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Functional polymorphisms in the *insulin-like binding protein-3* gene may modulate susceptibility to differentiated thyroid carcinoma in Caucasian Americans

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Abstract

Purpose—The insulin-like growth factor (IGF) pathway is believed to play a pivotal role in thyroid carcinogenesis. Polymorphisms of *IGF-1* and *IGF binding protein-3 (IGFBP-3)* have been associated with modulation of risk for the emergence of assorted common malignancies, but studies of the influence of such polymorphisms on risk of differentiated thyroid carcinoma (DTC) are lacking.

Methods—In a case-control study of 173 DTC patients, 101 patients with benign thyroid disease, and 401 controls, an unconditional logistical regression model adjusted for age and sex was applied to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between polymorphisms of *IGF-1* and *IGFBP-3* and DTC risk.

Results—*IGFBP-3* rs2132572 GA/AA genotypes were associated with a decreased risk of DTC (adjusted OR=0.6, 95% CI: 0.4–0.9), particularly multifocal DTC (adjusted OR=0.3, 95% CI: 0.1–0.7). The association with DTC was more evident in subjects with a first-degree family history of cancer (adjusted OR=0.4, 95% CI: 0.2–0.7, $P_{\text{interaction}}=0.013$) and nondrinkers (adjusted OR=0.4, 95% CI: 0.2–0.7, $P_{\text{interaction}}=0.028$). A 4 SNP haplotype of *IGFBP-3* was associated with a decreased risk of DTC (adjusted OR=0.7, 95% CI: 0.5–1.0, $P=0.030$).

Conclusions—Our study suggests that polymorphic *IGFBP-3* may be involved in susceptibility to DTC.

Keywords

IGF-1; multifocal; genetic susceptibility; papillary thyroid carcinoma; case-control study

Introduction

Thyroid cancer is the fifth most common cancer in US women.¹ The incidence of thyroid cancer is increasing faster than that of any other cancer in the US population, and thyroid

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cancer incidence has more than doubled since the 1990s, in large part due to improved diagnosis but also to unidentified factors.^{2, 3} Papillary thyroid carcinoma (PTC), follicular thyroid carcinoma, and Hürthle cell carcinoma are collectively termed differentiated thyroid carcinoma (DTC) and together account for approximately 90% of all thyroid cancers. Although development of DTC has been associated with medical and accidental radiation exposure during childhood,⁴ only a small proportion of exposed subjects develop DTC. Further, only a small proportion of DTCs develop on the basis of prior radiation exposure history. Therefore, these observations suggest that the emergence of DTC must be promulgated by various predisposing genetic factors; to-date, these factors remain largely unknown.

Insulin-like growth factor-1 (IGF-1) is believed to play a pivotal role in thyrocyte proliferation, differentiation, and malignant transformation.⁵ IGF-1 is a well-established mitogen for thyrocytes, which has been demonstrated by *in vitro* studies in human cell models as well as in animals.⁶ Within thyrocytes and through collaboration with thyroid-stimulating hormone, IGF-1 via activation of the signaling cascade downstream of its cognate receptor stimulates the activation of the MAPK and PI3K signaling pathways, which in turn can transcriptionally regulate critical genes and processes in carcinogenesis.⁷ Local production of IGF-1 has been found in thyroid cancer, and its in-situ expression level was higher than in normal thyroid tissue.⁸ IGF binding protein-3 (IGFBP-3) is the principal IGF-1 binding protein; in the peripheral blood, 70–90% of IGF-1 is bound to IGFBP-3 to form a ternary complex with an acid-labile subunit.⁶ This ternary complex controls the bioavailability of IGF-1 to target organs where IGF-1 can be released from IGFBP-3 by proteases to exert its biological function. IGFBP-3 exerts stimulatory or inhibitory actions on cell proliferation in an IGF-1-dependent or -independent manner, as well as in a cell system- and tissue-specific context.⁹ Like IGF-1, IGFBP-3 is produced in various tissues, including the thyroid gland.⁶

Although controversy exists regarding the role of IGF-1 and IGFBP-3 in carcinogenesis, a number of epidemiological studies have established a relationship between elevated circulating IGF-1 levels and/or altered levels of IGFBP-3 and increased risk for developing malignancy of several organs, including the breast, prostate, lung, colon, and rectum.^{10–12} Age, nutritional status, as well as metabolic and genetic factors have all been shown to affect circulating IGF-1 and IGFBP-3 levels.^{13, 14} A twin study has suggested that approximately 38% of the variation in circulating IGF-1 levels and 60% of the variation in circulating IGFBP-3 levels is genetically determined.¹⁵ It is then conceivable that genetic variation, especially polymorphisms located in the regulatory regions, may influence individual IGF-1 and IGFBP-3 levels and subsequent cancer risk. In fact, accumulating studies have reported associations between polymorphisms in the *IGF-1* and *IGFBP-3* genes and risk of common cancers and/or circulating IGF-1 and IGFBP-3 levels.^{11, 16, 17}

Because of the biological significance of the *IGF-1* and *IGFBP-3* genes and the lack of studies of the effect of polymorphisms in these genes on DTC risk, we aimed to evaluate whether polymorphisms in *IGF-1* and *IGFBP-3* contribute to predisposition to thyroid tumor. In the present study, we investigated a CA repeat polymorphism of the *IGF-1* promoter region and 4 single nucleotide polymorphisms (SNPs) in the *IGFBP-3* promoter region on the premise that they likely have an impact on protein expression. The frequency distributions of these polymorphisms were compared among DTC patients, patients with benign thyroid disease (BTD), and cancer-free controls to assess the association of these polymorphisms with disease risk. Additionally, clinicopathological characteristics of the disease were examined to explore the clinical relevance of these polymorphisms.

Materials and Methods

Study Subjects

This molecular epidemiological case-control study was conducted at The University of Texas MD Anderson Cancer Center. Case subjects were recruited from patients presenting to our institution between November 1999 and November 2005 with a final diagnosis of DTC or BTDC confirmed by histopathology. Patients who were less than 18 years old, received a blood transfusion during the 6 months prior to recruitment, had a prior malignancy (except for non-melanoma skin cancer), or were currently taking immunosuppressant medications were excluded from recruitment. To reduce potential ethnic confounding, only self-reported non-Hispanic Caucasians were included in the current analysis. Control subjects were non-Hispanic Caucasians derived from the control group accrued for an ongoing molecular epidemiological study of squamous cell carcinoma of the head and neck between November 1996 and March 2005. The same recruitment criteria were applied to these controls. In total, 173 patients with DTC, 101 patients with BTDC, and 401 cancer-free controls were included for genotyping analysis.

All recruited subjects signed an informed consent form approved by the Institutional Review Board of our institution prior to recruitment, completed a self-administered questionnaire, and donated 20 ml of blood for laboratory analysis. The detailed questionnaire collection has previously been reported.¹⁸ Study subjects also reported their height and weight at the time of recruitment. Body mass index was defined as weight in kilograms divided by height in meters squared (kg/m^2). Additionally, clinical-pathological information on case patients was obtained from the electronic medical records at our institution.

SNP Selection and Genotyping Analysis

A CA repeat polymorphism in the promoter region of the *IGF-1* gene was selected for analysis. Additionally, 4 SNPs in the promoter region of the *IGFBP-3* gene, rs2132571, rs2132572, rs2854744, and rs13241830, were included. These polymorphisms were selected as information about them is available in public databases (dbSNP and the SNP500Cancer project, maintained by the National Cancer Institute) and the minor allele frequency of these SNPs is >5% in Caucasian populations.

Buffy coat DNA was extracted using the QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA). Genotyping of the *IGF-1* CA repeat polymorphism was determined by PCR amplification followed by genetic fragment analysis using an ABI PRISM 377 DNA sequencer (Applied Biosystems, Foster City, CA). The SNPs in *IGFBP-3* were examined using the PCR-restriction fragment length polymorphism assay. The oligonucleotide primers (Integrated DNA Technologies Inc., Coralville, IA) and restriction endonucleases (New England Biolabs, Ipswich, MA) are reported in Supplementary Table 1. Laboratory personnel were blinded to case-control status. Repeated analysis was performed in a randomly selected 10% of samples and showed greater than 99% concordance between the results of initial and repeated analyses.

Statistical Analysis

Demographic and exposure characteristics were compared between cases and controls with chi-square test or Student's *t*-test as appropriate. Deviation from Hardy-Weinberg equilibrium assumption was examined by chi-square test in controls for each SNP. Association analysis was performed under different inheritance models—dominant, codominant, and recessive—to evaluate the risk associated with genotypes, and a final inheritance model was determined for each SNP on the basis of the Akaike information criterion. In the best model, odds ratio (OR) and 95% confidence intervals (CIs) were

calculated in a multivariate unconditional logistic regression model with adjustment for age (continuous) and sex. Other variables were included in final model if they changed the target odds ratio by 10 percent or more when added to the model with adjustment for age and sex.¹⁹ Additionally, a trend test was performed on the number of minor alleles (0, 1, or 2) in association with disease risk using a logistic regression model. Pairwise linkage disequilibrium measure (D' value) was calculated using Haploview software.²⁰ Individual haplotype frequencies were estimated with the expectation maximization algorithm using the SNPStats program (<http://bioinfo.iconcologia.net/SNPstats>²¹). Using the most frequent haplotype as the reference group, an unconditional regression model was applied to calculate ORs and 95% CIs with adjustment for age (continuous) and sex.

Subgroup analyses were performed on age (<48 years, ≥48 years, the median age of the controls), sex (male, female), body mass index (<25 kg/m², ≥25 kg/m²), first-degree family history of cancer (yes, no), smoking status (never, ever), and alcohol drinking status (never, ever), to examine whether the associations varied among different strata. Logistic regression model with adjustment for age (continuous) and sex was used to calculate ORs and 95% CIs for each subgroup. For the significance of interaction, a likelihood ratio test was used to compare the full model containing the interaction term with the model taking into account only the main effects.

To account for the multiple testing, the Bonferroni correction for the total number of polymorphisms tested was used. Additionally, we assessed the false-positive report probability (FPRP),²² which is calculated by incorporating the prior probability and the observed OR and 95% CI for each given comparison. Specifically, we assigned a prior probability of 0.25 when the biological plausibility was high and epidemiological supportive evidence was fair, a prior probability of 0.1 when the biological plausibility was high and the epidemiological evidence was lacking, and a prior probability of 0.01 when both were poor. A noteworthy association was accepted with a FPRP cut-off value of <0.5 and a cut-off of <0.2 was considered more stringent. All statistical tests were 2-sided, and $P < 0.05$ was considered statistically significant. All analyses were performed using SAS software version 9.2 (SAS Institute Inc., Cary, NC) unless noted otherwise.

Results

The characteristics of patients with DTC (N=173), patients with BTDC (N=101), and cancer-free controls (N=401) are summarized in Table 1. The proportion of older adults was higher in the BTDC group (median age=52 years) than in the DTC group (median age=45 years). There were similar rates of women, subjects with first-degree family history of thyroid cancer, and never-smokers in the BTDC and DTC groups, but the rates were lower in the control group. Most patients with DTC (156, 90.2%) were diagnosed with PTC, and most patients with DTC had T1 tumors (47.2%) and N0 lymph node status (60.3%), when they presented to our institution. Multifocal primary tumors and thyroiditis were detected in 30.2% and 20.9% of the patients with DTC, respectively.

For the *IGF-1* CA repeat polymorphism, 10 different alleles were detected: (CA)₁₁, (CA)₁₃, (CA)₁₄, (CA)₁₇, (CA)₁₈, (CA)₁₉, (CA)₂₀, (CA)₂₁, (CA)₂₂, and (CA)₂₃. The homozygous genotype 19/19 was the most frequently encountered in all groups—controls, patients with BTDC, and patients with DTC. The heterozygous 19/20 and 19/21 genotypes were the second and third most common genotypes (19/20: 25.1% in controls, 24.5% in BTDC, and 25.0% in DTC; 19/21: 8.3% in controls, 5.1% in BTDC, and 8.3% in DTC). All other genotypes were rare, occurring in well under 5% of subjects. As shown in Table 2 and 3, no significant association with either BTDC or DTC was found for *IGF-1* CA repeat genotypes when they were trichotomized as 19/19, 19/non-19, and non-19/non-19. We also dichotomized the

genotypes as 19/19 and others and grouped the genotypes in a fashion similar to that previously applied by Yu et al.,²³ who categorized alleles as a long allele (>19 CA repeats), a short allele (<19 CA repeats), and the “reference” allele (19 CA repeats); none of the association analyses revealed significant associations (data not shown).

The 4 *IGFBP-3* SNPs were in Hardy-Weinberg equilibrium ($P>0.05$). The minor allele frequency was 31% for rs2132571, 24% for rs2132572, 44% for rs2854744, and 31% for rs13241830; these frequencies are similar to the frequencies in the Caucasian populations according to the dbSNP database and/or the SNP500Cancer database.

Table 2 shows the genotype-specific risk of BTD associated with these SNPs under the best inheritance model according to the smallest value of Akaike information criterion.

Rs2132572 GA/AA genotypes were associated with a decreased risk of BTD (adjusted OR=0.6, 95% CI: 0.4–0.9, $P=0.022$, dominant model), while rs2854744 AA genotype was associated with an increased risk of BTD (adjusted OR=1.8, 95% CI: 1.1–2.9, $P=0.032$, recessive model).

Table 3 summarizes the genotype-specific risks of DTC and in the subsets of patients with DTC according to their clinicopathological characteristics. Under dominant inheritance model, rs2132572 GA/AA genotypes were associated with a decreased risk of DTC (adjusted OR=0.6, 95% CI: 0.4–0.9, $P=0.001$) and rs2854744 CA/AA genotypes were associated with an increased risk of DTC (adjusted OR=1.6, 95% CI: 1.0–2.4, $P=0.032$). After correction for multiple testing, only the association between rs2132572 GA/AA genotypes and DTC risk remained significant ($P_{\text{Bonferroni}}=0.005$). Additionally, an increasing number of rs2132572 minor alleles was significantly associated with a decreased risk of DTC ($P_{\text{trend}}=0.030$). Furthermore, case-control comparison revealed a significant reverse association between rs2132572 GA/AA genotypes and PTC risk (adjusted OR=0.6, 95% CI: 0.4–0.8, $P=0.005$, $P_{\text{Bonferroni}}=0.023$) and an allele-dose effect for rs2132572 ($P_{\text{trend}}=0.014$). Interestingly, when DTC cases were grouped by their multifocality status, rs2132572 GA/AA genotype displayed a strong protective effect against multifocal DTC (adjusted OR=0.3, 95% CI: 0.1–0.7, $P=0.002$, $P_{\text{Bonferroni}}=0.010$). For rs2854744, the CA/AA genotypes were associated with a 2.3-fold increase of multifocal DTC risk (95% CI: 1.0–5.4), although this was not statistically significant ($P=0.055$).

Table 4 summarizes the subgroup risk of DTC in association with *IGFBP-3* SNPs. The protective effect of rs2132572 on DTC risk was more evident in subjects with a first-degree family history of cancer (adjusted OR=0.4, 95% CI: 0.2–0.7) and nondrinkers (adjusted OR=0.4, 95% CI: 0.2–0.7), showing significant heterogeneity across strata ($P_{\text{interaction}}=0.013$ by family history of cancer, $P_{\text{interaction}}=0.028$ by drinking status). When the cancer patients were limited to those with PTC, the protective effect of rs2132572 GA/AA genotypes against cancer risk was again observed in subjects with a first-degree family history of cancer (adjusted OR=0.3, 95% CI: 0.2–0.6) and nondrinkers (adjusted OR=0.4, 95% CI: 0.2–0.6); the interaction effect was statistically significant ($P_{\text{interaction}}=0.007$ by family history of cancer, $P_{\text{interaction}}=0.015$ by drinking status). The association between rs2854744 and DTC risk was seemingly confined to older adults (age ≥ 48 years) (adjusted OR=2.6, 95% CI: 1.4–5.2, $P_{\text{interaction}}=0.038$). The association of rs2854744 CA/AA genotypes with DTC risk was somewhat stronger in subjects with a first-degree family history of cancer (adjusted OR=1.9, 95% CI: 1.0–3.5), but the interaction term was not statistically significant ($P_{\text{interaction}}=0.272$).

Strong linkage disequilibrium between the 4 *IGFBP-3* SNPs in controls were found ($D'>0.80$). A total of 13 haplotypes of the *IGFBP-3* gene were found, 3 of which accounted for approximately 95% of all haplotypes. The sex- and age-adjusted haplotype-specific risks are

shown in Table 5. Haplotype analysis identified a protective haplotype (G-A-C-C) that was associated with a decreased risk of DTC ($P=0.030$). This haplotype exhibited a similar protective effect against BTDC risk ($P=0.036$).

To estimate how likely these results would be expected by chance, we calculated FPRP, in which each statistically significant OR (95% CI) was examined assuming a range of prior probabilities (0.001~0.25), as indicated in Table 6. For a prior probability of 0.1, the likelihood of a false-positive result was below 50% for all of the significant results described above. For a prior probability of 0.01, the FPRP was below 20% for the association between rs2132572 and PTC risk in subjects with a first-degree family history of cancer and in nondrinkers and for the association between rs2132572 and DTC risk in nondrinkers.

Discussion

Although the effect of *IGF-1* and *IGFBP-3* genetic polymorphisms on risk of development of various cancers has been studied by several groups over recent years, a study on such an effect on DTC risk is lacking-to-date. In this case-control study in non-Hispanic Caucasians, we evaluated the association between DTC and an *IGF-1* CA repeat polymorphism and 4 *IGFBP-3* functional SNPs. In addition, we included BTDC as an intermediate group for comparison (in addition to DTC and cancer-free controls), because BTDC-associated parenchymal lesions (inflammatory [e.g., thyroiditis] or neoplasm [e.g., goiters and adenomas]) may represent a proliferative state that would be considered as an intermediate between normal thyroid epithelium and DTC. In the present study, 2 *IGFBP-3* SNPs, rs2132572 and rs2854744, were found to be associated with DTC with diverse effects: rs2132572 was associated with a decreased risk, whereas rs2854744 was associated with an increased risk. A similar but less marked genotype-disease risk association was observed between these SNPs and BTDC. These results support an association between polymorphic *IGFBP-3* and development of DTC in non-Hispanic Caucasians. Of note, our study had over 80% power to detect an OR of 1.7 (or its reciprocal, i.e., 0.6) for DTC with a risk genotype frequency of 0.43 in controls.

All of the studied *IGFBP-3* SNPs lie within the promoter region of the gene, either within or near the conserved domain containing putative response elements for various hormone receptors and transcriptional factors.²⁴ An *in vitro* study has reported that the transcriptional activity associated with the C allele of rs2854744 was approximately 50% that associated with the A allele (using a chloramphenicol acetyl transferase report system), reflecting the influence of a single SNP on the promoter activity, and hence, expression of the *IGFBP-3* gene.²⁴ In accordance with this finding, a dose-dependent relationship between the A allele of rs2854744 and higher circulating levels of IGFBP-3 has been consistently reported.²⁴⁻²⁷ For rs2132572, the opposite effect is possible, as evidenced by the observation of an association between the presence of the A allele of rs2132572 and lower circulating levels of IGFBP-3.²⁷

Several groups have previously examined the role of *IGFBP-3* SNPs in cancer susceptibility. The results from these studies have been inconsistent. For example, rs2854744 has been reported to be associated with an increased risk of non-small cell lung cancer,²⁸ a decreased risk of bladder cancer,²⁹ and no significant change in the risk of colorectal cancer.^{30, 31} For rs2132572, the minor A allele has been reported to be associated with an increased risk of colorectal cancer³¹ and no significant change in the risk of esophageal adenocarcinoma.³² In contrast with our results, a previous study reported that the minor A allele of SNP rs2132572 was associated with increased risk of breast cancer, whereas the minor A allele of SNP rs2854744 was associated with decreased risk of breast cancer.²⁷ In addition to the diverse genetic background and the difference in study design, the discrepancy between our results

and these results may be explained by the complicated biological actions of IGFBP-3, which are known to vary by site/tissue type.⁶ IGFBP-3 is recognized to exert a complex array of functions, displaying both stimulatory and inhibitory effect on cell proliferation; the net effect would be determined by the specific target cell and its microenvironment/stroma.⁹ The direct influence of these *IGFBP-3* polymorphisms (rs2854744 and rs2132572) on the mechanisms of thyroid carcinogenesis on a molecular/cellular level remains unknown and requires further elucidation.

In this study, rs2132572 GA/AA genotypes were associated with an approximately 40% reduction of DTC risk. Notably, this protective effect was more evident for multifocal DTC (adjusted OR=0.3), and there was a significant interaction with first-degree family history of cancer. For rs2854744, the CA/AA genotypes were associated with an approximately 50% increase of DTC risk. The level of risk was more pronounced in patients with multifocal DTC than those with non-multifocal DTC. Also, the association between rs2854744 and an increased risk of DTC remained significant in subjects with a family history of cancer. Multifocal loci of DTC often arise from independent clonal events, implicating predisposing influences of inherited and/or environmental factors across different locations within the thyroid gland.³³ A family history of cancer is suggestive of inherited susceptibility to cancer and/or environmental influences shared within families as well. In this case, *IGFBP-3* SNPs were in linkage with both DTC multifocality and cancer family history, suggesting the involvement of genetic factors in predisposition to thyroid cancer.

We did not observe an apparent difference in risk estimates between BTD and DTC risk associated with *IGFBP-3* rs2854744 and rs2132572 polymorphisms, suggesting that BTD and DTC may share common predisposition factors. We should not exclude the possibility that marginal effect in malignant transformation expected by the polymorphic *IGFBP-3* has not been detected in such sample size study. History of BTD is a risk factor for DTC, but it is not clear whether BTD predisposes to DTC or BTD is at intermediate stage of DTC development. More research is warranted to evaluate the role of polymorphic *IGFBP-3* in thyroid tumorigenesis.

These results ought to be interpreted with caution. The statistically significant results may be due to chance because a large number of association-seeking statistical tests have been performed. The association of rs2854744 with disease risk was no longer significant after application of the Bonferroni correction. In contrast, the Bonferroni correction did not alter the significance of the association between rs2132572 and cancer risk overall or when tested in various subgroups. On the other hand, because SNPs within the same gene may be in linkage disequilibrium, the Bonferroni correction may be too conservative and inadvertently negate a valid and biologically true association. To appropriately evaluate the issue of multiple testing in this study, we additionally applied the FPRP approach.²² As described above, several studies have found an association between these 2 significant SNPs and altered activity of IGFBP-3 and/or circulating IGFBP-3 level.^{24–27} This effect increases the prior probability to identify an association; thus, a prior probability of 0.1 (or less) should be appropriate. The association between rs2854744 and DTC risk was still significant at the FPRP threshold of 0.5 for a prior probability of 0.1.

In the present study, *IGF-1* CA repeat polymorphism was not associated with risk of DTC and BTD. The molecular basis for this polymorphism is variations in the length of a simple tandem CA repeat in the *IGF-1* promoter region; these variations have frequently been included in previous epidemiological studies of cancer risk. We identified 10 alleles, and (CA)₁₉ was the most common allele. This is in agreement with a previous systematic review, which found the 19 CA repeat allele to be the most common allele in Caucasian populations with a frequency between 59% and 70%.¹⁷ Although this polymorphism has

been speculated to alter the promoter activity,^{32, 34} no *in vitro* studies have confirmed its effect on *IGF-1* expression. Also, epidemiological studies on associations between the CA repeat polymorphism and circulating IGF-1 levels and/or cancer risk have yielded conflicting results.^{35–38} It was proposed that fractionation of the population by the large number of alleles of CA repeat polymorphism makes statistical analysis difficult.²⁵ A recent meta-analysis suggested that *IGF-1* CA repeat polymorphism is unlikely to be a major determinant of susceptibility to cancer in Caucasians.¹⁶ In general, our results do not support *IGF-1* CA repeat polymorphism as a genetic biomarker for thyroid cancer susceptibility, although this needs to be further confirmed in larger studies.

This study has several limitations. First, although it had an 80% power to detect a moderate OR associated with DTC risk, the sample size was not large enough to rule out the possibility of type II error in the analysis. The sample size also limited the statistical power in stratification analysis. Second, this was a hospital-based case-control study, and therefore, there are potential selection biases in generalizing the results to the population at large. In addition, we included only non-Hispanic Caucasian subjects, and hence, the results are not generalizable to other ethnicities. Nonetheless, whereas population stratification is usually a major source of confounding in genetic association analysis, such a problem is very unlikely in our study. Finally, it is noted that select polymorphisms may not directly affect the function of a given gene, and the observed association with disease may be due to SNPs that are in linkage disequilibrium with such polymorphisms rather than the polymorphisms themselves.

To the best of our knowledge, this is the first study of the association between *IGFBP-3* SNPs and thyroid cancer risk. The present findings suggest that *IGFBP-3* SNP rs2132572 is associated with DTC, particularly multifocal DTC. This SNP exhibited a stronger protective effect against DTC in subjects with a first-degree family history of cancer and nondrinkers. In conclusion, we now have genetic epidemiology evidence for a possible role of polymorphic *IGFBP-3* in DTC development. Further studies are needed to explore the mechanistic relevance of such observations and to further elucidate the role of the IGFBP3/IGF-1 system on thyroid carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BTD	benign thyroid disease
CI	confidence interval

DTC	differentiated thyroid carcinoma
FPRP	false-positive report probability
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
OR	odds ratio
PTC	papillary thyroid carcinoma
SNP	single nucleotide polymorphism

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin.* 2010; 60(5):277–300. [PubMed: 20610543]
2. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973–2002. *JAMA.* 2006; 295(18):2164–2167. [PubMed: 16684987]
3. Morris LG, Myssiorek D. Improved detection does not fully explain the rising incidence of well-differentiated thyroid cancer: a population-based analysis. *Am J Surg.* 2010; 200(4):454–461. [PubMed: 20561605]
4. Ron E, Lubin JH, Shore RE, et al. Thyroid cancer after exposure to external radiation: a pooled analysis of seven studies. *Radiat Res.* 1995; 141(3):259–277. [PubMed: 7871153]
5. Vella V, Sciacca L, Pandini G, et al. The IGF system in thyroid cancer: new concepts. *Mol Pathol.* 2001; 54(3):121–124. [PubMed: 11376121]
6. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. *Endocr Rev.* 2000; 21(3):215–244. [PubMed: 10857553]
7. Ciampolillo A, De Tullio C, Giorgino F. The IGF-I/IGF-I receptor pathway: implications in the pathophysiology of thyroid cancer. *Curr Med Chem.* 2005; 12(24):2881–2891. [PubMed: 16305477]
8. 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; 119(2):940–942. [PubMed: 2874015]
9. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev.* 2002; 23(6):824–854. [PubMed: 12466191]
10. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet.* 2004; 363(9418):1346–1353. [PubMed: 15110491]
11. Chen W, Wang S, Tian T, et al. Phenotypes and genotypes of insulin-like growth factor 1, IGF-binding protein-3 and cancer risk: evidence from 96 studies. *Eur J Hum Genet.* 2009; 17(12):1668–1675. [PubMed: 19491931]
12. Chen B, Liu S, Xu W, Wang X, Zhao W, Wu J. IGF-I and IGFBP-3 and the risk of lung cancer: a meta-analysis based on nested case-control studies. *J Exp Clin Cancer Res.* 2009; 28:89. [PubMed: 19549343]
13. Holmes MD, Pollak MN, Hankinson SE. Lifestyle correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev.* 2002; 11(9):862–867. [PubMed: 12223430]
14. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. *Cancer Epidemiol Biomarkers Prev.* 2002; 11(9):852–861. [PubMed: 12223429]
15. Harrela M, Koistinen H, Kaprio J, et al. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J Clin Invest.* 1996; 98(11):2612–2615. [PubMed: 8958225]

16. Chen X, Guan J, Song Y, et al. IGF-I (CA) repeat polymorphisms and risk of cancer: a meta-analysis. *J Hum Genet.* 2008; 53(3):227–238. [PubMed: 18188667]
17. Fletcher O, Gibson L, Johnson N, et al. Polymorphisms and circulating levels in the insulin-like growth factor system and risk of breast cancer: a systematic review. *Cancer Epidemiol Biomarkers Prev.* 2005; 14(1):2–19. [PubMed: 15668470]
18. Ho T, Li G, Lu J, Zhao C, Wei Q, Sturgis EM. Association of XRCC1 polymorphisms and risk of differentiated thyroid carcinoma: a case-control analysis. *Thyroid.* 2009; 19(2):129–135. [PubMed: 19191745]
19. Mickey RM, Greenland S. The impact of confounder selection criteria on effect estimation. *Am J Epidemiol.* 1989; 129(1):125–137. [PubMed: 2910056]
20. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics.* 2005; 21(2):263–265. [PubMed: 15297300]
21. Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics.* 2006; 22(15):1928–1929. [PubMed: 16720584]
22. Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N. Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. *J Natl Cancer Inst.* 2004; 96(6):434–442. [PubMed: 15026468]
23. Yu H, Li BD, Smith M, Shi R, Berkel HJ, Kato I. Polymorphic CA repeats in the IGF-I gene and breast cancer. *Breast Cancer Res Treat.* 2001; 70(2):117–122. [PubMed: 11768601]
24. Deal C, Ma J, Wilkin F, et al. Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. *J Clin Endocrinol Metab.* 2001; 86(3):1274–1280. [PubMed: 11238520]
25. Canzian F, McKay JD, Cleveland RJ, et al. Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. *Br J Cancer.* 2006; 94(2):299–307. [PubMed: 16404426]
26. Ren Z, Cai Q, Shu XO, et al. Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP-3 protein levels among Chinese women. *Cancer Epidemiol Biomarkers Prev.* 2004; 13(8):1290–1295. [PubMed: 15298948]
27. Al-Zahrani A, Sandhu MS, Luben RN, et al. IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. *Hum Mol Genet.* 2006; 15(1):1–10. [PubMed: 16306136]
28. Moon JW, Chang YS, Ahn CW, et al. Promoter -202 A/C polymorphism of insulin-like growth factor binding protein-3 gene and non-small cell lung cancer risk. *Int J Cancer.* 2006; 118(2):353–356. [PubMed: 16049980]
29. Safarinejad MR, Shafiei N, Safarinejad SH. The association between bladder cancer and a single nucleotide polymorphism (rs2854744) in the insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) gene. *Arch Toxicol.* 2011; 10.1007/s00204-011-0671-8
30. Pechlivanis S, Wagner K, Chang-Claude J, Hoffmeister M, Brenner H, Forsti A. Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer. *Cancer Detect Prev.* 2007; 31(5):408–416. [PubMed: 18031946]
31. Xiang H, Liu L, Chu GD, et al. Association between two functional polymorphisms of insulin-like growth factor binding protein 3 and colorectal cancer risk in a Chinese population. *J Toxicol Environ Health A.* 2009; 72(11–12):706–711. [PubMed: 19492232]
32. McElholm AR, McKnight AJ, Patterson CC, Johnston BT, Hardie LJ, Murray LJ. A population-based study of IGF axis polymorphisms and the esophageal inflammation, metaplasia, adenocarcinoma sequence. *Gastroenterology.* 2010; 139(1):204–212. [PubMed: 20403354]
33. Shattuck TM, Westra WH, Ladenson PW, Arnold A. Independent clonal origins of distinct tumor foci in multifocal papillary thyroid carcinoma. *N Engl J Med.* 2005; 352(23):2406–2412. [PubMed: 15944425]
34. Zecevic M, Amos CI, Gu X, et al. IGF1 gene polymorphism and risk for hereditary nonpolyposis colorectal cancer. *J Natl Cancer Inst.* 2006; 98(2):139–143. [PubMed: 16418517]
35. DeLellis K, Ingles S, Kolonel L, et al. IGF1 genotype, mean plasma level and breast cancer risk in the Hawaii/Los Angeles multiethnic cohort. *Br J Cancer.* 2003; 88(2):277–282. [PubMed: 12610514]

36. Jernstrom H, Deal C, Wilkin F, et al. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. *Cancer Epidemiol Biomarkers Prev.* 2001; 10(4):377–384. [PubMed: 11319179]
37. Sarkissyan M, Mishra DK, Wu Y, Shang X, Sarkissyan S, Vadgama JV. IGF gene polymorphisms and breast cancer in African-American and Hispanic women. *Int J Oncol.* 2011; 38(6):1663–1673. [PubMed: 21455574]
38. Rietveld I, Janssen JA, Hofman A, Pols HA, van Duijn CM, Lamberts SW. A polymorphism in the IGF-I gene influences the age-related decline in circulating total IGF-I levels. *Eur J Endocrinol.* 2003; 148(2):171–175. [PubMed: 12590635]

Table 1

Characteristics of DTC cases, BTD cases, and cancer-free controls

Variable	Controls (No. = 401)		BTD Cases (No. = 101)		DTC Cases (No. = 173)	
	No.	(%)	No.	(%)	No.	(%)
Age, years						
<30	11	(2.7)	8	(7.9)	24	(13.9)
30–45	133	(33.2)	25	(24.8)	63	(36.4)
>45	257	(64.1)	68	(67.3)	86	(49.7)
Sex						
Male	200	(49.9)	23	(22.8)	69	(39.9)
Female	201	(50.1)	78	(77.2)	104	(60.1)
Body mass index, kg/m ²						
Underweight (18.5)	4	(1.1)	2	(2.1)	4	(2.4)
Normal weight (18.5–24.9)	112	(29.6)	33	(34.0)	54	(32.7)
Overweight (25–29.9)	148	(39.1)	36	(37.1)	62	(37.6)
Obese (30)	115	(30.3)	26	(26.8)	45	(27.3)
First degree family history of cancer						
Yes	219	(56.2)	63	(62.4)	95	(54.9)
No	171	(43.9)	38	(37.6)	78	(45.1)
First degree family history of thyroid cancer						
Yes	5	(1.3)	7	(6.9)	13	(7.5)
No	385	(98.7)	94	(93.1)	160	(92.5)
Smoking status						
Ever (100 cigarettes/lifetime)	176	(44.4)	35	(34.7)	58	(33.5)
Never (<100 cigarettes/lifetime)	220	(55.6)	66	(65.4)	115	(66.5)
Alcohol drinking status						
Ever (1 drink/week for 1 year)	209	(52.8)	49	(48.5)	77	(44.5)
Never	187	(47.2)	52	(51.5)	96	(55.5)
Radiation exposure						
Yes	6	(1.6)	3	(3.0)	7	(4.1)
No	372	(98.4)	98	(97.0)	166	(96.0)

Abbreviations: BTD, benign thyroid disease; DTC, differentiated thyroid carcinoma.

* P values were calculated using chi-square test or Fisher's exact test as appropriate.

Table 2
Odds ratios of benign thyroid disease associated with *IGF-I* and *IGFBP-3* polymorphism

Polymorphism	Model*	Genotype	Controls (No.=401)		BTD cases (No.=101)		Adjusted OR [†] (95% CI)
			No.	%	No.	%	
IGF-1 CA repeat	/	19/19	179	(45.0)	47	(47.5)	Reference
		19/non-19	173	(43.5)	43	(43.4)	1.0 (0.6–1.5)
		non-19/non-19	46	(11.6)	9	(9.1)	0.8 (0.3–1.7)
IGFBP-3							
rs2132571	Dominant	GG	183	(45.6)	57	(56.4)	Reference
		GA/AA	218	(54.4)	44	(43.6)	0.7 (0.4–1.0)
rs2132572	Dominant	GG	229	(57.1)	71	(70.3)	Reference
		GA/AA	172	(42.9)	30	(29.7)	0.6 (0.4–0.9) [‡]
rs2854744	Recessive	CC/CA	324	(80.8)	70	(69.3)	Reference
		AA	77	(19.2)	31	(30.7)	1.8 (1.1–2.9) [‡]
rs13241830	Dominant	CC	186	(46.4)	56	(55.5)	Reference
		CT/TT	215	(53.6)	45	(44.5)	0.7 (0.5–1.1)

Abbreviations: BTD, benign thyroid disease; CI, confidence interval; *IGF-I*, insulin-like growth factor-1; *IGFBP-3*, insulin-like growth factor binding protein-3; OR, odds ratio.

^{*}The best inheritance model was determined by Akaike information criterion, and only the OR estimates for the best model for each SNP are provided.

[†]ORs and 95% CIs were calculated using unconditional logistic regression model adjusted for sex and age.

[‡]*P*value<0.05.

Table 3
Odds ratios for differentiated thyroid carcinoma associated with *IGF-I* and *IGFBP-3* polymorphism

Polymorphism	Model *	Genotype	Controls (No.=401)		DTC cases (No.=173)		Adjusted OR [†] (95% CI)		PTC cases (No.=156)		Adjusted OR [†] (95% CI)		Multifocal DTC (No.=42)		Adjusted OR [†] (95% CI)	
			No.	%	No.	%			No.	%			No.	%		
IGF-I	CA repeat	19/19	179	(45.0)	74	(44.0)	Reference		64	(42.4)	Reference		20	(48.8)	Reference	
		19/non-19	173	(43.5)	80	(47.6)	1.1 (0.7–1.6)		76	(50.3)	1.2 (0.8–1.8)		19	(46.3)	0.9 (0.5–1.7)	
		non-19/non-19	46	(11.6)	14	(8.3)	0.7 (0.4–1.4)		11	(7.3)	0.6 (0.3–1.3)		2	(4.9)	0.4 (0.1–1.6)	
IGFBP-3	rs2132571	Dominant	183	(45.6)	88	(50.9)	Reference		78	(50.0)	Reference		19	(45.2)	Reference	
		GA/AA	218	(54.4)	85	(49.1)	0.8 (0.6–1.1)		78	(50.0)	0.8 (0.6–1.2)		23	(54.8)	1.0 (0.5–2.0)	
	rs2132572	Dominant	229	(57.1)	120	(69.4)	Reference		111	(71.2)	Reference		34	(81.0)	Reference	
		GA/AA	172	(42.9)	53	(30.6)	0.6 (0.4–0.9) [‡]		45	(28.9)	0.6 (0.4–0.8) [‡]		8	(19.1)	0.3 (0.1–0.7) [‡]	
	rs2854744	Dominant	125	(31.2)	40	(23.1)	Reference		37	(23.7)	Reference		7	(16.7)	Reference	
		CA/AA	276	(68.8)	133	(76.9)	1.6 (1.0–2.4) [‡]		119	(76.3)	1.5 (1.0–2.3)		35	(83.3)	2.3 (1.0–5.4)	
rs13241830	Dominant	CC	186	(46.4)	87	(50.3)	Reference		77	(49.4)	Reference		19	(45.2)	Reference	
		CT/TT	215	(53.6)	86	(49.7)	0.8 (0.6–1.2)		79	(50.6)	0.9 (0.6–1.2)		23	(54.8)	1.1 (0.6–2.0)	

Abbreviations: CI, confidence interval; DTC, differentiated thyroid carcinoma; *IGF-I*, insulin-like growth factor-1; *IGFBP-3*, insulin-like growth factor binding protein-3; OR, odds ratio; PTC, papillary thyroid carcinoma.

* The best inheritance model was determined by Akaike information criterion, and only the OR estimates for the best model for each SNP are provided.

[†] ORs and 95% CIs were calculated using unconditional logistic regression model adjusted for sex and age.

[‡] $P < 0.05$.

Table 4
Stratification analysis of differentiated thyroid carcinoma risk associated with *IGFBP-3* polymorphism*

	Cases/Controls	rs2132571	rs2132572	rs2854744	rs13241830
Age					
< 48 years	98/186	0.8 (0.5–1.4)	0.6 (0.4–1.0)	1.1 (0.6–1.9)	0.9 (0.5–1.4)
48 years	75/215	0.8 (0.4–1.3)	0.6 (0.4–1.1)	2.6 (1.4–5.2) [‡]	0.8 (0.5–1.3)
<i>P</i> _{Interaction}		0.81	0.96	0.038	0.72
Sex					
Female	104/201	0.8 (0.5–1.3)	0.6 (0.3–1.0)	1.6 (0.9–2.7)	0.9 (0.5–1.4)
Male	69/200	0.8 (0.4–1.3)	0.7 (0.4–1.2)	1.6 (0.8–3.1)	0.8 (0.4–1.3)
<i>P</i> _{Interaction}		0.92	0.68	0.96	0.69
Body mass index					
< 25 kg/m ²	58/116	0.8 (0.4–1.5)	0.4 (0.2–0.9) [‡]	1.5 (0.7–3.2)	1.0 (0.5–1.8)
25 kg/m ²	107/263	0.8 (0.5–1.3)	0.7 (0.4–1.1)	1.7 (1.0–2.8)	0.8 (0.5–1.3)
<i>P</i> _{Interaction}		0.87	0.33	0.84	0.64
First-degree family history of cancer					
Yes	95/219	0.7 (0.4–1.2)	0.4 (0.2–0.7) [‡]	1.9 (1.0–3.5) [‡]	0.8 (0.5–1.2)
No	78/171	0.9 (0.5–1.5)	1.1 (0.6–1.9)	1.2 (0.7–2.2)	0.9 (0.5–1.5)
<i>P</i> _{Interaction}		0.55	0.013	0.27	0.69
Smoking status					
Never	115/220	0.9 (0.6–1.5)	0.6 (0.4–1.0)	1.5 (0.9–2.6)	0.9 (0.6–1.4)
Ever	58/176	0.6 (0.3–1.1)	0.6 (0.3–1.2)	1.5 (0.7–3.1)	0.7 (0.4–1.3)
<i>P</i> _{Interaction}		0.31	0.94	0.98	0.60
Alcohol drinking status					
Never	96/187	1.0 (0.6–1.7)	0.4 (0.2–0.7) [‡]	1.5 (0.9–2.6)	1.0 (0.6–1.6)
Ever	77/209	0.6 (0.4–1.0)	1.0 (0.6–1.7)	1.6 (0.9–3.1)	0.7 (0.4–1.1)
<i>P</i> _{Interaction}		0.17	0.028	0.82	0.28

Abbreviations: *IGFBP-3*, insulin-like growth factor binding protein-3.

* Values in table are adjusted ORs (95% CIs), under the dominant inheritance model, using wild-type genotype as the reference group, adjusted for age and sex.

[‡] *P*<0.05.

Table 5

Haplotype frequencies in *IGFBP-3* and haplotype-specific risk

Haplotype [*]	Frequency		Adjusted OR [†] (95% CI)	P	Frequency		Adjusted OR [†] (95% CI)	P
	Controls	BTD cases			DTC cases			
G-G-A-C	0.42	0.53	Reference	-	0.51	Reference	-	-
A-G-C-T	0.29	0.26	0.7 (0.5–1.1)	0.098	0.28	0.8 (0.6–1.1)	0.126	0.126
G-A-C-C	0.23	0.18	0.6 (0.4–1.0)	0.036	0.17	0.7 (0.5–1.0)	0.030	0.030
Others [‡]	0.06	0.02	0.4 (0.1–0.9)	0.034	0.03	0.5 (0.2–1.0)	0.039	0.039

Abbreviations: BTD, benign thyroid disease; CI, confidence interval; DTC, differentiated thyroid carcinoma; *IGFBP-3*, insulin-like growth factor binding protein-3; OR, odds ratio.

^{*} *IGFBP-3* haplotypes were in the following order of SNPs rs2132571, rs2132572, rs2854744, and rs13241830.

[†] ORs and 95% CIs were calculated using unconditional logistic regression model adjusted for sex and age.

[‡] Others are the combination of rare haplotypes with frequency <0.03.

Table 6

False-positive report probability for the significant results

Association analysis	Models*	OR (95% CI)	FPRP			
			PP=0.25	PP=0.1	PP=0.01	PP=0.001
rs2132572, DTC risk	Dominant	0.6 (0.4–0.9)	0.062	0.167	0.687	0.957
rs2132572, multifocal DTC risk	Dominant	0.3 (0.1–0.7)	0.023	0.066	0.438	0.887
rs2132572, PTC risk	Dominant	0.6 (0.4–0.8)	0.029	0.084	0.501	0.910
rs2132572, BTD risk	Dominant	0.6 (0.4–0.9)	0.125	0.299	0.825	0.979
rs2132572, DTC risk, (+)family history of cancer	Dominant	0.4 (0.2–0.7)	0.009	0.026	0.224	0.745
rs2132572, DTC risk, nondrinkers	Dominant	0.4 (0.2–0.7)	0.006	0.019	0.177	0.685
rs2132572, DTC risk, BMI<25 kg/m ²	Dominant	0.4 (0.2–0.9)	0.149	0.344	0.852	0.983
rs2132572, PTC risk, (+)family history of cancer	Dominant	0.3 (0.2–0.6)	0.003	0.008	0.079	0.463
rs2132572, PTC risk, nondrinkers	Dominant	0.4 (0.2–0.6)	0.003	0.008	0.084	0.481
rs2854744, DTC risk	Dominant	1.6 (1.0–2.4)	0.175	0.389	0.875	0.986
rs2854744, BTD risk	Recessive	1.8 (1.1–2.9)	0.157	0.358	0.860	0.984
rs2854744, DTC risk, (+)family history of cancer	Dominant	1.9 (1.0–3.5)	0.186	0.406	0.883	0.987
rs2854744, DTC risk, 48 years old	Dominant	2.6 (1.4–5.2)	0.028	0.079	0.487	0.906

Abbreviations: BTD, benign thyroid disease; CI, confidence interval; DTC, differentiated thyroid carcinoma; FPRP, false-positive report probability; OR, odds ratio; PP, prior probability; PTC, papillary thyroid carcinoma.

*The inheritance model was determined by Akaike information criterion in genotype association analysis.