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AXIN2 is Associated With Papillary Thyroid Carcinoma

Xin Liu,[1] Shuang Li,[1] Xuejun Lin,[1] Kangkang Yan,[1] Longyu Zhao,[1] Qiong Yu,[1,*] and Xiaodong Liu[2,3,*]

[1]Department of Epidemiology and Biostatistics, School of Public Health, Jilin University, Changchun, China
[2]Department of Radiology and Radiation Oncology, China-Japan Union Hospital, Changchun, China
[3]Key Laboratory of Radiobiology, School of Public Health, Jilin University, Changchun, China

*Corresponding Authors: Qiong Yu, Department of Epidemiology and Biostatistics, School of Public Health, Jilin University, Changchun, China. Tel: +86-43185619451, Fax: +86-43185619163, E-mail: yujoan_2001@163.com; Xiaodong Liu, Key Laboratory of Radiobiology, School of Public Health, Jilin University, Changchun, China. Tel: +86-43185619418, Fax: +86-43185619418, E-mail: liuxiaod@jlu.edu.cn

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Abstract

Background: Findings of recent studies have demonstrated a rapid increase of the incidence of papillary thyroid carcinoma (PTC), which accounts for nearly 80% of thyroid cancers.

Objectives: The aim of this study was to explore the association between *AXIN2* gene polymorphism and papillary thyroid carcinoma (PTC).

Patients and Methods: 106 blood samples (56 PTC patients and 50 healthy controls) were drawn from China-Japan Union Hospital in Jilin province, China, during October 2010 to March 2011. A case-control study was designed to examine the association between *AXIN2* and PTC. Seven tag single nucleotide polymorphisms (tag SNPs) in *AXIN2* were selected and genotyped. Frequencies of different genotypes and alleles were analyzed between the patients and the controls, using the R × C column contingency table χ^2 test. The possible association of haplotypes constructed by the combined effects of two or more loci with PTC was analyzed through the UNPHASED 3.1.4 program.

Results: Rs11655966, rs3923086 and rs7591 of *AXIN2* showed significant associations with PTC ($P < 0.05$). The result of haplotypes analysis showed that rs11655966-rs3923086-rs4791169 had statistically significant differences between the two groups ($P < 0.05$).

Conclusions: Together with the functions of the target genes, we further elucidated that *AXIN2* is associated with papillary thyroid carcinoma in the Chinese Han population.

Keywords: Thyroid Cancer, Papillary, Polymorphism, Single Nucleotide, *AXIN2*

1. Background

Recent studies have demonstrated the increasing incidence of papillary thyroid carcinoma all over the world; papillary thyroid carcinoma (PTC) is the most common malignancy in four main types of thyroid cancers, accounting for 80% of human thyroid cancers (1, 2). It has been described in details that microRNAs (miRNAs) are potential biomarkers in papillary thyroid carcinogenesis (3). According to our recent study, hsa-miR-222, hsa-miR-15a and their putative target gene, Axis inhibition protein 2 (AXIN2), were described to be associated with PTC (4). The *AXIN2* gene is a negative regulator gene of Wnt/β-catenin signaling pathway and it is a putative tumor suppressor gene in a negative feedback loop that limits the duration and intensity of Wnt-initiated signals (5, 6). This preliminary result leads us to question whether *AXIN2* is involved in the development of papillary thyroid carcinoma.

2. Objectives

The purpose of this study was to explore the association between *AXIN2* gene polymorphism and PTC.

3. Patients and Methods

We analyzed 106 samples collected from China-Japan Union Hospital in Jilin province, China during October 2010 to March 2011. The case group included all patients with confirmed clinical pathological diagnosis of thyroid papillary carcinoma during October 2010 to March 2011 in hospitals and their diagnosis was confirmed by two doctors at the same time. The control group included people who had physical examinations at the hospital during October 2010 to March 2011, and had normal thyroid confirmed by eight thyroid function tests (free triiodothyronine (FT3), free thyroxine (FT4), thyroid stimulating hormone (TSH), three iodine in serum thyroid nitric acid (TT3), total serum thyroxine (TT4), thyroid peroxidase antibody (TPOAb), thyrotropin receptor antibodies (TRAb), and thyroglobulin (Tg)) and the thyroid gland color Doppler ultrasound examination. Furthermore, individuals of the control group were those without other endocrine diseases and positive family history of thyroid disease and were age and sex matched with the patients group. Our study was examined and approved by the medical ethics committee of school

of public health of Jilin University during September 2010 (code: 2010-09-03). We genotyped seven tagSNPs of *AXIN2* in the Chinese population. The tagSNPs were predicted using the Haploview 4.2 with Hapmap data (CHB, MAF > 0.1, r^2 > 0.8), covering 100% of the SNPs. We used a case-control design to examine the association between *AXIN2* and PTC. All subjects were from the North China Han population. Blood samples were drawn from 56 patients (45 females and 11 males; average age of 45.13 ± 10.97) and 50 healthy controls (38 females and 12 males; average age of 41.9 ± 10.22).

Genomic DNA of all subjects was extracted from the whole blood sample using the Promega liquid extraction and purification of DNA Kit (Promega, USA). The genotyping analysis of the tagSNPs was done using the mechanism of assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS) of MassARRAY platform (Sequenom, USA). At the same time, 20% of the total sample was extracted by the random sampling method and used in duplicate detection by the blind method.

The goodness-of-fit chi-square test was used to test deviations of the patients and controls from the Hardy-Weinberg equilibrium (HWE). Data were analyzed using the SPSS 16.0 program for windows (SPSS Inc. Chicago, IL, USA). The difference in the allele frequencies and genotype frequencies between cases and controls were analyzed using the R × C column contingency table χ^2 test. The possible association of haplotypes constructed by the combined effects of two or more loci with PTC was analyzed through the UNPHASED 3.1.4 program.

4. Results

There was no statistically significant difference in the sex distributions (χ^2 = 0.245, P = 0.621) of the two groups. All studied SNPs in both the patient and control group had a HWE (P > 0.05). Results of single locus association analysis are shown in Table 1. TagSNPs rs11655966, rs3923086, and rs7591 of *AXIN2* showed significant associations with PTC (P < 0.05). The result of haplotypes analysis showed that rs11655966-rs3923086-rs4791169 had statistically significant differences between the two groups (P < 0.05). Among the five possible combinations of this haplotype (A-G-G, A-T-A, A-T-G, T-T-A, and T-T-G) T-T-G showed significant differences (frequency of cases = 36 (33.96%), frequency of controls = 17.45 (18.56%), OR = 4.126, χ^2 = 6.72, P = 0.009536) after 1000 permutations.

Table 1. The Association Between Single Nucleotide Polymorphisms and Papillary Thyroid Carcinoma

SNP	N	Allelic Frequency [a], %		χ^2	P	OR (95% CI)	Genotypic Frequency [a], %			χ^2	P
		1	2				1/1	1/2	2/2		
rs11655966											
Case group	54	70	38	6.029	0.014 [b]	0.493 (0.280 - 0.870)	24	22	8	4.877	0.087
Control group	48	47	49	NA	NA	NA	12	23	13	NA	NA
rs2240308											
Case group	53	78	28	2.885	0.089	0.597 (0.329 - 1.085)	27	24	2	3.327	0.190
Control group	50	63	37	NA	NA	NA	17	29	4	NA	NA
rs3923086											
Case group	55	102	8	4.518	0.034 [b]	0.391 (0.161 - 0.950)	47	8	0	4.990	0.082
Control group	50	83	17	NA	NA	NA	34	15	1	NA	NA
rs4074947											
Case group	56	64	48	0.161	0.689	0.894 (0.515 ~ 1.549)	19	26	11	0.242	0.886
Control group	50	60	40	NA	NA	NA	18	24	8	NA	NA
rs4791169											
Case group	56	79	33	0.323	0.570	0.844 (0.470 - 1.515)	29	21	6	0.715	0.699
Control group	49	65	33	NA	NA	NA	24	17	8	NA	NA
rs7210356											
Case group	55	96	14	0.000	0.994	0.997 (0.444 ~ 2.239)	42	12	1	0.636	0.727
Control group	50	87	13	NA	NA	NA	39	9	2	NA	NA
rs7591											
Case group	55	81	29	4.674	0.031 [b]	0.525 (0.292 - 0.945)	29	23	3	4.687	0.096
Control group	50	60	40	NA	NA	NA	17	26	7	NA	NA

Abbreviation: NA, not available; SNP, single nucleotide polymorphisms.
[a] Note: 1 wild allele, 2 mutational allele; 1/1 homozygous wild genotype, 1/2 heterozygous genotype, 2/2 homozygous mutational genotype.
[b] P < 0.05.

5. Discussion

To our knowledge, this was the first study to find an association between *AXIN2* polymorphisms and PTC in the Chinese population. In the present study, we identified seven tagSNPs on *AXIN2*, among them, rs2240308 belonged to the non-synonymous region, rs7591 was located on 3'untranslated regions (3'UTRs) and other sites were located in the intron. There was no significant difference shown in the distribution of the genotype loci through the statistical analysis of the case and the control group ($P > 0.05$); prompting rs11655966, rs3923086 and rs7591 were independently associated with PTC susceptibility. However, the result of haplotypes showed statistical significance. Taking into consideration the above findings, we further concluded that *AXIN2* is likely relevant in genetic susceptibility to PTC.

AXIN2 is located on chromosome 17q23-24, its cDNA is about 2.5 KB in length, consisting of 10 exons, coding AXIN-2α (843 amino acids) and AXIN-2β (778 amino acids) (7). *AXIN2* gene is involved in embryonic development, tumor formation, programmed cell death, glycogen metabolism and other physiological and pathological processes, and plays an important role in the Wnt signaling pathways, stress activated protein kinase (SAPK) signaling pathway, transforming growth factors (TGF)–β signaling pathways and insulin signal transduction pathway (8). *AXIN2* has relationships with colorectal cancer, lung cancer, adrenal cortical tumors, medulloblastomas, astrocytoma, hepatoblastoma, autosomal dominant ectodermal dysplasia and neoplastic syndrome, yet its association with prostate cancer has not yet been found (9).

However, there are many uncontrollable confounding factors in this case-control study, which may lead to bias in the results. According to the above reason, we strictly followed the inclusion criteria of the case and control group when we selected the study subjects, especially when screening thyroid papillary carcinoma in the control group.

Owing to the limited number of samples in our study, further research is recommended to further validate the accuracy and specificity of the association between *AXIN2* and PTC. Our findings can provide data for better understanding of the genetic and molecular pathogenesis of PTC. In the future, studies with larger samples and different ethnic groups are required. Such studies should enhance the efficiency of the statistical analysis and increase the number of SNPs that are to be detected. Moreover, learning the molecular mechanisms of *AXIN2* in PTC is fundamentally important in developing new molecular markers for earlier diagnosis and novel therapeutic targets.

Footnotes

Authors' Contribution: Study concept and design: Xin Liu, Qiong Yu, and Xiaodong Liu; analysis and interpretation of data: Qiong Yu; drafting of the manuscript: Xin Liu and Shuang Li; critical revision of the manuscript for important intellectual content: Qiong Yu and Xiaodong Liu; statistical analysis: Qiong Yu; administrative, technical, and material support: Xuejun Lin, Kangkang Yan, and Longyu Zhao.

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