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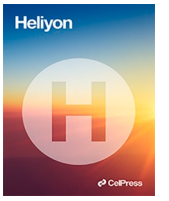

Research article

Underlying effect of SMAD4 gene polymorphism on risk prediction of papillary thyroid carcinoma in Chinese population

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ABSTRACT

Objective: This research intends to explore how variations in the SMAD4 gene impact papillary thyroid carcinoma (PTC) among patients in China.
Methods: The rs10502913 and rs12968012 polymorphisms were genotyped in 405 subjects using SNP-scan high-throughput technology. Differential mRNA expression of SMAD4 was analyzed using data from TCGA and GSE33630, and protein level expression differences were analyzed using immunohistochemistry.
Results: The results showed that SMAD4 mRNA expression was lower in thyroid cancer (THCA) tissues than in normal tissues. Immunohistochemical results showed that the expression level of SMAD4 in normal tissue, thyroid papillary carcinoma tissue and poorly differentiated tissue was significantly different. We found that SMAD4 mismatch variants (rs10502913 and rs12968012) were associated with PTC susceptibility. Specifically, the SMAD4-rs10502913 genotypes (GA and AA) showed a notable correlation with a lower likelihood of PTC in comprehensive and segmented studies (genotype GA: OR (95% CI) = 0.270 (0.077–0.950), p = 0.041; genotype AA: OR (95% CI) = 0.103 (0.025–0.416), p = 0.001). We categorized the immunohistochemical results according to genotype and found that rs10502913-GG protein level was expressed at the lowest level, and both GA and AA were higher than GG (GG vs. AA, $P < 0.05$), and rs12968012-CG protein level was expressed at the lowest level, and both GG and CC were higher than CG (GG vs. CG, $P < 0.01$).
Conclusion: Two missense variants of SMAD4 (rs10502913 and rs12968012) are associated with reduced risk of papillary thyroid carcinoma, possibly by reducing protein expression leading to susceptibility to papillary thyroid carcinoma.

1. Introduction

The incidence of thyroid cancer continues to rise worldwide [1]. Papillary thyroid cancer (PTC) is the main causative agent of overall thyroid cancer and is the only histological subtype that is systematically increasing in the countries studied [2]. Widespread

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population-based screening of asymptomatic thyroid cancer patients, not only for the diagnosis of the cancer, but also for the treatment of the cancer, may be harmless over a lifetime [3]. Therefore, it is crucial to understand the causes and risk factors of thyroid cancer to develop scientific and effective thyroid cancer prevention and treatment strategies.

The SMAD4 gene consists of 12 exons and 10 introns, and the protein encoded by this gene consists of 552 amino acids with a molecular weight of 60 KD [4]. SMAD4 plays a key role in the Transforming growth factor-β (TGFβ) signaling pathway that regulates proliferation, apoptosis, and genome stability [5]. TGFβ and other members of the TGFβ superfamily have been shown to play an important role in thyroid proliferative disorders [6]. Moreover, it has been shown that SMAD4 promoter mutations play a regulatory role in thyroid carcinogenesis [7].

Associations between SMAD4 gene polymorphisms and susceptibility to colorectal, gastric, or other cancers have been reported [8–10]. However, no study has yet reported an association between SMAD4 gene polymorphisms and papillary thyroid cancer. In this study, we compared the differences in the expression levels of SMAD4 mRNA and protein and assessed whether there is a correlation between SMAD4 gene polymorphisms and PTC susceptibility to provide new ideas for the diagnosis and treatment of PTC.

2. Materials and methods

2.1. Study subjects and ethics

A total of 405 participants were included in this study, all from the Affiliated Hospital of Guilin Medical University from August 2022 to April 2023, including 153 patients with PTC and 252 healthy controls. The races of the included population were all Han Chinese. No statistically significant difference existed in the age and sex of the subjects in the normal and PTC groups ($P > 0.05$). The subjects participating in this study were all independent individuals. The Medical Ethics Committee of the Affiliated Hospital of Guilin Medical University has reviewed and approved the study protocol (2023QTLL-36). Written Informed consent was obtained for enrollment of all subjects with a clear pathologic diagnosis and no history of other tumors in patients with PTC, and healthy individuals with no history of cancer in the control group. The diagnostic criteria for papillary thyroid carcinoma are the Diagnostic Guidelines for Thyroid Nodules and Differentiated Thyroid Cancer developed by the American Thyroid Association (ATA) in 2009 [11]. The following databases were used for bioinformatics analysis of differences in SMAD4 expression in this study: TCGA database (https://portal.gdc.cancer.gov/) and GEO database (https://www.ncbi.nlm.nih.gov/geo/).

2.2. Immunohistochemistry (IHC)

Experiments involving human tissues were approved by the Institutional Review Board of the Affiliated Hospital of Guilin Medical University and conducted in accordance with the principles of the Declaration of Helsinki (2023QTLL-36). The following primary antibody and antigen recovery protocol was used: anti-smad4 (ZM-0097, ZSGB-BIO, Beijing, China). Calculate IOD/area ratio using ImageJ.

Embedded wax blocks were sectioned and subjected to deparaffinization with graded alcohol and xylene. After rinsing in clean water, they were first soaked in hydrogen peroxide to remove endogenous catalase interference, followed by antigen repair by adding EDTA in an autoclave. After cooling at room temperature, the plates were rinsed with PBS buffer and added with serum and placed in a 37 °C incubator for 30min for containment. After incubation of primary and secondary antibodies, respectively, DAB was used to develop the coloration as well as the re-staining of hematoxylin.

2.3. Baseline information and lab experiments

Baseline information was gathered from the medical records of all researchers, encompassing details like age, gender, common tumor markers (CA199, CEA, AFP), blood glucose (Glu), lipid profile (triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c)), Hepatic function indexes (total protein (TP), albumin (ALB), globulin (GLO)), Renal function indexes (glomerular filtration rate (GFR) and Cystation C (Cysc)). Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald equation [12]. GFR was calculated using 2012 CKD-EPI cystatin C [13]. Blood glucose is measured as fasting blood glucose. All tumor markers and biochemical indices were measured using the Roche Cobas E701 or E801 analyzer.

2.4. SMAD4 polymorphism analysis

We used the same reagents and methodology as our previous study for DNA isolation, primer design, and genotyping [14]. The PCR primer sequence information are as follows: SMAD4 rs10502913 G/A (Forward: 5′-AGCATAGACTAGCCAATCCTGACTGATTCG-3′, Reverse: 5′-AGCATAGACTAGCCAATCCTGACTGATCCA-3′); SMAD4 rs12968012 C/G (Forward:5′-CTGAAGGAAAATGATGAGGAAAAGCATAG-3′, Reverse: 5′-CTGAAGGAAAATGATGAGGAAAAGCATAC-3′).

2.5. Statistical analysis

All statistical evaluations were conducted using the Statistical Product and Service Solutions (SPSS) 26.0 software (IBM Corp., Armonk, NY, USA). For normal distribution of continuous statistics, Kolmogorov-Smirnov normality tests were presented as mean ±

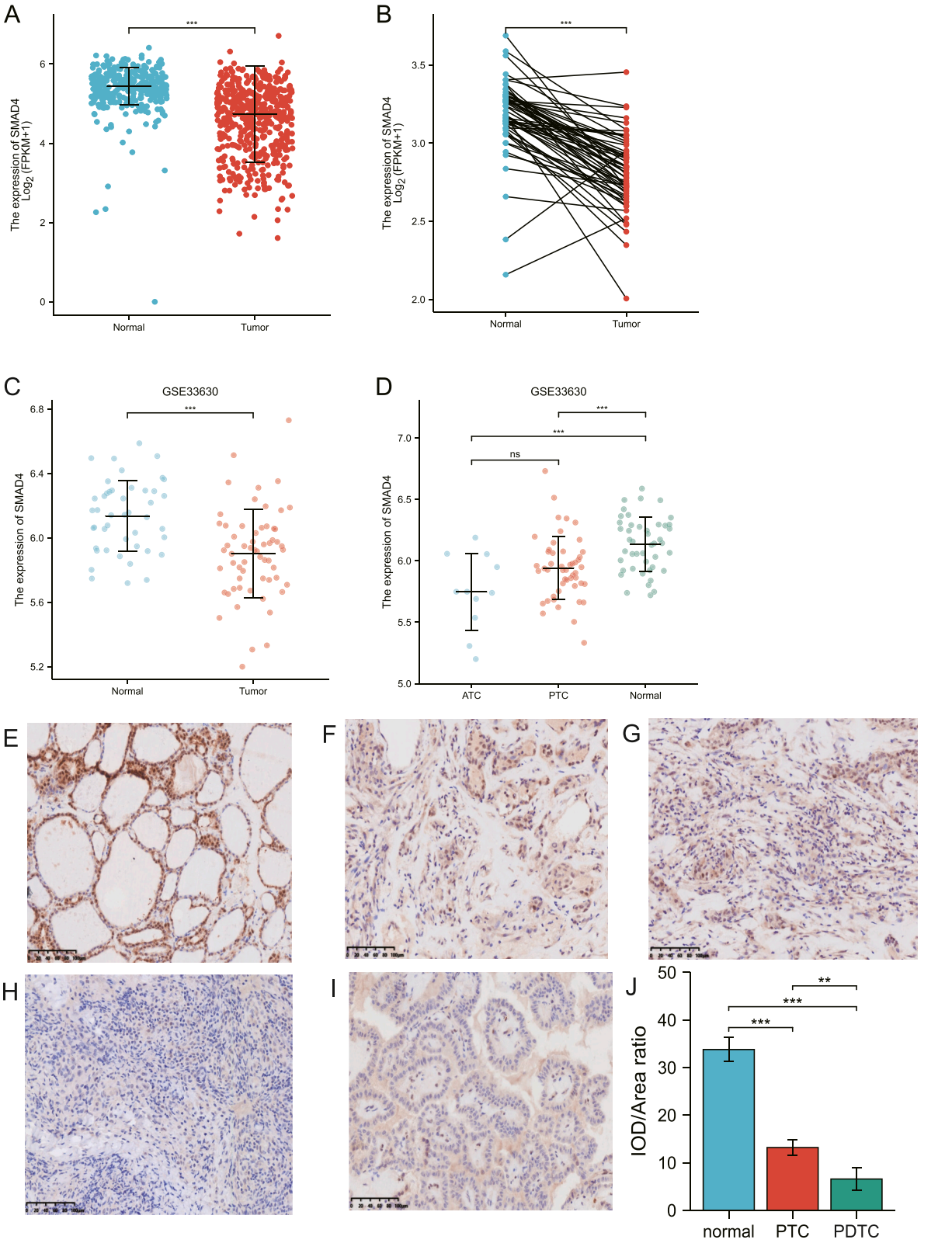

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Fig. 1. SMAD4 mRNA and protein expression in THCA patients. Immunohistochemistry images have a line scale of 20 μm of tissue size represented by 1 cm of the image. (A) SMAD4 expression in THCA tissues and normal tissues from TCGA (***, $P < 0.001$). (B) SMAD4 expression in THCA tissues and adjacent tissues (***, $P < 0.001$). (C) SMAD4 expression in THCA tissues and normal tissues from GSE33630 (***, $P < 0.001$). (D) SMAD4 expression in anaplastic thyroid carcinoma (ATC), papillary thyroid carcinoma (PTC) and normal tissues from GSE33630 (ns, $P > 0.05$ ***, $P < 0.001$). (E) Exemplary immunohistochemical images depicting the expression of SMAD4 in typical thyroid tissues. (F–G) Exemplary immunohistochemical images depicting the expression of SMAD4 in tissues of highly differentiated thyroid cancer (HDTC). (H–I) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in tissues of Poorly differentiated thyroid cancer (PDTC). (J) The ratio of IOD to area in the specified immunohistochemistry images.

standard deviation (SD), and an independent sample *t* test was employed to compare the two groups. Conversely, the Mann-Whitney *U* test facilitated the comparison of continuous measurement data that deviated from a normal distribution between the groups, denoted as a quartile [M (Q1-Q3)]. The χ^2-test was employed to examine the differences between categorical variables and Hardy-Weinberg genetic balance. Gene haplotypes were constructed using the online software SHEsis [15] (http://analysis.bio-x.cn/myAnalysis.php).The immunohistochemical analysis employed the Tukey HSD post hoc test, a type of multivariate hypothesis test. A p-value below 0.05 was deemed to hold statistical significance (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$).

3. Results

3.1. Expression of SMAD4 in patients with PTC

Our examination of SMAD4 transcription levels, utilizing the TCGA database and GSE33630, revealed a notable increase in SMAD4 mRNA expression in normal tissues compared to thyroid cancer (THCA) tissues (Fig. 1A–D). Additionally, the presence of SMAD4 protein in thyroid cancer was verified through immunohistochemical methods (Fig. 1E–J). Findings verified a reduced expression of SMAD4 in thyroid cancer tissues relative to normal thyroid tissues. Normal thyroid tissues and typical papillary thyroid carcinoma tissues were taken from the inflamed and diseased sides of the same patient, and poorly differentiated thyroid carcinoma specimens were taken from both sides of the same patient.

3.2. Diagnostic value of SMAD4 expression in THCA

We used the ROC curve to assess the diagnostic potential of SMAD4 expression in THCA. Findings indicated that SMAD4's area under the curve (AUC) stood at 0.868. Our assessment of SMAD4 expression's diagnostic potential at different phases revealed similar diagnostic effectiveness, evidenced by AUC values of 0.866, 0.861, 0.877, and 0.929 for stages I, II, III, and IV, respectively (Fig. 2A–E).

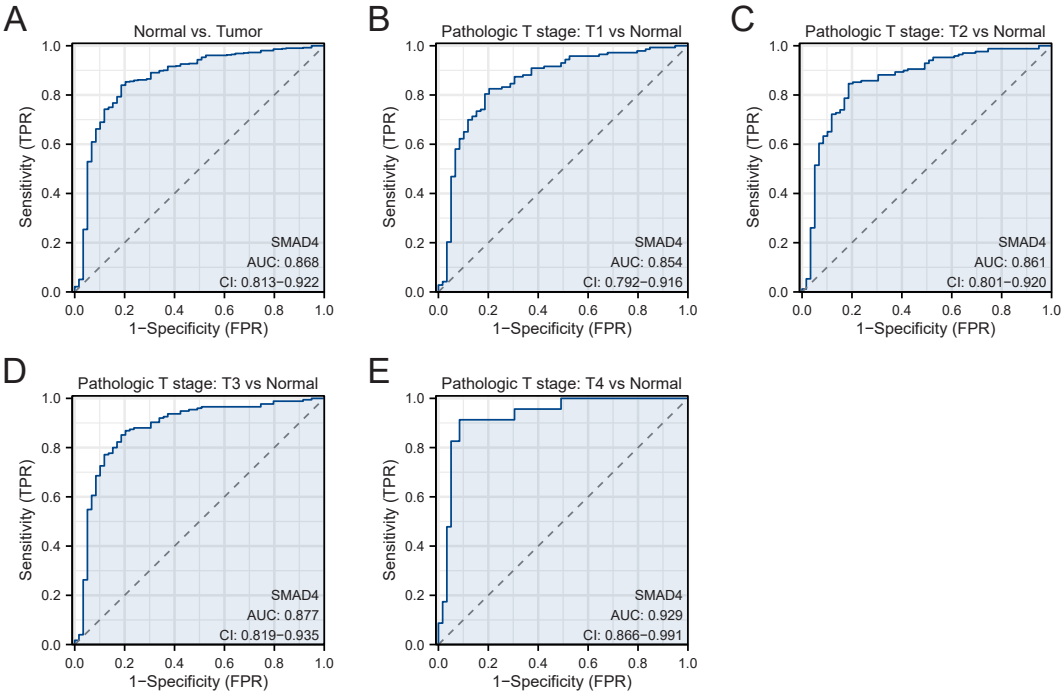


Fig. 2. ROC graph depicting SMAD4 expression in a group of thyroid cancer patients. (A) ROC graph depicting SMAD4 expression in healthy and cancerous cases, (B–E) Analyzing subgroups at stages I, II, III, and IV, in that order.

3.3. Study subjects' clinical and biochemical traits

Table 1 encapsulates the clinical and biochemical characteristics of patients categorized by their crowd state (252 Normal and 153 PTC). All liver and kidney function indices of PTC patients were statistically different from normal controls, suggesting that liver and kidney function may be impaired in PTC patients.

3.4. The relation between SMAD4 gene polymorphisms and PTC

The Hardy-Weinberg equilibrium test (both $P > 0.05$) was conducted to assess the allele frequencies of rs10502913 and rs12968012 in the control group, revealing that the genotype frequency of both sites in this population was in a state of equilibrium and exhibited representativeness. SMAD4 genotypes and allele distributions are shown in Fig. 3A–B, Table 2. The frequency of rs10502913 (GG vs. GA vs. AA, $\chi^2 = 11.961$, $p = 0.003$) as well as the allele frequency (G vs. A, $\chi^2 = 3.934$, $p = 0.047$) and allele frequency of rs12968012 (G vs. C, $\chi^2 = 4.090$, $p = 0.043$) showed significant correlations between groups, except for a significant correlation with the rs12968012's frequency (GG vs. CG vs. CC, $\chi^2 = 4.559$, $p = 0.102$). And the recessive models were all statistically different (AA vs. GA + GG, $\chi^2 = 11.604$, $p = 0.001$; GG vs. GA + GG, $\chi^2 = 4.247$, $p = 0.039$).

The incidence rate of papillary thyroid cancer was calculated using binary logistic regression, considering various complicating factors like age, gender, dyslipidemia, blood glucose, and liver and kidney function. The results of binary logistic regression (with [Normal vs. PTC] group as the dependent variable and age, gender, smoking status, alcohol consumption, CA199, CEA, AFP, TP, ALB, GLO, A/G, TG, CHOL, HDL-c, LDL-c, GRF, Cysc, Glu, and SMAD4 genotypes as covariates) showed that there was a significant association between SMAD4 gene polymorphisms and papillary-like thyroid cancer after correction for confounders (Figure-3C).

3.5. Relationship between SMAD4 protein expression levels and polymorphisms

We classified the immunohistochemical findings based on genotype and discovered that the expression of rs10502913-GG protein was at its minimum, with both GA and AA surpassing GG (GG vs. AA, $P < 0.05$, Fig. 4A–D), and the protein level of rs12968012-CG was the lowest, with both GG and CC surpassing CG (GG vs. CG, $P < 0.01$, Fig. 4E–H).

3.6. Haplotype analysis of SMAD4 gene

In this study, haplotypes were constructed at the rs10502913 and rs12968012 loci of the SMAD4 gene by SHEsis software, and haplotypes with a frequency of 3% or more were analyzed. The results, as found in Table 3, showed that the difference in the distribution of AG and GC frequencies among the three constructed haplotypes was statistically significant between PTC patients and normal controls (AG $P = 0.047$, OR (95% CI): 1.356 (1.003–1.834); GC $P = 0.043$, OR (95% CI): 0.746 (0.561–0.991)).

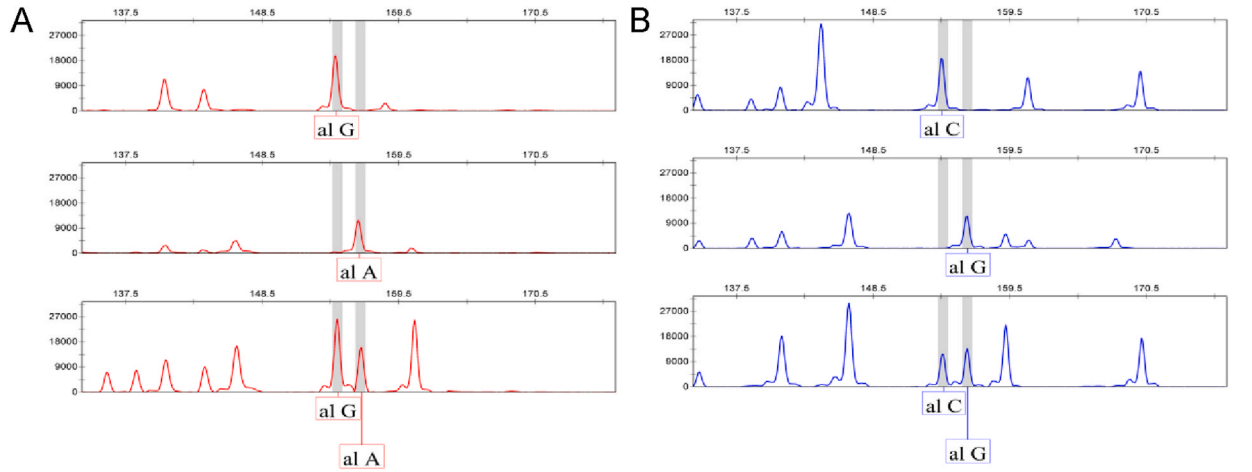
4. Discussion

Our research focused on the link between two missense forms of SMAD4 and the vulnerability to PTC in 405 subjects. Combined

Table 1
Clinical characteristics of the study subjects.

Variables	Total	Normal	PTC	P value
n	405	252	153	–
Age (years)	47 (38.00–56.00)	48.00 (38.50–56.50)	47.00 (36.00–56.00)	0.600
M:F	110/295	69/183	41/112	0.917*
Smoking status	13/392	10/242	3/150	0.269*
Drinking status	4/401	3/249	1/152	0.914*
CA199(U/L)	15.195 (10.663–23.085)	17.630 (11.320–27.820)	13.080 (9.680–18.105)	<0.001
CEA (U/L)	1.26 (0.81–1.95)	1.26 (0.78–2.03)	1.27 (0.89–1.85)	0.627
AFP(U/L)	2.800 (1.938–3.830)	3.005 (2.220–4.208)	2.465 (1.780–3.458)	<0.001
TP (g/L)	75.30 (72.15–77.65)	75.00 (72.05–76.95)	75.85 (72.35–79.48)	<0.001
ALB (g/L)	44.97 ± 2.86	45.48 ± 2.42	44.11 ± 3.30	<0.001**
GlO (g/L)	29.85 ± 3.69	28.84 ± 2.84	31.53 ± 4.29	<0.001**
A/G	1.53 (1.40–1.66)	1.57 (1.45–1.69)	1.42 (1.27–1.58)	<0.001
TG (mmol/L)	1.37 (0.89–2.01)	1.31 (0.86–1.85)	1.46 (0.98–2.26)	0.028
CHOL (mmol/L)	5.07 (4.51–5.83)	5.05 (4.47–5.73)	5.12 (4.60–6.13)	0.124
HDL-c (mmol/L)	1.37 (1.15–1.64)	1.39 (1.15–1.67)	1.34 (1.11–1.61)	0.208
LDL-c (mmol/L)	3.16 (2.63–3.73)	3.12 (2.58–3.69)	3.28 (2.71–4.14)	0.139
GRF (ml/min)	97.13 (82.83–111.54)	98.56 (84.70–115.29)	93.07 (79.53–109.75)	0.013
Cysc (mg/L)	0.82 (0.73–0.94)	0.81 (0.71–0.93)	0.85 (0.74–0.97)	0.015
Glu (mmol/L)	5.40 (5.08–5.87)	5.40 (5.13–5.83)	5.35 (4.88–6.04)	0.336

All variables were calculated by Kolmogorov-Smirnov test, data with normal distribution was indicated by mean ± standard deviation (SD), otherwise, it was presented by median (inter-quartile range, P25–P75).
The P values were calculated by * Pearson chi-square test, ** Independent-sample T test and blank Mann-Whitney *U* test, separately.



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Characteristics	Normal(n%)	PTC(n%)	HR(95%CI)	P value
rs10502913				
GG	119(47.2)	68(44.4)	Reference	
GA	117(46.4)	59(38.6)	0.270(0.077-0.950)	0.041
AA	16(6.3)	26(17.0)	0.103(0.025-0.416)	0.001
G	355(70.4)	195(63.7)	Reference	
A	149(29.6)	111(36.3)	0.961(0.557-1.690)	0.89
AA	16(6.3)	26(17.0)	Reference	
GA+GG	236(93.7)	127(83.0)	2.073(1.483-17.351)	0.01
rs12968012				
GG	50(19.8)	44(28.8)	Reference	
CG	125(49.6)	71(46.4)	0.825(0.285-2.385)	0.735
CC	77(30.6)	38(24.8)	0.471(0.201-1.101)	0.079
G	225(44.6)	159(52.0)	Reference	
C	279(55.4)	147(48.0)	0.829(0.492-1.396)	0.48
GG	50(19.8)	44(28.8)	Reference	
CG+CC	202(80.2)	109(71.2)	1.292(0.495-3.370)	0.601

Fig. 3. Capillary electrophoresis peak map and analysis of regression. (A) rs10502913 (B) rs12968012 (C) P Value was adjusted by age, gender, smoking status, alcohol consumption, CA199, CEA, AFP, TP, ALB, GLO, A/G, TG, CHOL, HDL-c, LDL-c, GRF, Cysc, Glu and SMAD4 genotype as covariates.

Table 2
SMAD4 gene polymorphism in Chinese patients with papillary thyroid carcinoma.

Genotype and allele		Total	Normal	PTC	comparsion	χ^2	P value
SMAD4 rs10502913	GG	187	119 (47.2)	68 (44.4)	GG vs GA + AA	0.296	0.587
	GA	176	117 (46.4)	59 (38.6)	AA vs GA + GG	11.604	0.001
	AA	42	16 (6.3)	26 (17.0)	GA vs GG + AA	2.398	0.122
	Total	405	252 (100)	153 (100)	GG vs GA vs AA	11.916	0.003
	G	550	355 (70.4)	195 (63.7)	G vs A	3.934	0.047
	A	260	149 (29.6)	111 (36.3)			
SMAD4 rs12968012	GG	94	50 (19.8)	44 (28.8)	GG vs CG + CC	4.247	0.039
	CG	196	125 (49.6)	71 (46.4)	CC vs CG + GG	1.531	0.216
	CC	115	77 (30.6)	38 (24.8)	CG vs CC + GG	0.390	0.532
	Total	405	289 (100)	168 (100)	GG vs CG vs CC	4.559	0.102
	G	384	225 (44.6)	159 (52.0)	G vs C	4.090	0.043
	C	426	279 (55.4)	147 (48.0)			

SMAD4, Mothers against decapentaplegic homolog 4; PTC, papillary thyroid carcinoma; SNP, single nucleotide polymorphism.Date are shown as n (percent).The P values were calculated by chi-square test.

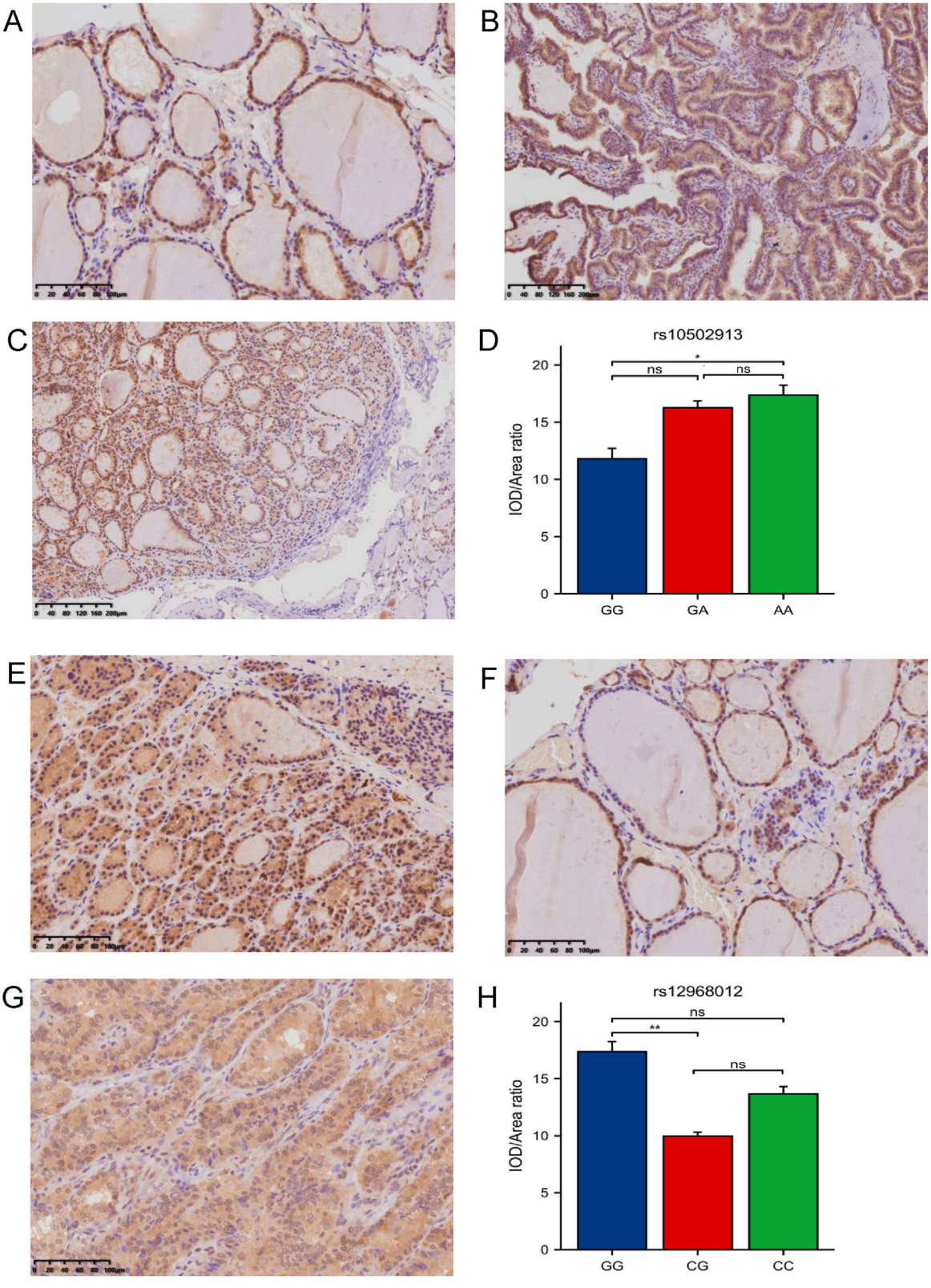

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Fig. 4. Relationship between protein expression and genotype of SMAD4 in PTC patients. Immunohistochemistry images have a line scale of 20/40 μm of tissue size represented by 1 cm of the image. (A) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs10502913 GG PTC tissues. (B) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs10502913 GA PTC tissues. (C) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs10502913 AA PTC tissues. (D) The ratio of IOD to area in the specified immunohistochemistry images of GG, GA, and AA tissues from PTC patients (ns, $P > 0.05$ *, $P < 0.05$). (E) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs12968012 GG PTC tissues. (F) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs12968012 CG PTC tissues. (G) Exemplary immunohistochemical visuals depicting the expression of SMAD4 in rs12968012 CC PTC tissues. (H) The ratio of IOD to area in the specified immunohistochemical images of GG, CG, and CC tissues from PTC patients (ns, $P > 0.05$ **, $P < 0.01$).

Table 3
Haplotype analysis of SMAD4 (rs10502913-rs12968012) gene in Chinese patients with papillary thyroid carcinoma.

Haplotype	Normal	PTC	P value	OR (95% CI)
AG	111 (36.3)	149 (29.6)	0.047	1.356 (1.003–1.834)
GC	147 (48.0)	279 (55.4)	0.043	0.746 (0.561–0.991)
GG	48 (15.7)	76 (15.0)	0.816	1.048 (0.707–1.552)

Date are shown as n(percent).The P values were calculated by Pearson test.

correlation analysis and comparison of expression differences revealed that two candidate missense variants of SMAD4 (rs10502913 and rs12968012) were associated with PTC risk. In overall and stratified analyses, SMAD4-rs10502913 genotypes GA and AA were significantly associated with reduced PTC risk, and SMAD4-rs12968012 genotype GG was also significantly associated with reduced PTC risk in the recessive model. This implies that SMAD4-rs10502913 and -rs12968012 might act as genetic safeguards against thyroid cancer. As far as we are aware, this research pioneers the discovery of a link between SMAD4 gene polymorphisms and the risk of thyroid cancer in the Chinese population.

Early diagnosis and screening of thyroid cancer and overmedication have been controversial, mainly due to the posteriority of imaging methods and tumor markers, which are mostly detected after the presence of nodules and masses or clinical symptoms [16,17]. Testing for polymorphic loci can provide personalized care based on the genetic background of the population and can guide clinical risk assessment and drug use [18]. Consequently, investigating the link between genetic variations and the emergence of diseases holds substantial clinical importance. Thyroid hormones, as key hormones in the endocrine system, play an important role in the functioning of the body's liver and kidneys [19,20] and in the metabolism of blood glucose [21] and lipids [20]. Meanwhile, whether there is an association between smoking and alcohol consumption and the development of thyroid cancer has also been the subject of ongoing research. Some studies [22] have shown reduced thyroid incidence in smoking men, not found in female patients. Our study also found a much smaller composition in smoking men than in non-smoking men. However, studies [23] indicate a correlation between healthier living habits, such as abstaining from smoking and alcohol, and reduced incidences of thyroid cancer. Therefore, we analyzed these potential confounders by putting them into covariates in the regression analysis. Merging prior research with our results, we theorized that the two missense forms of SMAD4 pose a risk for PTC among the Chinese, suggesting that the genetic risk factors linked to SMAD4 might remain unaffected by these possible risk elements.

In recent years, SMAD4 has received special attention in cancer research, mainly because it is associated with Wnt [24], TP53 [25], and TGF-β signaling pathways as a tumor suppressor, and serves as a fundamental gene in the TGF-β signaling route [4]. In addition, SMAD4 mutations occur in a variety of tumors, and thyroid tumors exhibit the presence of complex Smad4 mutations and aberrant splicing patterns [26], in which the ubiquitinated protease system is the main reason for the under-expression of SMAD4 proteins and the loss of tumor suppressor function [27]. The analysis of the TCGA database and GSE33630 validation in this study confirmed the significant down-regulation of SMAD4 mRNA in patients with thyroid cancer, while immunohistochemistry was performed for further validation at the protein level. Furthermore, our analysis of the database revealed a strong correlation between low SMAD4 expression and the diagnosis of THCA patients, and the ROC curve indicated a high level of diagnostic accuracy. While there's a strong link between SMAD4 expression and thyroid cancer, the way it regulates thyroid cancer remains uncertain. Alterations in the sequence of amino acids lead to modifications in the structure of proteins, which is intricately linked to their functionality [28]. Our results of classifying immunohistochemical results according to genotype also confirmed the differences in SMAD4 protein expression in PTC patients with different genotypes. Therefore, our research suggests that the missense variants could modify the sequence of amino acids, resulting in changes to the configuration of SMAD proteins, thereby impacting the functioning of pathways linked to cancer, like the TGF-β signaling route, and possibly elevating the probability of PTC. Nevertheless, the aforementioned is mere conjecture, necessitating additional mechanistic investigations to explore the impact of these two potential missense variants on PTC susceptibility through their influence on SMAD4 expression in Chinese. No matter what, this research provides a dependable theoretical basis for understanding how SMAD4 contributes to the advancement of PTC. In the meantime, it presents novel concepts for evaluating risks and tailoring personalized approaches to prevent and treat thyroid cancer in the Chinese population.

It should be noted that our research and analysis have certain constraints. Initially, our research concentrated on the SMAD4 polymorphic site, and future investigations should delve deeper into SMAD4's mechanisms in thyroid cancer cells and PDX mice, including its impact on follicular and poorly differentiated thyroid cancer patients. Additionally, there was a decrease in the case count for certain indicators, attributed to the absence of foundational clinical data. Ultimately, our research was structured as a cross-sectional, single-center study, necessitating additional longitudinal research.

5. Conclusion

In summary, two missense variants of SMAD4 (rs10502913 and rs12968012) are associated with reduced risk of papillary thyroid carcinoma, possibly by reducing protein expression leading to susceptibility to papillary thyroid carcinoma.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Guilin Medical University Hospital (2023QTLL-36). Written informed consent to participate in this study was provided by the participants. Written informed consent was obtained from individuals for the release of any potentially recognizable images or data included in this article. All methods were performed in accordance with relevant guidelines and regulations.

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Data availability statement

The supporting data for this study's conclusions can be obtained from the corresponding author (Yongchao Qiao, qiaoyc@glmc.edu.cn), if requested sensibly.

CRediT authorship contribution statement

Chao Zuo: Writing – original draft, Resources, Formal analysis, Conceptualization. **Yi Liu:** Formal analysis, Data curation. **Yu Wang:** Formal analysis, Data curation. **Ziqiang Wang:** Formal analysis, Data curation. **Hongyu Ma:** Formal analysis, Data curation. **Feng Wang:** Investigation, Data curation. **Yongchao Qiao:** Writing – review & editing, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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