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Long non-coding RNA POLR2E rs3787016 is associated with the risk of papillary thyroid carcinoma in Chinese population

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ABSTRACT

Long non-coding RNAs (LncRNAs) have been shown to be involved in cancer tumorigenesis and progression. Single nucleotide polymorphisms (SNPs) in the lncRNAs also play a vital role in carcinogenesis. We here explored the association between POLR2E rs3787016 and risk of papillary thyroid carcinoma (PTC) in a Chinese population, which was followed by a meta-analysis of POLR2E rs3787016 and cancer risk in Chinese population. A total of 409 PTC patients and 800 healthy individuals were enrolled in the present study. The POLR2E rs3787016 was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and was confirmed by sequencing. The POLR2E rs3787016T allele increased the PTC risk in Chinese population, particular in Chinese females. The meta-analysis further revealed that POLR2E rs3787016T allele was associated with an increased cancer risk in Chinese population. Collectively, the POLR2E rs3787016 may be used as a genetic biomarker to predict cancer risk in Chinese population.

1. Introduction

The papillary thyroid carcinoma (PTC), accounting for approximately 80% of differentiated thyroid carcinoma (DTC), has become a great threat to human health [1,2]. With the rapid development of modern medicine, efficient treatment such as the surgical resection [1] and radioactive iodine (RAI) therapy have been applied in PTC therapies [3,4]. However, most PTC patients are diagnosed at a late stage, which results in the limited treatment options and poor prognosis. In this regard, a rapid and sensitive diagnosis method of the PTC would be particularly necessary. Currently, the single nucleotide polymorphism (SNP), which can affect the expression and function of genes through altering the process of splicing and stability of mRNA conformation, has been an important genetic marker in gene mapping related to complex diseases (e.g. human cancer) [5,6]. Therefore, the identification of certain SNPs associated with PTC susceptibility should facilitate the early diagnosis and radical cure for PTC.

Long non-coding RNAs (LncRNAs), a new class of regulatory non-coding RNAs ranges from 200 bp to 100 kb. Although they have no open reading frame and the capacity of potential protein translation, they play vital roles in different biological processes including cell cycle control, cell differentiation, gene imprinting, chromatin remodeling, dosage compensation, genome rearrangement and regulation of gene expression [7]. Therefore, SNPs in lncRNAs are likely to modify functions of various biological pathways involved in PTC carcinogenesis.

The SNP rs3787016 was a newly identified prostate cancer risk locus from genome-wide association study in Caucasian population. It localizes to an intron of long non-coding RNA POLR2E, which encodes a subunit of RNA polymerase II and is responsible for synthesizing messenger RNA [8]. Interestingly, three subsequent studies in Chinese population reported that rs3787016 is significant associated with the risk of prostate cancer [9], esophageal cancer [10] and breast cancer [11], separately. Therefore, we doubted whether POLR2E rs3787016 might also be a genetic factor for PTC. To this end, a case-control study was conducted to evaluate the association between POLR2E rs3787016 and PTC risk in a Chinese population of Hubei province. Furthermore, a meta-analysis was further performed to get a more precise estimation of the association between POLR2E rs3787016 and cancer risk in Chinese population.

2. Materials and methods

A total of 409 PTC patients and 800 cancer-free individuals were enrolled in this case-control study. All participants are living in Hubei

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province but biologically unrelated Chinese. The PTC patients were enrolled from Wuhan Xinzhou District People's Hospital between January 2015 and September 2016, while the normal controls were recruited from visitors who came to Wuhan Xinzhou District People's Hospital for regular physical examinations between September 2014 and December 2016. This study was approved by the Ethical Committees of Wuhan University of Technology. Participants or their guardians provided written informed consent for the genetics analysis.

The TIANamp Blood DNA Kit (DP348, TianGen Biotech, Beijing) was used to extract genomic DNA from venous blood of all participants following the manufacturer's instructions, and the genomic DNA samples were stored at −20 °C before used. Since the transition of T > C at rs3787016 locus produces a *NLaIII* restriction site, we used the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique to genotype the POLR2E rs3787016. The PCR primers were 5′-CATCAACATCACGCAGCACG-3′(forward) and 5′-CCCTGTCCT CCAAGCA

CTCAT-3′(reverse), and annealing temperature was 60 °C. The PCR reaction produced a DNA fragment of 147 bp, which was then digested with *NLaIII* (Takara Biotechnology Co. Ltd, Dalian, China) overnight at 37 °C. After digestion, the 147 bp DNA fragment with the rs3787016C allele produced two bands (127 bp and 20 bp), while with the rs3787016 T allele produced one band (147 bp). For strict quality control, genotyping analysis was repeated twice. Furthermore, 20% randomly selected PCR-amplified DNA samples were examined with direct sequencing, and the results were 100% concordant.

All statistical analysis was conducted by SPSS 15.0 software (SPSS, Chicago, IL) and all tests were two-sided. Genotypic frequencies were tested against Hardy-Weinberg equilibrium (HWE) for rs3787016 in normal controls. Associations of rs3787016 with PTC risk was examined using logistic regression models. Odds ratios (ORs) together with their corresponding 95% confidence intervals (CIs) were calculated. A p value < 0.05 was considered to be statistically significant and Bonferroni correction for multiple testing was applied.

Without language restriction, all publications updated to October 2017 from the EMBASE, PubMed and ISI Web of Science data bases were searched by the key words including"RNA polymerase II polypeptide E or POLR2E"; "rs3787016"; "cancer" and "Chinese population". For getting missing information; references listed in retrieved articles were also checked. Finally; 3 relevant articles were included [9–11]. The departure from HWE for the controls in each study was calculated using Pearson's χ^2 test. The meta-analysis was performed with Review Manager 5.3 software (Cochrane Collaboration); The overall strength of an association between POLR2E rs3787016 and cancer risk was assessed by combined ORs together with their corresponding 95% Cls; which were calculated using fixed- or random-effects models. Statistical heterogeneity was evaluated with the χ^2 test. The fixed-effects model was used in the absence of statistically significant heterogeneity ($p \geq 0.1$) [12]; while the random-effects model was chosen when heterogeneity was found ($p < 0.1$) [13]. The significance of combined OR was determined by the Z test.

3. Results

The characteristics of PTC patients and normal controls were summarized in Table 1. No significant difference was observed for the distributions of age, smoking status and alcohol status between PTC patients and normal controls, while the gender distribution was significantly different between PTC patients and normal controls ($p < 0.001$). PTC has become the most common endocrine malignancy, and females are affected more often than males [14]. Consistently, the female PTC patients were about three times as many as the male PTC patients (74.8% *vs.* 25.2%) in this study.

The allele and genotype distributions of POLR2E rs3787016 and their association with PTC risk were shown in Table 2, and a p value < 0.008 (0.05/6) was considered to be statistically significant after Bonferroni correction for multiple testing. No significant deviations from HWE were found for the genotypic frequency of rs3787016 in normal controls, suggesting that the control participants enrolled were representative. As shown in Table 2, the frequency of rs3787016 T allele was significant higher in PTC patients than in normal controls ($p = 0.002$, OR = 1.33, 95% = 1.11–1.58), indicating that rs3787016 T allele conferred an increased risk to PTC. Similarly, the T variant genotypes of rs3787016 were also demonstrated to be associated with an increased risk of PTC in two genetic models (TT *vs.* CC, $p = 0.002$, OR = 1.80, 95% = 1.24–2.60; TT + TC *vs.* CC, $p = 0.006$, OR = 1.62, 95% = 1.15–2.28)

Table 1
Characteristics of PTC patients and normal controls.

Variable		PTC patients ($n = 409$)	normal control ($n = 800$)	p-Value[b]
Age	≤60 years	232 (56.7%)[a]	434 (54.3%)	0.413
	> 60 years	177 (43.3%)	366 (45.7%)	
Gender	Male	103 (25.2%)	558 (69.7%)	< 0.001
	Female	306 (74.8%)	242 (30.3%)	
Smoking	Ever	123 (30.1%)	209 (26.1%)	0.146
Status	Never	286 (69.9%)	591 (73.9%)	
Drinking	Ever	110 (26.9%)	237 (29.6%)	0.321
Status	Never	299 (73.1%)	563 (70.4%)	

PTC = Papillary thyroid cancer.
[a] Numbers in parentheses, percentage.
[b] Age, gender, smoking status, and drinking status distributions of PTC patients and normal controls were compared using a two-sided χ^2 test.

To explore the effect of gender differences on the association of rs3787016 with PTC risk, a stratified analysis in males and females was performed (Table 2). After Bonferroni correction for multiple testing ($p < 0.008$, 0.05/6), no association was found between rs3787016 and PTC risk in males, while the T allele and T variant genotypes of rs3787016 were significant associated with an increased risk of PTC in females (T *vs.* C, TT *vs.* CC, TT *vs.* TC + CC and TT + TC *vs.* CC). These results indicated a potential interaction of gender with POLR2E rs3787016 in the etiology of PTC.

In the following meta-analysis, 3 previous studies and current study were included, and their main features were demonstrated in Table 3. As shown in Table 4, the POLR2E rs3787016 was significant associated with cancer risk in 4 genetic models after Bonferroni correction: T *vs.* C, TT *vs.* TC, TT *vs.* CC and TT *vs.* TC + CC (Fig. 1), suggesting that the rs3787016T allele and TT genotype carriers had a significant increased cancer risk compared with C allele and TC/CC genotypes, respectively.

4. Discussion

PTC is the most frequent subtype of thyroid cancer and has become the most common type of cancer in female patients [14,15]. We here demonstrated that POLR2E rs3787016 is a susceptibility factor to PTC in Chinese population, particular in the Chinese females. A female patient carrying the rs3787016 T allele is more susceptible to PTC compared with those carrying the rs3787016C allele. The SNP rs3787016 localizes to the fourth intron of POLR2E, which encodes a subunit of RNA polymerase II and is responsible for synthesizing messenger RNA (mRNA) in eukaryotes [11]. Recently, there are an increasing number of new pathogenic SNPs located in introns, some of which have been reported to be responsible for aberrant splice processes [16,17]. Therefore, we hypothesized that the SNP rs3787016 may modulate the splice processes of POLR2E gene and produce a defective POLR2E, which would affect the transcriptional signature in normal cell, and thereby increase the risk of cancer (such as PTC). On the other side, it's known to all that estradiol (E2) is an estrogen steroid hormone and the major female sex hormone [18]. A previous *in vitro* study by using PTC cell lines have shown that exposure to estradiol increases cell proliferation via the estrogen receptor (ER) [19]. In this regard, the