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RESEARCH ARTICLE

Are the SNPs of NKX2-1 associated with papillary thyroid carcinoma in the Han population of Northern China?

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Abstract **Papillary thyroid carcinoma (PTC) is one of the most common tumors of the thyroid gland. The common risk factors of PTC include ionizing radiation, positive family history, and thyroid nodular disease. PTC was identified in Europeans by conducting a genome-wide association study, and a strong association signal with PTC was observed in rs944289 and *NKX2-1* (located at the 14q13.3 locus), which was probably the genetic risk factor of PTC. This study aimed to examine the association of this gene with PTC in Chinese. A total of 354 patients with PTC and 360 healthy control subjects from the Han population of Northern China were recruited in the study. These individuals were genotyped to determine rs12589672, rs12894724, rs2076751, and rs944289. The association of rs944289 with PTC was obtained (C vs. T, $P = 0.027$, OR = 1.264, 95% CI = 1.026 – 1.557; and C/C – C/T vs. T/T, $P = 0.034$, OR = 1.474, 95% CI = 1.028 – 2.112). Conducting a subgroup analysis, we found a marginal difference in the allele frequency distribution of rs944289 (adjusted $P = 0.062$) between the patients with PTC and multi-nodular goiter and the control subjects. We also observed an interaction ($P = 0.029$; OR = 2.578, 95% CI = 1.104 – 6.023) between rs944289 and diabetes in patients with PTC. In conclusion, rs944289 was associated with an increased risk of PTC in the Han population of Northern China, but no clear association was observed in either of the tag single nucleotide polymorphisms of *NKX2-1*.**

Keywords *NKX2-1*; papillary thyroid carcinoma; the Han population of Northern China; association

Introduction

Thyroid carcinoma (TC) is a common malignant tumor of the endocrine system and occurs more often in females than in males [1]. The incidence of TC has increased globally for several decades. At present, the incidence rates of TC are 5.3 and 17.7 per 100 000 Asian males and females, respectively (http://seer.cancer.gov/). However, information on the pathological mechanism of TC is insufficient, but genetic factors have strongly contributed to the incidence of TC [2].

TC can be classified into four main types: papillary (PTC), follicular (FTC), medullary, and undifferentiated TCs. PTC is the most common among these TCs, accounting for approximately 80% to 85% [3,4]. The established risk factors of PTC include ionizing radiation, positive family history, and thyroid nodular disease [3,5,6]. Studies have shown that abnormal iodine intake is a possible risk factor of cancer development [5]. Other studies have proposed that diabetes may also be a potential risk factor of PTC [7].

Three confirmed thyroid-specific transcription factors are important in thyroid function: *NKX2-1,FOXE1,* and *PAX8*; the mutations of *NKX2-1* gene on 14q13.3 and *FOXE1* gene on 9q22.33 contribute to an increased risk of PTC [8]. *NKX2-1*, also called *TITF1*, is expressed during embryonic development and differentiation; this gene is also associated with hypothyroidism, cancer, and nervous system diseases [9]. Common variants at rs944289 are also associated with PTC in Europeans, Japanese, and Chinese [10–12]. *NKX2-1* is identified as a genetic risk factor of PTC because this gene is one of the closest genes to rs944289 and participates in thyroid development [10,11]. This study aimed to examine the association of *NKX2-1* gene with PTC in the Han population of Northern China. This study also aimed to analyze the interaction between this gene and the exposed risk factors.

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Materials and methods

Study subjects

A total of 354 patients with PTC (mean age = 42.7 ± 9.4 years, ranging from 20 years to 75 years; 69.8% women) were recruited from China-Japan Union Hospital of Jilin University. Histological diagnoses were performed from August 2010 to June 2012 according to the revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. A total of 360 healthy individuals (mean age = 42.5 ± 9.9 years, ranging from 23 years to 83 years; 68.1% women) were recruited as control subjects from the First Hospital of Jilin University. The control subjects were free of thyroid diseases, diabetes, and other endocrine system diseases. These control subjects were also matched with cases by age and gender. All of the subjects came from the Han population of Northern China. Blood samples were collected; the corresponding clinical data, including ionizing radiation treatment, family history, and associated diseases, were obtained.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood lymphocytes of each subject by using ClotBlood DNA Kit (Cwbio, Beijing) and then detected using an ultraviolet spectrophotometer (Beckman, USA), OD_{260}/OD_{280}: 1.6–1.9.

The data of single nucleotide polymorphisms (SNPs) located in the *NKX2-1* gene were chosen from a HapMap database. Three tag SNPs of the gene were obtained using Haploview4.2 and listed as follows: rs12589672; rs12894724; and rs2076751 (population: Chinese in Beijing; R^2 cutoff: 0.8; minor allele frequency cutoff: 0.1; D'= 1). The tag SNPs and rs944289, a genome-wide association study (GWAS) positive locus, were included in the study [10]. The primers were designed using AssayDesigner3.1 (Table 1).

Genetic polymorphisms were genotyped using an iPLEX Gold reagent set (Sequenom, San Diego, CA, USA) containing a PCR enzyme GPR, PCR accessory set, SpectroCHIP II resin kit, and iPLEX Gold reagent kit.

PCR reaction was performed in 5 μl of mixture containing 10 ng of genomic DNA template, 1 × PCR buffer, 1.625 mM $MgCl_2$, 500 μM deoxynucleotide (dNTP) mix, 0.1 μM primer mix, 1 Unit of HotStar Taq, and 1.8 μl of water under the following conditions: initial denaturation at 94°C for 15 min; 45 cycles of denaturation at 94°C for 20 s; annealing at 56°C for 30 s; extension at 72°C for 1 min; and subsequent terminal extension at 72 °C for 3 min. PCR products were purified using shrimp alkaline phosphatase (SAP) to eliminate redundant dNTP. SAP reaction was performed in 2 μl of mixture containing 0.5 U SAP enzyme, 1 × SAP buffer, and 1.53 μl of water under the following conditions: 37 °C for 40 min and 85 °C for 5 min. Single-base extension was performed in 2 μl of mixture containing 1 × iPLEX enzyme, 0.94 μl iPLEX extend primer mix, 1 × iPLEX termination mix, 1 × iPLEX Buffer Plus, and 0.619 μl of water under the following conditions: 94°C for 30 s; 94°C for 5 s; 40 cycles of 52°C for 5 s and 5 cycles of 80°C for 5 s; and 72°C for 3 min. After desalting, the product was detected to determine the SNPs by matrix-assisted laser desorption/ionization time of flight mass spectrometry.

Table 1 Primer sequences of SNPs

SNPs		Primer sequence
rs12589672	F	5′-ACGTTGGATGACTCTGGCCCTCATAGACG-3′
	R	5′-ACGTTGGATGGGCTTCTGCTTCTCCCTTTC-3′
rs12894724	F	5′-ACGTTGGATGGAAATAATGGCGAGTGAGAG-3′
	R	5′-ACGTTGGATGTCCCGAATGGGGTCTAATTG-3′
rs2076751	F	5′-ACGTTGGATGAGAGAGGCAGACAGACTGAC-3′
	R	5′-ACGTTGGATGAACAAATGAGCGAGCGAGTC-3′
rs944289	F	5′-ACGTTGGATGCTTGCAATTTAATTTGGTTG-3′
	R	5′-ACGTTGGATGAGCCTGTGAATGGACATTAG-3′

Statistical analysis

Pearson's χ^2 test was applied to examine Hardy-Weinberg equilibrium and compare the allele frequency distributions of each SNP between cases and control subjects. *P* value was adjusted for multiple tests based on false discovery rate (FDR). The best inheritance models of the SNPs were decided based on Akaike Information Criterion. The interactions between the gene and exposed risk factors were examined by the case-only approach by employing logistic regression. All of the statistical tests were two sided, and $P < 0.05$ was considered statistically significant. Statistical analyses were accomplished using SPSS 19.0 and SNPStats (http://bioinfo.iconcologia.net/SNPStats).

Results

Table 2 shows the allele frequency distributions of SNPs, which did not deviate from Hardy-Weinberg equilibrium in cases and control subjects. A significant association was obtained between PTC and rs944289 ($\chi^2 = 4.860$, $v = 2$, $P = 0.027$; OR = 1.264, 95% CI = 1.026 – 1.557), but no significant association was found in the other SNPs.

The patients were classified into two groups according to whether or not multi-nodular goiter (MNG) was present. The allele frequency distribution of each group was compared with that of the control subjects. A marginal difference between the patients with MNG and the control subjects with rs944289 (adjusted $P = 0.062$) was found, but no significant difference was observed between the patients with or without MNG and the control subjects of the SNPs of *NKX2-1*.

The genotypic distributions of SNPs in the patients were compared with those in the control subjects; the genotypes

Table 2 Association between allelic distributions of SNPs and PTC in a Northern Chinese Han population

		Reference allele frequency		Variant allele frequency		H-W equilibrium *P* value		χ^2	*P*	OR (95% CI)
		Case	Control	Case	Control	Case	Control			
rs12589672 (A/T[a])	All	627	637	81	83	0.214	0.252	0.003	0.959	0.991 (0.716 – 1.373)
	MNG (+)	467	637	51	83	0.732	0.252	0.883	0.347*	0.838 (0.580 – 1.212)*
	MNG (–)	160	637	30	83	0.208	0.252	2.510	0.226*	1.439 (0.858 – 2.413)*
rs12894724 (C/T[a])	All	633	642	75	78	0.255	0.672	0.022	0.883	0.975 (0.697 – 1.364)
	MNG (+)	473	642	45	78	0.972	0.672	1.551	0.213*	0.783 (0.532 – 1.152)*
	MNG (–)	160	642	30	78	0.208	0.672	3.530	0.120*	1.543 (0.917 – 2.598)*
rs2076751 (G/T[a])	All	585	586	123	134	0.220	0.390	0.371	0.543	0.919 (0.702 – 1.205)
	MNG (+)	426	586	92	134	0.436	0.390	0.146	0.702*	0.944 (0.704 – 1.266)*
	MNG (–)	159	586	31	134	0.269	0.390	0.534	0.702*	0.853 (0.523 – 1.391)*
rs944289 (C/T[a])	All	362	410	346	310	0.462	0.874	4.860	**0.027**	1.264 (1.026 – 1.557)
	MNG (+)	263	410	255	310	0.240	0.874	4.626	0.062*	1.282 (0.989 – 1.662)*
	MNG(–)	99	410	91	310	0.601	0.874	1.428	0.232*	1.216 (0.882 – 1.675)*

[a]The risk allele * adjusted based on false discovery rate (FDR).

were obtained using SNPStats based on the best inheritance models of SNPs. For rs944289, a significant association was detected in the recessive inheritance model (C/C – C/T vs. T/T; $\chi^2 = 4.493$, $v = 2$, $P = 0.034$; OR = 1.474, 95% CI = 1.028 – 2.112). By comparison, no significant association was detected for other SNPs (Table 3).

The interaction between the gene and the exposed risk factors of PTC was analyzed by performing a case-only study. The allele frequencies of SNPs and known risk factors, including radiation, family history, and diabetes, were included in the analysis. Among four SNPs, only rs944289 showed a significant interaction with diabetes ($P = 0.029$; OR = 2.578, 95% CI = 1.104 – 6.023; Table 4).

Discussion

NKX2-1 gene, as a homeodomain transcription factor, was first discovered in a functional experiment of thyroglobulin in rats. The high level expression in pulmonary adenocarcinoma and small cell lung carcinoma resulted in the consideration of *NKX2-1* as the molecular biomarker of lung cancer [8,13,14]. To understand the role of *NKX2-1* in thyroid adenoma in mice, a genetic toxicity study has been carried out using *N-bis (2-hydroxypropyl)-nitrosamine* [15]. *NKX2-1* may function in the control of follicular cell proliferation in the carcinogenic process of the thyroid, which suggests that the mutation and abnormal expression of *NKX2-1* in the thyroid may be among the factors causing increased incidence of thyroid cancer.

A GWAS using 192 cases and 37 196 controls confirmed that a common variant on 14q13.3 predisposed individuals to PTC in an Icelandic population [10]. rs944289 showed strong association signal ($P = 2.03 \times 10^{-9}$; OR = 1.37, 95% CI = 1.24 – 1.52), and the mutation from C to T contributes to an increased risk of both PTC and FTC [10]. A similar conclusion was obtained in a Japanese population ($P = 0.0121$; OR = 1.21, 95% CI = 1.04 – 1.39) for rs944289 associated with PTC, and in a Chinese population ($P =$

Table 3 Association results of the genotypic distributions of SNPs in patients with PTC and control subjects from Northern Chinese Han population

	Model	Genotype	Case	Control	χ^2	*P*	OR (95% CI)
rs12589672	Overdominant	A/A – T/T	287	291	0.007	0.935	1.000
		A/T	67	69			0.985 (0.678 – 1.431)
rs12894724	Overdominant	C/C – T/T	291	292	0.142	0.706	1.000
		C/T	63	68			0.930 (0.636 – 1.358)
rs2076751	Overdominant	G/G – T/T	259	246	2.012	0.156	1.000
		G/T	95	114			0.792 (0.573 – 1.094)
rs944289	Recessive	C/C – C/T	266	294	4.493	0.034	1.000
		T/T	88	66			1.474 (1.028 – 2.112)

Table 4 Interactions between allele frequencies of SNPs and exposed risk factors of patients with PTC from Northern Chinese Han population

	Ionizing radiation		Family history		Diabetes	
	P	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
rs12589672	0.779	1.102 (0.558 – 2.178)	0.064	0.152 (0.021 – 1.118)	0.162	2.057 (0.749 – 5.625)
rs12894724	0.562	1.225 (0.617 – 2.430)	0.078	0.166 (0.023 – 1.224)	0.106	2.307 (0.837 – 6.361)
rs2076751	0.419	0.773 (0.414 – 1.444)	0.883	1.084 (0.511 – 2.302)	0.685	0.799 (0.270 – 2.366)
rs944289	0.463	0.845 (0.539 – 1.325)	0.250	0.705 (0.389 – 1.278)	**0.029**	2.578 (1.104 – 6.023)

2.028e – 10; OR = 1.53, 95% CI = 1.34 – 1.74) [11,12]. Another study has also shown that rs944289 may predispose to PTC by PTC susceptibility candidate 3(PTCSC3), a non-coding RNA gene, which was reported as a tumor suppressor [16]. However, the effects of PTCSC3 on the biological characteristics of TC cells remain uncertain. Among the genes closest to rs944289, *NKX2-1* is probably the best source of the association signal, which exhibits a major function in the development of the thyroid gland [10,11]. *NKX2-1* is also considered as a marker of thyroid differentiation because of the expression of this gene at lower levels in malignant thyroid compared with normal thyroid tissue [17]. In this study, an association between rs944289 and PTC was found in the Han population of Northern China, and the results are consistent with the conclusions of previous studies. The results also revealed that individuals with T allele showed a higher risk of PTC than those with C allele (OR = 1.264, 95% CI = 1.026 – 1.557), and the variant allele of homozygous genotype T/T likely increased the risk compared with a combination of C/C and C/T (OR = 1.474, 95% CI = 1.028 – 2.112). However, a significant association of *NKX2-1* with PTC in the Chinese population was not obtained. This result may be attributed to the small sample size and multifactorial pathogenesis of TC. Hence, large samples are needed in future studies to determine whether or not *NKX2-1* is associated with PTC.

MNG can be considered as a risk factor of PTC. A missense mutation (1016C > T) in NKX2.1 was possibly responsible for a mutant TTF-1 protein (A339V). A339V, which likely contributes to the pathogenesis of PTC, was detected in patients with PTC showing a history of MNG, but this protein was not detected in the control subjects or the patients with PTC but no history of MNG [18]. In this study, a marginal association was observed in patients with PTC accompanied by MNG in rs944289 (adjusted $P = 0.062$); no significant association was observed in the Tag SNPs of *NKX2-1*. Further studies should be conducted to confirm whether or not a mutation of *NKX2-1* predisposes to MNG and/or PTC.

A significant interaction was found between rs944289 and diabetes ($P = 0.029$; OR = 2.578, 95% CI = 1.104 – 6.023). Diabetes and TC are diseases of the endocrine system. Studies have also shown that patients with TC usually suffer from diabetes. Epidemiological studies have further revealed that the risk of TC significantly increases in diabetic women [7]. The *PSMA6* gene on 14q13.2 is possibly the susceptibility gene of type 2 diabetes; the position of *PSMA6* is close to rs944289 and *NKX2-1* (14q13.3); this result suggests that rs944289 may be associated with diabetes in the pathogenesis of PTC [19].

In conclusion, rs944289 was associated with PTC in the Han population of Northern China, but no significant association was obtained in the SNPs of *NKX2-1*. In addition, rs944289 may be related to diabetes in the pathogenesis of PTC. Further studies should be conducted whether or not *NKX2-1* contributes to the predisposition of PTC.

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Compliance with ethics guidelines

Lizhe Ai, Yaqin Yu, Xiaoli Liu, Chong Wang, Jieping Shi, Hui Sun, and Qiong Yu declare that they have no conflict of interest. The study was approved by the ethics committees of Jilin University. Written informed consent was obtained from the subjects.

References

1. Lang BH, Lo CY, Chan WF, Lam KY, Wan KY. Staging systems for papillary thyroid carcinoma: a review and comparison. Ann Surg 2007; 245(3): 366–378
2. Czene K, Lichtenstein P, Hemminki K. Environmental and heritable causes of cancer among 9.6 million individuals in the Swedish Family-Cancer Database. Int J Cancer 2002; 99(2): 260–266
3. Kondo T, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. Nat Rev Cancer 2006; 6(4): 292–306
4. DeLellis RA. Pathology and genetics of thyroid carcinoma. J Surg Oncol 2006; 94(8): 662–669
5. Meinhold CL, Ron E, Schonfeld SJ, Alexander BH, Freedman DM, Linet MS, Berrington de González A. Nonradiation risk factors for thyroid cancer in the US Radiologic Technologists Study. Am J Epidemiol 2010; 171(2): 242–252
6. Leux C, Truong T, Petit C, Baron-Dubourdieu D, Guénel P. Family history of malignant and benign thyroid diseases and risk of thyroid

cancer: a population-based case-control study in New Caledonia. Cancer Causes Control 2012; 23(5): 745–755

7. Shih SR, Chiu WY, Chang TC, Tseng CH. Diabetes and thyroid cancer risk: literature review. Exp Diabetes Res 2012; 2012: 578285
8. Kimura S. Thyroid-specific transcription factors and their roles in thyroid cancer. J Thyroid Res 2011; 2011: 710213
9. Katoh R, Kawaoi A, Miyagi E, Li X, Suzuki K, Nakamura Y, Kakudo K. Thyroid transcription factor-1 in normal, hyperplastic, and neoplastic follicular thyroid cells examined by immunohistochemistry and nonradioactive in situ hybridization. Mod Pathol 2000; 13(5): 570–576
10. Gudmundsson J, Sulem P, Gudbjartsson DF, Jonasson JG, Sigurdsson A, Bergthorsson JT, He H, Blondal T, Geller F, Jakobsdottir M, Magnusdottir DN, Matthiasdottir S, Stacey SN, Skarphedinsson OB, Helgadottir H, Li W, Nagy R, Aguillo E, Faure E, Prats E, Saez B, Martinez M, Eyjolfsson GI, Bjornsdottir US, Holm H, Kristjansson K, Frigge ML, Kristvinsson H, Gulcher JR, Jonsson T, Rafnar T, Hjartarsson H, Mayordomo JI, de la Chapelle A, Hrafnkelsson J, Thorsteinsdottir U, Kong A, Stefansson K. Common variants on 9q22.33 and 14q13.3 predispose to thyroid cancer in European populations. Nat Genet 2009; 41(4): 460–464
11. Matsuse M, Takahashi M, Mitsutake N, Nishihara E, Hirokawa M, Kawaguchi T, Rogounovitch T, Saenko V, Bychkov A, Suzuki K, Matsuo K, Tajima K, Miyauchi A, Yamada R, Matsuda F, Yamashita S. The FOXE1 and NKX2-1 loci are associated with susceptibility to papillary thyroid carcinoma in the Japanese population. J Med Genet 2011; 48(9): 645–648
12. Wang YL, Feng SH, Guo SC, Wei WJ, Li DS, Wang Y, Wang X, Wang ZY, Ma YY, Jin L, Ji QH, Wang JC. Confirmation of papillary thyroid cancer susceptibility loci identified by genome-wide association studies of chromosomes 14q13, 9q22, 2q35 and 8p12 in a Chinese population. J Med Genet 2013; 50(10): 689–695
13. Tan D, Li Q, Deeb G, Ramnath N, Slocum HK, Brooks J, Cheney R, Wiseman S, Anderson T, Loewen G. Thyroid transcription factor-1 expression prevalence and its clinical implications in non-small cell lung cancer: a high-throughput tissue microarray and immunohistochemistry study. Hum Pathol 2003; 34(6): 597–604
14. Moldvay J, Jackel M, Bogos K, Soltész I, Agócs L, Kovács G, Schaff Z. The role of TTF-1 in differentiating primary and metastatic lung adenocarcinomas. Pathol Oncol Res 2004; 10(2): 85–88
15. Hoshi S, Hoshi N, Okamoto M, Paiz J, Kusakabe T, Ward JM, Kimura S. Role of NKX2-1 in N-bis(2-hydroxypropyl)-nitrosamine-induced thyroid adenoma in mice. Carcinogenesis 2009; 30 (9): 1614–1619
16. Jendrzejewski J, He H, Radomska HS, Li W, Tomsic J, Liyanarachchi S, Davuluri RV, Nagy R, de la Chapelle A. The polymorphism rs944289 predisposes to papillary thyroid carcinoma through a large intergenic noncoding RNA gene of tumor suppressor type. Proc Natl Acad Sci USA 2012; 109(22): 8646–8651
17. Fabbro D, Di Loreto C, Beltrami CA, Belfiore A, Di Lauro R, Damante G. Expression of thyroid-specific transcription factors TTF-1 and PAX-8 in human thyroid neoplasms. Cancer Res 1994; 54(17): 4744–4749
18. Ngan ES, Lang BH, Liu T, Shum CK, So MT, Lau DK, Leon TY, Cherny SS, Tsai SY, Lo CY, Khoo US, Tam PK, Garcia-Barceló MM. A germline mutation (A339V) in thyroid transcription factor-1 (TITF-1/NKX2.1) in patients with multinodular goiter and papillary thyroid carcinoma. J Natl Cancer Inst 2009; 101(3): 162–175
19. Sjakste T, Kalis M, Poudziunas I, Pirags V, Lazdins M, Groop L, Sjakste N. Association of microsatellite polymorphisms of the human 14q13.2 region with type 2 diabetes mellitus in Latvian and Finnish populations. Ann Hum Genet 2007; 71(6): 772–776