wiersinga W. Thyroid size and thyroid function during pregnancy: an analysis. Eur J Endocrinol. 1998 May;138(5):536-42.

- Hegedüs L, Karstrup S, Rasmussen N. Evidence of cyclic alterations of thyroid size during the menstrual cycle in healthy women. Am J Obstet Gynecol. 1986 Jul;155(1):142-5.
- 79. Chen GG, Vlantis AC, Zeng Q, van Hasselt CA. Regulation of cell growth by estrogen signaling and potential targets in thyroid cancer. Curr Cancer Drug Targets. 2008 Aug;8(5):367-77.
- 80. Xhaard C, Rubino C, Cléro É, Maillard S, Ren Y, Borson-Chazot F, Sassolas G, Schvartz C, Colonna M, Lacour B, Danzon A, Velten M, Buemi A, Bailly L, Mariné Barjoan E, Schlumberger M, Orgiazzi J, Adjadj E, de Vathaire F. Menstrual and reproductive factors in the risk of differentiated thyroid carcinoma in young women in France: a population-based case-control study. Am J Epidemiol. 2014 Nov 15;180(10):1007-17.
- Negri E, Dal Maso L, Ron E, et al. A pooled analysis of case-control studies of thyroid cancer. II. Menstrual and reproductive factors. Cancer Causes Control. 1999;10(2):143–155.
- 82. Horn-Ross PL, Canchola AJ, Ma H, et al. Hormonal factors and the risk of papillary thyroid cancer in the California Teachers Study cohort. Cancer Epidemiol Biomarkers Prev. 2011;20(8):1751–1759.
- 83. Zivaljevic VR, Vlajinac HD, Marinkovic JM, et al. Case-control study of anaplastic thyroid cancer: goiter patients as controls. Eur J Cancer Prev. 2008;17(2):111–115.
- 84. Truong T, Orsi L, Dubourdieu D, et al. Role of goiter and of menstrual and reproductive factors in thyroid cancer: a population-based case-control study in New Caledonia (South Pacific), a very high incidence area. Am J Epidemiol. 2005;161(11):1056–1065.
- 85. Brindel P, Doyon F, Rachédi F, et al. Menstrual and reproductive factors in the risk of differentiated thyroid carcinoma in native women in French Polynesia: a population-based case-control study. Am J Epidemiol. 2008;167(2):219–229.
- 86. Sakoda LC, Horn-Ross PL. Reproductive and menstrual history and papillary thyroid cancer risk: the San Francisco Bay Area thyroid cancer study. Cancer Epidemiol Biomarkers Prev. 2002;11(1):51–57.
- 87. Zhou Y, Zhou Z, Qian M, Gong T, Wang J. Association of thyroid carcinoma with pregnancy: a meta-analysis. Mol Clin Oncol 2014:341–346.
- 88. Ron E, Kleinerman RA, Boice JD Jr, LiVolsi VA, Flannery JT, Fraumeni JF Jr. A population-based case-control study of thyroid cancer. J Natl Cancer Inst. 1987;79:1-12.

- Preston-Martin S, Bernstein L, Pike MC, Maldonado AA, Henderson BE. Thyroid cancer among young women related to prior thyroid disease and pregnancy history. Br J Cancer. 1987;55:191-195.
- 90. Preston-Martin S1, Jin F, Duda MJ, Mack WJ. A case-control study of thyroid cancer in women under age 55 in Shanghai (People's Republic of China). Cancer Causes Control. 1993;4:431–440.
- 91. Sack J. Thyroid function in pregnancy maternal-fetal relationship in health and disease. Pediatr Endocrinol Rev. 2003;1(Suppl 2):170-176.
- 92. Adami HO, Hunter D, Trichopoulos D, eds. Textbook of Cancer Epidemiology.2008;USA: Oxford University Press.
- 93. Haymart MR, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC, Chen H. Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. J Clin Endocrinol Metab. 2008 Mar;93(3):809-814.
- 94. de Vathaire F, Drozdovitch V, Brindel P, Rachedi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Paoaafaite J, Teuri J, Iltis J, Bouville A, Cardis E, Hill C, Doyon F. Thyroid cancer following nuclear tests in French Polynesia. Br J Cancer. 2010;103(7):1115–1121.
- 95. Galanti MR, Sparén P, Karlsson A, Grimelius L, Ekbom A. Is residence in areas of endemic goiter a risk factor for thyroid cancer? Int J Cancer. 1995;61:615-21.
- 96. Boltze C, Brabant G, Dralle H, Gerlach R, Roessner A, Hoang-Vu C. Radiationinduced thyroid carcinogenesis as a function of time and dietary iodine supply: An in vivo model of tumorigenesis in the rat. Endocrinology. 2002;143:2584–92.
- 97. Riesco G, Taurog A, Larsen R, Krulich L. Acute and chronic responses to iodine deficiency in rats. Endocrinology. 1977;100:303–13.
- 98. Hill RN, Erdreich LS, Paynter OE, Roberts PA, Rosenthal SL, Wilkinson CF. Thyroid follicular cell carcinogenesis. Fundam Appl Toxicol. 1989;12:629–97.
- 99. Ward JM, Ohshima M. The role of iodine in carcinogenesis. Adv Exp Med Biol. 1986;206:529–42.
- 100. Kaplan MM. Progress in Thyroid-Cancer. Endocrin Metab Clin. 1990;19:469–78.
- Zimmermann MB, Boelaert K. Iodine deficiency and thyroid disorders. Lancet Diabetes Endocrinol. 2015;3:286–95.
- 102. Uyttersprot N, Pelgrims N, Carrasco N, Gervy C, Maenhaut C, Dumont JE, et al. Moderate doses of iodide in vivo inhibit cell proliferation and the expression of

thyroperoxidase and Na+/I- symporter mRNAs in dog thyroid. Mol Cell Endocrinol. 1997;131:195–203.

- 103. Kolonel LN, Hankin JH, Wilkens LR, Fukunaga FH, Hinds MW. An epidemiologic study of thyroid cancer in Hawaii. Cancer Causes Control. 1990;1:223–34.
- 104. Truong T, Baron-Dubourdieu D, Rougier Y, Guenel P. Role of dietary iodine and cruciferous vegetables in thyroid cancer: a countrywide case–control study in New Caledonia. Cancer Causes Control. 2010;21:1183-92.
- 105. Cléro É, Doyon F, Chungue V, Rachédi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Dewailly E, Rubino C, de Vathaire F. Dietary iodine and thyroid cancer risk in French Polynesia: a case-control study. Thyroid. 2012;22:422-9.
- 106. Zimmermann MB, Galetti V. Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. Thyroid Res. 2015;8:8.
- 107. British Geological Survey 2003 Database of the iodine content of food and diets populated with data from published literature. British Geological Survey Commissioned Report, Keyworth, Nottingham, United Kingdom.
- Haldimann M, Alt A, Blondeau ABK 2005 Iodine content of food groups. J Food Compost Anal 18:461–471.
- Rose M, Miller P, Baxter M, Appleton G, Crews H, Croasdale M 2001 Bromine and iodine in 1997 UK total diet study samples. J Environ Monit 3:361–365.
- 110. Leufroy A, Noël L, Bouisset P, Maillard S, Bernagout S, Xhaard C, de Vathaire F, Guérin T 2015 Determination of total iodine in French Polynesian foods: method validation and occurrence data. Food Chem 169:134–140.
- 111. Liu ZT, Lin AH. Dietary factors and thyroid cancer risk: a meta-analysis of observational studies. Nutr Cancer. 2014;66(7):1165-78.
- 112. Ren Y, Kitahara CM, Berrington de Gonzalez A, Cléro É, Brindel P, Maillard S, Cote S, Dewailly E, Rachedi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Xhaard C, Rubino C, de Vathaire F. Lack of association between fingernail selenium and thyroid cancer risk: a case-control study in French Polynesia. Asian Pac J Cancer Prev. 2014;15(13):5187-94.
- 113. Ren Y, Lence-Anta JJ, Pereda CM, Chappe M, Velasco M, Infante I, Bustillo M, Turcios S, Leufroy A, Guérin T, Noël L, Lesueur F, Maillard S, Cléro É, Xhaard C, Allodji RS, Rubino C, Rodriguez R, Ortiz RM, de Vathaire F. FOXE1 Polymorphism Interacts with Dietary Iodine Intake in Differentiated Thyroid Cancer Risk in the Cuban Population. Thyroid. 2016 Dec;26(12):1752-1760.
- 114. Cléro É, Leux C, Brindel P, Truong T, Anger A, Teinturier C, Diallo I, Doyon F, Guénel P, de Vathaire F. Pooled analysis of two case-control studies in New Caledonia and French Polynesia of body mass index and differentiated thyroid cancer: the importance of body surface area. Thyroid. 2010 Nov;20(11):1285-93.
- 115. Kitahara CM, Platz EA, Freeman LE, Hsing AW, Linet MS, Park Y, Schairer C, Schatzkin A, Shikany JM, Berrington de González A. Obesity and thyroid cancer risk among U.S. men and women: a pooled analysis of five prospective studies. Cancer Epidemiol Biomarkers Prev. 2011 Mar;20(3):464-72.
- 116. Rinaldi S, Lise M, Clavel-Chapelon F, Boutron-Ruault MC, Guillas G, Overvad K, Tjønneland A, Halkjær J, Lukanova A, Kaaks R, Bergmann MM, Boeing H, Trichopoulou A, Zylis D, Valanou E, Palli D, Agnoli C, Tumino R, Polidoro S, Mattiello A, Bueno-de-Mesquita HB, Peeters PH, Weiderpass E, Lund E, Skeie G, Rodríguez L, Travier N, Sánchez MJ, Amiano P, Huerta JM, Ardanaz E, Rasmuson T, Hallmans G, Almquist M, Manjer J, Tsilidis KK, Allen NE, Khaw KT, Wareham N, Byrnes G, Romieu I, Riboli E, Franceschi S. Body size and risk of differentiated thyroid carcinomas: findings from the EPIC study. Int J Cancer. 2012 Sep 15;131(6):E1004-14.
- 117. Xu L, Port M, Landi S, Gemignani F, Cipollini M, Elisei R, Goudeva L, Müller JA, Nerlich K, Pellegrini G, Reiners C, Romei C, Schwab R, Abend M, Sturgis EM. Obesity and the risk of papillary thyroid cancer: a pooled analysis of three case-control studies. Thyroid. 2014 Jun;24(6):966-74.
- 118. Peterson E, De P, Nuttall R. BMI, diet and female reproductive factors as risks for thyroid cancer: a systematic review. PLoS One. 2012;7(1):e29177.
- 119. Kitahara CM, McCullough ML, Franceschi S, Rinaldi S, Wolk A, Neta G, Olov Adami H, Anderson K, Andreotti G, Beane Freeman LE, Bernstein L, Buring JE, Clavel-Chapelon F, De Roo LA, Gao YT, Gaziano JM, Giles GG, Håkansson N, Horn-Ross PL, Kirsh VA, Linet MS, MacInnis RJ, Orsini N, Park Y, Patel AV, Purdue MP, Riboli E, Robien K, Rohan T, Sandler DP, Schairer C, Schneider AB, Sesso HD, Shu XO, Singh PN, van den Brandt PA, Ward E, Weiderpass E, White E, Xiang YB, Zeleniuch-Jacquotte A, Zheng W, Hartge P, de González AB. Anthropometric Factors and Thyroid Cancer Risk by Histological Subtype: Pooled Analysis of 22 Prospective Studies. Thyroid. 2016 Feb;26(2):306-18.
- 120. Renehan AG, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. Lancet. 2008 Feb 16;371(9612):569-78.

- Nyrnes A, Jorde R, Sundsfjord J. Serum TSH is positively associated with BMI. Int J Obes (Lond). 2006 Jan;30(1):100-5.
- 122. Tramontano D, Cushing GW, Moses AC, Ingbar SH. Insulin-like growth factor-I stimulates the growth of rat thyroid cells in culture and synergizes the stimulation of DNA synthesis induced by TSH and Graves'-IgG. Endocrinology. 1986 Aug;119(2):940-2.
- 123. Food and Agriculture Organization of the United Nations (FAO)/International Network of Food Data Systems (INFOODS) Food Composition Databases. Available at: http://www.fao.org/infoods/infoods/tables-and-databases/faoinfoods-databases/en/ (accessed January 17, 2017).
- 124. Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail 2013 French food composition table Ciqual version 2013. Available at: https://pro.anses.fr/tableciqual/index.htm (accessed September 23, 2015).
- 125. Comite Europeen de Normalisation 2007 Foodstuffs Determination of trace elements –Determination of iodine by ICP-MS (inductively coupled plasma mass spectrometry). Comite Europeen de Normalisation 15111, Brussels.
- 126. Association française de normalisation 2010 NF V03–110 Analyse des produits agricoles et alimentaires –Protocole de caractérisation en vue de la validation d'une méthode d'analyse quantitative par construction du profil d'exactitude. Association française de normalisation, La Plaine Saint-Denis.
- 127. Mermet JM, Granier G 2012. Potential of accuracy profile for method validation in inductively coupled plasma spectrochemistry. Spectrochimica Acta–Part B 76:214–220.
- 128. Taga I, Sameza ML, Kayo AV, Ngogang J. Iodine levels in food and soil in different regions in Cameroon. Sante. 2004;14:11–15.
- 129. Takahashi T, Fujimori K, Simon SL, Bechtner G, Edwards R, Trott KR. Thyroid nodules, thyroid function and dietary iodine in the Marshall Islands. Int J Epidemiol. 1999 Aug;28(4):742-9.
- 130.
 Field
 Epidemiology
 Manual.
 Available
 at:

 https://wiki.ecdc.europa.eu/fem/w/wiki/principles-of-matching#freq (accessed March 29, 2017).
- 131. Truong T, Rougier Y, Dubourdieu D, Guihenneuc-Jouyaux C, Orsi L, Hémon D, Guénel P. Time trends and geographic variations for thyroid cancer in New Caledonia, a very high incidence area (1985-1999). Eur J Cancer Prev. 2007 Feb;16(1):62-70.
- 132. Guignard R, Truong T, Rougier Y, Baron-Dubourdieu D, Guénel P. Alcohol drinking, tobacco smoking, and anthropometric characteristics as risk factors for thyroid cancer: a

countrywide case-control study in New Caledonia. Am J Epidemiol. 2007 Nov 15;166(10):1140-9.

- 133. Leux C, Truong T, Petit C, Baron-Dubourdieu D, Guénel P. Family history of malignant and benign thyroid diseases and risk of thyroid cancer: a population-based casecontrol study in New Caledonia. Cancer Causes Control. 2012 May;23(5):745-55.
- 134. Tcheandjieu C, Lesueur F, Sanchez M, Baron-Dubourdieu D, Guizard AV, Mulot C, Laurent-Puig P, Schvartz C, Truong T, Guenel P. Fine-mapping of two differentiated thyroid carcinoma susceptibility loci at 9q22.33 and 14q13.3 detects novel candidate functional SNPs in Europeans from metropolitan France and Melanesians from New Caledonia. Int J Cancer. 2016 Aug 1;139(3):617-27.
- 135. "Présentation" (in French). Nouvelle-caledonie.gouv.fr. Archived from the original on
 30 October 2012. Retrieved 2013-01-30. Available at: https://web.archive.org/web/20121030174803/http://www.nouvelle-caledonie.gouv.fr/site/La-Nouvelle-Caledonie/Presentation (accessed January 29, 2017).
- 136. "Présentation L'Outre-Mer". Outre-mer.gouv.fr. Retrieved 2013-01-30. Available at: <u>http://www.outre-mer.gouv.fr/?presentation-nouvelle-caledonie.html</u> (accessed January 29, 2017).
- 137. "268 767 habitants en 2014.". ISEE. Retrieved 2014-11-16. Available at: <u>http://www.isee.nc/population/recensement/structure-de-la-population-et-evolutions</u> (accessed January 29, 2017).
- Hedinger C, Williams ED, Sobin LH. Histological typing of thyroid tumours. In: Hedinger C,Williams ED, Sobin LH, eds. International histological classification of tumours. 2nd ed. Berlin, Germany: Springer-Verlag, 1988.
- Lucas F, Niravong M, Villeminot S, Kaaks R, Clavel-Chapelon F. Estimation of food portion size using photographs: validity, strength, weaknesses and recommendations. J Hum Nutr Diet. 1995;8:65–74.
- Van Liere MJ, Lucas F, Clavel F, Slimani N, Villeminot S. Relative validity and reproducibility of a French dietary history questionnaire. Int J Epidemiol. 1997; 26:S128– 136.
- Gleize L, Laudon F, Yen Kai Sun L, et al (2000). Cancer registry in French Polynesia: results for the 1990-1995 period for native and immigrant populations. Eur J Epidemiol, 16, 661-7.
- 142. Brindel P, Doyon F, Rachédi F, Boissin JL, Sebbag J, Shan L, Chungue V, Bost-Bezeaud F, Petitdidier P, Paoaafaite J, Teuri J, de Vathaire F. Anthropometric factors in

differentiated thyroid cancer in French Polynesia: a case-control study. Cancer Causes Control. 2009 Jul;20(5):581-90.

- 143. Drozdovitch V, Bouville A, Doyon F, Brindel P, Cardis E, de Vathaire F. Reconstruction of individual radiation doses for a case-control study of thyroid cancer in French Polynesia. Health Phys. 2008 May;94(5):418-33.
- 144. Cléro É, Doyon F, Chungue V, Rachédi F, Boissin JL, Sebbag J, Shan L, Rubino C, de Vathaire F. Dietary patterns, goitrogenic food, and thyroid cancer: a case-control study in French Polynesia. Nutr Cancer. 2012;64(7):929-36.
- 145. Xhaard C, Ren Y, Clero E, Maillard S, Brindel P, Rachedi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Drozdovitch V, Doyon F, Rubino C, de Vathaire F. Differentiated thyroid carcinoma risk factors in French Polynesia. Asian Pac J Cancer Prev. 2014;15:2675-2680.
- 146. "Population des communes de Polynésie française". INSEE. Retrieved 13 October 2013. Available at: <u>https://www.insee.fr/fr/statistiques/2122859</u> (accessed January 29, 2017).
- 147. "Total Population Both Sexes". World Population Prospects, the 2015 Revision. United Nations Department of Economic and Social Affairs, Population Division, Population Estimates and Projections Section. July 2015. Retrieved 1 June 2016.
- 148. "French Polynesia". United Nations. Retrieved 19 August 2016. Available at: <u>http://data.un.org/CountryProfile.aspx?crName=FRENCH%20POLYNESIA#Economic</u> (accessed January 29, 2017).
- 149. Bricas N, Etienne J, Mou Y. Résultats de l'enquête de consommation alimentaire réalisée en Polynésie Française en 1995. Tahiti: Direction de la santé, Cirad, 2001.
- 150. Stsjazhko VA, Tsyb AF, Tronko ND, Souchkevitch G, Baverstock KF. Childhood thyroid cancer since accident at Chernobyl. BMJ. 1995 Mar 25;310(6982):801.
- 151. Cardis E, Krewski D, Boniol M, Drozdovitch V, Darby SC, Gilbert ES, Akiba S, Benichou J, Ferlay J, Gandini S, Hill C, Howe G, Kesminiene A, Moser M, Sanchez M, Storm H, Voisin L, Boyle P. Estimates of the cancer burden in Europe from radioactive fallout from the Chernobyl accident. Int J Cancer. 2006 Sep 15;119(6):1224-35.
- 152. Xhaard C, de Vathaire F, Cléro É, Maillard S, Ren Y, Borson-Chazot F, Sassolas G, Schvartz C, Colonna M, Lacour B, Danzon A, Velten M, Marrer E, Bailly L, Barjoan EM, Schlumberger M, Orgiazzi J, Adjadj E, Rubino C. Anthropometric Risk Factors for Differentiated Thyroid Cancer in Young Men and Women From Eastern France: A Case-Control Study. Am J Epidemiol. 2015 Aug 1;182(3):202-14.

- 153. Xhaard C, Lence-Anta JJ, Ren Y, Borson-Chazot F, Sassolas G, Schvartz C, Colonna M, Lacour B, Danzon A, Velten M, Cléro É, Maillard S, Marrer E, Bailly L, Mariné Barjoan E, Schlumberger M, Orgiazzi J, Adjadj E, Pereda CM, Turcios S, Velasco M, Chappe M, Infante I, Bustillo M, García A, Salazar S, Rodriguez R, Benadjaoud MA, Ortiz RM, Rubino C, de Vathaire F. Recreational Physical Activity and Differentiated Thyroid Cancer Risk: A Pooled Analysis of Two Case-Control Studies. Eur Thyroid J. 2016 Jul;5(2):132-8.
- 154. Constance Xhaard, Agnes Dumas, Vincent Souchard, Yan Ren, Françoise Borson-Chazot, Geneviève Sassolas, Claire Schvartz, Marc Colonna, Brigitte Lacour, Anne-Sophie Wonoroff, Michel Velten, Enora Cléro, Stéphane Maillard, Emilie Marrer, Laurent Bailly, Eugènia Mariné Barjoan, Martin Schlumberger, Jacques Orgiazzi, Elisabeth Adjadj, Carole Rubino, André Bouville, Vladimir Drozdovitch, Florent de Vathaire. Are dietary reports in a case-control study on thyroid cancer biased by risk perception of Chernobyl fallout? Accepted by EPIDEMIOLOGY AND PUBLIC HEALTH (REVUE D'EPIDÉMIOLOGIE ET DE SANTÉ PUBLIQUE, January 29, 2017).
- 155. "The World Factbook Field Listing Area". CIA.gov. Central Intelligence Agency. Retrieved 1 November 2015. Available at: https://www.cia.gov/library/publications/theworld-factbook/fields/2147.html (accessed January 29, 2017).
- 156. Bailly L, Giusiano B, Barjoan EM, Michiels JF, Ambrosetti D, Lacombe S, Granon C, Viot A, Dunais B, Daurès JP, Pradier C. Investigating the completeness of a histopathological cancer registry: estimation by capture-recapture analysis in a French geographical unit Alpes-Maritimes, 2008. Cancer Epidemiol. 2011 Dec;35(6):e62-e68.
- 157. Bailly L, Mariné-Barjoan E, Ambrosetti D, Roussel JF, Caissotti C, Ettore F, Diebolt E, Grill JM, Sattonnet C, Michiels JF, Pradier C. Data quality of cancer registration by Adicap codes, used by French pathologists from Paca, 2005-2006. Ann Pathol. 2009 Apr;29(2):74-79.
- 158. Cuban National Bureau of Statistics, Havana, Cuba 2014. Available at: www.one.cu/aec2013/esp/20080618_tabla_cuadro.htm (accessed September 23, 2015).
- 159. Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, Bai Y, Zhu C, Guo GL, Rothman N, Zhang Y 2009 International patterns and trends in thyroid cancer incidence, 1973–2002. Cancer Causes Control 20:525–531.
- 160. Ministerio de Salud Pública-Dirección Nacional de Registros Médicos y Estadísticas de Salud 2015 Anuario estadístico de salud 2014. Available at

http://files.sld.cu/dne/files/2015/04/anuario-estadistico-de-salud-2014.pdf (accessed September 23, 2015).

- 161. Lence-Anta JJ, Xhaard C, Ortiz RM, Kassim H, Pereda CM, Turcios S, Velasco M, Chappe M, Infante I, Bustillo M, García A, Cléro É, Maillard S, Salazar S, Rodriguez R, de Vathaire F. Environmental, lifestyle, and anthropometric risk factors for differentiated thyroid cancer in cuba: a case-control study. Eur Thyroid J. 2014 Sep;3(3):189-96.
- 162. Turcios S, Lence-Anta JJ, Santana JL, Pereda CM, Velasco M, Chappe M, Infante I, Bustillo M, García A, Cléro É, Maillard S, Rodriguez R, Xhaard C, Ren Y, Rubino C, Ortiz RM, de Vathaire F. Thyroid volume and its relation to anthropometric measures in a healthy cuban population. Eur Thyroid J. 2015 Mar;4(1):55-61.
- 163. "Cuba profile: Facts". BBC News. Retrieved March 26, 2013. Available at: http://www.bbc.com/news/world-latin-america-19583446 (accessed January 29, 2017).
- 164. "Cuba". Available at: <u>https://en.wikipedia.org/wiki/Cuba#cite_note-BBC_profile-14</u> (accessed January 29, 2017).
- 165. Rangel, Carlos (1977). The Latin Americans: Their Love-Hate Relationship with the United States. New York: Harcourt Brace Jovanovich. pp. 3–5. ISBN 978-0-15-148795-0. Skidmore, Thomas E.; Peter H. Smith (2005). Modern Latin America (6 ed.). Oxford and New York: Oxford University Press. pp. 1–10. ISBN 978-0-19-517013-9.
- 166. "Human Development Report 2015 "Rethinking Work for Human Development""
 (PDF). HDRO (Human Development Report Office) United Nations Development Programme. Available at: http://hdr.undp.org/sites/default/files/2015_human_development_report.pdf (accessed January 29, 2017).
- 167. "GHO By category Life expectancy Data by country". Available at: <u>http://apps.who.int/gho/data/node.main.688?lang=en</u> (accessed January 29, 2017).
- 168. Field Listing: Literacy Archived November 24, 2016, at the Wayback Machine.. CIA World Factbook. Available at: <u>https://www.cia.gov/library/publications/the-world-factbook/fields/2103.html#136</u> (accessed January 29, 2017).
- 169. Acosta LF 2015 Los dilemas de la sal. Available at: <u>www.granma.cu/cuba/2015-03-</u> <u>20/los-dilemas-de-la-sal?page=1</u> (accessed May 23, 2016).
- 170. Parlato R, Rosica A, Rodriguez-Mallon A, Affuso A, Postiglione MP, Arra C, Mansouri A, Kimura S, Di Lauro R, De Felice M. An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol. 2004 Dec 15;276(2):464-75.

- 171. Zannini M1, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R. TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. EMBO J. 1997 Jun 2;16(11):3185-97.
- 172. Zhang P, Zuo H, Nakamura Y, Nakamura M, Wakasa T, Kakudo K. Immunohistochemical analysis of thyroid-specific transcription factors in thyroid tumors. Pathol Int. 2006 May;56(5):240-5.
- 173. Perna MG, Civitareale D, De Filippis V, Sacco M, Cisternino C, Tassi V. Absence of mutations in the gene encoding thyroid transcription factor-1 (TTF-1) in patients with thyroid dysgenesis. Thyroid. 1997 Jun;7(3):377-81.
- 174. Denny JC, Crawford DC, Ritchie MD, Bielinski SJ, Basford MA, Bradford Y, Chai HS, Bastarache L, Zuvich R, Peissig P, Carrell D, Ramirez AH, Pathak J, Wilke RA, Rasmussen L, Wang X, Pacheco JA, Kho AN, Hayes MG, Weston N, Matsumoto M, Kopp PA, Newton KM, Jarvik GP, Li R, Manolio TA, Kullo IJ, Chute CG, Chisholm RL, Larson EB, McCarty CA, Masys DR, Roden DM, de Andrade M. Variants near FOXE1 are associated with hypothyroidism and other thyroid conditions: using electronic medical records for genome- and phenome-wide studies. Am J Hum Genet. 2011 Oct 7;89(4):529-42.
- Canman CE, Lim DS. The role of ATM in DNA damage responses and cancer. Oncogene. 1998 Dec 24;17(25):3301-8.
- 176. Schrauder M, Frank S, Strissel PL, Lux MP, Bani MR, Rauh C, Sieber CC, Heusinger K, Hartmann A, Schulz-Wendtland R, Strick R, Beckmann MW, Fasching PA. Single nucleotide polymorphism D1853N of the ATM gene may alter the risk for breast cancer. J Cancer Res Clin Oncol. 2008 Aug;134(8):873-82.
- 177. Barrett JH, Iles MM, Harland M, Taylor JC, Aitken JF, Andresen PA, et al. Genomewide association study identifies three new melanoma susceptibility loci. Nat Genet. 2011 Oct 9;43(11):1108-13.
- 178. Akulevich NM, Saenko VA, Rogounovitch TI, Drozd VM, Lushnikov EF, Ivanov VK, Mitsutake N, Kominami R, Yamashita S. Polymorphisms of DNA damage response genes in radiation-related and sporadic papillary thyroid carcinoma. Endocr Relat Cancer. 2009 Jun;16(2):491-503.
- 179. Damiola F, Byrnes G, Moissonnier M, Pertesi M, Deltour I, Fillon A, Le Calvez-Kelm F, Tenet V, McKay-Chopin S, McKay JD, Malakhova I, Masyakin V, Cardis E, Lesueur F, Kesminiene A. Contribution of ATM and FOXE1 (TTF2) to risk of papillary thyroid

carcinoma in Belarusian children exposed to radiation. Int J Cancer. 2014 Apr 1;134(7):1659-68.

- 180. Nguyen-Dumont T, Calvez-Kelm FL, Forey N, McKay-Chopin S, Garritano S, Gioia-Patricola L, De Silva D, Weigel R, Sangrajrang S, Lesueur F, Tavtigian SV; Breast Cancer Family Registries (BCFR); Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab). Description and validation of highthroughput simultaneous genotyping and mutation scanning by high-resolution melting curve analysis. Hum Mutat. 2009 Jun;30(6):884-90.
- 181. Li M, Eastman CJ. The changing epidemiology of iodine deficiency. Nat Rev Endocrinol. 2012 Apr 3;8(7):434-40.
- Lee SM, Lewis J, Buss DH, Holcombe GD, Lawrance PR. Iodine in British foods and diets. Br J Nutr. 1994 Sep;72(3):435-46.
- 183. Dunn OJ. Multiple comparisons among means. JASA. 1961;56:52–64.
- Choi WJ, Kim J. Dietary factors and the risk of thyroid cancer: a review. Clin Nutr Res. 2014 Jul;3(2):75-88.
- 185. Michikawa T, Inoue M, Shimazu T, Sawada N, Iwasaki M, Sasazuki S, Yamaji T, Tsugane S; Japan Public Health Center-based Prospective Study Group. Seaweed consumption and the risk of thyroid cancer in women: the Japan Public Health Centerbased Prospective Study. Eur J Cancer Prev. 2012 May;21(3):254-60.
- 186. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. Systematic populationbased assessment of cancer risk in first-degree relatives of cancer probands. J Natl Cancer Inst. 1994 Nov 2;86(21):1600-1608.
- 187. Pal T, Vogl FD, Chappuis PO, Tsang R, Brierley J, Renard H, Sanders K, Kantemiroff T, Bagha S, Goldgar DE, Narod SA, Foulkes WD. Increased risk for nonmedullary thyroid cancer in the first degree relatives of prevalent cases of nonmedullary thyroid cancer: a hospital-based study. J Clin Endocrinol Metab. 2001 Nov;86(11):5307-5312.
- 188. Hemminki K, Vaittinen P. Effect of paternal and maternal cancer on cancer in the offspring: a population-based study. Cancer Epidemiol Biomarkers Prev. 1997 Dec;6(12):993-997.
- 189. Bignell GR, Canzian F, Shayeghi M, Stark M, Shugart YY, Biggs P, Mangion J, Hamoudi R, Rosenblatt J, Buu P, Sun S, Stoffer SS, Goldgar DE, Romeo G, Houlston RS, Narod SA, Stratton MR, Foulkes WD. Familial nontoxic multinodular thyroid goiter locus maps to chromosome 14q but does not account for familial nonmedullary thyroid cancer. Am J Hum Genet. 1997 Nov;61(5):1123-30.

- 190. Canzian F, Amati P, Harach HR, Kraimps JL, Lesueur F, Barbier J, Levillain P, Romeo G, Bonneau D. A gene predisposing to familial thyroid tumors with cell oxyphilia maps to chromosome 19p13.2. Am J Hum Genet. 1998 Dec;63(6):1743-1748.
- 191. Lesueur F, Stark M, Tocco T, Ayadi H, Delisle MJ, Goldgar DE, Schlumberger M, Romeo G, Canzian F. Genetic heterogeneity in familial nonmedullary thyroid carcinoma: exclusion of linkage to RET, MNG1, and TCO in 56 families. NMTC Consortium. J Clin Endocrinol Metab. 1999 Jun;84(6):2157-2162.
- 192. Malchoff CD, Sarfarazi M, Tendler B, Forouhar F, Whalen G, Joshi V, Arnold A, Malchoff DM. Papillary thyroid carcinoma associated with papillary renal neoplasia: genetic linkage analysis of a distinct heritable tumor syndrome. J Clin Endocrinol Metab. 2000 May;85(5):1758-1764.
- 193. McKay JD, Lesueur F, Jonard L, Pastore A, Williamson J, Hoffman L, Burgess J, Duffield A, Papotti M, Stark M, Sobol H, Maes B, Murat A, Kääriäinen H, Bertholon-Grégoire M, Zini M, Rossing MA, Toubert ME, Bonichon F, Cavarec M, Bernard AM, Boneu A, Leprat F, Haas O, Lasset C, Schlumberger M, Canzian F, Goldgar DE, Romeo G. Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. Am J Hum Genet. 2001 Aug;69(2):440-446. Epub 2001 Jul 2.
- 194. Cavaco BM, Batista PF, Sobrinho LG, Leite V. Mapping a new familial thyroid epithelial neoplasia susceptibility locus to chromosome 8p23.1-p22 by high-density single-nucleotide polymorphism genome-wide linkage analysis. J Clin Endocrinol Metab. 2008 Nov;93(11):4426-4430.
- 195. Capezzone M, Cantara S, Marchisotta S, Filetti S, De Santi MM, Rossi B, Ronga G, Durante C, Pacini F. Short telomeres, telomerase reverse transcriptase gene amplification, and increased telomerase activity in the blood of familial papillary thyroid cancer patients. J Clin Endocrinol Metab. 2008 Oct;93(10):3950-3957.
- 196. Figlioli G, Köhler A, Chen B, Elisei R, Romei C, Cipollini M, Cristaudo A, Bambi F, Paolicchi E, Hoffmann P, Herms S, Kalemba M, Kula D, Pastor S, Marcos R, Velázquez A, Jarząb B, Landi S, Hemminki K, Försti A, Gemignani F. Novel genome-wide association study-based candidate loci for differentiated thyroid cancer risk. J Clin Endocrinol Metab. 2014 Oct;99(10):E2084-2092.
- 197. Bonora E, Rizzato C, Diquigiovanni C, Oudot-Mellakh T, Campa D, Vargiolu M, Guedj M; NMTC Consortium, McKay JD, Romeo G, Canzian F, Lesueur F. The FOXE1 locus is a major genetic determinant for familial nonmedullary thyroid carcinoma. Int J Cancer. 2014 May 1;134(9):2098-2107.

- 198. Zhuang Y, Wu W, Liu H, Shen W. Common genetic variants on FOXE1 contributes to thyroid cancer susceptibility: evidence based on 16 studies. Tumour Biol. 2014 Jun;35(6):6159-6166.
- 199. Terry-Berro CB, Quintana-Jardines I, de la Paz-Luna M, García J, Fernández-Bohórquez R, Silvera-Téllez D, Díaz-Fuentes Y, Ferret-Martínez A, Reyes-Fernández D. Impact of the actions for the sustainable elimination of iodine deficiency in Cuba. Rev Peru Med Exp Salud Publica. 2014;31(1):24-29.
- 200. Bell BA, Morgan GB, Schoeneberger JA, Loudermilk BL, Kromrey JD, Ferron JM. Dancing the Sample Size Limbo with Mixed Models: How Low Can You Go? SAS Global Forum 2010. Paper 197-2010.
- 201. Li B, Lingsma HF, Steyerberg EW, Lesaffre E. Logistic random effects regression models: a comparison of statistical packages for binary and ordinal outcomes. BMC Med Res Methodol. 2011 May 23;11:77.
- 202. Breslow NE, Day NE. Statistical methods in cancer research. Volume 1 The analysis of case-control studies. Lyon, 1980.
- 203. Bouyer J, Hémon D, Cordier S, et al. Épidémiologie Principes et methods quantitatives. Paris, 2009.
- 204. Cuban National Bureau of Statistics, Havana, Cuba 2012. Available at: www.one.cu/EstadisticaPoblacion/EstadisticaPoblacion.asp (accessed January 17, 2017).
- 205. Favier JC, Ireland-Ripert J, Toque C, Feinberg M. Repertoire general des aliments. Table de composition [Food composition tables] CIQUAL-REGAL. Paris: Tec & Doc Lavoisier and INRA. 1995.
- 206. Bleichrodt N, Born M.P. A meta-analysis of research on iodine and its relationship to cognitive development. In: Stanbury J.B., editor. The damaged brain of iodine deficiency: Cognitive, Behavioral, Neuromotor and Educative Aspects. Cognizant Communication Corporation; New York, NY, USA: 1994. pp. 195–200.
- 207. Qian M, Wang D, Watkins WE, Gebski V, Yan YQ, Li M, Chen ZP. The effects of iodine on intelligence in children: a meta-analysis of studies conducted in China. Asia pacific J Clin Nutr. 2005;14:32-42.
- 208. Mackerras DE, Eastman CJ. Estimating the iodine supplementation level to recommend for pregnant and breastfeeding women in Australia. Med J Aust. 2012;197:238-42.

- 209. Gago-Dominguez M. Marine n-3 fatty acid intake, glutathione S-transferase polymorphisms and breast cancer risk in post-menopausal Chinese women in Singapore. Carcinogenesis. 2004;25, 2143-2147.
- Fradet V, Cheng I, Casey G, Witte JS. Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. Clin. Cancer Res. 2009;15, 2559–2566.
- 211. Hedelin M, Chang ET, Wiklund F, Bellocco R, Klint A, Adolfsson J, Shahedi K, Xu J, Adami HO, Grönberg H, Bälter KA. Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. Int. J. Cancer 2007;120, 398–405.
- 212. Poole EM, Hsu L, Xiao L, Kulmacz RJ, Carlson CS, Rabinovitch PS, Makar KW, Potter JD, Ulrich CM. Genetic variation in prostaglandin E2 synthesis and signaling, prostaglandin dehydrogenase, and the risk of colorectal adenoma. Cancer Epidemiol. Biomark. Prev. 2010;19, 547–557.
- 213. Lian W, Wang R, Xing B, Yao Y. Fish intake and the risk of brain tumor: a metaanalysis with systematic review. Nutr J. 2017;16:1.
- 214. Haslam A, Robb SW, Bonner MR, Lindblad W, Allegra J, Shen Y, Vena JE. Polychlorinated biphenyls and omega-3 fatty acid exposure from fish consumption, and thyroid cancer among New York anglers. J Environ Sci (China). 2016;41:270-7.
- 215. Vena JE, Buck GM, Kostyniak P, Mendola P, Fitzgerald E, Sever L, Freudenheim J, Greizerstein H, Zielezny M, McReynolds J, Olson J. The New York angler cohort study: exposure characterization and reproductive and developmental health. Toxicol Ind Health. 1996;12:327-34.
- 216. Bosetti C, Kolonel L, Negri E, Ron E, Franceschi S, Dal Maso L, Galanti MR, Mark SD, Preston-Martin S, McTiernan A, Land C, Jin F, Wingren G, Hallquist A, Glattre E, Lund E, Levi F, Linos D, La Vecchia C. A pooled analysis of case-control studies of thyroid cancer. VI. Fish and shellfish consumption. Cancer Causes Control. 2001 May;12(4):375-82.
- 217. Aaseth J, Frey H, Glattre E, Norheim G, Ringstad J, Thomassen Y. Selenium concentrations in the human thyroid gland. Biol Trace Elem Res. 1990;24:147-52.
- Jung HJ, Seo YR. Current issues of selenium in cancer chemoprevention. Biofactors. 2010;36:153-8.
- 219. Fairweather-Tait SJ, Bao Y, Broadley MR, Collings R, Ford D, Hesketh JE et al. Selenium in human health and disease. Antioxid Redox Signal. 2011;14:1337-83.

- 220. Willett WC, Polk BF, Morris JS, Stampfer MJ, Pressel S, Rosner B et al. Prediagnostic serum selenium and risk of cancer. Lancet. 1983;2:130-4.
- 221. Clark LC, Combs GF, Turnbull BW, Slate EH, Chalker DK, Chow J et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional prevention of cancer study group. JAMA. 1996;276:1957-63.
- 222. Duffield-Lillico AJ, Reid ME, Turnbull BW, Combs GF, Slate EH, Fischbach LA et al. Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer Trial. Cancer Epidemiol Biomarkers Prev. 2002;11:630-9.
- 223. Guo KF, Zhang Z, Wang JY, et al. Variation of urinary and serum trace elements (Ca, Zn, Cu, Se) in bladder carcinoma in China. Asian Pac J Cancer Prev. 2012;13, 2057-61.
- 224. Kato MA, Finley DJ, Lubitz CC, Zhu B, Moo TA, Loeven MR et al. Selenium decreases thyroid cancer cell growth by increasing expression of GADD153 and GADD34. Nutr Cancer. 2010;62:66-73.
- 225. Glattre E, Thomassen Y, Thoresen SO, Haldorsen T, Lund-Larsen PG, Theodorsen L et al. Prediagnostic serum selenium in a case-control study of thyroid cancer. Int J Epidemiol. 1989;18:45-9.
- 226. Moore MA, Baumann F, Foliaki S, et al. Cancer epidemiology in the Pacific Islands past, present and future. Asian Pac J Cancer Prev. 2010;11, 99-106.
- 227. Dewailly E, Château-Degat L, Suhas E. Fish consumption and health in French Polynesia. Asia Pac J Clin Nutr. 2008;17:86-93.
- 228. Elwaer N, Hintelmann H. Comparative performance study of different sample introduction techniques for rapid and precise selenium isotope ratio determination using multi-collector inductively coupled plasma mass spectrometry (MC-ICP/MS). Anal Bioanal Chem. 2007;389:1889-99.
- 229. Dewailly E, Suhas E, Mou Y, Dallaire R, Chateau-Degat L, Chansin R. High fish consumption in French Polynesia and prenatal exposure to metals and nutrients. Asia Pac J Clin Nutr. 2008;17:461-70.
- 230. World Health Organization. Diet, food supply and obesity in the Pacific. Geneva: Regional Office for the Western Pacific, World Health Organization; 2003.
- 231. Satia JA, King IB, Morris JS, Stratton K, White E. Toenail and plasma levels as biomarkers of selenium exposure. Ann Epidemiol. 2006;16:53-8.
- 232. Fleckman P. Anatomy and physiology of the nail. Dermatol Clin. 1985;3:373-81.

- 233. Hunter DJ, Morris JS, Chute CG, Kushner E, Colditz GA, Willett WC et al. Predictors of selenium concentration in human toenails. Am J Epidemiol. 1990;132:114-22.
- Arnaud J, Bertrais S, Roussel AM, Arnault N, Ruffieux D, Favier A et al. Serum selenium determinants in French adults: the SU.VI.M.AX study. Brit J Nutr. 2006;95:313-20.
- 235. Méplan C, Crosley LK, Nicol F, Beckett GJ, Howie AF, Hesketh JE et al. Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). FASEB J. 2007;21:3063-74.
- 236. Ortega RM, Rodríguez-Rodríguez E, Aparicio A, Jiménez-Ortega AI, Palmeros C, López-Sobaler AM et al. Young children with excess of weight show an impaired selenium status. Int J Vitam Nutr Res. 2012;82:121-9.
- 237. Bleys J, Navas-Acien A, Laclaustra M, Pastor-Barriuso R, Menke A, Guallar E et al. Serum selenium and peripheral arterial disease: results from the national health and nutrition examination survey, 2003-2004. Am J Epidemiol. 2009;169:996-1003.
- 238. Karimi G, Shahar S, Homayouni N, et al (2012). Association between trace element and heavy metal levels in hair and nail with prostate cancer. Asian Pac J Cancer Prev, 13, 4249-53.
- 239. Singh P, Kapil U, Shukla NK, Deo S, Dwivedi SN. Association between breast cancer and vitamin C, vitamin E and selenium levels: results of a case-control study in India. Asian Pac J Cancer Prev. 2005;6, 177-80.
- 240. Moncayo R, Kroiss A, Oberwinkler M, Karakolcu F, Starzinger M, Moncayo H et al. The role of selenium, vitamin C, and zinc in benign thyroid diseases and of selenium in malignant thyroid diseases: low selenium levels are found in subacute and silent thyroiditis and in papillary and follicular carcinoma. BMC Endocr Disord. 2008;8:2.
- 241. Garland M, Morris JS, Rosner BA, Stampfer MJ, Spate VL, Hunter DJ et al. Toenail trace element levels as biomarkers: reproducibility over a 6-year period. Cancer Epidemiol Biomarkers Prev. 1993;2:493-7.
- 242. Cann SA, van Netten JP, van Netten C. Hypothesis: iodine, selenium and the development of breast cancer. Cancer Cause Control. 2000;11:121-7.
- 243. Brauer VF, Schweizer U, Köhrle J, Paschke R. Selenium and goiter prevalence in borderline iodine sufficiency. Eur J Endocrinol. 2006;155:807-12.
- 244. Köhrle J, Gärtner R. Selenium and thyroid. Best Pract Res Clin Endocrinol Metab. 2009;23:815-27.

- 245. Lloyd B, Lloyd RS, Clayton BE. Effect of smoking, alcohol and other factors on the selenium status of a healthy population. J Epidemiol Community Health. 1983;37:213-17.
- 246. Meinhold CL, Ron E, Schonfeld SJ, Alexander BH, Freedman DM, Linet MS et al. Nonradiation risk factors for thyroid cancer in the US Radiologic Technologists Study. Am J Epidemiol. 2010;171:242-52.
- 247. Galanti MR, Hansson L, Bergström R, Wolk A, Hjartåker A, Lund E, Grimelius L, Ekbom A. Diet and the risk of papillary and follicular thyroid carcinoma: a population-based case-control study in Sweden and Norway. Cancer Causes Control 1997;8:205-14.
- 248. Mack WJ, Preston-Martin S, Bernstein L, Qian D. Lifestyle and other risk factors for thyroid cancer in Los Angeles County females. Ann Epidemiol. 2002;12:395-401.
- 249. Daniel CR, Cross AJ, Graubard BI, Hollenbeck AR, Park Y, Sinha R. Prospective investigation of poultry and fish intake in relation to cancer risk. Cancer Prev Res (Phila). 2011;4:1903-11.
- 250. Markaki I, Linos D, Linos A. The influence of dietary patterns on the development of thyroid cancer. Eur J Cancer. 2003;39:1912-9.
- 251. Bandurska-Stankiewicz E, Aksamit-Białoszewska E, Rutkowska J, Stankiewicz A, Shafie D. The effect of nutritional habits and addictions on the incidence of thyroid carcinoma in the Olsztyn province of Poland. Endokrynol Pol. 2011;62:145-50.
- 252. Jung SK, Kim K, Tae K, Kong G, Kim MK. The effect of raw vegetable and fruit intake on thyroid cancer risk among women: a case-control study in South Korea. Br J Nutr. 2013;109:118-28.
- 253. Park Y, Leitzmann MF, Subar AF, Hollenbeck A, Schatzkin A. Dairy food, calcium, and risk of cancer in the NIH-AARP Diet and Health Study. Arch Intern Med. 2009;169:391-401.
- 254. Takezaki T, Hirose K, Inoue M, Hamajima N, Kuroishi T, Nakamura S, Koshikawa T, Matsuura H, Tajima K. Risk factors of thyroid cancer among women in Tokai, Japan. J Epidemiol. 1996;6:140-7.
- 255. Rossing MA, Cushing KL, Voigt LF, Wicklund KG, Daling JR. Risk of papillary thyroid cancer in women in relation to smoking and alcohol consumption. Epidemiology. 2000;11:49-54.
- 256. Nagano J, Mabuchi K, Yoshimoto Y, Hayashi Y, Tsuda N, Land C, Kodama K. A case-control study in Hiroshima and Nagasaki examining nonradiation risk factors for thyroid cancer. J Epidemiol. 2007;17:76-85.

- 257. Navarro Silvera SA, Miller AB, Rohan TE. Risk factors for thyroid cancer: a prospective cohort study. Int J Cancer. 2005;116:433-438.
- 258. Allen NE, Beral V, Casabonne D, Kan SW, Reeves GK, Brown A, Green J; Million Women Study Collaborators. Moderate alcohol intake and cancer incidence in women. J Natl Cancer Inst. 2009;101:296-305.
- 259. Meinhold CL, Park Y, Stolzenberg-Solomon RZ, Hollenbeck AR, Schatzkin A, Berrington de Gonzalez A. Alcohol intake and risk of thyroid cancer in the NIH-AARP Diet and Health Study. Br J Cancer. 2009;101:1630-4.
- 260. Kabat GC, Kim MY, Wactawski-Wende J, Rohan TE. Smoking and alcohol consumption in relation to risk of thyroid cancer in postmenopausal women. Cancer Epidemiol. 2012;36:335-40.

APPENDIXES

1 Résumé Substantiel En Français

L'apport alimentaire en iode, sélénium dans les ongles, et risque du cancer différencié de la thyroïde: Analyses d'études cas-témoins du consortium Epi-Thyr

1.1 Contexte

Le cancer différencié de la thyroïde est un cancer à évolution lente et à bon pronostique, qui frappe des individus relativement jeunes. On distingue 5 types histologiques de cancer de la thyroïde: le cancer papillaire, le cancer folliculaire, le cancer peu différencié, le cancer médullaire, et le cancer anaplastique.

L'interprétation des variations temporelles et spatiales des données d'incidence de ce cancer rapportées dans les registres est très difficile car le facteur explicatif le plus important de ces variations est le sur-dépistage. Il semble cependant que cette incidence soit plus élevées dans les îles du pacifique qu'ailleurs.

Plusieurs facteurs de risque de cancer de la thyroïde ont émergé d'études épidémiologiques, mais le seul facteur étiologique clairement démontré est exposition aux rayonnements ionisants durant l'enfance. Par ailleurs, une histoire familiale du cancer de la thyroïde, les facteurs anthropométriques, et la résidence dans les régions d'endémie goitreuse caractérisées par un apport d'iode insuffisant, sont des facteurs de risque confirmés dans plusieurs études épidémiologiques. L'apport alimentaire en iode, les perturbateurs endocriniens, l'activité physique, un nombre élevé de grossesses, et certains facteurs génétiques, sont des facteurs de risque potentiels du cancer de la thyroïde.

Le cancer différencié de la thyroïde est le type de cancer (hors les rares cancers « héréditaires ») dont l'héritabilité est la plus élevée, il est le seul pour lequel la contribution des facteurs héréditaires est considéré comme plus importante que celle des facteurs environnementaux. Selon certaines études familiales et génétiques, il s'agirait d'une transmission autosomique dominante à pénétrance réduite. Les analyses pan-génomiques réalisées dans les populations

européennes depuis 2009 ont permis d'identifier un certain nombre de variants génétiques associés au risque de cancer thyroïdien, et, récemment, d'autres variants dans des études menées chez d'autres ethnies, de nouveaux variants ont été associés au risque de cancer de la thyroïde. Cependant les SNPs mis en évidence dans ces GWAS, même si la signification statistique de leur association était forte, étaient associés à des risques relatifs de cancer différencié de la thyroïde considérés comme modérés à faibles.

L'identification des facteurs environnementaux de risque du cancer différencié de la thyroïde, est rendu complexe du fait du sur-diagnostic important car il est très difficile de séparer les facteurs liés à la fréquence des consultations médicales, et donc de diagnostic, des facteurs liés à l'incidence réelle de ces cancers.

Il est établi que, dans les régions d'endémie goitreuse caractérisées par un apport d'iode insuffisant, la fréquence des goitres et des cancers différenciés de la thyroïde de type folliculaire est plus élevée qu'ailleurs. En revanche, l'influence de l'apport alimentaire en iode sur le risque de cancer papillaire, forme représentant actuellement plus de 80% des cancers différenciés de la thyroïde, dans la plupart des pays, ne fait pas l'objet d'un consensus.

Plusieurs auteurs ont affirmé qu'un apport excessif d'iode pouvait augmenter le risque de cancer thyroïdien de type papillaire. Ceci est clairement démontré pour les goitres. Dans tous les pays dans lesquels une supplémentation en iode a été introduite, une réduction de l'incidence des cancers différenciés de la thyroïde a été observée pour les formes vésiculaires et anaplastiques, mais une augmentation a été observée dans certain cas pour les formes papillaires. Il est cependant nécessaire de faire très attention dans l'interprétation de ces données, car, dans un grand nombre de pays, la supplémentation en iode a été initiée durant les années 1990, c'est-à-dire durant la période de généralisation de la biopsie à l'aiguille fine, moyen diagnostic qui a entrainé une augmentation de l'incidence observée, et probablement un sur-diagnostic important.

Une meilleure compréhension du rôle de l'apport alimentaire en iode dans le risque de cancer de la thyroïde, et des facteurs influençant ce rôle est un enjeu important pouvant avoir des applications en santé publique.

Par ailleurs, le sélénium est un oligo-élément essentiel présent principalement dans les céréales, la viande, le poisson, les œufs et les produits laitiers. De même que pour l'iode, la teneur en sélénium d'un aliment donné dépend fortement de l'emplacement géographique où il

est produit. Les propriétés de protection redox du sélénium peuvent jouer un rôle important lors de la production d'hormones thyroïdiennes. En raison de sa capacité à protéger la glande thyroïde et à maintenir la production d'hormones thyroïdiennes, il existe une forte concentration de sélénium dans la thyroïde, et l'approvisionnement en sélénium de la thyroïde est prioritaire même pendant les périodes de carence en sélénium. La carence en sélénium a été associée à un risque accru de plusieurs types de cancer, mais la relation entre le sélénium et le cancer de la thyroïde n'avait été que très peu étudiés.

1.2 Objectifs

Les objectifs généraux de ce travail de thèse étaient d'explorer la relation entre l'apport alimentaire en iode et le risque de cancer différencié de la thyroïde dans un ensemble d'études cas-témoins, réalisées dans des régions d'incidence très variables, afin d'identifier les facteurs de risque environnementaux et génétiques qui interagissent avec cette relation et d'étudier la possibilité d'utiliser le sélénium présent dans les ongles comme biomarqueur potentiel dans les études de cas-témoins sur le cancer de la thyroïde.

Les objectifs spécifiques étaient les suivants:

 Estimer la relation entre des groupes d'aliments, ou l'apport alimentaire en iode, et le risque de CDT, dans une étude de cas-témoins réalisée à Cuba.

2) Établir ou de rétablir l'apport alimentaire en iode, en énergie et en sélénium, pour les participants de l'ensemble de 5 études cas-témoins du consortium Epi-Thyr réalisées en Nouvelle Calédonie, en Polynésie Française, en France métropolitaine (2 études) et à Cuba, selon les différentes tables de composition alimentaire.

3) Étudier la relation entre l'apport alimentaire en iode et le risque de CDT, ainsi d'évaluer la possibilité d'interaction entre cet apport et 4 polymorphismes identifiés dans la plupart des études d'association génomes entier (GWAS) comme associés au risque de CDT, dont deux sur le gène *FOXE1*, un sur le gène *ATM* et un sur le gène *NKX2-1*, et d'explorer l'interaction avec les facteurs de risque environnementaux, dans une méta-analyse sur des données individuelles de l'ensemble des 5 études cas-témoins.

4) Évaluer la possibilité d'utiliser le sélénium présent dans les ongles comme bio-marqueur potentiel du risque de CDT, dans l'étude cas-témoins de Polynésie française.

1.3 Matériel et Méthodes

Ces travaux ont été réalisés à partir de 5 études cas-témoins réalisées en France, Polynésie Française, Nouvelle Calédonie, et à Cuba (tableau 1).

Ils s'inscrivent dans le cadre du consortium Epi-Thyr réalisé conjointement par les Equipes Cancer et Radiations et Cancer et Environnent du Centre de recherche en Epidémiologie et Santé des Populations (CESP, Unité 1018 Inserm), L'objectif du consortium Epi-Thyr est d'étudier les facteurs de risque environnementaux et génétiques du risque de cancer différencié de la thyroïde selon un protocole standardisé, dans des populations d'origine ethnique différente, dont Européenne, Kanak, Polynésiennes, Africaine.

	Polynésie Française	Est de la France métropolitaine	Cuba	Nouvelle Calédonie	France Métropolitaine
		YOUNG-THYR			CATHY
Équipe	Cancer et Radiations	Cancer et Radiations	Cancer et Radiations	Cancer et Environnement	Cancer et Environnement
Cas / Témoins	229 / 371	827 / 877	203 / 212	332 / 412	621 / 707
DNA (salive)	177 / 225	727 / 712	203 / 212	231/270	517 / 568
Période de diagnostic	1986-2004	2002-2006	2005-2010	1993-1999	2002-2007
Age au diagnostic	39 (10-62)	27 (9-35)	38 (17-60)	48 (17-80)	52 (25-83)
Critère d'inclusion	Résident et né en PF après 1950 (> 15 ans en 1966, date du 1 ^{er} essai nucléaire en PF	Résident dans l'Est de la France et né après 1971 (<15 ans à l'accident de Tchernobyl)	Résident à Cuba et < 60 ans à l'entretien	Résident en Nouvelle Calédonie depuis 5 ans ou + avant le diagnostic	Résident dans un des 3 départements choisis et âgé de 25 ans ou + au diagnostic
Groupe Ethnique					
Européens		701 / 794		42 / 134	593 / 690
Polynésiens	200 / 320	/ 2		24 / 43	
Mélanésiens				244 / 189	
Africains				244 / 189	
Autres	28 / 52	124 / 61	93 / 101	24 / 46	28 / 17

Tableau 1 - Description des 5 études cas-témoins du consortium Epi-Thyr

Les questionnaires alimentaires de ces 5 études cas-témoins étaient dérivés de celui de l'étude de cohorte E3N. Ils étaient donc relativement homogènes quant à leur méthodologie (fréquence et portions basées sur des photographies). Cependant le pain, source importante d'iode, n'avait pas été pris en compte dans les questionnaires des études réalisées à Cuba et dans l'Est de la France (Young-Thyr).

Des mesures de l'iode, du sélénium et d'autres éléments dans les aliments traditionnels de Polynésie Françaises et de Cuba ont été effectuées par l'ANSES (FPFCT 2013 et CFCT 2014), à partir d'échantillons collectés par l'IRSN en Polynésie Française, et par nos partenaires à Cuba.

Avant le début de ce travail de thèse, le rôle des facteurs alimentaires et de l'iode avait été étudié pour les études réalisées en Polynésie Française et en Nouvelle Calédonie, mais à en utilisant une table de composition alimentaire élaborée à partir d'aliments collectés en France (Table Ciqual 205), et donc peu adaptée à ces régions du Pacifique. Aucune analyse de ces facteurs n'avait encore été réalisée pour les études réalisées à Cuba et en France métropolitaine.

Les génotypages des 4 SNPs investigués sont faites par les collaborations avec CIRC et CNG.

Par ailleurs, des mesures de l'iode, du sélénium et d'autres oligoéléments métalliques avaient été réalisées par l'université du Québec dans les ongles des sujets de l'étude réalisée en Polynésie Française.

1.4 Programme de travail

Chronologiquement, dans le cadre de cette thèse de doctorat, mon travail a consisté à:

- Etudier, à partir des données de l'étude réalisée en Polynésie Française, la différence entre la teneur en sélénium dans les ongles des mains des cas de cancer de la thyroïde et les témoins.
- 2) Reconstituer à partir des données des questionnaires alimentaires, l'apport alimentaire en iode, en sélénium, à partir de la nouvelle table de composition alimentaire des mesures dans les aliments traditionnels cubains (CFCT 2014), et en énergie à partir des bases de données de FAO, la table de composition alimentaire du CIQUAL pour les autres aliments, pour les sujets de l'étude réalisée à Cuba. Ceci a nécessité

préalablement que j'effectue un nettoyage et des corrections importantes dans ces questionnaires alimentaires.

- Analyser les données alimentaires de l'étude cas-témoins réalisée à Cuba, et étudier l'interaction avec les 4 SNPs qui avaient été génotypés.
- Collecter et standardiser les données des questionnaires alimentaires des 5 études castémoins du consortium Epi-Thyr.
- 5) Reconstituer à partir des données des questionnaires alimentaires, des mesures de l'iode et du sélénium dans les aliments traditionnels polynésiens, pour les études réalisées en Polynésie Française et en Nouvelle Calédonie, et des données de la table de composition alimentaire du CIQUAL pour les autres aliments de ces mêmes études; pour tous les aliments pour les études réalisées en France, l'apport alimentaire en iode et en sélénium des cas et des témoins des 4 autres études du Consortium Epi-Thyr.
- Etudier comment prendre en considération le fait qu'il n'y avait pas d'item Pain, dans les questionnaires alimentaire de certaines études.
- 7) Analyser le rôle de l'apport alimentaire en iode dans les 5 études cas-témoins du Consortium Epi-Thyr, et l'interaction entre ce rôle et celui des 4 SNPs génotypés dans les 5 études, ainsi que des facteurs alimentaires.

1.5 Résultats

1.5.1 Apport alimentaire en iode et risque de cancer thyroïdien à Cuba

Le risque de DTC était plus faible chez les sujets qui avaient déclaré une consommation plus importante de poisson (p=0,005), ce résultat restant significatif après ajustement sur les facteurs suspectés de risque de DTC (p = 0,04). En revanche, il n'a pas été possible de mettre en évidence de relation avec la consommation de fruit de mer (*Tableau 2*).

	Cas / témoins	OR ¹ (95% CI)	p-tendance
Poissons (g/ jour)			
< 4	103/77	1.0 (référence)	0.04
4–14	50/65	0.60 (0.36-1.0)	
≥15	50/70	0.55 (0.34-0.92)	
Fruits de mers (g/ jour)			
0	128/102	1.00 (référence)	0.2
1–4	21/44	0.31 (0.17-0.58)	
≥ 5	54/66	0.70 (0.43-1.1)	
Ensemble des produits de la mer (g/jour)			
< 5	89/64	1.00 (référence)	0.1
5–19	60/81	0.58 (0.36-0.96)	
≥ 20	54/67	0.58 (0.35-0.98)	

Tableau 2 - Consommation de poisson et de fruits de mer à Cuba et risque de DTC.

¹ Régression logistique conditionnelle multivariée stratifiée sur l'âge et le sexe et ajusté sur l'apport énergétique alimentaire, la surface corporelle, le tabagisme, le groupe ethnique, le niveau d'éducation, le nombre de grossesses pour les femmes.

Nous n'avons pas mis en évidence d'association significative entre l'apport alimentaire en iode te le risque de DTC. Ce résultat restait identique après ajustement sur les facteurs suspectés de risque de DTC. En outre, nous n'avons pas mis en évidence d'interaction avec l'âge, le tabagisme, l'apport alimentaire en sélénium ou l'appartenance ethnique (toutes les interactions p > 0,05). Une interaction presque significative avec l'IMC a cependant été mise en évidence, une diminution du risque de DTC avec l'augmentation

Tableau 3 - Apport alimentaire en iode et risque de DTC dans l'étude réalisée à Cuba.

Apport alimentaire en Iode, en quartiles de la distribution chez les témoins (µg/jour)							
	< 164.9	(164.9–186.5)	(186.5–237.5)	(≥237.5)	p-tendance		
Cas /témoins	62/53	27/53	57/53	57/53			
OR ¹ (95% CI)	1.1 (0.62–2.0)	0.56 (0.30-1.1)	1.2 (0.69–2.2)	1.0*	0.7		

¹ Régression logistique conditionnelle multivariée stratifiée sur l'âge et le sexe et ajusté sur l'apport énergétique alimentaire, la surface corporelle, le tabagisme, le groupe ethnique, le niveau d'éducation, le nombre de grossesses pour les femmes.

Nous n'avons trouvé aucune interaction entre l'apport en iode alimentaire et les polymorphismes suivants, rs944289, rs1867277 et rs71369530 dans le risque de développer un DTC. À l'inverse, une forte interaction (p-interaction multivariée = 0,005) a été identifiée entre l'apport en iode alimentaire et le rs965513 (situé près du gène FOXE1 sur le chromosome 9q22.33). Une forte augmentation du risque de cancer différencié de la thyroïde a été observée avec le nombre d'exemplaires de l'allèle mineur (A) de ce SNP parmi les personnes dont l'apport alimentaire en iode était inférieure à la médiane (<186 μ g/jour). Le

risque était 3,4 fois plus élevé pour les sujets portant une copie de l'allèle A et 6,7 fois plus élevé pour les sujets portant deux copies de l'allèle A, que chez les sujets sans copie de l'allèle mineur (A).

Apport alime	ntaire en iode	Polymorphisme	e of SNP rs965513	(près de FOXE1)	P-tendance
		G/G	G/A	A/A	
		0,0	0,11		
<i>(</i> 1'	G (F) :	21/60	45/01	11/2	
< médiane	Cas / Temoins	31/68	45/31	11/3	
	OR^{1} (95% CI)	1.0 (référence)	3.4 (1.7-6.5)	6.7 (1.6–27.3)	0.0005
> médiane	Cas / Témoins	50/48	44/46	16/9	
> mediane		50/10	11/10	10/ 2	
	$OP^{1}(050/CI)$	24(1244)	24(1245)	22(1200)	0.4
	OK (95% CI)	2.4 (1.3-4.4)	2.4 (1.3-4.3)	3.3 (1.2-8.8)	0.4

Tableau 4 - Apport alimentaire en iode, polymorphisme de rs965513 et risque de cancer de la thyroïde.

¹ Régression logistique conditionnelle multivariée stratifiée sur l'âge et le sexe et ajusté sur l'apport énergétique alimentaire, la surface corporelle, le tabagisme, le groupe ethnique, le niveau d'éducation, le nombre de grossesses pour les femmes.

1.5.2 Apport alimentaire en iode et risque de cancer thyroïdien dans les 5 études du consortium Epi-Thyr

Parmi les 2162 cas des 5 études du consortium Epi-Thyr, 762 étaient des microcancers (plus long diamètre inférieur à 10 mm). Il n'avait été réalisé d'échographie thyroïdienne systématique chez les témoins, qui aurait surement permis de mettre en évidence des microcarcinomes, dans aucune des études du consortium. J'ai donc décidé de vérifier les résultats des analyses en éliminant ces microcarcinomes.

Il n'a pas été possible d'imputer la consommation de pain pour les études Cuba et Young-Thyr, dans lesquelles elle n'avait pas été collectée. En effet, l'analyse des données de l'étude Cathy (les comportements alimentaires des sujets vivants en Polynésie Française et en Nouvelle Calédonie étant trop différents de celui des ceux vivants en France ou à Cuba) a montré que l'âge, le sexe, et l'ensemble des autres aliments ne permettaient de prédire qu'une faible proportion de la variance de la consommation de pain dans cette étude.

J'ai donc décidé valider les analyses portant sur l'iode présent dans tous les items recueillies par des analyses excluant l'iode provenant du pain.

Le tableau 5 présente les principales sources de l'apport alimentaire en iode, chez les 2162 cas et 2571témoins des 5 études du consortium Epi-Thyr. Il montre l'importance des produits laitiers et des produits de la mer dans cet apport.

T	Cas	Témoins	Ensemble	T	Cas	Témoins	Ensemble
Item	(µg/jour)	(µg/ jour)	(µg/ jour)	Item	(µg/ jour)	(µg/ jour)	(µg/ jour)
Paint (3 études)	39,1	35,1	36,7	Fruits	4,5	3,0	4,2
Yoghourt	24,2	22,7	23,4	Charcuterie	3,2	3,4	3,3
Poisson	13,7	13,9	13,8	Riz	3,3	3,3	3,3
Fromage	11,3	11,0	11,1	Tomates	2,2	2,2	2,2
Fruits de mer	8,7	9,7	9,3	Poulet	2,2	2,1	2,2
Lait	8,8	8,5	8,6	Pates	1,8	1,7	1,7
Dessert	8,2	7,0	7,6	Pommes de terre	1,5	1,4	1,4
Œufs	6,9	7,2	7,1	Légumineuses	1,2	1,1	1,1
Autres végétaux	5,9	6,7	6,3	Alcool	0,7	1,0	0,9
Viande	4,3	4,4	4,4	Choux	0,3	0,3	0,3
Chips	4,6	4,1	4,3				
Ensemble	117,5	115,8	116,5				

Tableau 5 - Apport alimentaire en iode des sujets du consortium Epi-Thyr, moyenne par type d'aliment

La majorité des cas (45%) et les témoins (45%) ont été considérés comme présentant une carence en iode alimentaire légère selon la classification Iodine Global Network (IGN). Les populations des études réalisées en Nouvelle Calédonie, en Polynésie et à Cuba avaient un alimentaire en iode inférieur à celui de celles réalisées en France Métropolitaine et étaient plus souvent en insuffisance légère ou sévère.

Dans l'analyse globale, il n'a pas été possible de mettre en évidence d'association significative entre l'apport en iode alimentaire et le risque de cancer de la thyroïde, que cet apport soit classé, selon la classification IGN, qu'il soit analysé comme une variable continue dans un modèle log linéaire ou linéaire, ou en quartiles dans chaque étude (tableau 6). Ce résultat été confirmé dans les analyses excluant l'apport du au pain, ainsi en excluant les microcarcinomes.

Apport alimentaire en lode, en quartile de la distribution chez les temoins de chaque étude							
	Q1	Q2	Q3	Q4	p-tendance		
Ensemble EpiThyr							
OR ¹ (95% CI)	0,98 (0,78–1,2)	0,99 (0,81–1,2)	0,89 (0,73–1,1)	1,0 (ref)	0,5		
Nouvelle Calédonie							
OR ¹ (95% CI)	0,63 (0,35–1,14)	0,61 (0,33–0,95)	0,54 (0,32–0,93)	1,0 (ref)	0,1		
Polynésie Française							
OR ¹ (95% CI)	1,7 (0,86–3,5)	1,6 (0,81–3,0)	1,2 (0,64–2,2)	1,0 (ref)	0,05		
Young-Thyr							
OR ¹ (95% CI)	1,0 (0,68–1,6)	1,2 (0,81–1,7)	0,96 (0,69–1,3)	1,0 (ref)	0,7		
Cathy							
OR ¹ (95% CI)	0,94 (0,56–1,6)	1,1 (0,72–1,7)	0,87 (0,60–1,3)	1,0 (ref)	0,4		
Cuba							
OR ¹ (95% CI)	1,1 (0,62–2,0)	0,56 (0,30–1,1)	1,2 (0,69–2,2)	1,0 (ref)	0,7		

Tableau 6 - Apport alimentaire en io	de et le risque de CDT, sel	on les quartiles de la distribution	chez les
témoins de chaque étude, d	es cas et des témoins des 5	études du consortium Epi-Thyr	

¹ Régression logistique conditionnelle multivariée stratifiée sur l'étude, l'âge et le sexe et ajustée sur l'apport énergétique alimentaire, la surface corporelle, le tabagisme, le groupe ethnique, le niveau d'éducation, le nombre de grossesses pour les femmes

Une hétérogénéité significative ou presque significative, selon les variables et modèles de relation dose effet, a été mise en évidence entre les études pour la relation dose-effet entre l'apport alimentaire en iode et le risque de cancer de la thyroïde. Cette hétérogénéité était due principalement à l'étude réalisée en Polynésie Française, seule étude pour laquelle une relation significative existait, le risque diminuant avec l'augmentation de l'apport alimentaire en iode, même après exclusion des microcancers.

L'hétérogénéité entre études était due à une hétérogénéité entre groupes ethniques, qui était due principalement à une différence de relation dose effet entre les Polynésiens et les autres groupes ethniques. Chez les Polynésiens, un important apport alimentaire était associé à une réduction du risque de cancer de la thyroïde, alors que cette relation n'existait pas pour les autres groupes ethniques. D'une manière générale, ce résultat se maintenait quel que soit le critère choisi et même si on éliminait les microcarcinomes.

Par ailleurs, sur l'ensemble des femmes de l'analyse globale, une interaction significative (p=0.05) a été mise en évidence entre l'effet de l'apport alimentaire en iode et ceux du nombre de grossesses, facteur connu de risque de cancer différencié de la thyroïde: l'augmentation du risque de cancer thyroïdien avec le nombre de grossesses était nettement plus importante chez les femmes qui avaient un apport alimentaire en iode inférieure à la médiane de cet apport dans leur étude (figure 1).



Figure 1: Interaction entre le nombre de grossesses, l'apport alimentaire en iode et le risque de cancer de la thyroïde

Il n'a pas été possible de mettre en évidence d'interaction significative entre l'apport en sélénium et l'apport en iode dans le risque de cancer de différencié de la thyroïde.

Enfin, Il n'a pas été possible de retrouver, sur l'ensemble des études d'Epi-Thyr le résultat que nous avions mis en évidence dans l'étude réalisée à Cuba, à avoir une interaction entre l'apport alimentaire en iode et les variants du polymorphisme rs965513, ni de mettre en évidence de relation entre l'un des 3 autres SNPs étudiés et l'apport en iode dans le risque de cancer différencié de la thyroïde.

1.5.3 Sélénium présent dans les ongles

Le sélénium, l'iode et 4 polluants métalliques avaient été dosés dans les ongles des sujets de l'étude réalisée en Polynésie Française. Ces dosages avaient été effectués par l'Université du Québec, par spectrographie de masse après digestion dans des conditions basiques utilisant de l'hydroxyde de triméthylammonium pour l'iode, et dans des conditions acides avec de l'acide nitrique pour le sélénium.

L'analyse statistique par régression logistique conditionnelle n'a pas permis de mettre en évidence une association significative entre le taux de sélénium dans des ongles et le risque de cancer de la thyroïde. Ce résultat a été confirmé après ajustement sur l'âge au diagnostic du cancer, l'appartenance ethnique, le niveau éducation, l'indice de masse corporelle (IMC), le tabagisme et les antécédents de radiothérapie (*tableau 7*). Cette absence de relation n'était pas différente selon le sexe, le tabagisme, l'IMC, le taux d'iode des ongles, l'apport alimentaire en iode, ou le type histologique des cancers, (p-interaction>0,05).

Tableau 7 - Sélénium dans les ongles et risque de cancer différencié de la thyroïde en Polynésie Française

Sélénium (µg/g): Quartiles						
	(0.00-0.68)	(0.68-0.76)	(0.76-0.86)	(0.86-1.30)	p-tendance	
Cas témoins	51/93	56/81	50/79	58/78		
OR (95% CI) ¹	1 (référence)	1.1 (0.62-1.9)	1.1 (0.62-2.0)	1.0 (0.56-1.8)	0.6	

¹ Régression logistique conditionnelle multivariée stratifiée sur l'âge et le sexe et ajusté sur l'apport énergétique alimentaire, la surface corporelle, le tabagisme, le groupe ethnique, le niveau d'éducation, le nombre de grossesses pour les femmes.

Une interaction juste significative (p=0,04) a cependant été mise en évidence avec la taille de la tumeur : le taux de sélénium était plus important chez les cas de cancers de la thyroïde de taille supérieure à 10mm que chez leurs témoins (p=0,09). De même, bien qu'aucune interaction significative n'ait été mise en évidence, le taux de sélénium dans les ongles était presque significativement (p=0.1) associé au risque de cancer de la thyroïde chez les sujets qui avaient moins de 3 ppm d'iode dans les ongles, et chez ceux dont l'apport alimentaire en iode était inférieur à 117 mg /1 000 kcal / jour.

En conclusion, bien que réalisée dans un région insulaire du Pacifique, sur la quasi-totalité des cas de cancer de la thyroïde de cette région, et des témoins sélectionnés à partir du registre d'état civil de actes de naissance, région dont les habitants sont parmi les plus grands consommateurs de poissons (une importante source alimentaire de sélénium), il n'a pas été possible de mettre en évidence un intérêt du sélénium comme biomarqueur prédictif du risque de cancer différencié de la thyroïde.

1.6 Discussion et Conclusion

Lorsqu'elles portent sur l'alimentation, les études cas témoins les limitations des études castémoins portant sur rôle de l'alimentation cumulent les limitations propres aux études castémoins (biais d'anamnèse, de confusion, de sélection des témoins) avec les celles dues aux imprécisions des réponses aux questionnaires alimentaires. Concernant l'étude de la relation entre l'apport d'iode et en sélénium d'origine alimentaire et le risque de cancer de la thyroïde, une autre source d'incertitude s'ajoute, celle autours de la valeur de la teneur en iode des aliments, qui peut être très variable, pour un même aliment.

Cependant, 3 des études cas-témoins du consortium avaient été réalisées dans des régions où l'alimentation n'est pas très variée, soit à cause de l'importance que continue à y avoir l'alimentation traditionnelle (Polynésie Française et Nouvelle Calédonie), soit à cause d'un niveau de vie très bas et d'un isolement du à un embargo (Cuba). De plus des mesures de l'iode présent dans l'alimentation traditionnelle en Polynésie Française et à Cuba, spécialement réalisée à cet effet.

Ces particularités étaient une des motivations lors du choix de ce sujet de thèse, outre son intérêt majeur en santé publique.

D'une manière générale, ce travail n'a pas permis de mettre en évidence de relation entre l'apport en iode d'origine alimentaire et le risque de cancer de la thyroïde, ni d'interaction avec l'apport alimentaire en sélénium, ni d'utilisation possible du sélénium dans les ongles comme biomarqueur prédictif du risque de cancer de la thyroïde. Il n'a pas non plus permis de mettre évidence d'interaction entre les 4 polymorphismes choisis et l'apport alimentaire en iode, à l'exception de rs965513 dans la population cubaine.

L'interprétation du résultat concernant la différence observée entre les Polynésiens, chez lesquels un apport alimentaire en iode était associé à une réduction risque de cancer de la thyroïde, et les autres groupes ethniques, chez lesquels une telle relation n'a pas observée, est compliquée. En effet, même si l'apport alimentaire en iode des Polynésiens est légèrement ou modérément insuffisant, cela est vrai aussi des Mélanésiens vivant en Nouvelle Calédonie, chez lesquels ce phénomène n'a pas été observé.

Le résultat concernant le risque associé au nombre de grossesse élevée chez les femmes ayant un apport alimentaire insuffisant en iode pourrait être important en santé publique. Etant donné l'importante de l'iode dans le développement du fœtus, il est certain que les femmes qui ont un grand nombre de grossesse ont un état thyroïdien plus surveillé que les autres, et que ceci explique en partie l'association observée dans la plupart des études entre le nombre de grossesses et le risque de cancer différencié de la thyroïde. Il ne s'agit probablement pas de la seule explication car, par exemple, Epi-Thyr, cette relation reste très significative (p<0.001), même si les 762 microcancers sont exclus. Une autre raison pourrait venir des plus grands besoins en iode durant la grossesse, ce qui pourrait expliquer notre résultat.

1.7 Perspectives

D'une manière générale, mon travail souligne la difficulté de l'utilisation des enquêtes cas témoins pour étudier le rôle de l'apport alimentaire en iode dans l'occurrence d'une pathologie, en particulier en l'absence de biomarqueur validé tel que l'iodurie ou l'iodémie. Les principales perspectives de mon travail concernent l'approfondissent de mes résultats concernant la spécificité des populations Polynésiens, qui pourra être réalisé grâce à une extension de l'étude Polynésienne est en cours, et de celui concernant le risque associé au nombre de grossesse élevée chez les femmes ayant un apport alimentaire insuffisant en iode. La validation de ce résultat nécessitera des études spécifiques.

2 Supplemental Table 1 - Details used common food groups and items for the pooled analysis

Pool	New	French	Young-Thyr	Cathy	Cuba
	Caledonia	Polynesia			
Milk	Milk	Local fresh	Fresh cow's	Cow's milk	Fresh cow's
		milk	milk		milk
		Other milk	Fresh goat's or	Soy milk	Fresh goat's or
			sheep's milk		sheep's milk
Alcohol	Alcohol	Wine	Wine	Wine	Wine
		Beer	Beer	Beer	Beer
		Punch	Whisky	Champagne	Whisky
			Other aperitif	Cider	Other aperitif
			(fortified		(fortified
			wine, pastis)		wine, pastis)
		Whisky or	Alcohol,	Whisky, pastis	Alcohol,
		alcohol	liqueur		liqueur
				Aperitif (20°)	
				liqueur (40°)	

Pool	New	French	Young-Thyr	Cathy	Cuba
	Caledonia	Polynesia			
Sausage or	Cured or	Saucisse et	Cured or	Pâté, rillettes,	Cured or
charcuterie	cooked ham,	charcuterie	cooked ham,		cooked ham,
	Sausage (pâté,		Sausage (pâté,	Saucisson	Sausage (pâté,
	saucisson,		saucisson,		saucisson,
	rillettes)		rillettes)		rillettes)
				Boudin,	
				andouillette	
	Hot sausage (Hot sausage (Fresh broiled	Hot sausage (
	blood sausage,		blood sausage,	sausage	blood sausage,
	andouillette)		andouillette)		andouillette)
				Frankfurter	
				Cured ham	
				Cooked ham	
Egg	Hard-boiled	Egg	Hard-boiled	Hard-boiled	Hard-boiled
	egg, soft-		egg, soft-	egg, soft-	egg, soft-
	boiled, omelet		boiled, omelet	boiled, omelet	boiled, omelet
	~			~	
Dessert	Cream dessert	Chocolate	Ice cream	Custard,	Ice cream
	(chocolate or			cream dessert,	
	caramel cream)			pudding	
				Ice cream	
				Pastry	
				Fruitscake	
	Pastry	Cake	Cream dessert	Dark	Cream dessert
			(chocolate or	chocolate bar	(chocolate or
			caramel		caramel
			cream)		cream)
			,	Milk	,
				chocolate bar	
	Ice cream			Bar of	
				chocolate (Ex:	
				Mars, Nuts)	
		Spread		Honey	
	Chocolate	_		Jam	
				Candy or other	
				sugary	
				-	
Pasta	Pasta, samolina	Pasta	Pasta,	Pasta	Pasta,
			samolina		samolina

Pool	New	French	Young-Thyr	Cathy	Cuba
	Caledonia	Polynesia			
				Other whole	
				cereal (Pasta,	
				samolina)	
Rice	Rice	Rice	Rice	Rice	Rice
				Whole rice	
Potato	Potato	Potato	Potato	Potato	Potato
Chips	Fry, chips	Fry, chips	Fry, chips	Fry	Fry, chips
Pulses	Dried pulse	Dried pulse	Dried pulse	Dried pulse	Dried pulse
	(lentil, navy		(lentil, navy	(lentil, navy	(lentil, navy
	bean, split pea,		bean, split pea,	bean,	bean, split pea,
	cassoulet)		cassoulet)	chickpea,)	cassoulet)
				Cassoulet	
Bread	Bread	Bread		White bread	
				Whole bread	
				Other bread	
				(rye, bran)	
Shellfish	Other shellfish	Clam	Shellfish	Mussel	Shellfish
			(oyster,		(oyster,
			mussel,)		mussel,)
				Oyster	
	Octopus		Lobster, crab	Other shellfish	Lobster, crab
				(ex: whelk,	
				winkle,	
		~ ~ ~ 1		clam)	
	Lobster,	Seafood		Shrimp	
	Caledonian		Shrimp		
	Mitten Lobster				01
	Shrimp				Shrimp
				Crab	
	Crab		Other	Lobster	
Fruits	Orange	Lemon	Orange,	Apricot	Orange,
			mandarin,		grapefruit,
			grapefruit,		mandarin,
			banana,		lemon
			pineapple,		
			mango, melon,		
			watermelon,		
			kiwi		

Pool	New	French	Young-Thyr	Cathy	Cuba
	Caledonia	Polynesia			
	Mandarin	Orange		Strawberry,	
				raspberry	
	Grapefruit	Apple, pear			
	Pineapple				
	Mango	Peach, apricot,		Cherry	
		nectarine			
	Yellow papaya			Plum	
	Red papaya			Melon	Mango
	Corossol, apple			Grape	
	cinnamon				
	Apple liana	Passion fruit		Banana	Guava
	Guava		Grape,	Orange	
			strawberry		
	Litchi	Miraculous		Clementine,	Banana
		fruit		mandarin	
	Apple				
	Pear	Papaya		Grapefruit	Pineapple
	Grape	Guava		Pineapple	
	Kiwi	Mango	Apple, pear	Kiwi	Papaya
	Strawberry	Watermelon		Peach,	
				nectarine	
	Melon	Banana		Apple	Melon
	Watermelon			Pear	
Yoghurt	Yoghurt,	Yoghurt	Yoghurt,	Fromage blanc	Yoghurt,
	fromage		fromage	(plain or	fromage blanc,
	blancs, petit		blancs, petit	flavored)	petit suisse
	suisse		suisse		
				Yogurt (plain	
				or flavored)	
				Soy yogurt	
Cheese	Soft cheese	Cheese	Cow's cheese	Fresh cheese	Cow's cheese
				Blue cheese	
			Goat's cheese	Roquefort	Goat's cheese
				Goat's cheese	
	Other cheese		Sheep's cheese	Hard cheese	Sheep's cheese
				Raw hard	
				cheese	

Pool	New	French	Young-Thyr	Cathy	Cuba	
	Caledonia	Polynesia				
			Soft cheese	Soft rind cheese	Soft cheese	
Tomatoes	Raw tomatoe	Tomatoe	Raw tomatoe	Raw tomatoe	Raw tomato	
	Cooked		Cooked	Cooked	Cooked	
	tomatoe		tomatoe	tomatoe	tomato	
Cabbages	Raw cabbage	Cabbage	Raw cabbage	Raw cabbage	Raw cabbage	
	Cooked		Cooked		Cooked	
	european		cabbage		cabbage	
	cabbage					
	Cooked		Cooked	Raw	Cooked	
	cauliflower		brussels sprout	cauliflower	brussels sprout	
	Cooked			Cooked		
	brussels sprout			cabbage		
	Cooked chinese		Cooked	Cooked	Cooked	
	cabbage		cauliflower	cauliflower	cauliflower	
	Cooked kanak			Cooked		
	cabbage			brussels sprout		
Other	Vegetable soup	Salade russe	Raw lettuce	Soup	Raw lettuce	
vegetables				Raw chicory		
	Raw lettuce		Raw carrot	Raw lettuce	Raw soy	
	Raw carrot		Raw soy	Raw carrot		
	Other raw		Raw	Raw celeriac	Raw	
	vegetable		mushroom		mushroom	
			Aother raw	Raw pepper		
			vegetable			
	Cooked carrot			Raw	Cooked carrot	
	1 1			cucumber		
	cooked		Cooked carrot	Raw radish		
	Cooked brocoli	Haricot long		Raw sov	Cooked	
	Cooked brocom	Thancot long		Raw Soy	pumpkin	
	Cooked beet		Cooked	Onion		
			pumpkin			
	Cooked			Vegetable	Cooked	
	dandelion			soup	brocoli	
	Cooked		Cooked	Cooked carrot		
	pumpkin leaf		brocoli			

Pool	New	French	Young-Thyr	Cathy	Cuba
	Caledonia	Polynesia			
	Cooked taro			Cooked	Cooked turnip
	leaf			artichoke	
	Cooked brèdes	Other green	Cooked turnip	Cooked	
		vegetables		asparagus	
	Other cooked			Cooked	Cooked beet
	green leaf			brocoli	
			Cooked beet	Cooked turnip	
				Cooked	Cooked leek
				spinach, beet	
	Cooked		Cooked leek	Cooked	
	chouchoutes			chicory	
	Cooked leek			Cooekd green	Cooked celery
				bean	2
	Cooked celery		Cooked celery	Cooked	
				marrow,	
				zucchini	
	Cooked green Cooked		Cooked		
	pea			eggplant	spinach
	Cooked peas		Cooked	Cooked celery	
			spinach		
	Cooked			Cooked fennel	Cooked green
	eggplan				pea, peas, corn
	Cooked	Chinese soup	Cooked green	Cooked	
	marrow		pea, peas, corn	pepper	
	Cooked pepper			Cooked peas	
	Cooked corn		Cooked	Cooked leek	Cooked
			eggplant,		eggplant,
			marrow		marrow
	Cooked turnip			Cooked red	
				beet	
	Cooked seaweed			Ratatouille	
Fish	Fresh sea fish	Fafaru	Fresh sea fish	Blue sea fish	Fresh sea fish
	Fresh game fish			White sea fish	
	Fresh brackish		Fresh game	Blue game	Fresh game
	fish		fish	fish	fish
		Offshore fish		White game	
				fish	

Pool	New	French	Young-Thyr	Cathy	Cuba	
	Caledonia	Polynesia				
	Smoked, dried		Canned	Fish stick	Canned	
	or salted fish		sardine		sardine	
	Canned sardine		Canned tuna	Tuna, sardine	Canned tuna	
				or anchovy in		
				oil		
	Canned tuna	Lagoon fish	Other canned	Mackerel in	Other canned	
			fish	oil	fish	
	Canned			Canned tuna,		
	pilchard			sardine or		
				anchovy		
	Other canned		Frozen fish	Canned	Frozen fish	
	fish			mackerel		
				Fish soup		
Poultry	Poultry	Poultry	Poultry	Poultry, rabbit	Poultry	
	(chicken,		(chicken,		(chicken,	
	turkey, rabbit)		turkey, duck,		turkey, duck,	
			rabbit)		rabbit)	
Meats	Deer	Beef, calf	Deer	Meat (beef,	Deer	
				lamb, calf,		
				pork)		
	Beef		Beef	Game	Beef	
	Calf	Pork	Calf	Liver	Calf	
	Lamb		Lamb	Foie gras	Lamb	
	Porc, cochon	Sheep	Pork	Offal (brains,	Pork	
	sauvage			kidney)		
	Liver (calf,	Punu pua toro	Liver (calf,		Liver (calf,	
	deer, pork,	(corned beef)	pork, poultry)		pork, poultry)	
	poultry)					

3 Supplemental Table 2 - Adjusted Odds Ratio of DTC Associated with Dietary Iodine Intake According to IGN Classification in the Pooled Analysis

Dietary iodine intake, IGN classification (µg/day)						
	Severe or moderate	Mild deficiency	Optimal	More than adequate or excess	p- trend ³	
	$\frac{\text{deficiency}}{(\leq 74)}$	(75–149)	(150–299)	(≥300)		
Study						
Pooled Analysis [†]						
Cases/controls 2162/2571	443/542	966/1164	652/757	101/108		
Stratified OR ¹ (95% CI)	0.93 (0.77-1.11)	0.93 (0.81-1.07)	1.00*	1.23 (0.91–1.66)	0.1^{a}	
Multivariate OR ² (95% CI)	0.95 (0.75-1.20)	0.98 (0.83-1.16)	1.00*	1.28 (0.90-1.83)	0.5^{b}	
The New Caledonia study [‡]						
Cases/controls 332/412	174/211	125/173	30/26	3/2		
Stratified OR ¹ (95% CI)	0.79 (0.45-1.39)	0.68 (0.38-1.20)	1.00*	1.09 (0.17-7.08)	0.9^{a}	
Multivariate OR ² (95% CI)	0.68 (0.33-1.42)	0.71 (0.37-1.38)	1.00*	0.96 (0.12-7.55)	0.4^{b}	
The French Polynesia study						
Cases/controls 229/371	51/58	90/151	70/116	18/46		
Stratified OR ¹ (95% CI)	1.45 (0.89–2.37)	0.98 (0.66-1.46)	1.00*	0.65 (0.35-1.21)	0.04^{a}	
Multivariate OR ² (95% CI)	1.65 (0.90-3.02)	1.22 (0.77-1.95)	1.00*	0.72 (0.33-1.55)	0.07^{b}	
The Yong-Thyr study						
Cases/controls 805/876	118/147	450/501	217/219	20/9		
Stratified OR^1 (95% CI)	0.80 (0.59–1.08)	0.90 (0.71-1.12)	1.00*	2.25 (1.00-5.05)	0.03 ^a	
Multivariate OR ² (95% CI)	0.91 (0.57–1.43)	1.01 (0.75–1.37)	1.00*	2.04 (0.79-5.25)	0.6^{b}	
The Cathy study						
Cases/controls 593/700	18/33	223/262	295/355	57/50		
Stratified OR^1 (95% CI)	0.65 (0.36–1.19)	0.98 (0.77–1.25)	1.00*	1.53 (1.00–2.33)	0.05^{a}	
Multivariate OR ² (95% CI)	0.54 (0.27–1.10)	1.00 (0.74–1.37)	1.00*	1.58 (0.94–2.67)	0.2 ^b	
The Cuba study						
Cases/controls 203/212	82/93	78/77	40/41	3/1	_	
Stratified OR ¹ (95% CI)	0.92 (0.54–1.57)	1.10 (0.63–1.89)	1.00*	2.71 (0.27–27.17)	0.49^{a}	
Multivariate OR ² (95% CI)	2.15 (0.88-5.23)	1.74 (0.84–3.57)	1.00*	1.69 (0.13-22.79)	0.14 [°]	

[†] Bread item presented in the New Caledonia, the French Polynesia and the Cathy studies, but it was missing in the Young-Thyr and Cuba studies;

[‡] The value of dietary iodine intake for the New Caledonia study was underestimated by about 25 μ g/day due to some compound dishes had not been taken into account;

¹ Stratified by age class, sex and study;

² Stratified by age class, sex and study, and adjusted for smoking status, ethnicity, education level, number of full term pregnancies, body surface area, radiotherapy for previous cancer, first-degree relative thyroid cancer history, and energy intake;

³ Calculated using the Wald test for dietary iodine intake modeled as a continuous variable (per classification);

* *Reference category;*

^a Crude p-trend;

^b Adjusted p-trend.
4 Supplemental Table 3 - Dietary iodine intake and thyroid cancer risk: summary of the results from the published case-control studies

Author	Study	Ν,	Age,	Dietary	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	factor	low vs. high	OR (95% CI)	
country		(case/control)					
Kolonel	Case-control	191/441	≥ 18	Iodine intake	Mean [µg/d]	M: OR = 1.3	A positive
(1990) US	(Population-	(51M&140F/113M&328F)		from food	M: 469 vs.	(0.5-3.4);	monotonic trend
(Hawaii)	based)			sources and	447;	p-trend =	for iodine intake
(103)				from iodine	F: 368 vs.	0.48;	from
				containing	311	F: OR = 1.5	Foods in female
				supplements		(0.8-2.8);	
						p-trend =	
						0.22	
Horn-Ross	Case-control	608F/558F	20-74	Total iodine	[µg/d]	OR = 0.49	High intake of
(2001) US	(Population-			intake	< 273 vs. >	(0.29-0.84)	dietary iodine
(63)	based)			from food	537		inversely
				sources			associated with
							PTC risk in
							female
	1					1	

Author	Study	Ν,	Age,	Dietary	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	factor	low vs. high	OR (95% CI)	
country		(case/control)					
Truong	Case-control	293F/354F	≥18	Total iodine	[µg/d]	OR = 1.13	No association in
(2010)	(Population-			intake	<75.0 vs. \geq	(0.68-1.87);	all ethnic groups
France	based)			from food	112.6	p-trend =	
(New				sources		0.43	
Caledonia)							
(104)							
Cléro	Case-control	229/371	< 56	Total iodine	[µg/d]	OR = 0.39	Higher dietary
(2012)	(Population-	(26M&203F/47M/324F)		intake	≤ 105 vs.	(0.21-0.72);	iodine intake
France	based)			from food	106-175	p-trend =	inversely
(French				sources		0.03	associated with
Polynesia)							TC risk
(105)							
Ren (2016)	Case-control	203/212	17-60	Total iodine	[µg/d]	OR = 1.12	No association
France	(Population-	(24M&179F/39M/173F)		intake	< 164.9 vs. ≥	(0.62-2.03);	
(Cuba) (113)	based)			from food	237.5	p-trend = 0.7	
				sources			

5 Supplemental Table 4 - The association between fish, fruits, vegetables, meat, dairy food, and thyroid cancer risk: summary of the results from the published observational studies

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
Fish & Seafood							
Galanti	Case-control	Sweden	18-75	1. Freshwater	[portions/mo.]	1. $OR = 0.6$	A monthly intake
(1997)	(Population-	(35M/130F;50M/198F)		fish	1. < 1 vs. > 1	(0.4-1.1)	of freshwater
Sweden &	based)	Norway		2. Roe	$2. \le 1 \text{ vs.} > 1$	2. $OR = 0.4$	fish and roe
Norway		(24M/57F;57M/135F)				(0.2-0.7)	inversely
(247)							associated with
							TC risk, but not
							statistically
							significant
							in both Sweden
							and Norway

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
Horn-Ross	Case-control	608F/558F	20-74	Fish sauce/dried	[g/d]	OR = 2.3	High intake of
(2001)	(Population-			or salted fish	0.0 vs. > 2.0	(1.3-4.0)	fish sauce/dried
US	based)						or
(63)							salted fish
							positively
							associated TC
							risk
							in Asian women
Mack	Case-control	292F/292F	15-54	Saltwater fish	[frequency]	OR = 0.3	High intake of
(2002)	(Population-				< few/yr vs.	(0.1-0.7); p =	saltwater fish
US	based)				few/wk+	0.006	inversely
(248)							associated with
							PTC in adult
							female
Memon	Case-control	313/313	≤70	1. Fish	[frequency]	1. $OR = 0.6$	High intake of
(2002)	(Population-	(75M/238F)		2. Fish products	never/yr vs.	(0.3-1.0); p <	freshwater fish
Kuwait	based)				2-4 d or 5-7	0.05	inversely
(64)					d/wk	2. OR = 3.0	associated with

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
						(1.6-5.3); p <	TC risk
						0.01	High intake of
							processed/canned/
							frozen fish
							products
							positively
							associated with
							TC risk
Truong	Case-control	293F/354F	≥18	Brackish water	[g/d]	OR = 0.43	High intake of
(2010)	(Population-			fish	0.0 vs. > 10.0	(0.20-0.93);	brackish water
France	based)					p-trend =	fish inversely
(New						0.03	associated with
Caledonia)							TC risk
(104)							in all ethnic
							groups
							(Melanesian,
							European, and
							others)

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
Xhaard	Case-control	229/373	≤ 55	Total seafood	[g/d]	OR = 0.4	High intake of
(2014)	(Population-	(26M/203F TC)			\leq 45 vs. \geq 91	(0.3-0.8); p =	total food from
France	based)					0.0002	sea
(French							inversely
Polynesia)							associated with
(145)							TC risk
Cléro	Case-control	229/371	< 56	1. Fish	[g/d]	1. $OR = 0.47$	High intake of
(2012)	(Population-	(26M/203F;47M/324F)		2. Shellfish	$1. \leq 39 \text{ vs.} \geq$	(0.27-0.82);	fish, shellfish, and
France	based)			3. Total intake of	80	p = 0.008	total food from
(French				food from sea	2. 0 vs. \ge 8	2. $OR = 0.40$	sea inversely
Polynesia)					$3. \leq 45 \text{ vs.} \geq$	(0.22-0.72);	associated
(105)					91	p = 0.002	with TC risk
						3. $OR = 0.44$	
						(0.25-0.79);	
						p = 0.002	
Daniel	Cohort	492,186	50-71	Fish	[g/1000 kcal]	HR = 1.18	No association
(2011)	1) NIH-	(250M/333F TC)			3.6 vs. 21.4	(0.90-1.55);	
US	AARP					p = 0.38	

Author	Study	N,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
(249)	(1995-1996)						
	2) Follow-up						
	(9 yr)						
Michikawa	Cohort	52,679F	40-69	Seaweed	[frequency]	1. HR = 1.58	High intake of
(2012)	1) JPHCPS	(134TC)			$\leq 2 \text{ d/wk vs.}$	(0.91-2.73);	seaweed
Japan	(1993-1994)				almost daily	p-trend =	positively
(185)	2) Follow-up				1. TC	0.10	associated with
	(14.5 yr)				2. PTC	2. HR = 1.86	TC risk, but not
						(1.03-3.34);	statistically
						p-trend =	significant
						0.04	High intake of
							seaweed
							positively
							associated with
							TC and PTC risk
							in
							postmenopausal
							women
1	1	1	1	1	1	1	

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result				
(year)	characteristics	population	year		low vs. high	OR/HR/RR					
country		(case/control)				(95% CI)					
Fruits & Vegetables											
Galanti	Case-control	Sweden	18-75	Citrus fruits	[portions/mo.]	OR = 2.8	High intake of				
(1997)	(Population-	(35M/130F;50M/198F)			\leq 5 vs. > 21	(1.1-7.5); p =	citrus fruits				
Sweden &	based)	Norway				0.02	positively				
Norway		(24M/57F;57M/135F)					associated with				
(247)							TC risk in				
							Norway				
Mack	Case-control	292F/292F	15-54	Turnips or	[frequency]	1. $OR = 0.5$	High intake of				
(2002)	(Population-			Rutabagas	< few/yr vs.	(0.3-1.1); p =	turnips/rutabagas				
US	based)				at least	0.01	inversely				
(248)					monthly	2. OR = 0.6	associated with				
					1. TC	(0.3-1.2); p =	TC/PTC risk				
					2. PTC	0.03					
Memon	Case-control	313/313	≤70	Cabbage	[frequency]	OR = 1.9	High intake of				
(2002)	(Population-	(75M/238F)			never/yr vs.	(1.1-3.3);	cabbage				
Kuwait	based)				2-4 d or 5-7	p-trend =	positively				
(64)					d/wk	0.08	associated with				
							TC risk, but not				

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
							statistically
							significant
Markaki	Case-control	113/138	25-60	1. Tomato, fresh	[servings/mo.]	1. $OR = 0.32$	High intake of
(2003)	(Population-	(31M/82F;43M/95F)		2. Lemons	$1. \leq 0.5 \text{ vs.} >$	(0.10-1.01);	fresh tomato and
Greece	based)				28	p-trend =	lemon inversely
(250)					$2. \le 4 \text{ vs.} > 28$	0.002	associated with
						2. $OR = 0.53$	TC/
						(0.24-1.15);	PTC risk
						p-trend =	
						0.001	
Truong	Case-control	293F/354F	≥18	Cruciferous	[g/d]	OR = 1.86	Melanesian
(2010)	(Population-			vegetables	\leq 27.8 vs. \geq	(1.01-3.43);	women with high
France	based)				65.4	p-trend =	intake of
(New						0.06	cruciferous
Caledonia)							vegetables
(104)							with low iodine
							intake (< 96.0 µg/
							day) positively

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
							associated with
							TC risk
Bandurska-S.	Case-control	297/589	-	Cruciferous	[times/wk]	OR = 1.53	High intake of
(2011)	(Population-	(33M/264F;75M/514F)		vegetables	0-2 vs. 5-7/wk	(1.19-1.96)	cruciferous
Poland	based)						vegetables
(251)							positively
							associated with
							TC risk
Cléro	Case-control	229/371	< 56	Cassava	non-	OR = 0.62	High intake of
(2012)	(Population-	(26M/203F;47M/324F)			consumers	(0.39-0.99);	cassava inversely
France	based)				vs. consumers	p = 0.03	associated with
(French							TC risk
Polynesia)							
(144)							
Jung	Case-control	Malignant	20-70	1. Malignant	[g/d]	1.	High intake of
(2013)	(Hospital-	111F/111F		1) Raw	1.	1) $OR = 0.20$	raw vegetables &
S. Korea	based)	Benign		vegetables	1) 4.5 vs. 72.5	(0.07-0.62);	persimmons
(252)		115F/115F		2) Tangerine	2) 4.1 vs.79.3	p = 0.007	inversely

Author	Study	N,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
				3) Persimmons	3) 0.0 vs. 24.8	2) OR = 0.34	associated with
				2. Benign	2.	(0.13-0.86);	TC malignant and
				1) Raw	1) 6.0 vs. 77.3	p = 0.027	benign risk
				vegetables	2) 80.8 vs.	3) OR = 0.41	High intake of
				2) Total	422.2	(0.17-0.96);	tangerine
				vegetables	3) 0.0 vs. 24.8	p = 0.061	inversely
				3) Persimmons		2.	associated with
						1) $OR = 0.28$	TC malignant
						(0.10-0.76);	High intake of
						p = 0.007	total vegetables
						2) OR = 0.11	associated with
						(0.03-0.47);	TC benign
						p = 0.003	
						3) $OR = 0.35$	
						(0.15-0.83);	
						p = 0.014	

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
Meat			I	I		I	I
Galanti	Case-control	Sweden	18-75	All Meat	[portions/mo.]	OR = 0.8	No association
(1997)	(Population-	(35M/130F;50M/198F)		-	$\leq 12 \text{ vs.} > 20$	(0.5-1.3)	
Sweden &	based)	Norway		sausages/sausage			
Norway		(24M/57F;57M/135F)		dishes			
(247)				- pork/beef/lamb			
				- wild			
				(reindeer/elk)			
				- chicken/poultry			
				- liver/kidney			
				- blood pudding/			
				blood bread			
				- smoked meat			
Memon	Case-control	313/313	≤ 70	1. Chicken	[frequency]	1. OR = 3.0	High intake of
(2002)	(Population-	(75M/238F)		2. Mutton &	never/yr vs.	(1.3-6.8); p <	chicken or mutton
Kuwait	Based)			Lamb	2-4 d or 5-7	0.01	& lamb positively
(64)					d/wk	2. OR = 1.8	associated with
						(1.1-2.8); p <	TC

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
						0.01	risk
Markaki	Case-control	113/138	25-60	Pork	[servings/mo.]	OR = 2.82	High intake of
(2003)	(Population-	(31M/82F;43M/95F)			\leq 0.5 vs. > 3	(1.36-5.86);	pork positively
Greece	based)					p = 0.001	associated
(250)							with TC risk in
							male
Daniel	Cohort	492,186	50-71	Poultry	[g/1000 kcal]	HR = 1.74	High intake of
(2011)	1) NIH-	(250M/333F TC)			5.3 vs. 51.2	(1.14-2.67);	poultry positively
US	AARP					p = 0.005	associated with
(249)	(1995-1996)						TC risk in male
	2) Follow-up						
	(9 yr)						
Dairy Food			I		1		
Galanti	Case-control	Sweden	18-75	1. Cheese (slice)	[portions/mo.]	1. OR = 1.5	High intake of
(1997)	(Population-	(35M/130F;50M/198F)		2. Butter (tpsn)	$1. \le 40 \text{ vs.} >$	(1.0-2.4)	cheese & butter
Sweden &	based)	Norway			90	2. OR = 1.6	positively
Norway		(24M/57F;57M/135F)			2. ≤ 60 vs. >	(1.1-2.5)	associated with
(247)					120		TC risk in

Author	Study	Ν,	Age,	Food type	Referent unit,	Outcome,	Result
(year)	characteristics	population	year		low vs. high	OR/HR/RR	
country		(case/control)				(95% CI)	
							both Sweden and
							Norway
Truong	Case-control	293F/354F	≥ 18	Yoghurt,	[g/d]	OR = 1.03	No association
(2010)	(Population-			fromage blancs,	40.6 vs. ≥	(0.67-1.59);	
France	based)			petit suisse, soft	194.9	p = 0.85	
(New				cheese			
Caledonia)							
(104)							
Park	Cohort	36,965M (170TC)/	50-71	NC	[servings/1000	1. $RR = 0.78$	No association
(2009)	1) NIH-	16,605F (199TC)			kcal/d]	(0.45-1.37);	
US	AARP				0.2 vs.	p = 0.41	
(253)	(1995-2003)				1. 1.4 (male)	2. RR = 1.04	
	2) Follow-up				2. 1.6 (female)	(0.67-1.62);	
	(7 yr)					p = 0.74	

TC/PTC: thyroid/papillary thyroid cancer, NIH-AARP: the national institutes of health-american association of retired persons' diet and health study, JPHCPS: the japan public health center-based prospective study, M: male, F: female, NC: not classified.

6 Supplemental Table 5 - The association between alcohol consumption and thyroid cancer risk: summary of the results from the published observational studies

Author	Study	N,	Age,	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	low vs. high	OR/HR/RR	
country		(case/control)			(95% CI)	
Takezaki	Case-control	94F/22,666F	20-79	[frequency]	OR = 0.7	No association
(1996)	(Hospital-based)			sometimes/less	(0.3-1.5)	
Japan				vs.		
(254)				\geq 4 times/wk		
Rossing	Case-control	558F/574F	18-64	[drink/yr]	OR = 0.7	High intake of alcohol
(2000)	(Population-based)	(410PTC, 58FTC)		never (≤ 12)	(0.5-1.0)	inversely associated
US				vs. ≥ 12		with
(255)						PTC risk in female
Mack	Case-control	292F/292F	15-54	[drink/wk]	OR = 0.7	High intake of wine
(2002)	(Population-based)			none vs. >3	(0.3-1.5); p =	inversely associated
US					0.047	with
(248)						PTC risk in female

Author	Study	N,	Age,	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	low vs. high	OR/HR/RR	
country		(case/control)			(95% CI)	
Guignard	Case-control	332/412	≥18	[drink/wk]	1. $OR = 0.32$	No association
(2007)	(Population-based)	(39M/293F;58M/354F)		never vs. > 10	(0.05-1.95);	
France				1. male	p-trend =	
(New Caledonia)				2. female	0.39	
(132)					2. $OR = 0.92$	
					(0.24-3.45);	
					p-trend =	
					0.82	
Nagano	Case-control	57M/305F	< 75	[frequency]	OR = 0.59	High intake of alcohol
(2007)	(Population-based)			never vs. daily	(0.35-1.01);	inversely associated
Japan					p-trend =	with
(256)					0.032	TC risk in both male
						and female
Xhaard	Case-control	229/373	≤ 55	[frequency]	OR = 1.2	No association
(2014)	(Population-based)	(26M/203F TC)		none vs.	(0.3-4.5); p =	
France				regular	0.8	
(French Polynesia)						
(145)						

Author	Study	N,	Age,	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	low vs. high	OR/HR/RR	
country		(case/control)			(95% CI)	
Iribarren	Cohort	204,964	10-89	[drink/d]	RR = 0.95	No association
(2001)	1) KPMC (1964-	(73M/123F TC)		$1-2 \text{ vs.} \ge 6$	(0.30-3.02)	
US	1973)					
(65)	2) Follow-up (20 yr)					
Navarro Silvera	Cohort	89,835F (169TC)	40-59	[g/d]	1. HR = 0.80	No association
(2005)	1) NBSS (1980-			never vs. > 10	(0.45-1.42);	
Canada	1985)			1. TC	p = 0.56	
(257)	2) Follow up (15.9			2. PTC	2. HR = 0.80	
	yr)			3. PTC/FTC	(0.35-1.84);	
					p = 0.49	
					3. HR = 0.84	
					(0.44-1.58);	
					p = 0.64	
Allen	Cohort	68,775F (421TC)	-	[drink/wk]	RR=0.54	High intake of alcohol
(2009)	1) MWS (1996-			$\leq 2 \text{ vs.} > 15$	(0.31-0.92);	inversely associated
UK	2001)				p-trend =	with
(258)	2) Follow-up (7.2 yr)				0.005	TC risk
Meinhold	Cohort	490,159	50-71	[drink/d]	1. $RR = 0.57$	High intake of alcohol

Author	Study	N,	Age,	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	low vs. high	OR/HR/RR	
country		(case/control)			(95% CI)	
(2009)	1) NIH-AARP	(170M/200F TC)		1. never vs. \geq	(0.36-0.89);	inversely associated
US	(1995-1996)			2	p-trend =	with
(259)	2) Follow-up (> 7.5			2. none(beer)	0.01	TC risk in both male
	yr)			vs. $\geq 1/wk$	2. $RR = 0.42$	and female
					(0.21-0.83);	High intake of beer
					p-trend =	inversely associated
					0.01	with
						TC risk in male (p-
						trend = 0.03), but not
						in
						female (p-trend =
						0.40)
Kabat	Cohort	159,340F	50-79	[drink/wk]	1. TC/PTC	No association in
(2012)	1) WHI (1993-1998)	(331TC, 276PTC)		1. none vs. ≥ 7	HR = 0.66	postmenopausal
US	2) Follow-up (12.7			[g/d]	(0.44-1.01);	women
(260)	yr)			2. none vs. ≥ 4	p = 0.13	
					HR = 0.79	
					(0.44-1.11);	

Author	Study	N,	Age,	Referent unit,	Outcome,	Result
(year)	characteristics	population	year	low vs. high	OR/HR/RR	
country		(case/control)			(95% CI)	
					p = 0.37	
					2. TC/PTC	
					HR = 0.79	
					(0.60-1.05);	
					p = 0.17	
					HR = 0.87	
					(0.64-1.19);	
					p = 0.57	

TC/PTC/FTC: thyroid, papillary or follicular thyroid cancer, KPMC: kaiser permanente multiphasic cohort, NBSS: the canadian national breast scanning study, NIH-AARP: the national institutes of health-american association of retired persons' diet and health study, MWS: the million women study, WHI: the women's health Initiative, M: male, F: female.

Publications within the thesis

RESEARCH ARTICLE

Lack of Association between Fingernail Selenium and Thyroid Cancer Risk: A Case-Control Study in French Polynesia

Yan Ren^{1,2,3}, Cari Meinhold Kitahara⁴, Amy Berrington de Gonzalez⁴, Enora Clero^{1,2,3}, Pauline Brindel^{1,2,3}, Stephane Maillard^{1,2,3}, Suzanne Cote⁵, Eric Dewailly⁵, Frederique Rachedi⁶, Jean-Louis Boissin⁷, Joseph Sebbag⁸, Larrys Shan⁹, Frederique Bost-Bezeaud⁶, Patrick Petitdidier¹⁰, Constance Xhaard^{1,2,3}, Carole Rubino^{1,2,3}, Florent de Vathaire^{1,2,3}*

Abstract

Background: Numerous studies have suggested that selenium deficiency may be associated with an increased risk for several types of cancer, but few have focused on thyroid cancer. Materials and Methods: We examined the association between post-diagnostic fingernail selenium levels and differentiated thyroid cancer risk in a French Polynesian matched case-control study. Conditional logistic regression models were used to estimate odds ratios and 95% confidence intervals. <u>Results</u>: The median selenium concentration among controls was $0.76 \mu g/g$. Significantly, we found no association between fingernail selenium levels and thyroid cancer risk after conditioning on year of birth and sex and additionally adjusting for date of birth (highest versus lowest quartile: odds-ratio=1.12, 95% confidence interval: 0.66-1.90; p-trend=0.30). After additional adjustment for other covariates, this association remained non-significant (p-trend=0.60). When restricting the analysis to thyroid cancer of 10 mm or more, selenium in nails was non-significantly positively linked to thyroid cancer risk (p-trend=0.09). Although no significant interaction was evidenced between iodine in nails and selenium in nails effect (p=0.70), a non-significant (p-trend =0.10) positive association between selenium and thyroid cancer risk was seen in patients with less than 3 ppm of iodine in nails. The highest fingernail selenium concentration in French Polynesia was in the Marquises Islands (M=0.87 µg/g) and in the Tuamotu-Gambier Archipelago (M=0.86 µg/g). <u>Conclusions</u>: Our results do not support, among individuals with sufficient levels of selenium, that greater long-term exposure to selenium may reduce thyroid cancer risk. Because these findings are based on post-diagnostic measures, studies with prediagnostic selenium are needed for corroboration.

Keywords: Thyroid cancer - selenium - diet - fingernail - case-control study

Asian Pac J Cancer Prev, 15 (13), 5187-5194

Introduction

Thyroid cancer, the most common malignancy of the endocrine system, accounts for less than 2% of all cancers diagnosed worldwide (Ron and Schneider, 2006). Iodine and selenium are dietary factors for which there is the most information to play a role in the risk of differentiated thyroid cancer. Moreover, these elements and the family of molecules in which they are present (thyroid hormones, isothiocyanates, selenoproteines) are substrates of enzymes encoded by genes whose polymorphisms are most suspected to play a role in the risk of thyroid cancer: FOXE1, FOXE2 for iodine (Gudmundsson et al., 2009), and genetic family of Glutathione-S-transferases for selenium (Adjadj et al., 2009).

Selenium is an essential trace element present mainly in grains, meat, fish, eggs, and dairy products, as well as multivitamin supplements (Aaseth et al., 1990; Jung and Seo, 2010). Like iodine, the selenium content of a given food strongly depends upon the geographic location where it is produced, as plant uptake is largely influenced by the availability and chemical species of selenium in the soil (Aaseth et al., 1990). The redox-protective properties of selenium are important during oxidative thyroid hormone production, in which thyroid cells produce excess H_2O_2 and reactive oxygen species. Due to its ability to

¹Radiation Epidemiology Group, Centre for Research in Epidemiology and Population Health (CESP), UMR 1018 Inserm, ²Gustave Roussy Institute, Villejuif, ³Faculty of Medicine, University Paris Sud 11, Le Kremlin-Bicêtre, France, ⁴Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, USA, ⁵Area of population health and optimal health practices, CHU de Québec Research Center, Québec, Canada, ⁶Territorial Hospital Mamao, ⁷IPRAME, ⁸Paofai Clinic, ⁹Endocrinologist, ¹⁰Laboratoire Boz, Papeete, French Polynesia [&]Equal contributors *For correspondence: Florent. DEVATHAIRE@gustaveroussy.fr

Yan Ren et al

protect the thyroid gland and maintain thyroid hormone production, there is a high concentration of selenium in the thyroid (Aaseth et al., 1990), and supply of selenium to the thyroid is prioritized even during periods of selenium deficiency (Fairweather-Tait et al., 2011).

Although numerous studies have shown that selenium deficiency may be associated with an increased risk for several types of cancer (Willett et al., 1983; Clark et al., 1996; Duffield-Lillico et al., 2002; Guo et al., 2012), the relationship between selenium and thyroid cancer has been examined in few studies. One in vitro study showed that selenium causes growth inhibition of thyroid cancer cells accompanied by cell-cycle arrest in the S and G2/M phases (Kato et al., 2010). In addition, a small case-control study of 43 thyroid cancer cases showed a significant increased risk for low compared to high prediagnostic serum selenium concentrations (Glattre et al., 1989).

In the present study, we examined the association between fingernail selenium concentrations with thyroid cancer in a case-control study of men and women in French Polynesia, a country who has high incidence of thyroid cancer in females (Moore et al., 2010) also highly exposed to selenium mainly through fish consumption (Dewailly et al., 2008). In contrast to other biomarker measures of selenium, such as blood or urine, nail concentrations represent longer-term exposure, typically the previous three to six months. This case-control study has already shown that a high number of pregnancies (Brindel et al., 2008), tallness and obesity (Brindel et al., 2009; Clero et al., 2010), familial history of thyroid cancer (Brindel et al., 2010), low dietary intake of iodine (Clero et al., 2012), poor economic conditions (De Vathaire et al., 2010), and nuclear radiation fallout (De Vathaire et al., 2010), having a spring as the main source of drinking water (Xhaard et al., 2014) are thyroid cancer risk factors in French Polynesia.

Materials and Methods

Selection of cases and controls

The process for selection and interviews of thyroid cancers and controls has been extensively described elsewhere (De Vathaire et al., 2010). Eligible cases were born and resided in French Polynesia and had been diagnosed with differentiated thyroid cancer before the age of 56 between 1981 and 2003. Four patients between the ages of 56 and 62 at diagnosis, one case diagnosed in 1979, and two cases diagnosed in 2004 were included in the study inadvertently and were retained in the analysis. The cases were identified from the cancer registry in French Polynesia, medical insurance files, and/or four endocrinologists in Tahiti. Information on histology was obtained from the two histopathology laboratories in Tahiti and/or the endocrinologists' medical files. Of the 255 eligible differentiated thyroid cancer cases, 26(10%)were not interviewed because they had died (n=14), could not be located (n=6), refused to participate (n=5), or were too ill to participate in the interview (n=1).

For each case, two controls were randomly selected from the birth registry in French Polynesia and matched to each case by date of birth (± 3 months for the first interviewed controls, increased by ± 20 days afterwards) and sex. Of the 458 randomly selected controls, 85 (19%) were not interviewed for the study because they had died (n=9), could not be located (n=29), refused to participate (n=29), were too ill to participate in the interview (n=2), or they no longer resided in French Polynesia (n=16). After these exclusions, 35 cases had one and 25 cases had no matched controls. Each of the 25 cases with no matched control was then matched to a control from among cases that had two matched controls; the matching criteria for date of birth were relaxed if necessary.

In total, the study population included 229 interviewed cases and 373 interviewed controls, with 85 cases (37%) matched to one control and 144 cases (63%) matched to two controls. We further excluded 6 cases and 9 controls for missing data on fingernail selenium, and a batch of 8 cases and 33 controls for which all measures of any elements in the fingernails were returned as null. The final analytic population was 215 cases and 331 controls.

The French Polynesian Ethics Committee approved the study, and written informed consent to participate in the study and to contact his or her physician was obtained from each participant.

Detailed characteristics of cases and controls are, as well as thyroid cancer risk factor estimates are reported elsewhere (de Vathaire et al. 2010, Xhaard et al., 2014).

Data collection

Home addresses for cases and controls were obtained through the territorial medical insurance plan that offers coverage for all inhabitants. In-person interviews were conducted in the participants' homes (though some cases were interviewed at the hospital on the day of their usual follow-up consultation) by trained interviewers and medical staff using a structured questionnaire which included questions regarding ethnicity, education, smoking, weight at various ages, personal history of thyroid disease and cancer, places of residence, reproductive and hormonal exposures, history of medical X-rays and diet.

We used a French food composition table (Favier et al., 1995) for the calculation of iodine and selenium intake because data were lacking in the Pacific Islands food composition tables (Dignan et al., 2004). The overall iodine intake took into account all foods of the questionnaire, except iodized salt because we had no information on the amount of iodized salt consumption.

All participants provided fingernail clippings on the date of the interview. Clippings were stored in paper envelopes at room temperature before being transferred to the toxicology centre of the Institut National de Sante Publique du Quebec (INSPQ).

Fingernail selenium and iodine measurements

Nail samples were digested under basic conditions using trimethylammonium hydroxide for iodine and under acidic conditions with nitric acid for selenium. The digested material was directly analysed by ICP-MS (inductively coupled plasma mass spectrometry, Perkin Elmer Sciex, Elan DRCII with autosampler ESI SC-4 and work station Elan version 3.0) (Elwaer and Hintelmann, 2007). Certified reference materials used were: Human hair powder GBW 07601 and GBW 09101b.

Statistical analysis

Descriptive statistics and trend tests in univariate analysis were performed using non-parametric Jonckheere-Terpstra test for association between two quantitative variables; non-parametric Wilcoxon rank test for association between a qualitative variable with two classes and a quantitative variable; and non-parametric Kruskal-Wallis rank tests for association between a qualitative variable with more than two classes and a quantitative variable.

The association between selenium in nails and other parameters has been investigated using generalized linear models.

We used conditional logistic regression to estimate OR and 95%CI for thyroid cancer by fingernail selenium concentration. Selenium was categorized into quartiles based on the distribution among the controls. All models were conditioned on year of birth (<1945, 1945-1955, 1955-1965, \geq 1965) and sex to reduce the potential bias due to the matching process and adjusted for year of birth (continuous, per year) to control for residual confounding by age. Multivariable models were additionally adjusted for ethnicity (Polynesian, mixed, other), education (primary school or lower vs. middle school or higher), BMI at the moment of cancer (<25, 25-29.9, \geq 30 kg/m², missing), current smoking (yes vs. no), and radiotherapy treatment to head or neck (ever vs. never). These associations were also evaluated by sex, smoking status,

BMI, thyroid cancer histology, tumour size, fingernail iodine and dietary iodine. Effect modification of selenium levels by sex, smoking status, BMI, thyroid cancer histology, tumour size, fingernail iodine and dietary iodine was evaluated using the likelihood ratio test comparing a model with an interaction term to one without. We also investigated for potential bias due to the delay between thyroid cancer diagnosis and fingernails sampling, by performing separate analysis for various categories of delay.

All statistical analyses were conducted using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA).

Results

The cases and controls were generally comparable in terms of ethnicity and level of education. Thirty percent of the tumours were less than 10 mm. About 70% of cases had papillary thyroid cancer, and 30% had a follicular type.

Among controls, higher fingernail selenium levels were observed in older people (p<0.001), and people who have higher BMI (p=0.001) and in non-smokers (p<0.0001), but in a multivariate analysis, only age (p=0.01) and smoking (p<0.0001) remained significant (Table 1). The highest fingernail selenium levels were in inhabitants of the Marquises Islands (M=0.87 μ g/g) and of the Tuamotu-Gambier Archipelago (M=0.86 μ g/g) (Figure 1). Adjusting by other factors, including estimated selenium and iodine in diet, selenium in the fingernails of controls from the Tuamotu-Gambier Archipelago remained significantly higher than in the rest of French



Figure 1. Fingernail Selenium Concentration in Studied Population of French Polynesia

Yan Ren et al

Polynesian, but not from the Marquises Islands (Table 1). On the contrary, among cases, only selenium in fingernails of the Marquises Islands inhabitants remained higher than in the other inhabitants of French Polynesia (p=0.05) (Table 1).

We did not evidence a significant association between fingernail selenium levels and thyroid cancer risk in models that were conditioned on year of birth and gender and further adjusted for year of birth (highest versus lowest quartile: OR=1.12, 95%CI: 0.66-1.90; p-trend=0.30) (Table 2). After additional adjustment for age at the moment of the diagnosis of cancer, ethnicity, education, BMI, smoking status and radiotherapy treatment to the head or neck, this association with the risk of thyroid cancer comparing the highest to the lowest quartile was also non-significant (OR=1.02, 95%CI: 0.56-1.84; p-trend=0.60) (Table 2).

Among the studied population, average fingernail selenium concentration was $0.75 \ \mu g/g$ in smokers and $0.80 \ \mu g/g$ in non-smokers (p<0.0001). We did not observe evidence for a significant effect modification by sex, smoking status, BMI, histological type, fingernail iodine and dietary iodine (all p-interaction>0.05; Table 2). A significant interaction was evidenced between selenium and tumour size (p=0.04): when restricting the analysis to differentiated thyroid cancer of 10 mm or more, selenium in nails was nearly significantly positively (p=0.09) linked to thyroid cancer risk. Although no significant interaction

Table 1. Predictor of Selenium	Concentration in Fing	ernails in Thyroid	Cancer Patients and	Controls
	6			

	Cases	5	Controls		
	Regression Coefficient (SD [†])	p-value*	Regression Coefficient (SD [†])	p-value*	
Dietary selenium (mg/1,000 kcal)	<0.001 (<0.001)	0.60	-0.0002 (<0.001)	0.06	
Dietary iodine (mg/1,000 kcal)	-0.0002 (<0.001)	0.30	0.0001 (<0.001)	0.20	
Gender (women/men)	-0.01 (0.03)	0.80	0.04 (0.02)	0.06	
Age (years)	0.001 (0.001)	0.20	0.002 (<0.001)	0.01	
BMI (kg/m ²)	0.003 (0.002)	0.03	0.002 (0.001)	0.07	
Currently smoking (yes/no)	-0.05 (0.02)	0.01	-0.06 (0.01)	0.0001	
Marquises Islands (yes/no)	0.14 (0.07)	0.05	0.04 (0.06)	0.40	
Tuamotu-Gambier Archipelago (yes/no)	0.06 (0.04)	0.10	0.12 (0.03)	0.0002	

†Standard deviation; *Regression coefficient t-test

Table 2. Fingernail Selenium and Thyroid Cancer by Various Confounding Factors in French Polynesia, 1981-2003

				Quartiles of s	elenium (µg/g)		
		Q1	Q2	Q3	Q4	p-trend*	p-interaction**
		(0.00-0.68)	(0.68-0.76)	(0.76-0.86)	(0.86-1.30)	•	•
Overall	Cases/controls	51/93	56/81	50/79	58/78		
	OR (95% CI) 1	1.00	1.16 (0.70-1.94)	1.06 (0.64-1.77)	1.12 (0.66-1.90)	0.30	
	OR (95% CI) 2, 3	1.00	1.08 (0.62-1.90)	1.10 (0.62-1.96)	1.02 (0.56-1.84)	0.60	
Men	Cases/controls	2/15	8/9	6/5	7/11		
	OR (95% CI) 2, 3	1.00	?†	?†	752.6(<0.001->?†)	0.99	
Women	Cases/controls	49/78	48/72	44/74	51/67		
	OR (95% CI) 2, 3	1.00	0.95 (0.53-1.71)	0.95 (0.51-1.74)	0.90 (0.48-1.70)	0.90	0.20
Non-smoker	Cases/controls	15/33	20/40	27/47	32/49		
	OR (95% CI) 2, 3	1.00	0.91 (0.38-2.18)	1.02 (0.45-2.32)	1.07 (0.47-2.46)	0.50	
Current smoker	Cases/controls	36/60	36/41	23/32	26/29		
	OR (95% CI) 2, 3	1.00	1.29 (0.68-2.43)	1.08 (0.53-2.19)	1.19 (0.59-2.41)	0.40	0.70
BMI $<30 \text{ kg/m}^2$	Cases/controls	35/68	27/52	23/53	24/48		
0	OR (95% CI) 2, 3	1.00	0.95 (0.49-1.84)	0.81 (0.41-1.62)	0.76 (0.37-1.55)	0.80	
BMI \geq 30 kg/m ²	Cases/controls	16/25	28/28	27/26	34/30		
	OR (95% CI) 2, 3	1.00	1.65 (0.70-3.90)	1.74 (0.73-4.18)	1.97 (0.83-4.66)	0.15	0.30
Papillary	Cases/controls	40/58	39/56	37/66	49/56		
* •	OR (95% CI) 2, 3	1.00	0.95 (0.52-1.76)	0.81 (0.44-1.49)	1.18 (0.64-2.18)	0.30	
Follicular	Cases/controls	11/25	17/19	13/10	9/20		
	OR (95% CI) 2, 3	1.00	1.74 (0.59-5.08)	3.79 (1.12-12.8)	0.98 (0.29-3.29)	0.80	0.60
Tumours <10 mm	Cases/controls	9/25	15/20	17/25	18/24		
	OR (95% CI) 2, 3	1.00	1.84 (0.59-5.75)	1.79 (0.61-5.29)	1.84 (0.59-5.75)	0.30	
Tumours ≥10 mm	Cases/controls	32/59	30/49	28/43	33/39		
	OR (95% CI) 2, 3	1.00	1.04 (0.53-2.02)	1.19 (0.60-2.36)	1.37 (0.69-2.72)	0.09	0.04
Fingernail iodine <3 ppm	Cases/controls	24/49	23/43	25/45	27/39		
	OR (95% CI) 2, 3	1.00	0.98 (0.46-2.09)	1.18 (0.56-2.49)	1.44 (0.66-3.11)	0.10	
Fingernail iodine ≥3 ppm	Cases/controls	27/44	33/38	25/34	31/39		
	OR (95% CI) 2, 3	1.00	1.15 (0.56-2.37)	1.13 (0.52-2.47)	0.99 (0.47-2.06)	0.90	0.70
Dietary iodine <117	Cases/controls	29/38	29/49	23/35	35/36		
(mg/1,000 kcal)	OR (95% CI) 2, 3	1.00	0.83 (0.41-1.70)	0.95 (0.44-2.06)	1.40 (0.67-2.95)	0.10	
Dietary iodine ≥117	Cases/controls	22/55	27/32	27/44	23/42		
(mg/1,000 kcal)	OR (95% CI) 2, 3	1.00	1.71 (0.80-3.64)	1.31 (0.63-2.72)	0.94 (0.43-2.06)	0.90	0.50

*Calculated using the Wald test for selenium modelled as a continuous variable (per quartile); **Calculated using the likelihood ratio test comparing a model with an interaction term to one without; †Not estimable; 1Adjusted for year of birth (continuous); 2Adjusted for year of birth (continuous), age at the moment of the diagnosis of cancer (continuous), gender, ethnicity, education, BMI, smoking status, radiotherapy treatment to head or neck; 3One case and one control were excluded from the analysis due to missing data on BMI

Table 3. Fingernail Selenium and Thyroid Cancer bythe Time between Diagnosis and Interview in FrenchPolynesia, 1981-2003

	Tert	iles of selenium ((µg/g)	
	Q1	Q2	Q3	p-trend*
	(0.00-0.71) (0.71-0.82)	(0.82-1.30)	
<3 years				
Cases/controls	s 17/34	13/24	16/23	
OR (95% CI)	1 1.00	1.03 (0.38-2.80)	1.24 (0.48-3.23) 0.20
3-7 years				
Cases/controls	s 16/33	18/24	28/38	
OR (95% CI)	1 1.00	1.15 (0.43-3.04)	1.19 (0.49-2.90	0.80
7-11 years				
Cases/controls	s 21/37	20/23	18/31	
OR (95% CI)	1 1.00	1.82 (0.72-4.60)	1.03 (0.41-2.61) 0.50
≥11 years				
Cases/controls	s 13/18	17/27	18/19	
OR (95% CI)	1 1.00	0.93 (0.30-2.88)	1.75 (0.53-5.77) 0.40

*Calculated using the Wald test for selenium modelled as a continuous variable (per tertile); ¹Adjusted for year of birth (continuous), age at the moment of the diagnosis of cancer (continuous), gender, ethnicity, education, BMI, smoking status, radiotherapy treatment to head or neck

was evidenced between iodine in nails and selenium in nails effect (p=0.70), a nearly significant positive association (p=0.10) between selenium and thyroid cancer risk was seen in patients with less than 3 ppm of iodine in nails. A similar result also has been found in which a nearly significant positive association (p=0.10) between selenium and thyroid cancer risk was seen in patients with less than 117 mg/1,000 kcal of dietary iodine (Table 2).

There was no clear association of fingernail selenium for cases who were interviewed and provided fingernail samples less than 3 years, 3 to 7, 7 to 11 or more than 11 years after diagnosis (all p-trend >0.05; Table 3).

Discussion

Our objective was to examine the association between fingernail selenium concentrations and thyroid cancer in French Polynesia, a country highly exposed to selenium mainly through fish consumption and characterized by mild iodine deficiency (Clero et al., 2012).

Overall, we did not evidence a significant association between fingernail selenium levels and thyroid cancer risk. Fingernail selenium levels were higher in subjects with high BMI and in non-smokers, and among controls only, in older subjects. Furthermore, fingernail selenium levels were higher in inhabitants of the Marquises Islands and the Tuamotu-Gambier Archipelago. Selenium in nails was nearly significantly positively linked to the risk of thyroid cancer of 10 mm or more (p=0.09). Lastly, a nearly significant positive association (p=0.10) between selenium and thyroid cancer risk was seen in patients with less than 3 ppm of iodine in nails.

This analysis has important strengths including, the population-based design with virtually exhaustive identification of thyroid cancer cases in isolated Pacific Islands and a high participation rate among cases and population control subjects, which were from the population register of French Polynesia (Brindel et al., 2009). Another important strength is that French Polynesia ranks as one of world's leading consumers of fish (Dewailly et al., 2008), a major dietary source of selenium (Dewailly et al., 2008), which increased the power of our analysis. Furthermore, despite recent changes in lifestyle and nutrition, an important proportion of inhabitants (at least the ones living outside Tahiti), still have a traditional diet of fish, starchy roots and fruits, and consumed at the time of interview few imported foods that are high in fat and low in fibre (World Health Organization, 2003), and thus are more likely to accurately report their dietary intake.

This analysis also has some limitations. In regard to the statistical method, when we conditioned on age and sex using the original matching criteria (year of birth ± 6 months and sex), we observed similar results but reduced statistical power since matched groups that were missing either a case or control were dropped from the analysis.

Because of the transition in diet habits from a traditional Polynesian diet to a more Westernized one, the results of this case-control study about diet in adulthood could be confounded by diet in previous periods.

We did not find a correlation between selenium measured in fingernails and dietary selenium intake, as estimated using dietary questionnaire data and a French food composition. This lack of correlation was not surprising because the concentration of selenium in foods is largely variable and its quantification from food composition table data is uncertain. Indeed, its concentration in vegetables is dependent on the soil content in a particular geographical location (Aaseth et al., 1990), and its concentration in fishes is dependent on the type of cooking and variable between species (Favier et al., 1995). Therefore, our estimation of selenium in diet is probably uncertain. Thus, biomarker measures are essential in investigating the relationship between selenium exposure and chronic disease risk.

Selenium concentration in nails is a highly reproducible value. In a cohort on the investigation of dietary supplement use and cancer risk in Western Washington state, the concentration of toenail selenium without selenium supplement was about six times higher than the concentration of plasma selenium without selenium supplement, mean toenail and plasma concentrations were $1.02\pm0.21 \ \mu g/g$ and $161\pm29 \ \mu g/L$, respectively (Satia et al., 2006). There are advantages to measure selenium concentration in fingernails rather than blood: the quantity of selenium in nails is more stable, cumulative, and integrates exposure occurring from 12 to 18 months before sampling (Fleckman, 1985). Additionally, collecting, transporting and storing fingernails are easier than blood.

A previous cross-sectional study, including 195 adults aged 18 years old and over from Tahiti and the Moorea islands, found that serum selenium levels in French Polynesia in 2008 were similar to those observed in Inuit adults in 1992; however, these are four times lower than France (foods imported to Polynesia come from France), even considering the whole blood/plasma ratio (Dewailly et al., 2008).

We found that the highest fingernail selenium concentrations of French Polynesia were in the Marquises Islands and in the Tuamotu-Gambier Archipelago, which is similar to observations performed on cord blood selenium

Yan Ren et al

concentration in French Polynesian new-borns in 2008 (Dewailly et al., 2008). These highest values are probably mostly attributable to higher seafood consumption, but the results shown in Table 1, in which the higher fingernail selenium values in controls living in the Tuamotu-Gambier Archipelago remains when adjusting for estimated dietar 100.0 decrease, relatively to controls, when the delay from blood selenium intake, showing that other factors could interact, or that selenium level could be higher in the Tuamotu-Gambier Archipelago fishes. Because most of the fishes eaten in the atolls of the Tuamotu-Gambier Archipelago 75.0^{42} patients with follicular thyroid carcinoma and 1.02 come from the lagoon, rather than the open sea like other archipelagos of French Polynesia, this result could be due to an under-estimation of selenium contents for lagoon50.0 fishes in food composition tables.

Among the studied population, fingernail selenium concentration averages in French Polynesian, 0.75 ppm_{25.0} between cancer diagnosis and nails sample was less than in smokers and 0.80 ppm in non-smokers (p<0.0001), are very similar to those measured in other studies, and in particular in a large cohort study of US women, which have shown that toenail selenium was significantly reduced among cigarette smokers (M=0.75, SD=0.12, among 146 current smokers; and M=0.82, SD=0.16, among 311 never smokers; p<0.001) (Hunter et al., 1990).

We found a positive relationship between BMI and selenium in fingernails, both in cases and controls (Table 1). Our result is in opposition with most European surveys(Arnaud et al., 2006; Meplan et al., 2007; Ortega et al., 2012) or US populations (Bleys et al., 2009), in which obese patients have lower serum selenium concentration, but in agreement with Dewailly's survey of a French Polynesian sampling in which selenium concentration in blood was positively linked to BMI (Dewailly et al., 2008), the regression coefficient (β) being 0.02, i.e. ten times higher than in our study, among the controls (β =0.002). A possible explication is that in the general population, BMI has a trend to rise with age, if we do not consider extreme ages. Furthermore, older people have more possibilities to eat traditional food, which is rich in selenium.

There may be a range of selenium that offers optimal protection against cancer development in general, as a deficiency in selenium has been associated with an increased risk of cancer, this association having been evidenced for selenium in hair and nails and prostate cancer in Malaysia (Karimi et al., 2012), and selenium in serum and, respectively bladder carcinoma in China (Guo et al., 2012), and breast cancer in India (Singh et al., 2005). In the latter case-control study, it has been estimated that each increase of one µ mol/l of selenium was associated to decrease in 7% of breast cancer risk (Singh et al., 2005). Nevertheless, selenium supplementation may reduce cancer risk only in selenium-deficient individuals.

Few studies have examined the association between selenium and thyroid cancer risk, however, we haven't found association between fingernail selenium and thyroid cancer risk in our results. In a case-control study of 43 cases and 129 matched controls in Norway, prediagnostic serum selenium levels were inversely associated with thyroid cancer; levels ≤ 1.25 and $1.26-1.64 \mu mol/l$ were associated with a 7.70- and 6.10-fold increased risk of thyroid cancer compared to levels ≥1.65 µmol/l (Glattre et al., 1989). This case-control study also showed, when

considering the delay from blood sampling to diagnosis of the case, that the protective effect of high serum selenium concentrations was restricted to the seven-year period prior to the diagnosis of thyroid cancer, because the serum selenium concentration of cases tended to sampling to the diagnosis was shorter. Similarly, in an μ mol/l in 73 patients with papillary thyroid carcinom **100.0** (Monca **56.3**: al., 2008).

Our study did not evider 56 a significant association between fingernail selenium levels and thyroid cancer 75.0 30.0 even when considering only patients for which the delay seven or less than the years (Table 3). It has to be noted that over a period of six years, releniuil in toenails was 50.00.0 one of the most reproducible trace elements (Garland et al., 01993). Therefore, our non-significant results are probably not due te the fact that we inevestigated post-diagnosis 25.0 selenium evels rather than prediagnosiseones, except if thyroid cancer, by itself, modifies selenium level in nails. A possible explication for the lack of association could 0 be the iodene status of French Polynesians, who could be less frequently ioding deficient han Norwegians because selenium acts synergistically with iodine. These results could nevertheless also be due to the fact that fingernail sampling among ∉ases were performed after thyroid cancer diagnosis and therefore during thyroid hormone substitution treatment, such as levothyrox, which could interact with selenium. Indeed, all three mono-deiodinase enzymes are selenium-dependent and are involved in thyroid hormone regulation. In this way, selenium status may affect both thyroid hormone homeostasis and iodine availability (Cann et al., 2000; Brauer et al., 2006; Köhrle and Gärtner, 2009). Lastly, we evidenced a nearly significant positive association (p=0.15) between fingernail selenium and thyroid cancer risk in patients with more than 30 kg/m² BMI (Table 2). This result has to be considered with caution because it is nearly significant, but it could be due to some specificity of obese patients.

Because cigarette smoking has been shown to be inversely associated with both selenium status(Lloyd et al., 1983) and thyroid cancer risk (Meinhold et al., 2010), it was important to adequately control for these exposures in our analysis. Despite having evidenced a lower selenium status in smokers than in non-smokers, we failed to show an interaction between fingernail selenium levels, smoking habit and thyroid cancer risk: both in smokers and nonsmokers, selenium status did not influence thyroid cancer risk. Nevertheless, it should be noted that such interaction tests are not very powerful in our study, given the number of patients.

In summary, our results do not support, among individuals with sufficient levels of selenium, that greater long-term exposure to selenium may reduce thyroid cancer risk. Because these findings are based on post-diagnostic measures, studies with prediagnostic selenium are needed for corroboration.

12.8

6.3

30.0

Acknowledgements

This study was supported by the Association pour la Recherche contre le Cancer, the Ligue Nationale Contre le Cancer, the Direction Generale de la Sante, the Comite de radioprotection de Electricite de France, Agence Française de Securite Sanitaire et Environnementale et du Travail, CHILD-THYR EEC programme and the Fondation de France.

The first author Yan Ren has a scholarship from the Fondation de France for her Ph.D study.

References

- Aaseth J, Frey H, Glattre E, et al (1990). Selenium concentrations in the human thyroid gland. *Biol Trace Elem Res*, 24, 147-52.
- Adjadj E, Schlumberger M, de Vathaire F (2009). Germ-line DNA polymorphisms and susceptibility to differentiated thyroid cancer. *Lancet Oncol*, **10**, 181-90.
- Arnaud J, Bertrais S, Roussel AM, et al (2006). Serum selenium determinants in French adults: the SU.VI.M.AX study. *Brit J Nutr*, **95**, 313-20.
- Bleys J, Navas-Acien A, Laclaustra M, et al (2009). Serum selenium and peripheral arterial disease: results from the national health and nutrition examination survey, 2003-2004. *Am J Epidemiol*, **169**, 996-1003.
- Brauer VF, Schweizer U, Köhrle J, Paschke R (2006). Selenium and goiter prevalence in borderline iodine sufficiency. *Eur J Endocrinol*, **155**, 807-12.
- Brindel P, Doyon F, De Vathaire F, et al (2008). Menstrual and reproductive factors in the risk of differentiated thyroid carcinoma in native women in French Polynesia: a population-based case-control study. *Am J Epidemiol*, **28**, 219-29.
- Brindel P, Doyon F, De Vathaire F, et al (2009). Anthropometric factors in differentiated thyroid cancer in French Polynesia: a case-control study. *Cancer Causes Control*, **20**, 581-90.
- Brindel P, Doyon F, De Vathaire F, et al (2010). Family history of thyroid cancer and the risk of thyroid cancer in French Polynesia. *Thyroid*, **20**, 393-400.
- Cann SA, van Netten JP, van Netten C (2000). Hypothesis: iodine, selenium and the development of breast cancer. *Cancer Causes Control*, **11**, 121-7.
- Clark LC, Combs GF, Turnbull BW, et al (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional prevention of cancer study group. *JAMA*, 276, 1957-63.
- Clero E, Doyon F, De Vathaire F, et al (2012). Dietary iodine and thyroid cancer risk in French Polynesia: a case-control study. *Thyroid*, **22**, 422-9.
- Clero E, Leux C, De Vathaire F, et al (2010). Pooled analysis of two case-control studies in New Caledonia and French Polynesia of body mass index and differentiated thyroid cancer: the importance of body surface area. *Thyroid*, **20**, 1285-93.
- De Vathaire F, Drozdovitch V, Brindel P, et al (2010). Thyroid cancer following nuclear tests in French Polynesia. *Brit J Cancer*, **103**, 1115-21.
- Dewailly E, Château-Degat L, Suhas E (2008). Fish consumption and health in French Polynesia. *Asia Pac J Clin Nutr*, **17**, 86-93.
- Dewailly E, Suhas E, Mou Y, et al (2008). High fish consumption in French Polynesia and prenatal exposure to metals and nutrients. *Asia Pac J Clin Nutr*, **17**, 461-70.
- Dignan C, Burlingame B, Kumar S, Aalbersberg W (2004). The

Pacific Islands food composition tables - second edition. Rome: FAO.

- Duffield-Lillico AJ, Reid ME, Turnbull BW, et al (2002). Baseline characteristics and the effect of selenium supplementation on cancer incidence in a randomized clinical trial: a summary report of the nutritional prevention of cancer trial. *Cancer Epidemiol Biomarkers Prev*, **11**, 630-9.
- Elwaer N, Hintelmann H (2007). Comparative performance study of different sample introduction techniques for rapid and precise selenium isotope ratio determination using multicollector inductively coupled plasma mass spectrometry (MC-ICP/MS). *Anal Bioanal Chem*, **389**, 1889-99.
- Fairweather-Tait SJ, Bao Y, Broadley MR, et al (2011). Selenium in human health and disease. *Antioxid Redox Signal*, **14**, 1337-83.
- Favier JC, Ireland-Ripert J, Toque C, Feinberg M (1995). Repertoire general des aliments. Table de composition [Food composition tables] CIQUAL-REGAL. Paris: Tec & Doc Lavoisier and INRA.
- Fleckman P (1985). Anatomy and physiology of the nail. *Dermatol Clin*, **3**, 373-81.
- Garland M, Morris JS, Rosner BA, et al (1993). Toenail trace element levels as biomarkers: reproducibility over a 6-year period. *Cancer Epidemiol Biomarkers Prev*, 2, 493-7.
- Glattre E, Thomassen Y, Thoresen SO, et al (1989). Prediagnostic serum selenium in a case-control study of thyroid cancer. *Int J Epidemiol*, 18, 45-9.
- Gudmundsson J, Sulem P, Gudbjartsson D, et al (2009). Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. *Nat Genet*, **41**, 460-4.
- Guo KF, Zhang Z, Wang JY, et al (2012). Variation of urinary and serum trace elements (Ca, Zn, Cu, Se) in bladder carcinoma in China. *Asian Pac J Cancer Prev*, **13**, 2057-61.
- Hunter DJ, Morris JS, Willett WC, et al (1990). Predictors of selenium concentration in human toenails. *Am J Epidemiol*, 132, 114-22.
- Jung HJ, Seo YR (2010). Current issues of selenium in cancer chemoprevention. *Biofactors*, 36, 153-8.
- Karimi G, Shahar S, Homayouni N, et al (2012). Association between trace element and heavy metal levels in hair and nail with prostate cancer. Asian Pac J Cancer Prev, 13, 4249-53.
- Kato MA, Finley DJ, Lubitz CC, et al (2010). Selenium decreases thyroid cancer cell growth by increasing expression of GADD153 and GADD34. *Nutr Cancer*, **62**, 66-73.
- Köhrle J, Gärtner R (2009). Selenium and thyroid. *Best Pract Res Clin Endocrinol Metab*, **23**, 815-27.
- Lloyd B, Lloyd RS, Clayton BE (1983). Effect of smoking, alcohol and other factors on the selenium status of a healthy population. J Epidemiol Community Health, 37, 213-17.
- Meinhold CL, Ron E, Schonfeld SJ, et al (2010). Nonradiation risk factors for thyroid cancer in the US radiologic technologists study. *Am J Epidemiol*, **171**, 242-52.
- Meplan C, Crosley LK, Nicol F, et al (2007). Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). FASEB J, 21, 3063-74.
- Moncayo R, Kroiss A, Oberwinkler M, et al (2008). The role of selenium, vitamin C, and zinc in benign thyroid diseases and of selenium in malignant thyroid diseases: low selenium levels are found in subacute and silent thyroiditis and in papillary and follicular carcinoma. *BMC Endocr Disord*, **8**, 2.
- Moore MA, Baumann F, Foliaki S, et al (2010). Cancer epidemiology in the Pacific Islands - past, present and future. *Asian Pac J Cancer Prev*, **11**, 99-106.
- Ortega RM, Rodríguez-Rodríguez E, Aparicio A, et al (2012). Young children with excess of weight show an impaired

Yan Ren et al

selenium status. Int J Vitam Nutr Res, 82, 121-9.

- Ron E, Schneider AB (2006). Thyroid cancer. In: Schottenfeld D, Fraumeni J, editors. Cancer Epidemiology and Prevention. Oxford University Press, New York: pp. 975-94.
- Satia JA, King IB, Morris JS, Stratton K, White E (2006). Toenail and plasma levels as biomarkers of selenium exposure. *Ann Epidemiol*, **16**, 53-8.
- Singh P, Kapil U, Shukla NK, Deo S, Dwivedi SN (2005). Association between breast cancer and vitamin C, vitamin E and selenium levels: results of a case-control study in India. *Asian Pac J Cancer Prev*, **6**, 177-80.
- Willett WC, Polk BF, Morris JS, et al (1983). Prediagnostic serum selenium and risk of cancer. *Lancet*, **2**, 130-4.
- World Health Organization (2003). Diet, food supply and obesity in the Pacific. Geneva: regional office for the Western Pacific.
- Xhaard C, Ren Y, Clero E, et al (2014). Differentiated thyroid carcinoma risk factors in French Polynesia. Asian Pac J Cancer Prev, 15, 2675-80.

FOXE1 Polymorphism Interacts with Dietary Iodine Intake in Differentiated Thyroid Cancer Risk in the Cuban Population

Yan Ren,^{1,2,3} Juan J. Lence-Anta,⁴ Celia M. Pereda,⁴ Mae Chappe,⁴ Milagros Velasco,⁴ Idalmis Infante,⁴ Marlene Bustillo,⁴ Silvia Turcios,⁵ Axelle Leufroy,⁶ Thierry Guérin,⁶ Laurent Noël,⁷ Fabienne Lesueur,⁸ Stéphane Maillard,^{1,2,3} Enora Cléro,^{1,2,3} Constance Xhaard,^{1,2,3} Rodrigue S. Allodji,^{1,2,3} Carole Rubino,^{1,2,3} Regla Rodriguez,⁹ Rosa M. Ortiz,⁴ and Florent de Vathaire^{1,2,3}

Background: The incidence of differentiated thyroid cancer (DTC) is low in Cuba, and the contribution of dietary factors to DTC in this population has not been investigated so far. The aim of this study was to evaluate the relationship between dietary iodine intake and DTC with regard to the interaction with environmental factors or some common single nucleotide polymorphisms (SNPs), based on a case-control study carried out in Cuba. *Methods:* A total of 203 cases and 212 controls from the general population were interviewed face-to-face using the dietary intake questionnaire and the photo booklet from the E3N cohort. A specific food composition table was constructed for this study. For each parameter studied, the odds ratio (OR) was stratified on age group and sex, and further adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, and body surface area.

Results: The risk of DTC was significantly reduced with increasing consumption of fish (p = 0.04), but no association between total dietary iodine intake and DTC risk was evident (p = 0.7). This lack of significant association was true whatever the age, the smoking status, the dietary selenium intake, and the ethnicity (p > 0.05). DTC risk was positively and strongly associated with the number of copies in the minor allele (A) for SNP rs965513 near *FOXE1* among people who consumed less iodine than the median (p = 0.005).

Conclusion: Overall, the majority of the studied population had an optimal dietary iodine intake. DTC risk was inversely associated with high fish consumption. Furthermore, DTC risk was positively associated with the number of copies in the minor allele (A) of rs965513 among people who consumed less iodine than the median. Because these findings are based on post-diagnostic measures, studies with pre-diagnostic dietary iodine are needed for confirmation.

Keywords: thyroid cancer, dietary iodine intake, food composition table, DNA polymorphisms, case-control study

Introduction

I ODINE IS AN IMPORTANT trace element that is needed for the production of thyroid hormones. The body does not make iodine, so it is an essential part of the diet. Iodine is found in various foods, particularly dairy products, seafood, meat, and eggs, which are high in iodine and iodized salt (1–5). Iodine deficiency and iodine excess can cause or worsen hypothyroidism and hyperthyroidism. However, outside of endemic goiter areas, the association between dietary iodine intake and thyroid cancer is unclear (6,7).

The incidence of differentiated thyroid cancer (DTC) is lower in African populations and higher in island populations, particularly in volcanic regions of islands (8,9). Cuba is

¹Cancer and Radiations, Center for Research in Epidemiology and Population Health (CESP) - U1018, French National Institute of Health and Medical Research (INSERM), Villejuif, France.

²Department of Research, Gustave Roussy Institute, Villejuif, France.

³Faculty of Medicine, University of Paris XI, Le Kremlin-Bicêtre, France.

⁴Department of Clinical Research, Institute of Oncology and Radiobiology, Havana, Cuba.

⁵Medical Care, National Institute of Endocrinology, Havana, Cuba.

⁶Laboratory for Food Safety, Department of Chemical Contaminants in Food, Metallic Trace Elements and Minerals Unit, University of Paris-Est, Anses, Maisons-Alfort, France.

⁷The French Directorate General for Food, Ministry of Agriculture, Agro-16 Food and Forestry, Paris, France.

⁸Institut Curie, Mines ParisTech, U900, French National Institute of Health and Medical Research (INSERM), Paris, France.

⁹Department of Foreign Affairs, Public Health Ministry, Havana, Cuba.

FOXE1 SNP, DIETARY IODINE, AND THYROID CANCER RISK

an island of $100,000 \text{ km}^2$ where 35% of the population belong to the African ethnic group (10). Before colonization by Spain, Cuba was occupied by Native Americans who had migrated from the mainland of North, Central, and South America several centuries before (11). Although thyroid cancer is the sixth most common female cancer in Cuba, its incidence is quite low. Indeed, while in North America the world age-standardized rate (ASR) of DTC incidence was 10.0/100,000 in white females during the period 1998–2002 (12), the ASR of DTC incidence in Cuba was around 4.1/ 100,000 for females and was even sixfold less frequent in males in 2009 (13). For other Latin American countries such as Colombia, the ASR of DTC incidence was 9.4/100,000 in females during the period 1998-2002 (12). Why is DTC incidence low in Cuba? Is this situation related to dietary iodine intake or other dietary factors?

Up to now, no clear explanation for the low incidence of DTC in the Cuban population has been established. Globally, the only highlighted risk factors for developing thyroid cancer are radiation exposure, having a family history of thyroid cancer, residing in an iodine-deficient area, large body size, and a high number of pregnancies (14–16). The effect of alcohol, green tea, coffee, and other food groups or nutrients on the risk of developing thyroid cancer has also been investigated, but no significant associations have been identified (6,17).

It has been established that in endemic goiter areas with insufficient iodine intake, the incidence of follicular thyroid cancer is higher than elsewhere (18). However, this has not been replicated in papillary cancer, which currently represents >80% of DTCs in most countries, and 93% of the cases in this study, although overall goiter prevalence by palpation and inspection was 17.6% in Cuba, which corresponds to a mild endemic goiter area. However, the median urinary iodine concentration (UIC) of Cuban schoolchildren 6-11 years of age was $176 \,\mu\text{g/L}$ in 2011 and 2012 (19), which reflects adequate iodine intake (20), attributable to successful salt iodization. Nevertheless, until now, there has been no evidence to prove that iodine supplementation or excess dietary iodine intake in general decrease papillary DTC risk. The debate, however, is complicated because iodine content in food is very variable and, for the same food, depends on soil, agricultural techniques, and meal preparation. In addition, iodine is very volatile, and its measurement is still a challenge (4). For example, iodine content in cabbage was estimated to be 0.000427 mg/100 g in Cuba (ANSES 2014 food composition table), but 0.0007 mg/100 g in France (CIQUAL 2013 table) (21), and between 0.00026 and 0.00037 mg/100 g in Cameroon, depending on the samples (22). These variations exist for all foods, even those rich in iodine such as shellfish. The iodine content ranged from 0.056 mg/100 g depending on the type of shellfish in France (CIQUAL 2013 table) (21) to 0.50 mg/100 g in the Marshall Islands (23). In addition, iodine deficiency may also occur from interaction with other factors such as foods that are rich in isothiocyanates (24).

Traditional Cuban food includes staple food such as potato, taro, yam, vegetable banana, rice, and spaghetti; meat such as chicken, pork, beef, and ham; eggs; vegetables such as watercress, lettuce, tomato, red bean, and cabbage; fruits such as guava, mango, papaya, and banana; and fish or seafood such as sardine, tuna (canned or fresh), lobster, crab, shrimp, and shellfish. However, it is not clear whether dietary factors contribute to thyroid cancer risk in the Cuban population.

Both *NK2 homeobox 1* (*NKX2-1*, also called *TTF1* for *Thyroid Transcription Factor 1*) and *Forkhead factor E1* (*FOXE1*, also called *TTF2 for Thyroid Transcription Factor 2*) play a crucial role in the development of the thyroid gland, and their expression is modified in thyroid tumors (25–28). The single nucleotide polymorphism (SNP) rs944289 of the *NKX2-1* gene was reported to increase the risk of DTC (29, 30). The SNP rs965513, rs1867277, and rs71369530 near or within the *FOXE1* gene were identified in previous genome-wide association studies (GWASs) as being associated with an increased risk of DTC (29–33). This study thus sought to investigate the contribution of genetic variations at the *NKX2-1* and *FOXE1* loci to the risk of DTC in the Cuban population.

To the best of the authors' knowledge, this observational study is the first to investigate dietary factors in the Cuban population. The aims were to explore the role of specific food groups or items, especially in regard to iodine intake, in the risk of developing DTC, and to assess the possibility of interaction between environmental factors and some common SNPs previously associated with DTC in the Cuban population. It was hypothesized, first, that fish consumption may reduce the risk of DTC; second, that dietary iodine deficiency and excess dietary iodine could be associated with an increasing risk of DTC; and third, that dietary iodine deficiency may interact with *FOXE1* due to its role in thyroid hormone pathways.

Materials and Methods

This population-based case-control study was carried out in Havana, Cuba. A detailed methodology and the results of the analysis of non-dietary risk factors have already been published (34).

Consent

The Clinical Research Ethics Committee of the National Institute of Oncology and Radiobiology (INOR; Havana, Cuba) revised and approved this study. Informed written consent was obtained from all the study participants.

Study participants

All patients aged between 17 and 60 years who were living in Havana and its surrounding areas and who were treated for DTC between 2003 and 2010 at INOR and at the Institute of Endocrinology, Havana, were eligible for the study. Potential cases were selected from the National Cancer Registry databases (C73 ICD-10) and were cross-referenced with the INOR Pathology Register. On average, around 25–30 patients corresponding to these criteria were identified for each year of the study period. Among the 240 eligible DTC cases, 37 (15%) individuals were not interviewed because they had an unknown address (n=32) or refused to participate (n=5). The final study population consisted of 203 cases.

A total of 229 controls were selected from the general population living in Havana city and its surroundings using consultation files from primary-care units (family doctors) and from workplaces with a general practitioner. Each control was frequency matched with each case by age (± 5 years)

and sex. Of the 229 potential controls, 17 refused and 212 agreed to be interviewed (Fig. 1).

Between July 2009 and January 2012, both cases and controls were interviewed face-to-face by trained professionals (nursing and medical staff) using a structured questionnaire. A standardized questionnaire was used to collect data on demographic characteristics (age, sex, place of residence, occupation), blood group and rhesus factor, anthropometric parameters, reproductive and hormonal history, lifestyle (smoking habits, alcohol consumption), exposure to radiation or chemicals, personal medical history and family medical history, and dietary intake in first-degree relatives. The dietary intake questionnaire is divided into two parts. The first part focuses on the general dietary intake, and the second part addresses the typical Cuban dietary intake more specifically. This questionnaire quantifies consumption by frequency and portion sizes per food group or food item. In addition, the photo booklet from the E3N cohort was used, which provides more detailed qualitative information on separate items within one food group (35). A saliva sample was also collected from the study participants.

Assuming a prevalence of exposure close to 30%, the size of this study (about 200 cases and 200 controls) allowed us to reach a power of 80% for identifying the role of a risk factor associated with an odds ratio (OR) of 1.8 when accepting a type I error of 5%.

Measures of iodine and selenium in food

The composition of 20 (28%) traditional Cuban food items was measured by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). It is noteworthy that the ANSES uses an innovative technology for determining total iodine (4). This technology is based on a European standard (36) that involves alkaline extraction using tetra-

methylammonium hydroxide (TMAH), optimized with an additional enzymatic treatment for samples containing starch, and followed by inductively coupled plasma mass spectrometry (ICP-MS) analysis. A complete single-laboratory validation was performed according to the accuracy profile approach (37,38) based on tolerance intervals to select the best calibration function and to determine the validated concentration ranges. This method was used to determine the iodine and selenium content in the main foods consumed by Cubans (Supplementary Figs. S1 and S2; Supplementary Data are available online at www.liebertpub.com/thy).

The composition of the remaining 51 (72%) food items of other general food consumed by cases and controls, and included in the diet questionnaire, was estimated using the CIQUAL 2013 table (21).

For each food item in the diet questionnaire, total intake of iodine and selenium consumption were estimated by multiplying the daily consumption of each food by the iodine or selenium content in this food based on ANSES 2014 (Supplementary Table S1) or CIQUAL 2013 food composition tables (21) (if iodine or selenium content were measured from several similar foods in the composition tables, the mean of the iodine or selenium content in these similar foods was taken).

An item for quantifying iodized salt intake in the questionnaire was not included. In fact, since 2005, Cuba has established compulsory iodization of salt for human consumption for the whole Cuban population because a global strategy at the national level is the best solution for a cost-effective prevention and control of iodine deficiency (5). A fixed value of 10 g of iodized salt per person per day was included. This value was estimated to be the average consumption of iodized salt per inhabitant per day by the Center for Nutrition and Food Hygiene, the National Institute of Hygiene, Epidemiology, and Microbiology (Inhem) of Cuba.



FIG. 1. Participant flow chart of inclusion criteria, Cuba, 2009–2012.

TABLE 1	I. CHARACT	ERISTICS	OF DTC	CASES
IN TH	ie Studied	CUBAN]	POPULAT	ION

	Cases $(n=203)$		
Characteristics	n	%	
Sex			
Male	24	11.8	
Female	179	88.2	
Delay (years) between d	liagnosis and intervi	ews	
0-1	76	37.4	
2–4	72	35.5	
5-11	55	27.1	
Year of diagnosis			
2000-2005	50	24.6	
2006-2008	64	31.5	
2009-2011	89	43.9	
Age (years) at diagnosis			
17–20	10	4.9	
21–25	16	7.9	
26-30	12	5.9	
31–35	34	16.8	
36–40	46	22.7	
41–45	41	20.2	
46-50	30	14.8	
51–55	7	3.4	
56-60	7	3.4	
Histology			
Papillary	189	93.1	
Follicular	14	6.9	

DTC, differentiated thyroid cancer.

Analyzed parameters

For quantitative factors, such as fish, shellfish, and total seafood consumption, the study participants were categorized into tertiles according to the consumption value distribution in controls.

Overall, the study participants were classified into three categories according to the Iodine Global Network (IGN)

(39): mild iodine deficiency (75–149 μ g/day), optimal iodine intake (150–299 μ g/day), and more than adequate or excess iodine intake (\geq 300 μ g/day). Furthermore, the study participants were regrouped into quartiles according to the dietary iodine intake in controls.

The possible interaction between dietary iodine intake and the following polymorphisms was investigated: rs944289 (near *NKX2-1*), rs965513 (near *FOXE1*), rs1867277 (5' UTR of *FOXE1*), and rs71369530 (polyalanine length polymorphism in *FOXE1*), which were significantly associated with the risk of developing DTC in the same Cuban case-control study (40). For this purpose, the values of dietary iodine intake were classified into two categories: either less or more than the median (186 μ g/day) value observed in controls.

Statistical analysis

Nineteen strata were defined on the basis of age and sex: seven for men and twelve for women. For each parameter studied, the OR stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, and body surface area (BSA), was estimated using conditional logistic regression with SAS v9.3 (SAS Institute, Inc., Cary, NC). Linear trend tests were performed by creating a variable that assigned the median value for each category of the studied variable consumption, and this variable was then modeled as a continuous variable. Likelihood ratio tests comparing models with and without multiplicative interaction terms were performed to assess the significance of interactions. Sensitivity analyses were performed by excluding individuals diagnosed more than five years before the interviews.

Results

The majority of the population in this Cuban case-control study were females (88%) with DTC diagnosed between 2000 and 2011. The mean age at diagnosis was 38 years (range 17–60 years). Almost all (93.1%) DTCs were papillary carcinomas (Table 1). The risk of DTC reduced significantly with increasing consumption of fish (p=0.04; Table 2).

TABLE 2. ODDS RATIOS OF DTC ASSOCIATED WITH FISH AND SHELLFISH CONSUMPTION

	Cases/controls	Age and sex		Multivariate OR ^b [CI]	p-Value ^b
	(203/212)	stratified OR [CI]	p-Value ^a		
Fish consum	nption (g/day)				
<4	103/77	1.00°	0.005	1.00°	0.04
4-14	50/65	0.58 [0.36-0.95]		0.60 [0.36–1.00]	
≥15	50/70	0.52 [0.33–0.84]		0.55 [0.34–0.92]	
Shellfish co	nsumption (g/day)				
0	128/102	1.00°	0.04	1.00°	0.2
1–4	21/44	0.38 [0.21-0.69]		0.31 [0.17-0.58]	
≥5	54/66	0.68 [0.43–1.06]		0.70 [0.43–1.13]	
Total consu	mption of seafood (g/da	v)			
<5	89/64	1.00 ^c	0.02	1.00°	0.1
5-19	60/81	0.54 [0.34-0.87]		0.58 [0.36-0.96]	
≥20	54/67	0.57 [0.35–0.92]		0.58 [0.35–0.98]	

^aMantel–Haenszel chi-square test stratified on age group and sex.

^bMultivariate logistic regression stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, body surface area, and, for trend test, calculated by assigning the median value to each category and treating this variable as a continuous variable.

^cReference category.

OR, odds ratio; CI, confidence interval.

	Dietary iodine intake, IGN classification (µg/day)			
	Mild deficiency (75–149)	Optimal (150–299)	More than adequate or excessive (≥ 300)	p-Value ^a
Cases/controls Age and sex stratified OR [CI] Multivariate OR ^d [CI]	34/28 1.44 [0.83–2.51] 1.27 [0.70–2.30]	147/175 1.00 ^b 1.00 ^b	22/9 2.78 [1.24–6.25] 2.32 [0.98–5.48]	0.6 ^c 0.5 ^e

TABLE 3. ODDS RATIOS OF DTC ASSOCIATED WITH DIETARY IODINE INTAKE ACCORDING TO IGN CLASSIFICATION IN THE STUDIED CUBAN POPULATION

^aCalculated using the Wald test for dietary iodine intake modeled as a continuous variable (per classification). ^bReference category.

^cCrude *p*-trend.

^dStratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, and body surface area.

^eAdjusted *p*-trend.

It was observed that only 15% of the studied population samples were estimated as having mild iodine deficiency, and the majority (78%) of the studied population had an optimal dietary iodine intake according to the IGN classification. No significant association was identified between dietary iodine intake, when classified according to the IGN classification, and DTC risk in the entire Cuban sample (p=0.5; Table 3). This association was still not significant (p=0.7) when the population sample was regrouped by quartiles of dietary iodine intake in controls (Supplementary Table S2). Furthermore, this non-significant association was not modified by age, smoking status, dietary selenium intake, or ethnicity (p > 0.05; Supplementary Table S2).

No interaction was found between dietary iodine intake and the polymorphisms rs944289, rs1867277, and rs71369530 in the risk of developing DTC. Conversely, a strong interaction (p=0.005) was identified between dietary iodine intake and rs965513, near the *FOXE1* gene on chromosome 9q22.33 (Table 4). A strong increased DTC risk was observed with the number of copies in the minor allele (A) of rs965513 among people who consumed less iodine than the median (<186 μ g/ day). The risk of DTC was 3.4 times higher (p=0.0005 [confidence interval (CI) 1.7–6.5]) for subjects carrying one copy of the A allele, and 6.7 times higher (p=0.0005 [CI 1.6– 27.3]) for subjects carrying two copies of the A allele when compared with the reference group of subjects with no copies in the minor allele (A). This dose effect with regard to the DTC risk with the increasing number of copies in the A allele was not observed in subjects consuming more iodine than the median (>186 μ g/day). Independent of the number of copies in the minor allele (A), the risk of DTC was around three times higher than that of the reference group whose subjects were not carrying any A allele and had a dietary iodine intake below the median (Table 4).

An interaction that was close to significance was identified between dietary iodine intake and body mass index (BMI; p=0.08). Among people whose BMI was ≥ 25 kg/m², the risk of DTC was 2.47 times higher (multivariate odds ratio [OR] = 2.47 [CI 0.89–6.85]) in people who were in the lowest quartile of dietary iodine intake compared with those who

 TABLE 4. ODDS RATIOS OF DTC RISK ASSOCIATED WITH DIETARY IODINE INTAKE AND THE POLYMORPHISM

 OF SNP rs965513 NEAR FOXE1

	Polymorph			
Dietary iodine intake	G/G	G/A	A/A	p-Value
<median (186="" day)<="" td="" µg=""><td></td><td></td><td></td><td></td></median>				
Cases/controls	31/68	45/31	11/3	
OR [CI]	1.0^{a}	3.2 [1.7–5.9]	8.0 [2.1-30.9]	< 0.0001 ^b
Age and sex stratified OR [CI]	1.0^{a}	3.2 [1.7–6.2]	8.0 [1.6-40.3]	< 0.0001 ^c
Multivariate OR ^d [CI]	1.0^{a}	3.4 [1.7–6.5]	6.7 [1.6–27.3]	0.0005^{d}
>Median			L J	
Cases/controls	50/48	44/46	16/9	
OR [CI]	2.3 [1.3-4.1]	2.1 [1.2–3.8]	3.9 [1.6–9.8]	0.5^{b}
Age and sex stratified OR [CI]	2.6 [1.4-4.8]	2.3 [1.2-4.3]	4.8 [1.7–13.8]	0.3°
Multivariate OR ^d [CI]	2.4 [1.3–4.4]	2.4 [1.3–4.5]	3.3 [1.2–8.8]	0.4 ^d

Missing data: two cases and four controls with dietary iodine intake <median, and four cases and three controls with dietary iodine intake >median due to low quality of samples for genotyping.

p-Value between the number of A allele in FOXE1 and dietary iodine intake (+ or - median), crude=0.007, stratified=0.003, multivariate=0.005.

^aReference category.

^bMantel–Haenszel chi-square test.

^cMantel-Haenszel chi-square test stratified on age group and sex.

^dMultivariate logistic regression stratified on age group and sex, and adjusted for dietary energy, smoking status, ethnic group, level of education, number of pregnancies, body surface area, and for trend test, calculated by assigning the number of A allele in *FOXE1*.

were in the highest quartile, although this difference was not significant (p=0.1). This increase of DTC risk when decreasing iodine intake was not observed in people with a BMI <25 kg/m² (Supplementary Table S2).

Discussion

It was observed that the risk of DTC significantly decreased with increasing consumption of fish in the Cuban population. In addition, a strong increase in DTC risk was found with the number of copies in the minor allele (A) of rs965513 near *FOXE1* on chromosome 9q22.33 among people who consumed less than the median value of iodine in controls. Furthermore, the risk of DTC tended to be higher among people whose BMI $\geq 25 \text{ kg/m}^2$ and who were in the lowest quartile of dietary iodine intake.

The increasing number of eligible DTC cases in this study from the first six years (n = 50) to the last three years (n = 89) was not related to technical developments in thyroid cancer diagnosis (e.g., fine-needle aspiration). For cases diagnosed closer to the year of their interview (between July 2009 and January 2012), the reason was that their clinical and personal dossiers were more easily obtained. Furthermore, the charts contained more precise information, and therefore these cases were more likely to fulfill the inclusion criteria.

The present study is the first case-control study to focus on the risk factors of DTC in the Cuban population. The food photo booklet from the E3N cohort (35) was used in this study in order to estimate the portions of food and drink daily consumption by the participants, which complemented the quantificational questionnaire and gave us normative, accurate, and qualitative information on which to build the database. Another noteworthy point is that a special food composition table was constructed to measure the quantity of iodine in typical Cuban food for this study. However, only 28% of the food items in the questionnaire of this study were measured using this special and innovative table.

As with any case-control study, this study has some limitations. Self-reported food consumption is associated with imprecise measures and recall bias. Another limitation is that for a large part of the Cuban diet, iodine content was estimated from the CIQUAL 2013 food composition table, which had been established for French food, and may not be applicable to Cuban food. Moreover, because cases usually take more care of their health and are more motivated to participate in research studies than controls, the cases may have reported more precise information than the controls did, and this could have introduced a differential bias in the final results and overestimated the risk of DTC. Nevertheless, this potential bias cannot explain the significant relation between the number of copies in the minor allele (A) of rs965513 and the risk of DTC among people who consumed less iodine than the median. Indeed, it is very unlikely that the finding regarding an interaction between SNP rs965513 and iodine intake was due to chance because it remained present when correcting the p-value for the number of SNPs tested, which was four SNPs in the analyses (41). Additionally, this result was almost exactly identical when adjusting or not for other DTC risk factors and stratifying or not for age groups and sex. Lastly, recall bias was probably reduced by the fact that food import and export limitations exist in Cuba, which keeps the typical Cuban food more stable and less diversified than Western food.

The French CIQUAL 2013 food composition table was used in this study in order to evaluate the iodine content in fish and shellfish. This could introduce some uncertainties. Nevertheless, the iodine content in fish is highly variable even in one country (42). It depends strongly on the species and the places of capture, and most of the sea fish consumed in Cuba are from areas far away from Cuban coasts. Given the low number of fish captured by Cuban boats and low fish consumption in Cuba, the difficulty in measuring the iodine content in fish, and the cost-effectiveness of this study, the iodine content was not measured in either Cuban fish or shellfish. This study shows that in the Cuban population, the risk of DTC significantly decreased with the increasing consumption of fish at the time of the interview (Table 2). This important finding is in line with another case-control study, which was carried out in French Polynesia (43). A meta-analysis of 19 observational studies on dietary factors and thyroid cancer risk summarized the ORs for each risk factor. Based on the highest level of total consumption versus the lowest level, the aggregate OR [CI] of thyroid cancer was 0.79 [0.66–0.94] for fish consumption. Subgroup analysis showed that fish (OR=0.74 [CI 0.59-0.92]) and shellfish (OR = 0.46 [CI 0.27 - 0.75]) consumption has a protective effect in iodine-deficient areas (44). Another related review showed that iodine-rich food such as fish and shellfish may provide a protective role in populations with insufficient daily iodine intake (6). In contrast, a Japanese cohort where subjects were followed up for a mean of 14.5 years showed that a high intake of seaweed was positively associated with thyroid cancer risk, although this result was not statistically significant. In addition, a high intake of seaweed was positively associated with papillary thyroid cancer risk in postmenopausal women (especially for papillary carcinoma, the hazard ratio [HR] for almost daily consumption compared with ≤ 2 days/week was 3.81 [CI 1.67–8.68]; p < 0.01) (45).

First-degree relatives of DTC patients have an increased risk for developing thyroid cancer, and a positive family history is reported in approximately 5% of cases (46–49). These observations strongly suggest the contribution of genetic factors to the susceptibility to the disease. Six potential regions for harboring a high-risk DTC gene have been identified through family-based linkage studies in the early 2000s: MNG1 (14q32) (50), TCO (19p13.2) (51,52), fPTC/ *PRN* (1q21) (53), *NMTC1* (2q21) (54), *FTEN* (8p23.1-p22) (55), and the telomere-telomerase complex (56). More recently, population-based studies, particularly GWASs, have identified and replicated susceptibility loci at 9q22, 14q13, 2q35, and 8p12 (57). In a previous study, in the same sample of the Cuban population, the association was validated between DTC risk and variants of genes localized at 9q22.33 (FOXE1) and 14q13.3 (NKX2-1) (40). The FOXE1 locus is the most widely replicated susceptibility locus associated with DTC risk in a number of population-based studies of various ethnic origins, especially associated with sporadic and radiation-related papillary thyroid cancer and also with familial nonmedullary thyroid carcinoma (58,59).

This case-control study has for the first time identified a strong increase in DTC risk associated with the number of copies in the minor allele (A) of SNP rs965513, near *FOXE1* at 9q22.33, among people who consumed less iodine than the median, but no variation in people who consumed more (p=0.005). To the best of the authors' knowledge, gene–

environment interactions in DTC have only been investigated in relation to radiation exposure in a candidate gene approach, but no convincing association has been reported so far. In particular, no gene–environment interaction study has investigated an association with *FOXE1* (at 9q22.33) and dietary iodine intake.

According to the IGN classification, an optimal iodine intake corresponds to 150–299 μ g/day (39). The quartiles of the dietary iodine intake in the controls in this study were: <164.9, 164.9–186.5, 186.5–237.5, and \geq 237.5 µg/day. The last three quartiles cover the optimal iodine intake, as well as more than adequate or excess iodine intake. However, a casecontrol study using a food frequency questionnaire is not an adequate way to estimate iodine status (because a casecontrol study without iodine measurement in urine is not designed to estimate the iodine status of a population, but to perform comparisons between the cases and the controls). Nevertheless, the majority of the studied population had an optimal dietary iodine intake. If this result was confirmed by other studies, it would emphasize the effectiveness of the compulsory public health policy by using iodized salt in urban, rural, and mountainous areas of Cuba. A cross-sectional study to assess the effectiveness of this program through determination of UIC and goiter prevalence has been previously completed in Cuban schoolchildren aged 6-11 years in 2011-2012. The results of this study by Terry-Berro et al. showed that the median UIC was $176.3 \,\mu\text{g/L}$ (19). Only 7.6% of the children showed iodine deficiency (2.2% had severe deficiency $<50 \,\mu g/L$) and 15.3% had an UIC $>300 \,\mu g/L$. The prevalence of optimal iodine nutrition was 43.5% (52.5% in mountain areas). The overall goiter prevalence was 17.6%, which reflects mild endemic goiter. However, in mountain areas, the prevalence of goiter was 32.6% (severe endemic goiter). The authors concluded that iodine deficiency is no longer a public health problem in urban and rural areas of Cuba, which is attributable to successful salt iodization. Nevertheless, high goiter rates are still observed in mountain areas.

In a comprehensive synthesis of animal and human studies (7), Zimmermann *et al.* concluded that iodine deficiency increases DTC risk. However, the present study does not have enough participants in the moderate iodine deficiency group to verify this conclusion with sufficient statistical power.

In conclusion, the majority of the studied population had an optimal dietary iodine intake. Although DTC risk was not found to be linked to dietary iodine intake in this study, DTC risk significantly decreased with high fish consumption. Additionally, DTC risk significantly increased with an increasing number of copies in the minor allele (A) of rs965513 near *FOXE1* among people who consumed less iodine than the median. Because these findings are based on postdiagnostic measures, studies with pre-diagnostic dietary iodine are needed for confirmation.

Acknowledgments

We sincerely acknowledge the Ligue Nationale Contre le Cancer (LNCC), the Région IIe de France, and the Institut National du Cancer (INCA) for funding this research project. We extend special gratitude to Fondation de France for funding the first author, Yan Ren, for her PhD study, and we sincerely thank the Région IIe de France for the grant to Constance Xhaard.

REN ET AL.

Author Disclosure Statement

The authors state that no competing financial interests exist.

References

- 1. British Geological Survey 2003 Database of the iodine content of food and diets populated with data from published literature. British Geological Survey Commissioned Report, Keyworth, Nottingham, United Kingdom.
- Haldimann M, Alt A, Blondeau ABK 2005 Iodine content of food groups. J Food Compost Anal 18:461–471.
- Rose M, Miller P, Baxter M, Appleton G, Crews H, Croasdale M 2001 Bromine and iodine in 1997 UK total diet study samples. J Environ Monit 3:361–365.
- Leufroy A, Noël L, Bouisset P, Maillard S, Bernagout S, Xhaard C, de Vathaire F, Guérin T 2015 Determination of total iodine in French Polynesian foods: method validation and occurrence data. Food Chem 169:134–140.
- Acosta LF 2015 Los dilemas de la sal. Available at: www .granma.cu/cuba/2015-03-20/los-dilemas-de-la-sal?page=1 (accessed May 23, 2016).
- Choi WJ, Kim J 2014 Dietary factors and the risk of thyroid cancer: a review. Clin Nutr Res 3:75–88.
- Zimmermann MB, Galetti V 2015 Iodine intake as a risk factor for thyroid cancer: a comprehensive review of animal and human studies. Thyroid Res 8:8.
- Pellegriti G, De Vathaire F, Scollo C, Attard M, Giordano C, Arena S, Dardanoni G, Frasca F, Malandrino P, Vermiglio F, Previtera DM, D'Azzò G, Trimarchi F, Vigneri R 2009 Papillary thyroid cancer incidence in the volcanic area of Sicily. J Natl Cancer Inst **101**:1575–1583.
- Kristbjornsdottir A, Rafnsson V 2012 Incidence of cancer among residents of high temperature geothermal areas in Iceland: a census-based study 1981–2010. Environ Health 11:73.
- Cuban National Bureau of Statistics, Havana, Cuba 2014. Available at: www.one.cu/aec2013/esp/20080618_tabla_ cuadro.htm (accessed September 23, 2015).
- 11. Dacal R, Rivero de la Calle M 1996 Art and Archaeology of Pre-Columbian Cuba. University of Pittsburgh Press, Pittsburgh, PA.
- Kilfoy BA, Zheng T, Holford TR, Han X, Ward MH, Sjodin A, Zhang Y, Bai Y, Zhu C, Guo GL, Rothman N, Zhang Y 2009 International patterns and trends in thyroid cancer incidence, 1973–2002. Cancer Causes Control 20: 525–531.
- Ministerio de Salud Pública-Dirección Nacional de Registros Médicos y Estadísticas de Salud 2015 Anuario estadístico de salud 2014. Available at: http://files.sld.cu/dne/files/2015/04/ anuario-estadístico-de-salud-2014.pdf (accessed September 23, 2015).
- 14. Clero E, Leux C, Brindel P, Truong T, Anger A, Teinturier C, Diallo I, Doyon F, Guenel P, de Vathaire F 2010 Pooled analysis of two case-control studies in New Caledonia and French Polynesia of body mass index and differentiated thyroid cancer: the importance of body surface area. Thyroid 20:1285–1293.
- Peterson E, De P, Nuttall R 2012 BMI, diet and female reproductive factors as risks for thyroid cancer: a systematic review. PLoS One 7:e29177.
- 16. Xhaard C, Rubino C, Cléro E, Maillard S, Ren Y, Borson-Chazot F, Sassolas G, Schvartz C, Colonna M, Lacour B, Danzon A, Velten M, Buemi A, Bailly L, Mariné Barjoan E, Schlumberger M, Orgiazzi J, Adjadj E, de Vathaire F 2014 Menstrual and reproductive factors in the risk of

differentiated thyroid carcinoma in young women in France: a population-based case-control study. Am J Epidemiol **180**:1007–1017.

- Michikawa T, Inoue M, Shimazu T, Sasazuki S, Iwasaki M, Sawada N, Yamaji T, Tsugane S 2011 Green tea and coffee consumption and its association with thyroid cancer risk: a population-based cohort study in Japan. Cancer Causes Control 22:985–993.
- Galanti MR, Sparén P, Karlsson A, Grimelius L, Ekbom A 1995 Is residence in areas of endemic goiter a risk factor for thyroid cancer? Int J Cancer 61:615–621.
- Terry-Berro CB, Quintana-Jardines I, de la Paz-Luna M, García J, Fernández-Bohórquez R, Silvera-Téllez D, Díaz-Fuentes Y, Ferret-Martínez A, Reyes-Fernández D 2014 Impact of the actions for the sustainable elimination of iodine deficiency in Cuba. Rev Peru Med Exp Salud Publica 31:24–29.
- WHO, UNICEF, ICCIDD 2007 Assessment of the iodine deficiency disorders and monitoring their elimination: a guide for programme managers. Third edition. Available at: http://apps.who.int/iris/bitstream/10665/43781/1/ 9789241595827_eng.pdf (accessed March 14, 2016).
- Agence Française de Sécurité Sanitaire des Aliments 2013 French food composition table Ciqual version 2013. Available at: https://pro.anses.fr/tableciqual/index .htm (accessed September 23, 2015).
- Taga I, Sameza ML, Kayo AV, Ngogang J 2004 Iodine levels in food and soil in different regions in Cameroon. Sante 14:11–15.
- Takahashi T, Fujimori K, Simon SL, Bechtner G, Edwards R, Trott KR 1999 Thyroid nodules, thyroid function and dietary iodine in the Marshall Islands. Int J Epidemiol 28:742–749.
- Truong T, Baron-Dubourdieu D, Rougier Y, Guénel P 2010 Role of dietary iodine and cruciferous vegetables in thyroid cancer: a countrywide case-control study in New Caledonia. Cancer Causes Control 21:1183–1192.
- 25. Parlato R, Rosica A, Rodriguez-Mallon A, Affuso A, Postiglione MP, Arra C, Mansouri A, Kimura S, Di Lauro R, De Felice M 2004 An integrated regulatory network controlling survival and migration in thyroid organogenesis. Dev Biol 276:464–475.
- 26. Zannini M, Avantaggiato V, Biffali E, Arnone MI, Sato K, Pischetola M, Taylor BA, Phillips SJ, Simeone A, Di Lauro R 1997 TTF-2, a new forkhead protein, shows a temporal expression in the developing thyroid which is consistent with a role in controlling the onset of differentiation. EMBO J 16:3185–3197.
- Zhang P, Zuo H, Nakamura Y, Nakamura M, Wakasa T, Kakudo K 2006 Immunohistochemical analysis of thyroidspecific transcription factors in thyroid tumours. Pathol Int 56:240–245.
- Perna MG, Civitareale D, De Filippis V, Sacco M, Cisternino C, Tassi V 1997 Absence of mutations in the gene encoding thyroid transcription factor-1 (TTF-1) in patients with thyroid dysgenesis. Thyroid **7:**377–381.
- 29. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, He H, Blondal T, Geller F, Jakobsdottir M, Magnusdottir DN, Matthiasdottir S, Stacey SN, Skarphedinsson OB, Helgadottir H, Li W, Nagy R, Aguillo E, Faure E, Prats E, Saez B, Martinez M, Eyjolfsson GI, Bjornsdottir US, Holm H, Kristjansson K, Frigge ML, Kristvinsson H, Gulcher JR, Jonsson T, Rafnar T, Hjartarsson H, Mayordomo JI, de la Chapelle A, Hrafnkelsson J,

Thorsteinsdottir U, Kong A, Stefansson K 2009 Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet **41**:460–464.

- 30. Matsuse M, Takahashi M, Mitsutake N, Nishihara E, Hirokawa M, Kawaguchi T, Rogounovitch T, Saenko V, Bychkov A, Suzuki K, Matsuo K, Tajima K, Miyauchi A, Yamada R, Matsuda F, Yamashita S 2011 The *FOXE1* and *NKX2-1* loci are associated with susceptibility to papillary thyroid carcinoma in the Japanese population. J Med Genet **48:**645–648.
- 31. Jones AM, Howarth KM, Martin L, Gorman M, Mihai R, Moss L, Auton A, Lemon C, Mehanna H, Mohan H, Clarke SE, Wadsley J, Macias E, Coatesworth A, Beasley M, Roques T, Martin C, Ryan P, Gerrard G, Power D, Bremmer C; TCUKIN Consortium, Tomlinson I, Carvajal-Carmona LG 2012 Thyroid cancer susceptibility polymorphisms: confirmation of loci on chromosomes 9q22 and 14q13, validation of a recessive 8q24 locus and failure to replicate a locus on 5q24. J Med Genet **49**:158–163.
- 32. Landa I, Ruiz-Llorente S, Montero-Conde C, Inglada-Pérez L, Schiavi F, Leskelä S, Pita G, Milne R, Maravall J, Ramos I, Andía V, Rodríguez-Poyo P, Jara-Albarrán A, Meoro A, del Peso C, Arribas L, Iglesias P, Caballero J, Serrano J, Picó A, Pomares F, Giménez G, López-Mondéjar P, Castello R, Merante-Boschin I, Pelizzo MR, Mauricio D, Opocher G, Rodríguez-Antona C, González-Neira A, Matías-Guiu X, Santisteban P, Robledo M 2009 The variant rs1867277 in *FOXE1* gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors. PLoS Genet **5**:e1000637.
- 33. Bullock M, Duncan EL, O'Neill C, Tacon L, Sywak M, Sidhu S, Delbridge L, Learoyd D, Robinson BG, Ludgate M, Clifton-Bligh RJ 2012 Association of *FOXE1* polyalanine repeat region with papillary thyroid cancer. J Clin Endocrinol Metab **97:** E1814–1819.
- 34. Lence-Anta JJ, Xhaard C, Ortiz RM, Kassim H, Pereda CM, Turcios S, Velasco M, Chappe M, Infante I, Bustillo M, García A, Clero E, Maillard S, Salazar S, Rodriguez R, de Vathaire F 2014 Environmental, lifestyle, and anthropometric risk factors for differentiated thyroid cancer in Cuba: a case-control study. Eur Thyroid J 3:189–196.
- Van Liere MJ, Lucas F, Clavel F, Slimani N, Villeminot S 1997 Relative validity and reproducibility of a French dietary history questionnaire. Int J Epidemiol 26:S128–136.
- 36. Comite Europeen de Normalisation 2007 Foodstuffs— Determination of trace elements—determination of iodine by ICP-MS (inductively coupled plasma mass spectrometry). Comite Europeen de Normalisation 15111, Brussels.
- 37. Association française de normalisation 2010 NF V03– 110—analyse des produits agricoles et alimentaire protocole de caractérisation en vue de la validation d'une méthode d'analyse quantitative par construction du profil d'exactitude. Association française de normalisation, La Plaine Saint-Denis.
- Mermet JM, Granier G 2012. Potential of accuracy profile for method validation in inductively coupled plasma spectrochemistry. Spectrochimica Acta–Part B 76:214–220.
- 39. Li M, Eastman CJ 2012 The changing epidemiology of iodine deficiency. Nat Rev Endocrinol **8:**434–440.
- 40. Pereda CM, Lesueur F, Pertesi M, Robinot N, Lence-Anta JJ, Turcios S, Velasco M, Chappe M, Infante I, Bustillo M, García A, Clero E, Xhaard C, Ren Y, Maillard S, Damiola F, Rubino C, Salazar S, Rodriguez R, Ortiz RM, de Vathaire F 2015 Common variants at the 9q 22.33, 14q13.3

and ATM loci, and risk of differentiated thyroid cancer in the Cuban population. BMC Genet **1**:16–22.

- 41. Dunn OJ 1961 Multiple comparisons among means. JASA **56:**52–64.
- Lee SM, Lewis J, Buss DH, Holcombe GD, Lawrance PR 1994 Iodine in British foods and diets. Br J Nutr 72:435–446.
- 43. Cléro É, Doyon F, Chungue V, Rachédi F, Boissin JL, Sebbag J, Shan L, Bost-Bezeaud F, Petitdidier P, Dewailly E, Rubino C, de Vathaire F 2012 Dietary iodine and thyroid cancer risk in French Polynesia: a case-control study. Thyroid 22:422–429.
- Liu ZT, Lin AH 2014 Dietary factors and thyroid cancer risk: a meta-analysis of observational studies. Nutr Cancer 66:1165–1178.
- 45. Michikawa T, Inoue M, Shimazu T, Sawada N, Iwasaki M, Sasazuki S, Yamaji T, Tsugane S 2012 Japan Public Health Center-based Prospective Study Group. Seaweed consumption and the risk of thyroid cancer in women: the Japan Public Health Center-based Prospective Study. Eur J Cancer Prev 21:254–260.
- 46. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH 1994 Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J Natl Cancer Inst 86:1600–1608.
- 47. Pal T, Vogl FD, Chappuis PO, Tsang R, Brierley J, Renard H, Sanders K, Kantemiroff T, Bagha S, Goldgar DE, Narod SA, Foulkes WD 2001 Increased risk for nonmedullary thyroid cancer in the first degree relatives of prevalent cases of nonmedullary thyroid cancer: a hospital-based study. J Clin Endocrinol Metab **86**:5307–5312.
- Hemminki K, Vaittinen P 1997 Effect of paternal and maternal cancer on cancer in the offspring: a population-based study. Cancer Epidemiol Biomarkers Prev 6:993–997.
- Hemminki K, Li X 2003 Familial risk of cancer by site and histopathology. Int J Cancer 103:105–109.
- 50. Bignell GR, Canzian F, Shayeghi M, Stark M, Shugart YY, Biggs P, Mangion J, Hamoudi R, Rosenblatt J, Buu P, Sun S, Stoffer SS, Goldgar DE, Romeo G, Houlston RS, Narod SA, Stratton MR, Foulkes WD 1997 Familial nontoxic multinodular thyroid goiter locus maps to chromosome 14q but does not account for familial nonmedullary thyroid cancer. Am J Hum Genet **61:**1123–1130.
- 51. Canzian F, Amati P, Harach HR, Kraimps JL, Lesueur F, Barbier J, Levillain P, Romeo G, Bonneau D 1998 A gene predisposing to familial thyroid tumors with cell oxyphilia maps to chromosome 19p13.2. Am J Hum Genet 63:1743– 1748.
- 52. Lesueur F, Stark M, Tocco T, Ayadi H, Delisle MJ, Goldgar DE, Schlumberger M, Romeo G, Canzian F 1999 Genetic heterogeneity in familial nonmedullary thyroid carcinoma: exclusion of linkage to RET, MNG1, and TCO in 56 families. NMTC Consortium. J Clin Endocrinol Metab 84:2157–2162.

- 53. Malchoff CD, Sarfarazi M, Tendler B, Forouhar F, Whalen G, Joshi V, Arnold A, Malchoff DM 2000 Papillary thyroid carcinoma associated with papillary renal neoplasia: genetic linkage analysis of a distinct heritable tumor syndrome. J Clin Endocrinol Metab 85:1758–1764.
- 54. McKay JD, Lesueur F, Jonard L, Pastore A, Williamson J, Hoffman L, Burgess J, Duffield A, Papotti M, Stark M, Sobol H, Maes B, Murat A, Kääriäinen H, Bertholon-Grégoire M, Zini M, Rossing MA, Toubert ME, Bonichon F, Cavarec M, Bernard AM, Boneu A, Leprat F, Haas O, Lasset C, Schlumberger M, Canzian F, Goldgar DE, Romeo G 2001 Localization of a susceptibility gene for familial nonmedullary thyroid carcinoma to chromosome 2q21. Am J Hum Genet **69:**440–446.
- 55. Cavaco BM, Batista PF, Sobrinho LG, Leite V 2008 Mapping a new familial thyroid epithelial neoplasia susceptibility locus to chromosome 8p23.1-p22 by high-density single-nucleotide polymorphism genome-wide linkage analysis. J Clin Endocrinol Metab **93**:4426–4430.
- 56. Capezzone M, Cantara S, Marchisotta S, Filetti S, De Santi MM, Rossi B, Ronga G, Durante C, Pacini F 2008 Short telomeres, telomerase reverse transcriptase gene amplification, and increased telomerase activity in the blood of familial papillary thyroid cancer patients. J Clin Endocrinol Metab **93:**3950–3957.
- 57. Figlioli G, Köhler A, Chen B, Elisei R, Romei C, Cipollini M, Cristaudo A, Bambi F, Paolicchi E, Hoffmann P, Herms S, Kalemba M, Kula D, Pastor S, Marcos R, Velázquez A, Jarzab B, Landi S, Hemminki K, Försti A, Gemignani F 2014 Novel genome-wide association study-based candidate loci for differentiated thyroid cancer risk. J Clin Endocrinol Metab **99:**E2084–2092.
- Bonora E, Rizzato C, Diquigiovanni C, Oudot-Mellakh T, Campa D, Vargiolu M, Guedj M; NMTC Consortium, McKay JD, Romeo G, Canzian F, Lesueur F 2014 The *FOXE1* locus is a major genetic determinant for familial nonmedullary thyroid carcinoma. Int J Cancer 134:2098–2107.
- Zhuang Y, Wu W, Liu H, Shen W 2014 Common genetic variants on *FOXE1* contributes to thyroid cancer susceptibility: evidence based on 16 studies. Tumour Biol **35:**6159– 6166.

Address correspondence to: Florent de Vathaire, PhD Cancer and Radiations CESP—U1018 Inserm Institut Gustave Roussy 114, rue Edouard-Vaillant 94805 Villejuif Cedex France

E-mail: Florent.Devathaire@gustaveroussy.fr




Title : Dietary iodine intake, fingernail selenium, and risk of differentiated thyroid cancer: Analyses of case-control studies from Epi-Thyr consortium

Keywords : Differentiated thyroid cancer, dietary iodine intake, single-nucleotide polymorphism, fingernail selenium, case-control study, Epi-Thyr

Context : Iodine is a trace element derived from food. In endemic goiter areas characterized by dietary iodine deficiency, it is established that the incidence of goiter and differentiated thyroid cancers (DTC) of follicular type is higher than elsewhere. On the other hand, the influence of dietary iodine intake in the risk of papillary thyroid cancer which currently representing more than 80% of DTCs in most countries, has not achieved a consensus. It appears, however, that this incidence is higher in the Pacific islands than elsewhere. In addition, the redox protection properties of selenium could protect the thyroid gland and help to maintain the production of thyroid hormones, but few studies evaluated the relationship between selenium intake and DTC.

Objectives : The general objective of this thesis was to explore the relationship between dietary iodine intake and DTC risk. More specifically, it was 1) to study the relationship between dietary iodine intake and the risk of DTC in five case-control studies of Epi-Thyr consortium, carried out in New Caledonia, French Polynesia, Metropolitan France (two studies) and Cuba; 2) to investigate the interaction between this intake and four single-nucleotide polymorphisms (SNP) identified in most genome-wide association studies (GWAS) as associated with DTC risk, as well as the interaction with the well-established environmental risk factors; and 3) to evaluate the benefit of selenium present in fingernails as a potential bio-marker of DTC risk.

Materials and Methods : Conditional logistic regression was used to analyze the relationship between dietary iodine intake and DTC risk first in the Cuba study including 203 cases and 212 controls, and then in the pooled analysis of the five studies including a total of 2162 cases and 2571 controls. The dietary questionnaires of these five studies were derived from those in the E3N cohort study. Measurements of iodine and selenium in traditional Polynesian and Cuban foods were specifically carried out for this pooled analysis. Four SNPs were genotyped, including two of *FOXE1* gene, one of *ATM* gene and another near *NKX2-1* gene. Measurements of iodine, selenium and other metal trace elements were made in the fingernails of the participants of the French Polynesia study.

Results : There was no significant association between dietary iodine intake and DTC risk in the Cuba study. In the pooled analysis of the five studies, the majority of the cases and the controls were considered as being in mild dietary iodine deficiency according to the IGN classification. Although DTC risk was not found to be linked to dietary iodine intake, this risk significantly decreased with high fish consumption, this reduction in DTC risk per quartile of fish consumption was more important in the Cuba study than in the other studies. Additionally, higher dietary iodine intake significantly associated with lower DTC risk only in the French Polynesia study and in Polynesians. A strong increase in DTC risk associated with the number of the minor allele (A) of the SNP rs965513 near *FOXE1*, among participants who consumed less iodine than the median value in the Cuba study. For the women who had a high number of full term pregnancies and who were iodine deficient, increasing dietary iodine intake may reduce their risk of suffering from DTC. Moreover, it was not possible to demonstrate an interest in fingernail selenium as a biomarker that predicts DTC risk.



ÉCOLE DOCTORALE Santé Publique



Titre : L'apport alimentaire en iode, sélénium dans les ongles, et risque du cancer différencié de la thyroïde: Analyses d'études cas-témoins du consortium Epi-Thyr

Mots clés : Cancer différencié de la thyroïde, apport alimentaire en iode, polymorphisme nucléotidique, sélénium dans les ongles, étude cas-témoins, Epi-Thyr

Contexte : L'iode est un micronutriment provenant des aliments. Il est établi que, dans les régions d'endémie goitreuse caractérisées par un apport d'iode insuffisant, l'incidence des goitres et des cancers différenciés de la thyroïde (CDT) de type folliculaire est plus élevée qu'ailleurs. En revanche, l'influence de l'apport alimentaire en iode sur le risque de CDT de type papillaire, forme représentant actuellement plus de 80% des CDTs dans la plupart des pays, ne fait pas l'objet d'un consensus. Il semble cependant que cette incidence soit plus élevée dans les îles du pacifique qu'ailleurs. Par ailleurs, les propriétés de protection redox du sélénium pourraient protéger la glande thyroïdienne et aider à maintenir la production d'hormones thyroïdiennes, mais la relation entre l'apport en sélénium et le CDT n'avait été que très peu étudiée.

Objectifs : L'objectif général de ce travail de thèse était d'explorer la relation entre l'apport alimentaire en iode et le risque de CDT. Plus spécifiquement, il s'agissait 1) d'étudier la relation entre l'apport alimentaire en iode et le risque de CDT dans les cinq études cas-témoins du consortium Epi-Thyr, conduites en Nouvelle Calédonie, en Polynésie Française, en France métropolitaine (deux études) et à Cuba; 2) d'étudier l'interaction entre cet apport et quatre polymorphismes nucléotidiques (SNP) identifiés dans la plupart des études d'association pangénomiques (GWAS) comme associés au risque de CDT, ainsi qu'avec les facteurs de risque environnementaux connus; et 3) d'évaluer l'intérêt du sélénium présent dans les ongles comme un bio-marqueur potentiel du risque de CDT.

Matériel et Méthodes : La régression logistique conditionnelle a été utilisée pour analyser la relation entre l'apport alimentaire en iode et le risque de CDT d'abord dans l'étude Cuba incluant 203 cas et 212 témoins, puis dans l'ensemble des cinq études incluant au total 2162 cas et 2571 témoins. Les questionnaires alimentaires de ces cinq études étaient dérivés de ceux de l'étude de cohorte E3N. Les mesures de l'iode et du sélénium dans les aliments traditionnels polynésiens et cubains avaient été réalisées spécialement pour cette étude. Quatre SNPs avaient été génotypés, dont deux sur le gène *FOXE1*, un sur le gène *ATM* et un autre près du gène *NKX2-1*. Les mesures de l'iode, du sélénium et des autres oligoéléments métalliques avaient été réalisées dans les ongles des sujets de l'étude Polynésie Française.

Résultats : Il n'a pas été observée, une association significative entre l'apport alimentaire en iode et le risque de CDT dans l'étude réalisée à Cuba. Dans l'ensemble des cinq études, la majorité des cas et des témoins ont été considérée comme présentant une carence légère en iode selon la classification du Réseau Mondial d'Iode (IGN). Bien que le risque de CDT ne soit pas lié à l'apport alimentaire en iode, ce risque était significativement réduit avec l'augmentation de la consommation de poisson, ceci de manière plus importante dans l'étude réalisée à Cuba que dans les autres. En outre, une augmentation de l'apport alimentaire en iode a été significativement associée à une diminution du risque de CDT uniquement dans l'étude réalisée en Polynésie Française et chez les Polynésiens. Une augmentation significative du risque de CDT associée au nombre de l'allèle mineur (A) du SNP rs965513 près de *FOXE1* parmi les sujets qui ont consommé moins d'iode que la médiane dans l'étude réalisée à Cuba. Pour les femmes qui ont eu un nombre de grossesses élevé et qui étaient déficitaires en iode, l'augmentation de l'apport alimentaire en iode as l'étude réalisée à Cuba. Pour les femmes qui ont eu un nombre de CDT. Par ailleurs, il n'a pas été possible de mettre en évidence un intérêt du sélénium dans les ongles comme bio-marqueur prédictif du risque de CDT.