

# Thyroid-Stimulating Hormone, Thyroid Hormones, and Risk of Papillary Thyroid Cancer: A Nested Case-Control Study

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## Abstract

**Background:** The effects of thyroid-stimulating hormone (TSH) and thyroid hormones on the development of human papillary thyroid cancer (PTC) remain poorly understood.

**Methods:** The study population consisted of 741 (341 women, 400 men) histologically confirmed PTC cases and 741 matched controls with prediagnostic serum samples stored in the Department of Defense Serum Repository. Concentrations of TSH, total T3, total T4, and free T4 were measured in serum samples. Conditional logistic regression models were used to calculate ORs and 95% confidence intervals (CI).

**Results:** The median time between blood draw and PTC diagnosis was 1,454 days. Compared with the middle tertile of TSH levels within the normal range, serum TSH levels below the normal range were associated with an elevated risk of PTC among women (OR, 3.74; 95% CI, 1.53–9.19) but not men. TSH levels

above the normal range were associated with an increased risk of PTC among men (OR, 1.96; 95% CI, 1.04–3.66) but not women. The risk of PTC decreased with increasing TSH levels within the normal range among both men and women ( $P_{\text{trend}} = 0.0005$  and 0.041, respectively).

**Conclusions:** We found a significantly increased risk of PTC associated with TSH levels below the normal range among women and with TSH levels above the normal range among men. An inverse association between PTC and TSH levels within the normal range was observed among both men and women.

**Impact:** These results could have significant clinical implications for physicians who are managing patients with abnormal thyroid functions and those with thyroidectomy. *Cancer Epidemiol Biomarkers Prev*; 26(8); 1209–18. ©2017 AACR.

## Introduction

Thyroid cancer has the highest prevalence of all endocrine malignancies, and its incidence is increasing faster than any other malignancy in both men and women (1). In the United States, thyroid cancer is the ninth most common cancer, accounting for 3.8% of all malignancies and 0.3% of all deaths from cancer (2). The most common histologic type of thyroid cancer is papillary thyroid cancer (PTC), which accounts for more than 80% of all thyroid carcinomas (3). The causal factors underlying thyroid

cancer are poorly understood. The most well-established risk factors for thyroid cancer include increased age, female gender, exposure to ionizing radiation, history of benign thyroid disease, and a family history of thyroid cancer (4–7). Recent studies have identified higher body weight and height as risk factors for thyroid cancer (8, 9).

Thyroid-stimulating hormone (TSH) is the major growth factor for thyroid cells and regulator of thyroid functions. It controls the processes that lead to increased thyroid hormone production and secretion (10). Blood concentrations of thyroid hormones [i.e., triiodothyronine (T3) and its prohormone thyroxine (T4)] inversely regulate the release of TSH through a negative feedback loop at the pituitary levels. High TSH levels have been associated with PTC pathogenesis in a mouse model (11). Suppression of TSH is currently recommended to manage patients with differentiated thyroid cancer (DTC), which has shown benefits to patient survival (12). Thyroid hormones have also been suggested to have a tumor-promoting effect on several cancers, including pancreatic, breast, ovarian, and prostate cancers (13). However, findings of epidemiologic studies linking TSH and thyroid hormones to the risk of thyroid cancer have been inconsistent (14–40).

The majority of early studies reported an increased risk of thyroid cancer associated with elevated TSH levels (14–30), several studies found no significant association (31–39), and one reported a reduced risk (40). All studies that reported a positive association between TSH and thyroid cancer were cross-sectional (14–29) or case-control studies (30). Therefore, the possibility of

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**Note:** Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

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reverse causation or treatment effect could be of potential concern because the TSH levels were measured after diagnosis. There are only three previous prospective cohort studies. One reported a significantly reduced risk of thyroid cancer associated with elevated TSH levels (40). Two smaller studies reported lower, but not significant, TSH levels in thyroid cancer cases than in controls (38, 39). The relationship between thyroid hormones and risk of thyroid cancer has also been inconclusive (14–17, 37, 38, 40). Two studies found that lower thyroid hormone levels were associated with a higher risk of thyroid cancer (16, 17), whereas the remaining five reported no association (14, 15, 37, 38, 40).

In light of the inconclusive associations between TSH, thyroid hormones, and thyroid cancer, we conducted a nested case–control study using data from the Department of Defense (DoD) Automated Central Tumor Registry (ACTUR) and the Defense Medical Surveillance System (DMSS), with prediagnostic serum samples from the Department of Defense Serum Repository (DoDSR) to investigate the associations of PTC with TSH and thyroid hormones [total T3 (TT3), total T4 (TT4), and free T4 (FT4)].

## Materials and Methods

### Study population

Our study population was U.S. military personnel who had serum samples stored in the DoDSR (41). These stored samples were leftover sera collected for the routine HIV test of military personnel. The DoDSR is maintained by the Armed Forces Health Surveillance Center, U.S. Army Public Health Command. As of August 2013, the repository stored more than 55 million serum samples from more than 10 million individuals, most of whom were active-duty and reserve personnel. Serum samples from all military members were typically drawn at the time of service entry and, on average, every 2 years thereafter for mandatory HIV testing (42, 43).

We designed an individually matched nested case–control study. Cases were identified by linkage of the ACTUR with the DoDSR database. The ACTUR was established in 1986 and is the data collection and clinical tracking system for cancer cases diagnosed and treated at military treatment facilities among DoD beneficiaries, including active-duty military personnel, retired military personnel, and their dependents. The registry includes information on demographic variables, diagnostic factors, and tumor characteristics (44). Cases met the following criteria: (i) histologically confirmed with International Classification of Diseases for Oncology, third edition (ICD-O-3 for thyroid gland: C739) histology codes 8021, 8050, 8052, 8130, 8260, 8290, 8330–8332, 8335, 8340–8346, 8450, 8452, and 8510; (ii) at least 1.5-mL prediagnostic and 0.5-mL postdiagnostic serum samples stored in the DoDSR; (iii) diagnosis between 2000 and 2013; and (iv) age 21 years or older at diagnosis. Cases with any prior cancers (excluding non-melanoma skin cancer) recorded in the ACTUR at the date of diagnosis of thyroid cancer were excluded from the study. A total of 800 eligible cases were identified. The histology of all reported thyroid cancer cases was abstracted from ACTUR. Of these eligible cases, 742 (92.8%) were PTC (ICD-O-3: 8050, 8260, and 8340–8343). IDs for all cases were sent from ACTUR to Armed Forces Health Surveillance Center via encrypted methods. Those IDs were excluded from the eligible pool of controls. Controls' eligibility criteria were: having at least 4 serum samples in DoDSR, and according to the matching criteria, the midpoint of those 4

samples within 1 year of the control's matched case's midpoint of their 4 samples. Controls were randomly selected with replacement from the cohort of service members who were not diagnosed with any cancer (with the exception of non-melanoma skin cancer), as per query of the ACTUR. Controls were matched one-to-one to cases by date of birth ( $\pm 1$  year), gender, race/ethnicity (White, Black, Hispanic, and other), average date of the selected 4 samples drawn ( $\pm 1$  year), and component at diagnosis/matching. Demographic and military characteristics for all cases and controls were abstracted from DMSS. The DMSS now serves as the central repository of medical surveillance data for the U.S. armed forces and contains longitudinal records which have been continuously updated since 1990. The system includes demographic and military characteristics as well as military and medical experiences of service members throughout their military careers (41). All study procedures were approved by the Uniformed Services University Institutional Review Board, the Walter Reed National Military Medical Center, the DoD Joint Pathology Center, and The Human Investigation Committee of Yale University.

### Measurement of TSH and thyroid hormones

A calibrated Roche Cobas E601 analyzer was used to measure the serum concentrations of TSH and thyroid hormones using the manufacturer's reagents and calibrators. TSH was captured between 2 monoclonal antibodies (one was biotinylated and the other labeled with a ruthenium complex) which specific for sterically noninterfering epitopes of human TSH. TT3 and TT4 were dissociated from binding proteins using 8-anilino-1-naphthalene sulfonic acid (ANS) and competed with the exogenous biotinylated T3 or T4 for binding to a T3- or T4-specific antibody labeled with a ruthenium complex. FT4 directly competed with the exogenous biotinylated T4 for binding to a T4-specific antibody labeled with a ruthenium complex. All the antibodies were captured by streptavidin-coated magnetic micro-particles, which were then magnetically captured by an electrode and the application of voltage-induced emission of photons by the ruthenium complex. The intensities of the luminescence were inversely proportional to the serum concentrations of TSH and thyroid hormones. The normal ranges for serum concentrations of TSH, TT3, TT4, and FT4 were 0.3–4.2  $\mu\text{U}/\text{mL}$ , 79–149  $\text{ng}/\text{dL}$ , 5.0–10.6  $\mu\text{g}/\text{dL}$ , and 0.80–1.80  $\text{ng}/\text{dL}$ , respectively. All control samples were tested in the same batch as their matched case samples. On the basis of results obtained from quality control samples (5%), intrabatch coefficient of variation ranged from 3.9% to 7.7%.

### Statistical analyses

Measurements of TSH and thyroid hormones failed in one serum sample, leaving 741 pairs of PTC cases and matched controls included in the final analysis. The distributions of demographic and military characteristics between cases and controls were compared by  $\chi^2$  tests. The correlations between TSH, TT3, TT4, and FT4 were estimated using the Pearson correlation coefficients. Given the individual-matched case–control design, conditional logistic regression analyses were employed to calculate ORs and 95% confidence intervals (95% CI) for the association between TSH, thyroid hormones, and PTC. Serum concentrations of TSH, TT3, TT4, and FT4 were divided into 3 categories on the basis of the normal range (below, within, and above the normal range). The normal range group was further categorized into tertiles on the basis of the distributions of serum concentrations among controls. Thus, there were 5 categories for each hormone:

below the normal range, lower, medium, and higher levels within the normal range, and above the normal range. The middle tertile within the normal range was used as reference category for all analyses. All the conditional logistic regression models were adjusted for body mass index (BMI; <18.5, 18.5–24.9, 25–29.9, and  $\geq 30$  kg/m<sup>2</sup>) and branch of military service (army, air force, marines and coast guard, and navy). TT3, TT4, and FT4 models were also adjusted for serum concentrations of TSH. However, additional adjustment for serum concentrations of TT3, TT4, and FT4 in TSH models did not result in material changes in the observed associations and thus were not included in the final models. Dose–response relationship was further investigated using  $P_{\text{trend}}$ , estimated by treating serum concentrations of TSH and thyroid hormones as continuous variables. Stratified analyses were performed by gender, histologic subtype [classical PTC (ICD-O-3: 8050, 8260, 8341–8343) and follicular variant of PTC (ICD-O-3: 8340)], tumor size ( $\leq 10$  and  $>10$  mm), and years between serum samples drawn and PTC diagnosis (<3, 3–6, and  $>6$  years, based on sample size). Sensitivity analysis was also conducted among women younger than 50 years to see whether estrogen impacts the associations among premenopausal women. All tests were 2-sided with  $\alpha$  of 0.05. Statistical analyses were conducted using SAS software, version 9.3 (SAS Institute, Inc.).

## Results

PTC cases were more likely to have served in the Army or Air Force at time of diagnosis, whereas controls were more likely to have served in the Navy, Marines, or Coast Guard (Table 1). Cases had a slightly larger BMI than controls, but the difference was not statistically significant. Since the cases were individually matched to controls on the basis of age, gender, and race/ethnicity, the distributions of these variables were similar between cases and controls.

**Table 1.** Distributions of selected characteristics among PTC cases and matched controls

	Cases (N = 741) n (%)	Controls (N = 741) n (%)	P
Age at diagnosis, y			
<30	210 (28.3)	203 (27.4)	
30–39	309 (41.7)	322 (43.5)	
40–49	185 (25.0)	179 (24.2)	
$\geq 50$	37 (5.0)	37 (5.0)	0.92
Gender			
Male	400 (54.0)	400 (54.0)	
Female	341 (46.0)	341 (46.0)	1.00
Race			
White	467 (63.0)	466 (62.9)	
Black	131 (17.7)	132 (17.8)	
Hispanic	68 (9.2)	68 (9.2)	
Other	55 (7.4)	55 (7.4)	
Unknown	20 (2.7)	20 (2.7)	1.00
BMI, kg/m <sup>2</sup>			
<25	256 (34.6)	285 (38.5)	
25–29.9	148 (20.0)	129 (17.4)	
$\geq 30$	16 (2.2)	10 (1.4)	
Missing	321 (43.3)	317 (42.8)	0.23
Service			
Army	299 (40.4)	253 (34.1)	
Air Force	193 (26.1)	150 (20.2)	
Marines and Coast Guard combined	64 (8.6)	91 (12.3)	
Navy	185 (25.0)	247 (33.3)	<0.0001

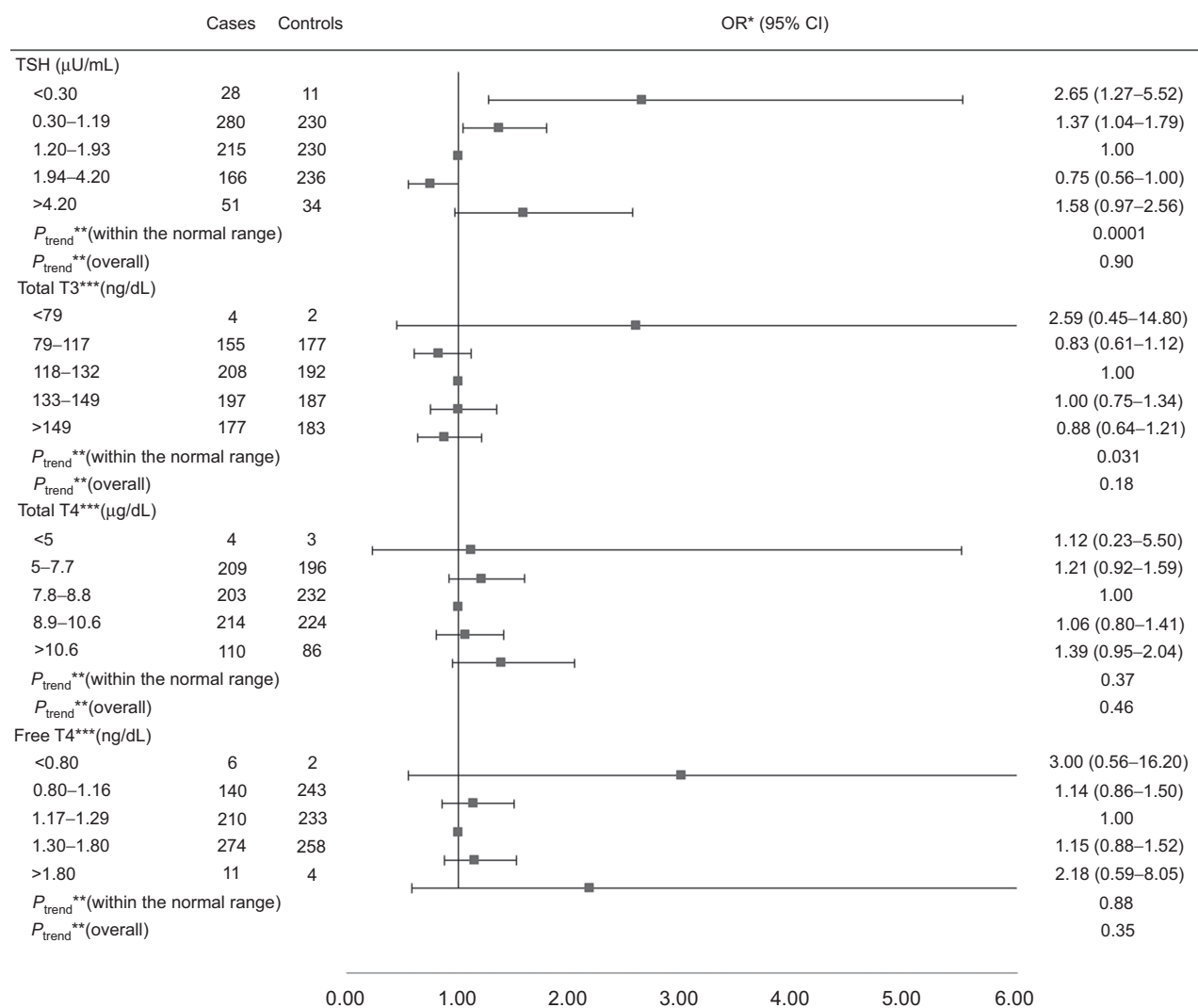
All serum samples were drawn between 83 and 4,232 days before the cases were diagnosed with PTC. As anticipated, there were statistically significant strong positive correlations between TT3, TT4, and FT4 ( $r = 0.68$  for TT3 and TT4,  $P < 0.0001$ ;  $r = 0.40$  for TT3 and FT4,  $P < 0.0001$ ; and  $r = 0.52$  for TT4 and FT4,  $P < 0.0001$ , respectively). TSH was weakly but statistically significantly correlated with TT3, TT4, and FT4 ( $r = -0.06$  for TSH and TT3,  $P = 0.022$ ;  $r = -0.17$  for TSH and TT4,  $P < 0.0001$ ; and  $r = -0.19$  for TSH and FT4,  $P < 0.0001$ , respectively). Female cases had lower mean TSH levels than their matched controls, whereas male cases had higher mean TSH levels than their matched controls. None of these differences were statistically significant. We also observed nonsignificantly higher mean levels of thyroid hormones among female cases as compared with female controls, but thyroid hormone levels were similar between cases and controls among men.

In the overall population, serum TSH levels below the normal range were associated with a significantly increased risk of PTC (OR, 2.65; 95% CI, 1.27–5.52; Fig. 1) compared with the middle tertile of the normal range. Paradoxically, TSH levels above the normal range were also associated with an increased risk of PTC (OR, 1.58; 95% CI, 0.97–2.56) with borderline significance. Serum concentrations of TT3, TT4, and FT4 below or above the normal range were not significantly related to an elevated risk of PTC. Within the normal ranges, the risk of PTC decreased with increasing TSH levels ( $P_{\text{trend}} = 0.0001$ ) and with decreasing TT3 levels ( $P_{\text{trend}} = 0.031$ ), but no dose–response relationships were observed for TT4 and FT4.

TSH levels below the normal range were associated with increased risk of PTC among women (OR, 3.74; 95% CI, 1.53–9.19) but not among men (OR, 1.07; 95% CI, 0.25–4.62; Fig. 2) compared with the middle tertile of the normal range. In addition, an increased risk of PTC in relation to TSH levels above the normal range was observed only among men (OR, 1.96; 95% CI, 1.04–3.66) but not among women (OR, 1.09; 95% CI, 0.49–2.46). The risk of PTC decreased with increasing TSH levels within the normal range among both men and women ( $P_{\text{trend}} = 0.0005$  and 0.041, respectively). However, lower TSH levels within the normal range were associated with an increased risk of PTC and the association was stronger in women (OR, 1.53; 95% CI, 1.03–2.28) than in men (OR, 1.17; 95% CI, 0.80–1.71). In contrast, higher TSH levels within the normal range were associated with a reduced risk of PTC among men (OR, 0.65; 95% CI, 0.44–0.95). An inverse association between TT3 levels above the normal range and risk of PTC was observed only among men (OR, 0.59; 95% CI, 0.36–0.98); while the risk of PTC increased with increasing serum concentrations of TT3 among women (overall  $P_{\text{trend}} = 0.019$ ), no significant associations with TT4 and FT4 were observed.

When the analyses were stratified by histologic subtype among men (Fig. 3), TSH levels above the normal range were borderline significantly associated with an increased risk of classical PTC (OR, 1.94; 95% CI, 1.00–3.77). A significantly inverse dose–response relationship was observed between TSH levels within the normal range and risk of classical PTC ( $P_{\text{trend}} = 0.0010$ ) but not follicular variant PTC ( $P_{\text{trend}} = 0.16$ ). TT3 levels above the normal range were significantly associated with a decreased risk of classical PTC (OR, 0.53; 95% CI, 0.30–0.94). When the analyses were stratified by histologic subtype among women (Fig. 4), TSH levels below the normal range were associated with a significantly increased risk of classical PTC (OR, 2.72; 95% CI, 1.09–6.78). The lower TSH levels within the normal range were associated with an

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**Figure 1.**

Risk of PTC associated with serum concentrations of TSH and thyroid hormones. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.

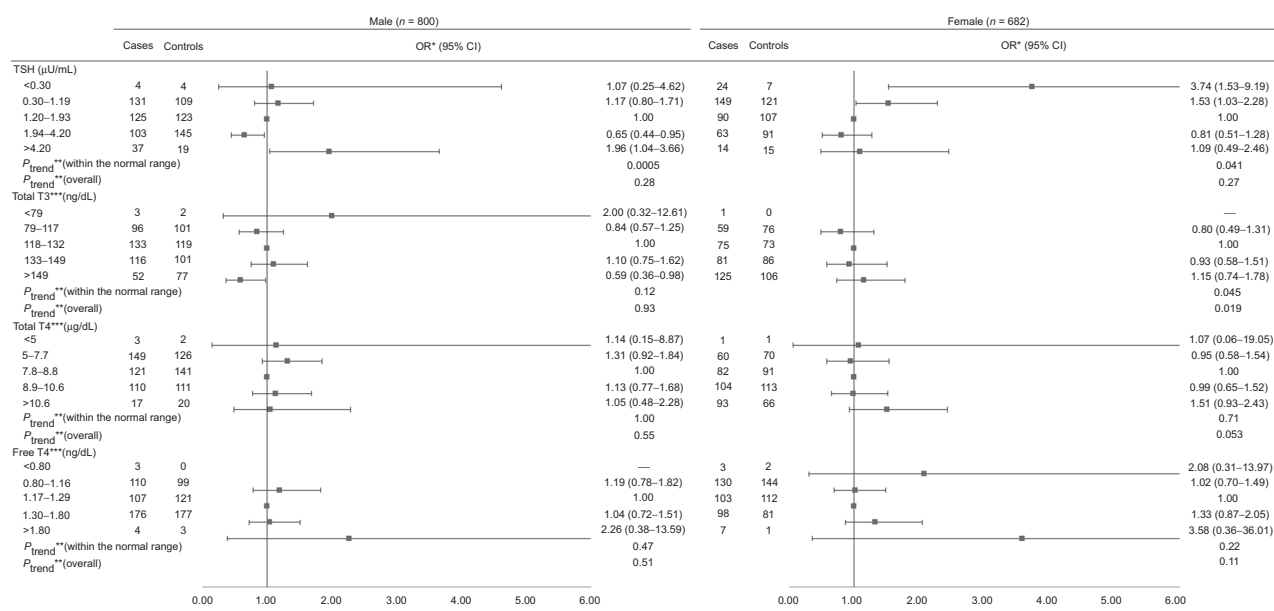
elevated risk of follicular variant of PTC (OR, 11.31; 95% CI, 3.10–41.31). There was an increasing trend in risk of classical PTC with increasing TT3 levels ( $P_{\text{trend}} = 0.021$ ), but no statistically significant association between TT3 levels and risk of follicular variant of PTC was observed.

When the analyses were stratified by tumor size among men (Fig. 5), the higher TSH levels within the normal range were associated with a reduced risk of PTC with tumor size greater than 10 mm (OR, 0.57; 95% CI, 0.35–0.92) but not PTC microcarcinoma ( $\leq 10$  mm; OR, 0.99; 95% CI, 0.47–2.12). TSH levels within the normal range were inversely associated with PTC >10 mm ( $P_{\text{trend}} = 0.0003$ ), whereas, there was no trend for PTC microcarcinoma ( $P_{\text{trend}} = 0.21$ ). When the analyses were stratified by tumor size among women (Fig. 6), TSH levels below the normal range were associated with a significantly increased risk of PTC >10 mm (OR, 4.98; 95% CI, 1.30–19.06) and a nonsignificantly elevated risk of PTC micro-

carcinoma (OR, 3.62; 95% CI, 0.85–15.48). The lower TSH levels within the normal range were associated with an elevated risk of PTC microcarcinoma (OR, 2.47; 95% CI, 1.10–5.55) but not PTC >10 mm (OR, 1.30; 95% CI, 0.79–2.14). TSH levels within the normal range were inversely associated with PTC >10 mm ( $P_{\text{trend}} = 0.027$ ). No significant associations were found for TT3, TT4, and FT4 for both PTC microcarcinoma and PTC > 10 mm among men and women.

Further stratified analyses were conducted by the years between serum samples drawn and PTC diagnosis (Supplementary Table S1). No clear pattern was observed with timing of the serum samples drawn, although the numbers of cases was small after stratification.

Sensitivity analyses showed that the associations between risk of PTC and serum concentrations of TSH, TT3, TT4, and FT4 did not change materially after restricting the analyses to women younger than 50 years.



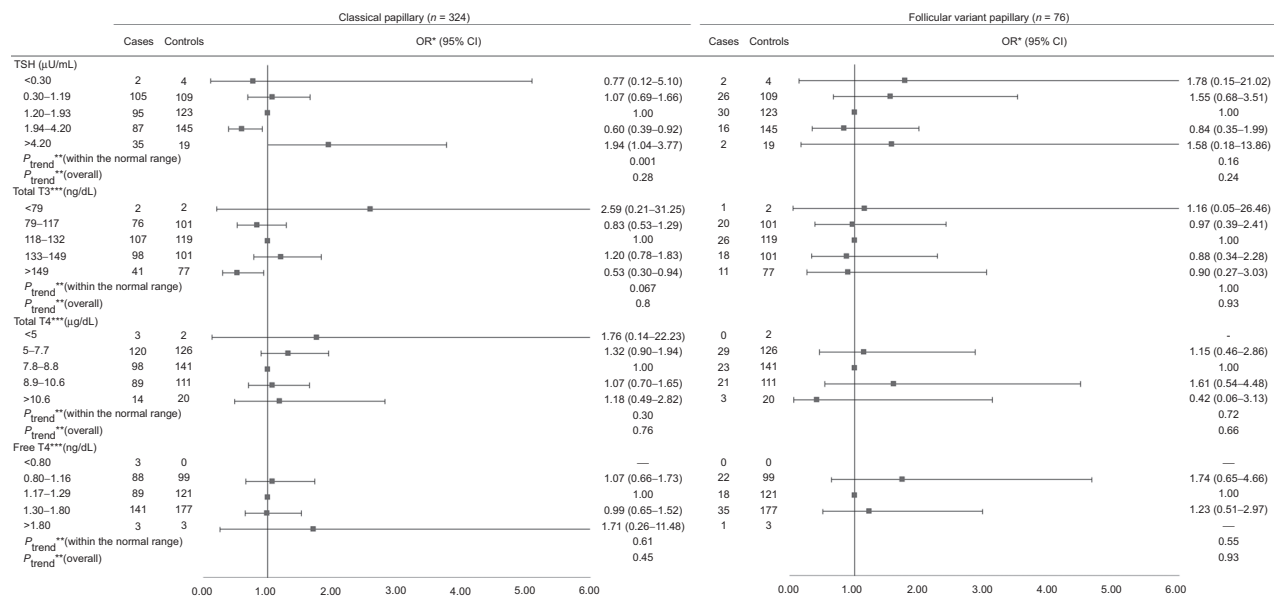
**Figure 2.**

Risk of PTC associated with serum concentrations of TSH and thyroid hormones, stratified by gender. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.

## Discussion

In this large case-control study based on prediagnostic serum measures and with sufficient power to stratify by gender, we found that serum TSH levels below the normal range were associated with an elevated risk of PTC among women but not men. TSH levels above the normal range were only associated with an

increased risk of PTC among men. There was an inverse association between PTC and TSH levels within the normal range among both men and women. The observed associations varied somewhat by histologic subtypes (classical vs. follicular variant PTCs) and by tumor size ( $\leq 10$  vs.  $>10$  mm) among men and women. The gender effect on the association between TSH and

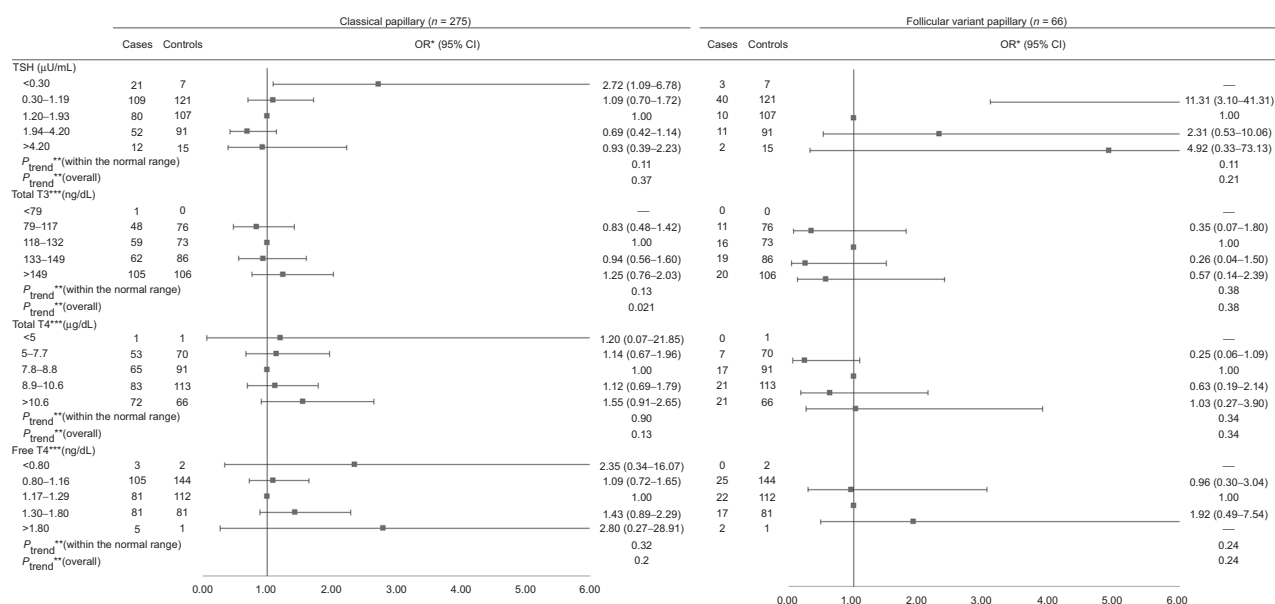


**Figure 3.**

Risk of PTC associated with serum concentrations of TSH and thyroid hormones among males, stratified by histological subtypes. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.



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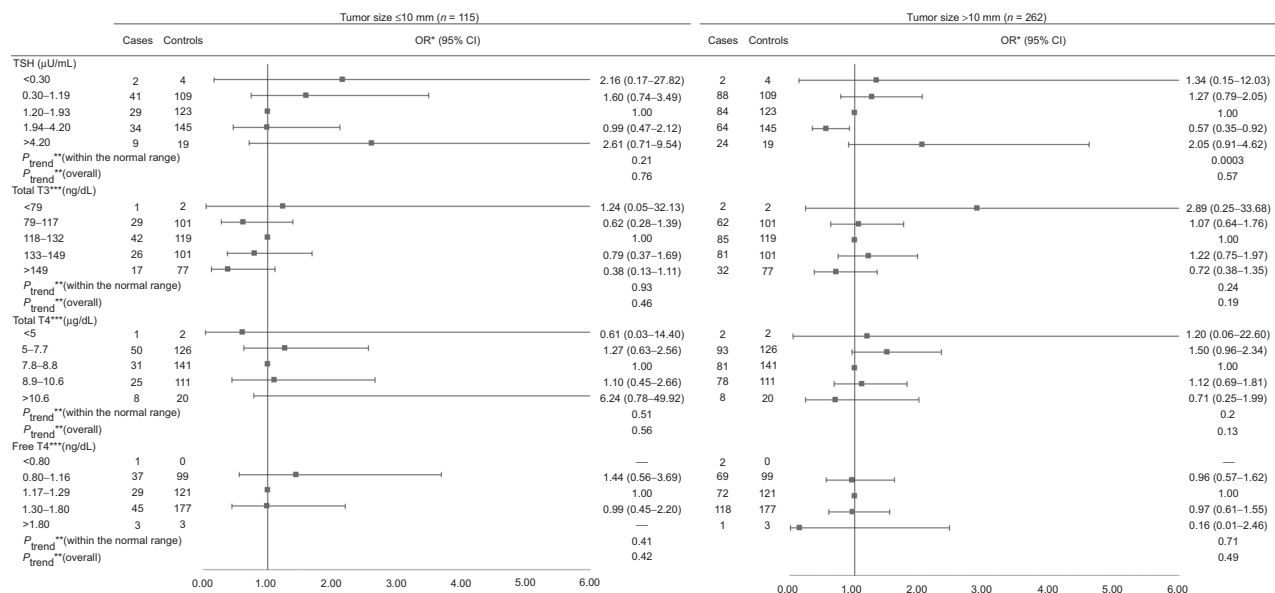


**Figure 4.** Risk of PTC associated with serum concentrations of TSH and thyroid hormones among females, stratified by histological subtypes. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.

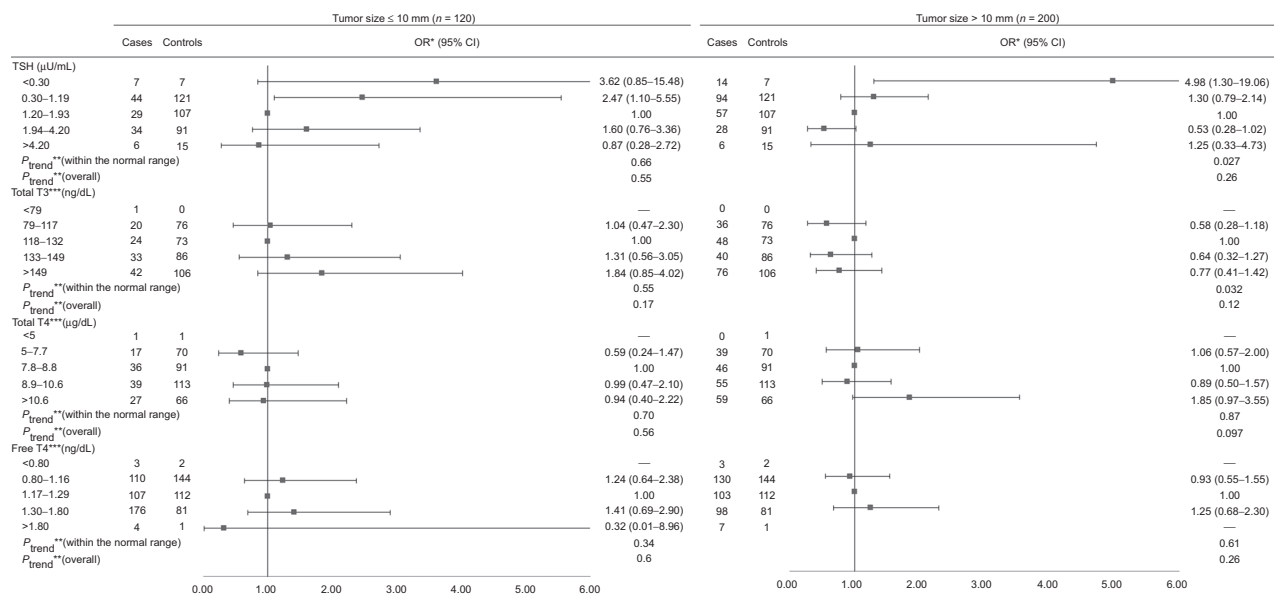
PTC was only observed among classical PTC cases. TSH levels showed a stronger association with PTC with larger tumor size. A suggestive inverse association between higher TT3 levels and risk of PTC was observed among men.

The inverse trends between TSH levels and risk of PTC observed in the present study were in accordance with results from a nested case-control study within a large population-based prospective cohort in Europe (40). The cohort consisted of approximately

520,000 healthy individuals of ages 35 to 69 years when recruited between 1992 and 1998 in 10 European countries. A total of 357 incident thyroid cancer cases (57 men and 300 women) diagnosed during 1992 to 2009 and 767 matched controls were included in the analyses. Blood samples were collected at enrollment. This European study found an inverse dose-response relationship between overall TSH levels and risk of differentiated thyroid cancer. The years between sample collection and thyroid



**Figure 5.** Risk of PTC associated with serum concentrations of TSH and thyroid hormones among males, stratified by tumor size. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.



**Figure 6.**

Risk of PTC associated with serum concentrations of TSH and thyroid hormones among females, stratified by tumor size. \*, Conditional logistic regression, adjusted for BMI and branch of military service. \*\*, Estimated by continuous variables. \*\*\*, Additionally adjusted for serum concentration of TSH.

cancer diagnosis were similar between the European study and our study. However, as compared with the European study, our population was younger and healthier (45), with participants of ages 17 to 56 years at blood samples collection. In addition, our study had a larger number of the male cases than the European study, which provides sufficient power to examine the associations among men. The present study observed inconsistent associations between TSH levels and risk of PTC among women as compared to men, whereas the European study reported similar associations among men and women. There were 2 other prospective studies with smaller sample size that investigated the association between TSH and risk of thyroid cancer (38, 39). Although no significantly inverse association was observed in these studies, both reported lower TSH levels among thyroid cancer cases than controls.

A previous meta-analysis showed that higher TSH levels were associated with an increased thyroid cancer risk (46). However, all 22 studies included in the meta-analysis were cross-sectional studies and measured TSH levels after treatment of thyroid cancer began. The cross-sectional design could not clarify whether elevated TSH levels preceded thyroid cancer diagnosis or were effects of treatment. The low levels of thyroid hormones due to dysfunction of the thyroid gland among thyroid cancer patients could cause the pituitary gland to release more TSH. In addition, higher TSH levels may promote the growth of already initiated thyroid cancer, making the cancer larger and more easily diagnosed. Therefore, the positive association seen in the cross-sectional studies could be due to ascertainment bias (10). On the other hand, controls in these studies were always patients with thyroid nodules or patients undergoing surgical treatment for a suspicious thyroid tumor. Some nodules can produce high levels of thyroid hormones, thus lowering TSH levels (40). Many patients with thyroid cancer also had additional benign thyroid nodules, and the mutual influence

between those nodules and TSH concentrations has not yet been determined (18).

TSH plays an important role in regulating thyroid function, including increasing number, size, and secretory activity of thyrocytes, increasing thyroid blood flow, and increasing thyroid hormone production and secretion from follicular thyroid cells (10). Classical TSH actions are mainly mediated through the  $G_{\alpha s}$ -adenylyl cyclase-protein kinase A-cyclic adenosine monophosphate (cAMP) pathway, which is associated with production of thyroid hormones and proliferation of thyroid epithelial cells (47). However, somatic mutations in thyroid epithelial cell can also activate the cAMP pathway, which facilitate the cell growth and clonal expansion, leading to the formation of an autonomously functioning thyroid adenoma. The adenoma can synthesize and secrete thyroid hormones autonomously, thereby suppressing TSH secretion (48). Therefore, constitutive activation of the cAMP pathway could be associated with an increased carcinogenic potential and a decreased TSH level. Because of the deprivation of TSH stimulation, the extranodular tissue would become quiescent. Depending on the iodine intake, growth potential, and other factors, it may take months to a decade or longer for an adenoma to grow large enough to cause hyperthyroidism (49).

While the underlying mechanism of lower TSH levels increasing the risk of PTC is currently unclear, two genome-wide association studies have found that 5 common variants [rs965513[A] on 9q22.23, rs944289[T] and rs116909374[T] on 14q13.3, rs966423[C] on 2q35, and rs2439302[G] on 8p12] were associated with both an increased risk of thyroid cancer and low TSH level (50, 51). According to Gudmundsson and colleagues, the 5 variants could refer to genes *FOXE1*, *NKX2-1*, *DIRC3*, and *NRG1*. The *FOXE1* gene can regulate the transcription of thyroglobulin and thyroperoxidase genes, and together with the *NKX2-1* gene, plays an essential role in thyroid gland formation, differentiation,

and function (52). Although the function of the *DIRC3* gene is unknown, it is presumed to have tumor suppressor activity (53). The gene *NRG1* encodes a signaling protein which mediates cell-cell interactions and plays a critical role in the growth and development of thyroid gland. The carriers of these 5 variants may be characterized by lower concentrations of TSH. The consequence of the lower TSH levels may result in less differentiation of the thyroid epithelium, causing a higher predisposition to malignant cell transformation (51).

The present study observed positive associations in a dose-response manner between serum concentrations of TT3 and risk of PTC only among women. The observed inverse association between TSH levels and risk of PTC among women still held after excluding those  $\geq 50$  years old. These findings suggest that women may be more sensitive to the effect of TSH and thyroid hormones as compared with men. Possible explanations including effect modification of estrogen and different exposure to endocrine-disrupting chemicals (e.g., birth control pills and personal care products) by gender that need to be explored in future studies.

The present study also noted different associations of TSH and thyroid hormones on risk of PTC by histologic subtype and tumor size. Lower TSH levels showed an association with increasing risk of the follicular variant of PTC and PTC  $> 10$  mm, whereas higher TSH levels were associated with a decreased risk of classical PTC and PTC microcarcinoma. These associations may support the hypothesis that follicular variant of PTC and papillary microcarcinoma are unique clinical entities with different etiologic profiles (54, 55).

In addition, the present study failed to find a clear pattern between TSH levels and risk of PTC with timing of the serum samples drawn. The relevant time window in which TSH exerts influence on development of thyroid cancer needs to be further studied.

The present study has several strengths. It included a relatively large number of male cases, providing sufficient statistical power to investigate and compare the associations by gender, which is important because women are much more likely to develop thyroid cancer than men. The study population was composed entirely of U.S. active-duty military personnel, minimizing the potential differences in effects of TSH and thyroid hormones among healthy people and people with high risk of thyroid cancer. Potential selection bias from difference in access to medical care was also minimized for our study population. The serum concentrations of TSH and thyroid hormones were prospectively assessed and were not influenced by the disease process or treatment, which provided an opportunity to estimate potentially causal relationships between TSH, thyroid hormones, and thyroid cancer. A limitation of this study is that there was a lack of

information on several potential confounding factors, such as ionizing radiation exposure, history of benign thyroid disease, family history of thyroid cancer, and smoking status. There were also a high percentage of participants with missing BMI data, which may have led to insufficient adjustment for BMI. The lack of data on medication use precludes us from carrying out sensitivity analyses excluding people who were taking thyroid hormones. Furthermore, the subgroup analyses, which were stratified by years between samples collection and diagnosis, histologic subtype, and tumor size, may have yielded unstable results due to the small subgroup counts.

In conclusion, the present study showed a significantly increased risk of PTC associated with TSH levels lower than the normal range among women and higher than the normal range among men. The observed associations varied by histologic subtype and tumor size. These results could have significant clinical implications for physicians who are managing patients with abnormal thyroid functions and those with thyroidectomy. Future studies are warranted to further understand these associations.

### Disclosure of Potential Conflicts of Interest

R. Udelsman is a director at the Endocrine Neoplasia Institute (Miami Cancer Institute). No potential conflicts of interest were disclosed by the other authors.

### Authors' Contributions

**Conception and design:** J. Rusiecki, R. Udelsman, Y. Zhang  
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### Disclaimer

The content of this publication is the sole responsibility of the author(s) and does not necessarily reflect the views or policies of the Uniformed Services University of the Health Sciences or the Department of Defense.

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