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Original Article

The investigation of foxe1 variations in papillary thyroid carcinoma

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Abstract: Background: Recent reports indicated that incidence of thyroid carcinoma is increasing throughout the worldwide. The aim of our study was to determine a possible relationship between Forkhead box E1 (FOXE1) gene variants and histopathological features of papillary thyroid carcinoma. Methods: FOXE1 gene variations; rs894673, rs1867277 and rs3758249 were analyzed in 57 Papillary thyroid carcinoma patients and 51 age matched healthy control subjects. Restriction fragment length polymorphism (RFLP) technique was used to specifically detect the variations. Results: There was a significant difference in the distribution of rs894673 genotypes in Papillary thyroid carcinoma cases (P=0.01). AA genotype presence of rs1867277 was more significantly associated with several histopathological parameters such as focal and diffuse capsular invasion, lymphatic invasion, P3 with P4 tumor grade and surgical margins. AA genotype presence in rs1867277 variation was significantly associated with the classical variant which is subtype of papillary thyroid carcinoma. Furthermore, the presence of the allel A was found to be related with lymph node invasion risk by 2.46 fold, capsular invasion risk by 2.97 fold, and pT3 with pT4 pathological stage risk by 4.13 fold and the presence of allele A in rs1867277 was significantly associated with classic variants. The presence of allele A in rs1867277 was more significantly associated with several histopathological parameters in classic variant in papillary thyroid carcinoma cases such as, the presence of the A allele was found relationship with lymph node invasion risk by 2.0 fold, capsular invasion risk by 2.39 fold , and pT3 with pT4 pathological stage risk by 3.57 fold. In addition, AATT, AAAA and GATT haplotypes (rs1867277 and rs894673) were evaluated for association with papillary thyroid carcinoma cases. Our results indicate that the significant difference according to two-allele haplotype distribution between papillary thyroid carcinoma cases and control groups. Conclusion: Our findings suggest that FOXE1 variations generate a higher risk for poor histopatological features of papillary thyroid carcinoma.

Keywords: Thyroid, carcinoma, foxe1

Introduction

Thyroid cancer is one of the major endocrine malignancies worldwide [1]. Thyroid carcinoma was found to be the 3th most common cancer type for the female in Turkey in 2006. The incidence of the disease has increased from 10.8 to 15.3 per 100,000 in 2008, representing approximately a 1.5-fold increase in Turkey [2]. Papillary thyroid carcinoma (PTC), which originate from thyroid epithelial cells, is the most common histopathological subtype of thyroid cancer and it is responsible for 80% of all thyroid cancers and it is usually metastase to lymphatic vessels [3-6].

Previous studies implicated that, several prognostic factors, such as, sex, age, tumor size, histologic type, tumor infiltration and vascular or lymphatic invasion effects clinical outcome of metastatic PTC. There is a significant relationship between survival rates in PTC patients and prognostic factors. Furthermore, clinicopathologic features and prognostic factors are also found to be important for determining treatment options [6]. Genetic factors are the main reason for the development of papillary thyroid cancer. Interaction of environmental factors and low-to-moderate penetrance genes initiates the development of thyroid cancer. One of the good candidate low-to-moderate penetrance gene is Forkhead box E1 (FOXE1), which belongs to a large family of transcription factors, previously known as a thyroid transcription factor 2 (TTF2) [7].

Table 1. Genotype and allele frequencies according to in papillary thyroid carcinoma cases, and controls

	Papillary thyroid carcinoma cases (n=57)	Controls (n=51)	Statistical value
FOXE1 rs894673			
Genotypes			P=0.018 X^2=8.068
TT	23 (67.6%)	11 (32.4%)	
TA	31 (43.7%)	40 (56.3%)	
AA	3 (100%)	0 (0%)	
Alleles			
T	77 (67.5%)	62 (60.8%)	
A	37 (32.5%)	40 (39.2%)	
FOXE1 rs1867277			
Genotypes			
AA	19 (57.6%)	14 (42.4%)	P=0.582 X^2=1.082
GA	24 (54.5%)	20 (45.5%)	
GG	14 (45.2%)	17 (54.8%)	
Alleles			
A	62 (54.3%)	48 (47.0%)	
G	52 (45.6%)	54 (52.9%)	
FOXE1 rs3758249			
Genotypes			
AA	16 (72.7%)	6 (27.3%)	P=0.182 X^2=3.412
GG	5 (41.7%)	7 (58.3%)	
AG	26 (55.3%)	21 (44.7%)	
Alleles			
A	58 (61.7%)	33 (48.5%)	
G	36 (38.3%)	35 (51.5%)	

FOXE1 is called as a specific core protein of thyroid gland [8], which is expressed during the embryonic stage in the thyroid primordium and persists throughout the development of the thyroid gland [9]. It is important for several processes in thyroid follicular cells. Thyroid-specific genes regulated by FoxE1 are essential for thyroid gland development, thyroid cell migration and differentiation [8, 10].

FOXE1 gene variations have been associated with development of several cancer types such as papillary thyroid cancer in numerious studies [8, 11]. The FOXE1 protein consists of 373-amino acids and its molecular weight is 42 kDa [9]. The gene encoding the FOXE1 protein is located at 9q22 [12]. One of the gene variants of FOXE1 is the (rs1867277) variant, which is located within the-283G>A promoter region, results with an alteration on transcription factor binding site where as FOXE1 rs894673 (A>T) and FOXE1 rs3758249 (A>G) are located on 5'UTR region of FOXE1 gene [7].

Current knowledge on FoxE1 and PTC indicates some polymorphisms located on this gene that variations of this gene maybe related with the development of papillary thyroid cancer. This study aimed to determine the incidence of the variations and the role likely played on papillary thyroid cancer.

Materials and methods

Three most common polymorphisims of the FoxE1 gene were investigated in 57 Papillary thyroid carcinoma patients (including 48 female and 9 male patients) and 51 age matched healthy control subjects (including 28 female and 23 male).

Subjects who were in the follow-up in Cerrahpasa Faculty of Medicine, Departments of General Surgery and Nuclear Medicine, Istanbul University. The specimens were obtained right after the informed consents and the study was conducted prospectively. The Medical Ethics Committee of Istanbul Medical Faculty approved this study. The protocol followed was consistent with the World Medical Association Declaration of Helsinki (Ethical Principles for Medical Research Involving Human Subjects). Blood specimens were collected in tubes containing EDTA, and DNA samples were extracted from whole blood by a salting out procedure [13].

FOXE1 gene variations were determined with the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques. For FOXE1 rs1867277 variation, the following primers were used to amplify the FOXE1 gene; 5'-AATCCTAAACTAGCGGGCACCACA-3'; 5'-AGACAGAGGCTCGGGAGTGA-3' and for FOXE1 rs894673 variation, 5'-ACCTGAGTTTCCTTCTCAGCCCAA3'; 5'-TCAGCCCTTCAAGTATTCCCAGCA-3' and FOXE1 rs3758249

Table 2. FOXE1 rs1867277 genotype frequencies according to histopathological parameters in papillary thyroid carcinoma cases

	rs1867277				
	AA	GA	GG	*p* value	χ^2
Papillary thyroid variant				0.038	6.514
Follicular	2 (13.3%)	6 (40%)	7 (46.7%)		
Classic	17 (40.5%)	18 (42.9%)	7 (16.7%)		
Pathological stage				0.001	14.89
pT1 and pT2	5 (14.7%)	20 (58.8%)	9 (26.5%)		
pT3 and pT4	14 (60.9%)	4 (17.4%)	5 (21.7%)		
Lymph node invasion				0.041	6.38
Present	6 (66.7%)	3 (33.3%)	0		
Absent	13 (27.1%)	21 (43.8%)	14 (29.2%)		
Capsular invasion				0.017	8.09
Absent	6 (18.2%)	17 (51.5%)	10 (30.3%)		
Focal and diffuse	13 (54.2%)	7 (29.2%)	4 (16.7%)		
Surgical margins				0.04	6.33
Absent	16 (29.6%)	24 (44.4%)	14 (25.9%)		
Present	3 (100%)	0 (0%)	0 (0%)		

variation, 5'-CGAAGAACGCGACTAAAACC-3'; 5'-GTATAGGATTCCGGGCCTTG-3'. Fordetection of the FoxE1 variations PCR was performed with, 50-100 ng genomic DNA and was amplification with 1× PCR) buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each primer and Taq polymerase in a 50 µl reaction volume. The PCR conditions were as follows, for FOXE1 rs1867277 variation: Initial denaturation step of 95 C0 for 5 min followed by 35 cycles of 72 C0 for 45 sec, 64 C0 for 45 secand 72 C0 for 45 secand. For FOXE1 rs894673: Initial denaturation step of 95 C0 for 5 min followed by 35 cycles of 72 C0 for 45 sec, 64 C0 for 45 sec, 72 C0 for 45 sec and 72 C0 for 5 min. And for FOXE1 rs3758249: Initial denaturation step of 95 C0 for 5 min followed by 35 cycles of 95 C0 for 45 sec, 61 C0 for 45 sec, 72 C0 for 45 sec and 72 C0 for 5 min. For the determination of FOXE1 rs1867277, rs894673 and rs3758249 variations, PCR products were digested with NruI, Hpy188III, MscI restriction enzymes and then electrophoresis was performed on 2% agarosegels and stained with ethidium bromide, respectively. Genotypes were determined as AA (homozygous mutant genotype) (347 bp), GG (wild type genotype) (214, 133 bp) or GA (347, 214, 133 bp) for FOXE1 rs1867277 variation and AA (196, 155 bp), TT (351 bp) or TT (351, 196, 155 bp) for FOXE1 rs894673 and GG (201 bp), AA (155, 46 bp) or AG (201, 155, 46 bp) for FOXE1 rs3758249.

Statistical analysis

Statistical analyses were performed using the SPSS software package (revision 11SPSS Inc., Chicago, IL, U.S.A.). Data is expressed as means + SD. Differences in the distribution of genotypes or alleles between cases and controls were tested using the chi-square and Tukey statistical methods. Values of $P<0.05$ were considered statistically significant. Haplotype frequencies, D' and r^2 were calculated using Haplo view 4.0 programme. All of the combination of haplotypes were compaired between all SNPs.

Results

The analysis included 57 Papillary thyroid carcinoma cases (48 female and 9 male) and 51 healthy controls (28 female and 23 male). Patients and control groups mean age were 44.92±12.93 and 31.63.48±7.47, respectively.

Genotype and allele frequencies of patients and control groups are shown in **Table 1**. There was a significant difference in the distribution of FOXE1 rs894673 genotypes between Papillary thyroid carcinoma and control cases (P=0.01) (**Table 1**). The genotype frequency for the FOXE1 rs1867277 and FOXE1 rs3758249 variations in study groups were not significantly different (P>0.05). The presence of FOXE1 rs1867277 AA genotype was also significantly associated with several histopathological parameters such as focal and diffuse capsular invasion (P=0.017, χ^2=8.09), lymphatic invasion (P=0.041, χ^2=6.38), P3 with P4 tumor grade (P=0.001, χ^2=14.89) and surgical margins (P=0.040, χ^2=6.33) (**Table 2**). The presence of AA genotype of FOXE1 rs1867277 variation was significantly associated with the classic variant in papillary thyroid carcinoma cases (P=0.03, χ^2=6.51). Furthermore, the A allel was found related with lymph node invasion risk by 2.46 fold (χ^2=5.34, P=0.020, OR=2.46 95%, CI=1.20-4.73), capsular invasion risk by 2.97 fold (χ^2=8.09, P=0.00, OR=2.97 95%, CI=1.30-6.71), pT3 with pT4 pathological stage risk by 4.13 fold (χ^2=13.15, P=0.00, OR=4.13 95%,

Table 3. FOXE1 rs1867277 allele frequencies according to histopathological parameters in papillary thyroid carcinoma cases

	rs1867277 GG+GA/AA				
	G	A	*p* value	χ^2	OR (95% CI)
Papillary thyroid variant					
Follicular	13 (86.7%)	2 (13.3%)	0.05	3.66	3.03 (0.79-11.60)
Classic	25 (59.5%)	17 (40.5%)			
	GG+GA	AA			
Pathological stage					
pT1 and pT2	29 (85.3%)	5 (14.7%)	0.00	13.15	4.13 (1.72-9.90)
pT3 and pT4	9 (39.1%)	14 (60.9%)			
Capsular invasion					
Absent	27 (81.8%)	6 (18.2%)	0.00	8.09	2.97 (1.3-6.71)
Focal and diffuse	11 (45.8%)	13 (54.2%)			
Lymph node invasion					
Absent	35 (72.9%)	13 (27.1%)	0.02	5.34	2.46 (1.2-4.73)
Present	3 (33.3%)	6 (66.7%)			
Surgical margins					
Absent	38 (70.4%)	16 (29.6%)	0.01	6.33	3.37 (2.23-5.1)
Present	0 (0%)	3 (100.0%)			

Table 4. FOXE1 rs1867277 allele frequencies according to histopathological parameters in classicvariant

	rs1867277 GG+GA/AA				
	G	A	*p* value	χ^2	OR (95% CI)
Lymph node invasion			0.07	3.26	2.0 (1.02-3.90)
Present	22 (66.7%)	11 (33.3%)			
Absent	3 (33.3%)	6 (66.7%)			
Pathological stage			0.002	9.531	3.57 (1.39-9.17)
pT1 and pT2	18 (81.8%)	4 (18.2%)			
pT3 and pT4	7 (35.0%)	13 (65.0%)			
Capsular invasion			0.028	4.84	2.39 (1.02-5.61)
Absent	16 (76.2%)	5 (23.8%)			
Focal and diffuse	9 (42.9%)	12 (57.1%)			

Table 5. FOXE1 haplotypes (rs1867277 and rs894673) frequencies according to classic variant of thyroid carcinoma cases, and controls

	AATT, AAAA, GATT	Others
Control	0 (0%)	51 (62.2%)
Classic variant of papillary thyroid carcinoma	11 (100%)	31 (37.8%)

P Value =0.000 χ^2=15.14 OR (95% CI)=2.64 (2.00-3.49).

CI=1.72-9.90) and the presence of FOXE1 rs1867277 A allele was significantly associated with classic variants (χ^2=3.66 P=0.05, OR=3.03 95%, CI=0.79-11.60) (**Table 3**).

The presence of A allel for the rs1867277 was significantly associated with several histopathological parameters in classic variant in papillary thyroid carcinoma cases such as, the A allel was found associated with lymph node invasion risk by 2.0 fold (χ^2=3.26, P=0.07, OR=2.0 95%, CI=1.02-3.90), capsular invasion risk by 2.39 fold (χ^2=4.84, P=0.02, OR =2.39 95%, CI=1.02-5.61), and pT3 with pT4 pathological stage risk by 3.57 fold (χ^2=9.53, P=0.02, OR =3.57 95%, CI=1.39-9.17) (**Table 4**). In addition, the AATT, AAAA, GATT haplotypes (rs1867277 and rs894673) were evaluated for association with papillary thyroid carcinoma cases. Our study results showed a significant difference according to two-allele haplotypes distribution between papillary thyroid carcinoma cases and control groups (*P* Value =0.000 χ^2= 15.14 OR=2.64 (95% CI=2.00-3.49)) (**Table 5**).

Discussion

We demonstrated the positive association of FOXE1 gene variants (rs894673, rs1867277, rs894673, rs3758249) with papillary thyroid carcinoma cases in Turkish population for the first time. Results indicated that, there was a significant difference in the distribution of FOXE1 rs894673 genotypes in Papillary thyroid carcinoma cases (P=0.01) but not for the other polmorphisms. FOXE1 protein is a transcription factor belonging to the family of forkhead proteins which is considered as an initiator of tran-

scription [7, 10]. Furthermore, FOXE1 protein is important for the development and differentiation of thyroid gland. FOXE1 is thought to be particularly significant for the development of a classic subtype of PTC, which constitutes the half of all PTC cases [7, 8, 14]. According to some studies, FOXE1 protein is effective in cancer development and progression. Therefore, FOXE1 protein is thought to be a potential marker for this cancer. Thyroid cancer is defined as a complex disease, which genetic and environmental factors can lead to the development of the disease [7, 15].

Some FOXE1 gene variants have been associated with 5.7 fold increase the risk of thyroid cancer development [16]. Not all thyroid nodules are malignant; the main risk factors are, under 30 or over 60 of age, gender and family history [16, 17]. Polyzos et al indicated a possible relationship between benign thyroid nodules and carcinoma development due to their frequency [17]. In another study, benign thyroid nodules were found in 6.4% of all women and 1.6% of all men with malignant thyroid tumors in Framingham study [17, 18]. Consequently, the patients we consider as healthy individuals may be at risk for thyroid cancer development. In this study, no significant difference has been detected between patients and controls for FOXE1 variant incidences since the relatively small sample size of patient and control group.

Sequeira et al. showed the FOXE1 expression frequency as 60% in human thyroid glands. Furthermore FOXE1 expression was found in 43-100% of benign thyroid lesions, 44% of follicular carcinoma and 65% of papillary thyroid carcinoma (PTC) cases [19]. Several studies have identified a relationship between thyroid cancers and FOXE1 variants. Additionally, Tomaz et al. found a relationship between FOXE1 rs965513 and rs1867277 gene variants and susceptibility to familial and sporadic non-medullary thyroid cancer in Portuguese population [20]. Bullock et al. implied a relationship between papillary thyroid cancer and the FOXE1 gene variations [21]. Landa et al. associated the FOXE1 gene with papillary thyroid carcinoma in the Spanish population. They implicated that, FOXE1 rs1867277 A allele is associated with an increased transcriptional activity and expression levels, which may result in tumor growth in thyroid gland [7]. Kula et al, reported that, FOXE1 rs1867277 A allele is associated with increased expression levels and may be related with metastasis of thyroid carcinoma [4]. Previous studies mentioned many unfavorable prognostic factors such as, capsular infiltration, extrathyroidal extension and lymph node metastases, which are critical for recurrence, are strongly associated with poor thyroid cancer prognosis [6, 22, 23]. Likewise, Bychkov et al, showed a relationship between FOXE1 rs1867277 variation and tumor multifocality, capsular invasion and tumor aggressiveness while the presence of the A allele in rs1867277 was related with elevated level of nuclear expression of FOXE1 in cancer cells [24]. In this study, the presence of the AA genotype of FOXE1 rs1867277 variation was significantly associated with follicular variant formation in the patient group. Therefore, the GA genotype was found to provide protection capsular invasion and early stage tumors. The protective effect of the GG genotype was determined in the lymph node invasion cases. Furthermore, the G allele was shown to provide protection against lymph node invasion, capsular invasion and early stage tumors, respectively. Due to protection against lymph node invasion, this will improve the prognosis such as seen at various gastrointestinal tumors [25-27]. In addition, the presence of the A allel for rs3758249 variation was found to be protective against the formation of follicular variant in patient groups.

Our findings suggest a role for FOXE1 variations which confer a higher risk for the histopathological features of papillary thyroid carcinoma. One of the potential limitations of this study is the relatively small sample size. A larger sample size would strengthen the present study findings and provide further verification.

Disclosure of conflict of interest

None.

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