OXFORD

Carcinogenesis, 2016, 1–8

doi:10.1093/carcin/bgw047
Advance Access publication April 28, 2016
Original Manuscript

ORIGINAL MANUSCRIPT

Selected single-nucleotide polymorphisms in *FOXE1*, *SERPINA5*, *FTO*, *EVPL*, *TICAM1* and *SCARB1* are associated with papillary and follicular thyroid cancer risk: replication study in a German population

Alice J.Sigurdson, Alina V.Brenner, James A.Roach, Lilia Goudeva[1], Jörg A.Müller[2], Kai Nerlich[3], Christoph Reiners[3], Robert Schwab[4], Liliane Pfeiffer[5,6], Melanie Waldenberger[5,6], Melissa Braganza, Li Xu[7], Erich M.Sturgis[7], Meredith Yeager[8], Stephen J.Chanock[8], Ruth M.Pfeiffer[9], Michael Abend[10,*] and Matthias Port[11]

Radiation Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, USA, [1]Department of Transfusion Medicine and [2]Department of Nuclear Medicine, Hannover Medical School, Hannover, Germany, [3]Department of Nuclear Medicine, University Hospital Würzburg, Würzburg, Germany, [4]Department of Surgery, Federal Armed Forces Hospital, Koblenz, Germany, [5]Research Unit of Molecular Epidemiology and [6]Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, [7]Department of Head and Neck Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA, [8]Laboratory of Translational Genetics and [9]Biostatistics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, USA, [10]Bundeswehr Institute of Radiobiology, University of Ulm, Neuherbergstr. 11, Munich 80937, Germany and [11]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany

*To whom correspondence should be addressed. Tel: +89 992692 2280; Fax: +89 992692 2255; Email: michaelabend@bundeswehr.org

Abstract

Several single-nucleotide polymorphisms (SNPs) have been associated with papillary and follicular thyroid cancer (PTC and FTC, respectively) risk, but few have replicated. After analyzing 17 525 tag SNPs in 1129 candidate genes, we found associations with PTC risk in *SERPINA5*, *FTO*, *HEMGN* (near *FOXE1*) and other genes. Here, we report results from a replication effort in a large independent PTC/FTC case–control study conducted in Germany. We evaluated the best tagging SNPs from our previous PTC study and additionally included SNPs in or near *FOXE1* and *NKX2-1* genes, known susceptibility loci for thyroid cancer. We genotyped 422 PTC and 130 FTC cases and 752 controls recruited from three German clinical centers. We used polytomous logistic regression to simultaneously estimate PTC and FTC associations for 79 SNPs based on log-additive models. We assessed effect modification by body mass index (BMI), gender and age for all SNPs, and selected SNP by SNP interactions. We confirmed associations with PTC and SNPs in *FOXE1/HEMGN*, *SERPINA5* (rs2069974), *FTO* (rs8047395), *EVPL* (rs2071194), *TICAM1* (rs8120) and *SCARB1* (rs11057820) genes. We found associations with SNPs in *FOXE1*, *SERPINA5*, *FTO*, *TICAM1* and *HSPA6* and FTC. We found two significant interactions between *FTO* (rs8047395) and *BMI* ($P = 0.0321$) and between *TICAM1* (rs8120) and *FOXE1* (rs10984377) ($P = 0.0006$). Besides the known associations with *FOXE1* SNPs, we confirmed additional PTC SNP associations reported previously. We also found several new associations with FTC risk and noteworthy interactions. We conclude that multiple variants and host factors might interact in complex ways to increase risk of PTC and FTC.

Received: November 12, 2015; **Revised:** April 1, 2016; **Accepted:** April 15, 2016

Abbreviations

BMI	body mass index
CI	confidence interval
DTC	differentiated thyroid cancer
FTC	follicular thyroid cancer
PTC	papillary thyroid cancer
SNP	single-nucleotide polymorphism

Introduction

Sporadic thyroid cancer is the most common endocrine malignancy for which incidence has increased worldwide during the last 40 years (1–3). Heightened detection with ultrasound has likely contributed to this trend, but additional reasons for the increased incidence rates remain unclear (3). Thyroid cancer is more common in women than men, with a female-to-male ratio in incidence rates of about three to one (4). Papillary thyroid cancer (PTC) is the most common histologic type (~80%), followed by follicular thyroid cancer (FTC; ~15%), with poorly differentiated (anaplastic; ~2%) and medullary cancers (~3%) constituting the remainder (5). PTC and FTC combined are often referred to as differentiated thyroid cancer (DTC). DTC likely has a greater familial risk than other cancers, with relative risk estimates of three to four or higher for a family history in first-degree relatives (6–8). Several genome-wide association studies (GWAS) (9–11) and a candidate gene study (12) have clearly implicated the gene *FOXE1* (forkhead box protein E1, formerly known as thyroid transcription factor 2 [*TTF2*]; 9q22.33) as a susceptibility locus for DTC both in persons unexposed (9,11) and exposed to ionizing radiation (10), a known risk factor for thyroid cancer (reviewed in ref. 13). The genes *NKX2-1* (NK2 homeobox 1, formerly known as thyroid transcription factor 1; 14q13.2) and *DIRC3* (disrupted in renal carcinoma 3; 2q35) have also been associated with increased DTC risk (9,11). The post-GWAS candidate loci continue to be examined (14); however, only a portion of the genetic determinants of risk has been validated and explained so far (14,15).

The main objective of the current study was to replicate the most significant findings for PTC that we reported previously from an analysis of 1129 candidate genes based on 17 525 tag single-nucleotide polymorphisms (SNPs) from the National Cancer Institute's (NCI) rare cancer i-Select study (16–21). In total, we aimed to validate findings for 100 SNPs including genes near or in the *FOXE1* or *NKX2-1* region in an independent case–control study of PTC and FTC conducted in Germany. In addition to the replication for PTC, we also evaluated the associations with 100 SNPs for FTC as well as explored their interaction with gender, age and body mass index (BMI); finally, for SNPs significantly associated with DTC, we explored pairwise SNP–SNP interactions.

Materials and methods

Case and control recruitment and data collection

Cases included individuals with PTCs (n = 465), FTCs (n = 150) and other histologic types of thyroid cancer (n = 12) diagnosed at three hospitals in Germany between February 2008 and March 2010 (Hannover Medical School, University Clinic Würzburg and the Central Hospital of the German Federal Armed Forces Koblenz). Controls included 813 individuals recruited from among healthy blood donors (Hannover) or surgery patients (Koblenz) who presented to these hospitals between February 2008 and March 2010.

Of the 1440 study subjects recruited, we excluded 7 who were not of European ancestry (6 cases, 1 control), 41 who were <19 years of age (27 cases, 14 controls), 11 who were >80 years of age (4 cases, 7 controls), 65 with SNP completion proportions <90% (26 cases, 39 controls) and 12 cases who had a histology other than PTC or FTC, leaving 1304 persons for analysis. All thyroid cancer cases included in this study were histologically confirmed. To ensure that cases and controls from the multiple recruiting medical centers were comparable, we evaluated the distribution of allele frequencies by recruiting hospital, gender and age among controls, and the distribution of tumor stage by hospital among cases. We found that the allele frequencies among the controls did not differ by hospital, gender or age after correction for multiple testing (Supplementary Table 1, available at *Carcinogenesis* Online). For cases, tumor stage distributions were similar by hospital. This study was reviewed and approved by the three Institutional Review Boards of the respective hospitals/clinics.

At the time of blood draw, basic demographic data were collected on the cases and controls, including date of birth, gender, ethnicity, height, weight and personal history of cancer. For cases, information on previous history of any radiation treatment and family history of cancer was additionally collected, whereas information on tumor, nodes and metastasis staging was available from medical records. Personal history of other thyroid conditions, such as Hashimoto's thyroiditis, was not collected systematically. For 45 cases and 5 controls, a history of prior cancer diagnosis was reported.

SNP selection and genotyping

From among 17 525 tag SNPs in 1129 candidate genes evaluated in the NCI's rare cancer i-Select study of PTC (16–21), the top 50 SNPs were selected for validation based on joint evaluation of lowest P values and per genotype odds ratio of 1.3 or greater. For greater coverage, we added 45 SNPs to the genes or regions with high priors, i.e. *SERPINA5* (serpin peptidase inhibitor clade A member 5), *FTO* (fat mass and obesity-associated), *SCARB1* (scavenger receptor class B type 1), and the 8q24 region. All selected SNPs had a minor allele frequency >5%. Three SNPs in *FOXE1* and two in *NKX2-1* genes were included based on information from literature reports (9–12) for a total of 100 *a priori* selected SNPs.

Whole blood samples collected by venipuncture were shipped overnight with a temperature stabilizing pack to the laboratory of Bundeswehr Institute of Radiobiology in Munich, Germany. DNA was extracted from peripheral blood leucocytes using Qiagen Mini Kits (Qiagen, Hilden, Germany) according to manufacturer instructions. DNA samples were stored at –80°C until transported to the Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, for genotyping.

Genotyping was performed using the MassARRAY iPLEX platform (Sequenom, San Diego, CA). Five nanograms of genomic DNA were amplified by PCR using HotStar Taq DNA polymerase (Qiagen) and PCR primers designed by Sequenom's Mass Array Assay Design program. About 20 nl of purified reaction products [using shrimp alkaline phosphatase and SpectroCLEAN resin (Sequenom) to remove salts] were transferred to a SpectroCHIP® using the MassARRAY® nanodispenser (Sequenom). A modified Bruker Biflex matrix-assisted laser desorption ionization–time of flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP®. Genotypes were called with the MassARRAY TYPER 4.0 software (Sequenom). Negative controls were included in all assays. Thirty-one aliquots from 16 cases and 15 controls were analyzed for 3 random SNPs in duplicates with their identity blinded to laboratory investigators. Concordance for these 31 duplicates was 100%.

Of the 100 selected SNPs, 90 assays were ultimately developed, 6 subsequently failed genotyping and 3 SNPs failed Hardy–Weinberg equilibrium test in controls ($P < 0.0001$), leaving 81 evaluable SNPs. In addition, two SNPs had call rates <95% (89 and 92%) and were excluded from the analyses. All remaining 79 SNPs had call rates of 95% or higher.

Statistical analysis

Characteristics of cases and controls, and controls by gender and hospital were compared using chi-square tests. The risks of PTC and FTC were evaluated relative to the common control group using polytomous logistic regression. The risk of DTC was evaluated using standard binary logistic regression. We estimated odds ratios (ORs), 95% confidence intervals (95% CIs) and P values of linear trend (P_{trend}) for each SNP genotype, coded as 0, 1, 2, with 0 denoting the homozygous common allele genotype as

the referent category (log-additive coding). All models were adjusted for sex, attained age in four categories (19–35, 35–44, 45–54 and 55–80 years) and hospital site in two categories (medical/university or armed forces). In selected analyses, where indicated, we also adjusted for BMI (calculated from height and weight in kg/m^2) in four categories (<18.5, 18.5–24.9, 25.0–29.9 and ≥30.0 kg/m^2) with cut-offs according to the WHO classification of 'underweight', 'normal weight', 'overweight' and 'obese' for adults (22). Given the strong priors for the selected SNPs, we did not formally adjust the *P* values of trend for multiple testing. In sensitivity analyses, we excluded cases with a prior cancer diagnosis. Because the results including and excluding such cases were similar, we present only results based on all cases and controls.

For DTC, we assessed effect modification (interaction) by sex (women versus men), age (under versus over age 45 years) and BMI [coded as normal/underweight (<25 kg/m^2) versus overweight/obese (≥25.0 kg/m^2)]. In these analyses, we used log-additive coding for the SNPs. The interaction tests were based on Wald chi-square statistics associated with multiplicative interaction terms added to the model including main effects of the SNP and the factor under investigation. Two-way SNP by SNP interactions were evaluated for all unlinked SNPs with DTC main effect *P* values <0.05. Each SNP was included in the model using log-additive coding and the interaction tests were based on Wald chi-square statistics for the multiplicative interaction term. The *P* values of interaction tests were corrected for multiple testing using a Bonferroni approach unless stated otherwise.

For unlinked SNPs significantly associated with DTC risk (n = 10), we assessed the ability of a combined SNP risk score for prediction. We calculated a conditional SNP risk score using additive SNP coding (0, 1, 2) for the number of minor alleles for 10 significant SNPs included as main effects in the logistic regression models in the presence and absence of other predictors, i.e. sex, age and BMI. We then compared the discriminatory ability measured by the areas under the receiver operator characteristics curves (AUCs) for three logistic regression models: one that included sex, age and BMI (in three categories), one that included the 10 SNPs and one that included age, sex, BMI and the 10 SNPs. All three models were adjusted for hospital site.

All statistical tests were two sided, and *P* value <0.05 was considered statistically significant, unless corrected for multiple testing. Statistical analyses were conducted in SAS version 9.3 (23) and *R* (24).

Results

Demographic characteristics of the PTC and FTC cases and the controls are presented in Table 1. Among cases, more were female than male, whereas among controls, males were more common than females (P < 0.001). About half of the PTC and FTC cases were recruited from Hannover Medical School with the other half from Würzburg and Koblenz combined. Most controls (88%) were recruited from Hannover Medical School (blood donors) and 12% from Koblenz (surgery patients). Controls were younger at recruitment than cases (P < 0.001). BMI differed between cases and controls with 31% of PTC cases and 32% of FTC cases in the normal BMI range compared with 52% of controls; thyroid cancer cases were more likely than controls to be obese (27% for PTC, 29% for FTC versus 10% for controls) (P < 0.001).

Complete results of polytomous logistic regression analyses for PTC and FTC and binary logistic regression for DTC for 79 SNPs are presented in Supplementary Table 2, available at *Carcinogenesis* Online, whereas the results for 20 SNPs significantly associated with at least 1 outcome (PTC, FTC or DTC) are summarized in Table 2. Six of 20 SNPs were in the *FOXE1/C9orf156/HEMGN* region, although only two (rs965513, rs10984377) were associated with thyroid cancer risk independently. There were also seven significant associations with SNPs in *SERPINA5* gene, six for FTC and one (rs2069974) for PTC, FTC and DTC. Only the effect of latter SNP appeared to be independent. The remaining seven SNPs significantly associated with at least one outcome were located in different genes. Of these, two SNPs in *FTO* (rs8047395) and *TICAM1* (rs8120) genes were significantly associated with risk of PTC, FTC and DTC; two SNPs in *EVPL* (rs2071194) and *SCARB1* (rs11057820) genes were associated with risk of PTC and DTC; one SNP in *HSAP6* (rs9427401) with risk of FTC and DTC; and two SNPs in *APC2* (rs11668593) and *MASP1* (rs3815623) with risk of DTC. Overall, 6 SNPs were significantly and independently associated with risk of PTC, 5 SNPs with risk of FTC and 10 SNPs with risk of DTC.

Of *a priori* interest for effect modification of DTC risk, we evaluated how the associations with SNPs in the *SCARB1* and *FTO* genes belonging to the metabolism pathway might vary according to BMI (Supplementary Table 3, available at *Carcinogenesis* Online). None of the interaction tests was significant except for one between *FTO* (rs8047395) and BMI (P = 0.0321). The association with the latter SNP among those normal or underweight was stronger ($OR_{per\ allele}$ = 1.67, 95% CI: 1.23–2.27) than among those overweight or obese ($OR_{per\ allele}$ = 1.09, 95% CI: 0.86–1.39). We found no evidence of effect modification by gender, age or BMI for other SNPs after multiple comparisons correction (data not shown). There was one significant, negative SNP by SNP interaction between *TICAM1* (rs8120) and *FOXE1* (rs10984377) that withstood correction for multiple testing (P < 0.0001, data not shown).

The AUC values for the three predictive models of DTC risk were 0.63 (95% CI: 0.60–0.66) for a model with 10 SNPs, 0.80 (95% CI: 0.77–0.82) for a model with BMI, age and sex, and 0.82 (95% CI: 0.79–0.84) for a model with sex, age, BMI and 10 SNPs.

Discussion

We confirmed the well-known association with DTC of the 9q22.31 region that includes the *FOXE1* gene (9–12). In this independent and separate case–control study conducted in Germany, we replicated associations with PTC risk that we reported previously in *SERPINA5* (21) and *FTO* (17) genes. Other candidate variants in *EVPL*, *TICAM1* and *SCARB1* previously identified (17,21) were also associated with PTC risk. We found several new associations between SNPs in *SERPINA5*, *FTO*, *TICAM1* and *HSPA6* genes, and risk of FTC. In contrast to several studies, we did not observe associations for *NKX2-1* SNPs (9,25,26). We also did not replicate earlier findings on the associations with *HDAC4* (16) and the 8q24 region SNPs for PTC (18).

The rs965513 SNP in 9q22 has been most consistently associated with risk of PTC or DTC in different populations (9–12). The association was observed in familial DTC (27,28) and PTC attributed to exposure to ionizing radiation (16). Here, we confirmed such an association in a previously unstudied German population. The rs965513 resides ~60 000 bases upstream of *FOXE1* gene known as thyroid transcription factor 2 that is important in thyroid development, differentiation and function (28). Although several functional studies demonstrated that AA risk genotype of rs965513 SNP is associated with decreased coexpression of *FOXE1*, *PTCSC2* and *TSHR* in unaffected thyroid tissue of PTC patients (29,30), one study suggested that this effect may be due to multiple, coinherited with rs965513 noncoding variants located in long-range enhancers that control *FOXE1* expression (31).

Previously, we reported strong associations with several *SERPINA5* SNPs in the US study of PTC (21). In the German study, we not only confirmed an association for PTC with the intronic rs2069974 SNP but also extended it to FTC. *SERPINA5* gene (chromosome 14q32) codes the protein C inhibitor, a member of the plasma serine protease inhibitor family. Although it was originally discovered as an inhibitor of activated protein C, today

Table 1. Descriptive characteristics of papillary, follicular and differentiated thyroid cancer cases and controls recruited from three German hospitals between February 2008 and March 2010

	PTC cases		FTC cases		DTC cases		Controls		
Characteristic	*N*	%	*N*	%	*N*	%	*N*	%	*P* value[a]
Total	422	100	130	100	552	100	752	100	
Gender									
Female	307	72.7	81	62.3	388	70.3	258	34.3	
Male	115	27.3	49	37.7	164	29.7	494	65.7	**<0.001**
Hospital									
Hannover Medical School[b]	212	50.2	59	45.4	271	49.1	662	88.0	
University Hospital Würzburg	140	33.2	51	39.2	191	34.6	0	0	
Armed Forces Hospital Koblenz[b]	70	16.6	20	15.4	90	16.3	90	12.0	**<0.001**
Age at diagnosis or blood draw									
19–34	85	20.1	23	17.7	108	19.6	392	52.1	
35–44	126	29.9	26	20.0	152	27.5	142	18.9	
45–54	107	25.4	37	28.5	144	26.1	125	16.6	
55–80	104	24.6	44	33.8	148	26.8	93	12.4	**<0.001**
BMI (kg/m^2)									
Underweight (<18.5 kg/m^2)	5	1.2	1	0.8	6	1.1	5	0.7	
Normal (18.5–24.9 kg/m^2)	132	31.3	42	32.3	174	31.5	368	48.9	
Overweight (25.0–29.9 kg/m^2)	168	39.8	48	36.9	216	39.1	285	37.9	
Obese (≥30.0 kg/m^2)	115	27.2	38	29.2	153	27.7	75	10.0	**<0.001**
Unknown[a]	2	0.5	1	0.8	3	0.5	19	2.5	

[a]P values based on chi-square differences in proportions for controls versus all cases combined.
[b]Controls from Hannover Medical School were healthy blood donors and controls from the Armed Forces Hospital Koblenz were surgery patients.

protein C inhibitor is known to play a role in many biological processes beyond hemostasis including inflammation, innate immunity, fertilization and carcinogenesis (32). The potential role of *SERPINA5* in thyroid cancer initiation and progression has not been studied extensively. However, one recent study found that expression of *SERPINA5* in tissue of PTC patients was decreased (through increased promotor methylation) and associated with the presence of *BRAF* mutation (33), believed to be one of the major drivers of thyroid carcinogenesis.

Another intronic SNP in *FTO* gene (chromosome 16q12), rs8047395, was also strongly associated with risk of PTC in both German and US studies (17). The *FTO* gene is involved in regulation of energy homeostasis, body size and fat accumulation, and genetic variants in this gene have been associated with obesity, diabetes and metabolic syndrome (reviewed in ref. 34). Increased BMI has emerged as a novel risk factor for thyroid cancer in several large and well-conducted studies (35–37). In the current study, we similarly found that PTC and FTC cases were significantly more likely to be overweight or obese. When the effect of rs8047395 and BMI was evaluated simultaneously, the magnitude of either association was not meaningfully affected by mutual adjustment and remained significant; however, the association with rs8047395 was significantly stronger in underweight or normal weight individuals than in overweight or obese individuals.

Based on association studies of SNPs with disease outcomes, it is not possible to infer whether the validated variants are causal or show associations due to linkage disequilibrium with undetected causal variants. Even though all of the above validated variants are intronic, there is accumulating evidence that introns may be a larger mutational target due to numerous functional elements located within them (38). It has been shown that intronic variants can exert their effects by altering DNA conformation (39), transcriptional activity, RNA secondary structure (40) or splicing efficiency of their host genes. In addition, disease-associated intronic SNPs may play a role in long-range gene regulation. To establish the biological relevance of these variants, functional follow-up studies are needed.

The significant associations with other SNPs replicated in the German study were less convincing statistically and the biological basis of these associations remains unclear. Further replication in other populations is important to establish robust relationships. Some genetic variants may be specific for papillary versus follicular thyroid tumors, but large studies are required to establish associations for the less common FTC.

To evaluate a predictive value of 10 unlinked SNPs significantly associated with risk of DTC in the current study, we added the 10 SNP risk score to a model that included sex, age and BMI to predict thyroid cancer risk. The AUC improved by 2%, which is a noticeable improvement in discriminatory performance (41). Thus, in the presence of these personal characteristics, the SNP score might improve predictive ability. However, this is only a proof of concept, and careful evaluation in independent, preferably prospective studies is needed.

Considering that there are few known risk factors for thyroid cancer besides ionizing radiation exposure, gender and age (reviewed in ref. 5), and given that family-related risk of thyroid cancer is high (6–8), it is likely that multiple environmental and genetic factors or their combination contribute to risk. Although studies of gene–environment interaction require large sample size, we explored interaction between each genotyped SNP and sex, age and BMI in an attempt to identify strong interactions, if such exist. However, none of the interaction tests survived correction for multiple testing. We also assessed SNP by SNP interaction among those SNPs showing significant associations with DTC; one such negative interaction between rs8120 (*TICAM1*) and rs10984377 (*FOXE1*) was significant after multiple comparisons correction suggesting that the joint effect of the two SNPs may be lower than expected based on the multiplicative interaction model.

Our study has several strengths. Selected tag SNPs had high priors based on associations found in the previous studies. The

Table 2. Associations between genotype and risk of papillary thyroid cancer, follicular thyroid cancer and differentiated thyroid cancer in a replication case–control study conducted in three hospitals in Germany between February 2008 and March 2010

Gene/region			PTC			FTC			DTC		
Location	db SNP[a]	Genotype	OR[b,c]	95% CI		OR	95% CI		OR	95% CI	
FOXE1/HEMGN	rs965513[d]	GG	1.00	Referent		1.00	Referent		1.00	Referent	
9q22.33		GA	1.45	1.07	1.97	1.21	0.79	1.86	1.38	1.04	1.83
		AA	2.07	1.36	3.15	1.22	0.65	2.30	1.83	1.23	2.72
		P[e]	**0.0004**			0.4045			**0.0014**		
	rs1867277	GG	1.00	Referent		1.00	Referent		1.00	Referent	
		GA	1.45	1.05	2.00	1.58	1.00	2.49	1.48	1.10	2.00
		AA	1.91	1.29	2.83	1.31	0.73	2.37	1.76	1.21	2.54
		P	**0.0009**			0.2355			**0.0018**		
	rs1912998	GG	1.00	Referent		1.00	Referent		1.00	Referent	
		GA	1.16	0.87	1.55	1.28	0.85	1.92	1.19	0.91	1.55
		AA	1.62	1.04	2.55	0.57	0.24	1.33	1.35	0.87	2.09
		P	**0.0355**			0.7890			0.1080		
C9orf156	rs6586	GG	1.00	Referent		1.00	Referent		1.00	Referent	
9q22.33		GA	0.93	0.69	1.24	1.28	0.84	1.95	1.00	0.77	1.32
		AA	0.57	0.36	0.90	0.71	0.37	1.39	0.60	0.39	0.91
		P	**0.0389**			0.7560			0.0748		
	rs10984377	AA	1.00	Referent		1.00	Referent		1.00	Referent	
		AG	1.15	0.82	1.61	1.79	1.15	2.81	1.29	0.95	1.77
		GG	3.13	0.95	10.28	2.18	0.39	12.24	2.90	0.95	9.87
		P	0.1020			**0.0104**			**0.0250**		
	rs4743146	AA	1.00	Referent		1.00	Referent		1.00	Referent	
		AT	0.64	0.41	1.01	0.68	0.35	1.32	0.65	0.42	0.98
		TT	0.59	0.10	3.43	0.85	0.09	8.00	0.66	0.12	2.99
		P	0.0523			0.2893			**0.0457**		
SERPINA5	rs10139508	AA	1.00	Referent		1.00	Referent		1.00	Referent	
14q32.13		AG	0.98	0.73	1.31	0.81	0.54	1.21	0.93	0.71	1.22
		GG	0.89	0.58	1.38	0.42	0.20	0.90	0.77	0.51	1.15
		P	0.6703			**0.0288**			0.2430		
	rs12887091	AA	1.00	Referent		1.00	Referent		1.00	Referent	
		AG	0.78	0.58	1.05	0.66	0.44	1.01	0.75	0.57	0.98
		GG	0.87	0.57	1.32	0.60	0.31	1.15	0.80	0.54	1.19
		P	0.2463			**0.0426**			0.0894		
	rs2069959	GG	1.00	Referent		1.00	Referent		1.00	Referent	
		GC	1.02	0.76	1.36	1.64	1.06	2.54	1.13	0.86	1.48
		CC	0.90	0.57	1.41	2.06	1.14	3.71	1.12	0.74	1.68
		P	0.7599			**0.0068**			0.4334		
	rs2069974	AA	1.00	Referent		1.00	Referent		1.00	Referent	
		AG	0.78	0.57	1.05	0.79	0.51	1.20	0.78	0.59	1.04
		GG	0.62	0.42	0.91	0.41	0.22	0.76	0.56	0.39	0.81
		P	**0.0122**			**0.0063**			**0.0022**		

Table 2. *Continued*

Gene/region			PTC			FTC			DTC		
Location	db SNP[a]	Genotype	OR[b,c]	95% CI		OR	95% CI		OR	95% CI	
	rs2069978	CC	1.00	Referent		1.00	Referent		1.00	Referent	
		CT/TT[f]	0.85	0.56	1.29	0.46	0.22	0.96	0.75	0.51	1.11
		P	0.4431			**0.0391**			0.1474		
	rs6108	TT	1.00	Referent		1.00	Referent		1.00	Referent	
		TA	0.79	0.58	1.06	0.67	0.44	1.01	0.75	0.57	0.99
		AA	0.75	0.50	1.13	0.41	0.21	0.81	0.66	0.45	0.97
		P	0.1002			**0.0049**			**0.0164**		
	rs1050013	AA	1.00	Referent		1.00	Referent		1.00	Referent	
		AG	1.09	0.79	1.51	1.62	0.97	2.71	1.19	0.88	1.63
		GG	1.17	0.80	1.71	1.88	1.06	3.31	1.30	0.91	1.86
		P	0.4354			**0.0336**			0.1522		
FTO	rs8047395[d]	AA	1.00	Referent		1.00	Referent		1.00	Referent	
16q12.2		AG	1.24	0.89	1.71	1.04	0.64	1.68	1.18	0.87	1.61
		GG	1.64	1.11	2.43	1.76	1.02	3.05	1.67	1.16	2.41
		P	**0.0143**			**0.0491**			**0.0066**		
SCARB1	rs11057820	CC	1.00	Referent		1.00	Referent		1.00	Referent	
12q24.31		CT	0.81	0.59	1.11	1.12	0.71	1.77	0.88	0.65	1.18
		TT	0.69	0.47	1.01	0.61	0.34	1.11	0.67	0.47	0.96
		P	**0.0474**			0.1516			**0.0310**		
APC2	rs11668593	GG	1.00	Referent		1.00	Referent		1.00	Referent	
19p13.3		GA	0.87	0.64	1.17	0.69	0.44	1.08	0.82	0.62	1.08
		AA	0.48	0.22	1.02	0.70	0.26	1.91	0.53	0.26	1.04
		P	0.0651			0.1056			**0.0333**		
EVPL	rs2071194	TT	1.00	Referent		1.00	Referent		1.00	Referent	
17q25.1		TG	1.43	1.07	1.91	1.32	0.88	1.98	1.40	1.07	1.84
		GG	1.88	1.20	2.95	0.72	0.32	1.63	1.57	1.02	2.43
		P	**0.0015**			0.8312			**0.0077**		
MASP1	rs3815623	CC	1.00	Referent		1.00	Referent		1.00	Referent	
3q27.3		CT	1.22	0.88	1.67	1.06	0.67	1.70	1.18	0.88	1.59
		TT	1.43	0.97	2.11	1.58	0.92	2.72	1.46	1.02	2.11
		P	0.0668			0.1093			**0.0381**		
TICAM1	rs8120	TT	1.00	Referent		1.00	Referent		1.00	Referent	
19p13.3		TG	1.31	0.98	1.75	1.44	0.95	2.18	1.34	1.02	1.76
		GG	1.60	0.90	2.85	2.11	0.99	4.49	1.72	1.01	2.93
		P	**0.0264**			**0.0204**			**0.0079**		
HSPA6	rs9427401	CC	1.00	Referent		1.00	Referent		1.00	Referent	
1q23.3		CG/GG[f]	0.76	0.55	1.05	0.57	0.34	0.95	0.71	0.52	0.97
		P	0.0962			**0.0312**			**0.0307**		

The bold values are significant at $P < 0.05$.

[a]Entrez SNP reference ID number (http://www.ncbi.nlm.nih.gov/snp).

[b]OR.

[c]Adjusted for age in four categories (19–35, 35–44, 45–54 and 55–80 years), gender and hospital (medical school or military).

[d]Not shown are rs7030241 (*FOXE1*) and rs9922047 (*FTO*) since these are in nearly perfect linkage disequilibrium with rs965513 and 8047395, respectively.

[e]Adjusted *P* trend, log-additive model.

[f]Due to the low frequency of homozygous variant allele genotype, ORs are based on the dominant inheritance model.

study population was independent and not evaluated before with respect to genetic susceptibility to thyroid cancer. There are also some limitations. Cases and controls came from different source populations and were not optimally matched on sex or age. Therefore, all analyses were controlled for hospital site, sex and age. Also, to assure genetic comparability, we evaluated the distribution of allele frequencies in controls by recruiting hospital, sex and age, and found no meaningful differences. Limited covariate information was available, and among controls, some covariates such as family cancer history were not collected. The moderate sample size limited our ability to assess weak and moderate interactions. Chance could be an explanation for some of our findings, particularly for FTC, although replication was our motivation for conducting the study of PTC in order to minimize chance associations.

In summary, we independently confirmed associations with PTC and *FOXE1/HEMGN* SNPs, as well as selected findings from our previous thyroid cancer i-Select study for *SERPINA5*, *FTO*, *EVPL*, *TICAM1* and *SCARB1* genes. We also found several new associations with FTC and noteworthy interactions. As more thyroid cancer risk variants are discovered and evaluated in conjunction with known risk factors, the pathogenesis of this disease will be better understood.

Supplementary material

Supplementary Tables 1–3 can be found at http://carcin.oxfordjournals.org/

Funding

Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health; German Ministry of Defense.

Acknowledgements

We are grateful to persons who participated in this project and to our laboratory technicians S.Doucha-Senf and E.Grumpelt, in particular, for DNA isolation from the blood samples. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services nor does the mention of trade names, commercial products or organizations imply endorsement by the US Government. We thank thyroid expert Dr E.Ron, whose vision, support and memory sustain us into the present.
Conflict of Interest Statement: None declared.

References

1. Sipos, J.A. et al. (2010) Thyroid cancer epidemiology and prognostic variables. Clin. Oncol. (R. Coll. Radiol.), 22, 395–404.
2. Davies, L. et al. (2006) Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA, 295, 2164–2167.
3. Enewold, L. et al. (2009) Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980-2005. Cancer Epidemiol. Biomark. Prev., 18, 784–791.
4. Rahbari, R. et al. (2010) Thyroid cancer gender disparity. Future Oncol., 6, 1771–1779.
5. Ron, E. et al. (2006) Thyroid cancer. In Schottenfeld, D. et al. (ed) Cancer Epidemiology and Prevention. Oxford University Press, New York, NY, pp. 975–994.
6. Xu, L. et al. (2012) Family history of cancer and risk of sporadic differentiated thyroid carcinoma. Cancer, 118, 1228–1235.
7. Goldgar, D.E. et al. (1994) Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J. Natl Cancer Inst., 86, 1600–1608.
8. Fallah, M. et al. (2013) Risk of thyroid cancer in first-degree relatives of patients with non-medullary thyroid cancer by histology type and age at diagnosis: a joint study from five Nordic countries. J. Med. Genet., 50, 373–382.
9. Gudmundsson, J. et al. (2009) Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat. Genet., 41, 460–464.
10. Takahashi, M. et al. (2010) The FOXE1 locus is a major genetic determinant for radiation-related thyroid carcinoma in Chernobyl. Hum. Mol. Genet., 19, 2516–2523.
11. Köhler, A. et al. (2013) Genome-wide association study on differentiated thyroid cancer. J. Clin. Endocrinol. Metab., 98, E1674–E1681.
12. Landa, I. et al. (2009) The variant rs1867277 in FOXE1 gene confers thyroid cancer susceptibility through the recruitment of USF1/USF2 transcription factors. PLoS Genet., 5, e1000637.
13. Sinnott, B. et al. (2010) Exposing the thyroid to radiation: a review of its current extent, risks, and implications. Endocr. Rev., 31, 756–773.
14. Figlioli, G. et al. (2015) Novel genetic variants in differentiated thyroid cancer and assessment of the cumulative risk. Sci. Rep., 5, 8922.
15. Liyanarachchi, S. et al. (2013) Cumulative risk impact of five genetic variants associated with papillary thyroid carcinoma. Thyroid, 23, 1532–1540.
16. Neta, G. et al. (2011) Common genetic variants related to genomic integrity and risk of papillary thyroid cancer. Carcinogenesis, 32, 1231–1237.
17. Kitahara, C.M. et al. (2012) Common obesity-related genetic variants and papillary thyroid cancer risk. Cancer Epidemiol. Biomark. Prev., 21, 2268–2271.
18. Neta, G. et al. (2012) Common genetic variants in the 8q24 region and risk of papillary thyroid cancer. Laryngoscope, 122, 1040–1042.
19. Schonfeld, S.J. et al. (2012) Common genetic variants in sex hormone pathway genes and papillary thyroid cancer risk. Thyroid, 22, 151–156.
20. Aschebrook-Kilfoy, B. et al. (2012) Common genetic variants in metabolism and detoxification pathways and the risk of papillary thyroid cancer. Endocr. Relat. Cancer, 19, 333–344.
21. Brenner, A.V. et al. (2013) Common single nucleotide polymorphisms in genes related to immune function and risk of papillary thyroid cancer. PLoS One, 8, e57243.
22. Report of a WHO Consultation (2000) Obesity: preventing and managing the global epidemic. World Health Organ. Tech. Rep. Ser., 894, i–xii, 1–253.
23. SAS Institute Inc (2012) SAS Version 9.3 for Windows: User's Guide. SAS Institute Inc., Cary, NC.
24. R Development Core Team (2008) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org (27 April 2016, date last accessed).
25. Matsuse, M. et al. (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.
26. Rogounovitch, T.I. et al. (2015) The common genetic variant rs944289 on chromosome 14q13.3 associates with risk of both malignant and benign thyroid tumors in the Japanese population. Thyroid, 25, 333–340.
27. Tomaz, R.A. et al. (2012) FOXE1 polymorphisms are associated with familial and sporadic nonmedullary thyroid cancer susceptibility. Clin. Endocrinol. (Oxf.), 77, 926–933.
28. Bonora, E. et al. (2014) The FOXE1 locus is a major genetic determinant for familial nonmedullary thyroid carcinoma. Int. J. Cancer, 134, 2098–2107.
29. He, H. et al. (2015) Genetic predisposition to papillary thyroid carcinoma: involvement of FOXE1, TSHR, and a novel lincRNA gene, PTCSC2. J. Clin. Endocrinol. Metab., 100, E164–E172.
30. Kallel, R. et al. (2010) Genetic investigation of FOXE1 polyalanine tract in thyroid diseases: new insight on the role of FOXE1 in thyroid carcinoma. Cancer Biomark., 8, 43–51.
31. He, H. et al. (2015) Multiple functional variants in long-range enhancer elements contribute to the risk of SNP rs965513 in thyroid cancer. Proc. Natl Acad. Sci. USA, 112, 6128–6133.
32. Meijers, J.C. et al. (2011) Protein C inhibitor. Semin. Thromb. Hemost., 37, 349–354.

33. Lee, E.K. et al. (2013) DNA methylation of MAPK signal-inhibiting genes in papillary thyroid carcinoma. Anticancer Res., 33, 4833–4839.
34. Zhao, X. et al. (2014) FTO and obesity: mechanisms of association. Curr. Diab. Rep., 14, 486.
35. Kitahara, C.M. et al. (2011) Obesity and thyroid cancer risk among U.S. men and women: a pooled analysis of five prospective studies. Cancer Epidemiol. Biomark. Prev., 20, 464–472.
36. Kitahara, C.M. et al. (2014) Childhood height and body mass index were associated with risk of adult thyroid cancer in a large cohort study. Cancer Res., 74, 235–242.
37. Xu, L. et al. (2014) Obesity and the risk of papillary thyroid cancer: a pooled analysis of three case-control studies. Thyroid, 24, 966–974.
38. Cooper, D.N. (2010) Functional intronic polymorphisms: buried treasure awaiting discovery within our genes. Hum. Genomics, 4, 284–288.
39. Lu, L. et al. (2013) An insulin-like growth factor-II intronic variant affects local DNA conformation and ovarian cancer survival. Carcinogenesis, 34, 2024–2030.
40. Lu, L. et al. (2012) Functional study of risk loci of stem cell-associated gene lin-28B and associations with disease survival outcomes in epithelial ovarian cancer. Carcinogenesis, 33, 2119–2125.
41. Pepe, M.S. et al. (2004) Limitations of the odds ratio in gauging the performance of a diagnostic, prognostic, or screening marker. Am. J. Epidemiol., 159, 882–890.