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ORIGINAL ARTICLE

Association between E-cadherin (CDH1) polymorphisms and papillary thyroid carcinoma risk in Han Chinese population

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Abstract The aim of this study is to investigate the associations between E-cadherin gene (CDH1) polymorphisms and papillary thyroid carcinoma (PTC) risk predisposition. We undertook a case–control study to analyze three CDH1 polymorphisms (+54T>C, −160C>A, and −347G→GA) in an Han Chinese population, by extraction of genomic DNA from the peripheral blood of 98 patients with PTC and 176 control participants, and performed CDH1 genotyping using DNA sequencing. The obtained results indicated that overall, no statistically significant association was observed in +54T>C. Nevertheless, −347G→GA genotype was at increased risk of PTC ($P = 0.001$; odds ratio (OR) = 2.12, CI 95%:1.24–3.34). Furthermore, −347GA/GA genotype thyroid cancers were more significantly common in patients with tumor size of ≥20 mm than G or G/GA genotypes PTC and in cases of advanced T stage. However, −160C>A genotype demonstrated a protective effect in PTCs ($P = 0.006$; OR = 0.59, CI 95%: 0.42–0.87). These findings led us to conclude that polymorphism in −347G→GA was observed to be associated with susceptibility of PTC. However, −160C>A polymorphism indicated to play a protective role in susceptibility to PTC. Nevertheless, further investigation with a larger sample size is needed to support our results.

Keywords Allele · E-cadherin · Papillary thyroid carcinoma · Polymorphism

Introduction

Papillary thyroid carcinoma (PTC) comprises the vast majority (90%) of all thyroid cancers. The yearly incidence of PTC has a 2.9-fold increase from 1988 to 2002, and this trend appears to be continuing [1, 2]. Therefore, enhancing our knowledge of molecular biology of the malignant thyroid disease will aid to cover the gaps in comprehending the pathogenesis of this neoplasm and potentially improve patients' clinical outcome, and currently, genetic susceptibility, with regard to specific genotypes, suggests the combination of some genetic alleles in the etiology of this kind of malignancy [3, 4].

E-cadherin is one of the major constituents of cell adhesion complexes in epithelial cells [5, 6]. It is a 97-kDa transmembrane glycoprotein encoded by the E-cadherin gene (CDH1) located on chromosome 16q22.1. It plays central roles in the establishment of adherent type junctions by mediating calcium-dependent cellular interactions, and is thought to be a tumor suppressor protein [7]. Loss of its function results in transition to an invasive phenotype in human epithelial cancers [8]. Besides its role in physical cell–cell adhesions, E-cadherin is also thought to be involved in intracellular signaling in normal epithelial cells, since downregulation of this molecule in epithelial cells is frequently associated with tumor formation and differentiation [9].

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Previous studies have reported that CDH1 expression is decreased in classic PTC [10–12] and its diffuse sclerosing variant and also in follicular carcinoma [13, 14]. Rarely, this decrease in expression is attributable to mutation of the CDH1 gene, loss of heterozygosity [15], or hypermethylation of the CDH1 promoter [16]. In most tumors, the mechanism of CDH1 down-regulation is, however, unknown.

Recently, the promoter region of CDH1 was reported to be highly polymorphic [17]. One of the polymorphisms is the −347G→GA (rs5030625) single nucleotide polymorphism (SNP) upstream from the transcriptional start site [18]. Just as nucleotide variations in the coding region of a gene can alter protein expression, the −347G→GA polymorphism within the promoter region may change the transcriptional efficiency of CDH1 [19]. For example, the GA-allele has a weak transcriptional factor-binding strength and transcriptional activity compared with the G-allele [18], suggesting that the GA-allele may be associated with tumor formation or differentiation. Moreover, several other SNPs, including +54T>C, −3159T>C, −160C>A, −2076C>T, and −616G>C, were studied in Japanese and Italian populations, which resulted in the identification of haplotypes associated with increased risk of carcinoma [19, 20].

The above studies have highlighted the ethnic variation in frequency and risk predisposition of these SNPs [21]. However, association of E-cadherin (CDH1) gene polymorphisms and PTC susceptibility has not been reported. Thus, in this study, in order to clarify association between three CDH1 gene polymorphisms (+54T>C, −160C>A, and −347G → GA) and PTC risk, we have performed a hospital-based case–control study on Han Chinese population.

Materials and methods

Subjects

A total of 98 cases of patients with PTC and 176 healthy controls were qualified for this study. All samples were collected before any kind of therapeutic measures between March 2008 and October 2010 at the Department of Endocrinology and Department of Surgery, The Affiliated Hospital of Binzhou Medical University. The patient samples were collected after the diagnosis was confirmed by histopathological exam using fine-needle aspiration. All patients were submitted to thyroidectomy. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of the Affiliated Hospital of Binzhou Medical University. The PTCs were staged according to the American Joint Committee on Cancer/International Union Against Cancer tumor-node-metastasis (TNM) staging system [22] and total-body radio-iodine scintigraphy was used for this purpose in all patients. The TNM is a system that describes the anatomic extent of the primary tumor (T), the involvement of regional lymph nodes (N), and distant metastasis (M). Although the system is applicable to all histologies of thyroid carcinoma, the stage grouping varies with different histologic types. PTC is being staged in the same way. It is the only staging system that regularly undergoes revision to keep up with prevailing changes in the field of thyroid carcinoma.

DNA extraction

Genomic DNA from whole blood cells was extracted using a QIAamp Blood kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. DNA concentration and purity of each sample were measured by ultraviolet spectrophotometer (Eppendorf, Hamburg, Germany). DNA samples were routinely stored at −20°C.

Genotyping

Analysis of the CDH1 SNPs, +54T>C, −160C>A, and −347G→GA, was performed using multiplex polymerase chain reaction (PCR) with an ABI premix. Genomic DNA from whole blood was used as a PCR template in a total reaction volume of 10 μl that contained 10 pmol designed primers: +54T>C (rs3743674): 5′-CCCCTGGTCTCATC ATTTC-3′ (forward) and 5′-AATTCCTCCAAGAATCCC CAG-3′ (reverse); −160C>A(rs16260): 5′-TGATCCCA GGTCTTAGTGAG-3′ (forward) and 5′-GCTCCTCAGG ACCCGAAC-3′ (reverse); and −347G→GA(rs5030625): 5′-GCCCCGACTTGTCTCTCTAC-3′ (forward) and 5′-GG CCACAGCCAATCAGCA-3′ (reverse). PCR was performed as follows: one cycle at 94°C for 10 min, 35 cycles at 94°C for 30 s, 59°C for 30 s, and 72°C for 30 s, followed by 72°C for 5 min. The final extension was at 72°C for 10 min. PCR products were analyzed on a 3% ethidium bromide-added agarose gel, and photographs were taken under ultraviolet light transilluminator. Subsequently, PCR product was sequenced in an ABI PRISM 3100 sequencer using BigDye Terminator v3.1 Cycle Sequencing method (Applied Biosystems, USA) as recommended by the manufacturer. Candidate SNP regions were detected and typed with the aid of DNA Star Software (DNASTAR, Madison, WI, USA).

Statistical analysis

Statistical calculations were performed using the SPSS Statistics 13.0 for Windows software package (SPSS Inc., Chicago, Ill). Frequency and susceptibilities of mutations were compared with the χ^2 test. The P values obtained were two-tailed, and the association of significance was

assumed to be less than 0.05. The Hardy–Weinberg equilibrium (HWE) was verified for the different polymorphisms studies; P value >0.05 was considered with no significant deviation from the equilibrium. The crude and adjusted odds ratio (ORs) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression.

Results

Characteristics of Subjects

This study comprised 98 patients and 176 controls with comparable sex and age, 62 patients were classified as stage I and II, and 36 cases as stage III and IV. All the cases and controls were randomly selected from the general Han Chinese population of China. Table 1 shows the main characteristics of case–control populations. The gender, age distribution, and smoking habits in case and control population group are not statistically different. The frequency of females was significantly higher, being in accordance with a worldwide estimation for PTC.

E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA Polymorphisms in PTC

The gene polymorphisms of E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA were successfully amplified in the majority of PTCs and control cases; however, 1–6 samples failed for PTC patients and 7–13 samples for control subjects, as shown in Table 2. The number of patients with E-cadherin polymorphisms of +54T>C, −160C>A, and −347G→GA were 35/95 cases, 61/92 cases, and 23/97 cases, respectively. The genotypic distributions of all the three gene polymorphisms in cases and controls were in HWE (all $P > 0.05$). Overall, no statistically significant association was observed in +54T>C. Individuals with −347G→GA genotype were more susceptible to PTC ($P = 0.001$, OR = 2.12). Although the variant allele frequency AA of −160C>A was higher in controls as compared with cases (23.9% versus 12.8%), this result was not statistically significant ($P = 0.082$). In case of alleles, association was observed with A allele of −160C>A with statistically significant reduced risk of PTC ($P = 0.006$, OR = 0.59).

Table 1 General characteristics for the PTC cases ($n = 98$) and control population ($n = 176$)

Characteristics	Cases, n (%) ($n = 98$)	Controls, n (%) ($n = 176$)	P value[c]
Gender			
Male	34 (34.7)	58 (33.0)	0.77
Female	64 (65.3)	118 (67.0)	
Age[a,b]			
≤30	5 (5.1)	8 (4.5)	0.96
31–49	34 (34.7)	63 (35.8)	
50–69	48 (49.0)	82 (46.6)	
≥70	11 (11.2)	23 (13.1)	
Smoking habits			
Never	80 (81.6)	155 (86.6)	0.51
Current	15 (15.3)	21 (11.7)	
Missing	3 (3.1)	3 (1.7)	

[a] Age of diagnosis for cases

[b] Age of control population at the time of diagnosis for the matched case

[c] P value obtained by χ^2 (cases vs. control group)

Relationship between E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA polymorphisms and known clinicopathological variables

Table 3 depicts the association of +54T>C, −160C>A, and −347G→GA polymorphism with clinicopathological characteristics, including gender, age at diagnosis, tumor size, pathological diagnosis, capsule invasion, lymph node metastasis, and pathological stage of the cancer. The CHD1 −160AA genotype was observed to be significantly associated with reduced risk with capsule invasion, lymph node metastasis, and pathological stage ($P = 0.04$, $P = 0.02$ and $P = 0.02$, respectively).

For CHD1 −347G→GA, the GA/GA genotype thyroid cancers were significantly more common in cancers of higher pathological stages (stages 3&4 versus 1&2, $P = 0.02$). The polymorphism was not related to the gender of the patients and pathological features (histological variant, multicentricity capsule invasion, lymph node metastasis and pathological stage) of the cancer.

Our data indicated that CHD1 −160AA polymorphism may be protective genotype for PTC development and may decrease the risk of PTC among Han Chinese population. However, the −347G→GA promoter polymorphism in CDH1 gene may be a susceptibility factor of PTC.

Discussion

A classical tumor suppressor, E-cadherin expression has been shown to be frequently reduced or lost among epithelial carcinomas [23, 24]. The altered expression of E-cadherin results in the suboptimal regulation of cell–cell adhesion, loss of cellular polarity, tissue disorganization, tumor progression, and metastasis [24–26]. Several functional polymorphisms that diminish E-cadherin expression

Table 2 Association between E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA polymorphisms and PTC

Genotype	Cases[a], *n* (%)	Controls[a], *n* (%)	*P* value	Crude OR (95% CI)	Adjusted OR (95% CI)[b]
+54T>C	*n* = 95	*n* = 163			
TT	60 (63.2)	100 (61.3)		1 (Reference)	1 (Reference)
TC	27 (28.4)	51 (31.3)	0.664	1.14 (0.66–1.99)	1.06 (0.67–1.78)
CC	8 (8.4)	12 (7.4)	0.828	0.91 (0.45–2.87)	1.15 (0.42–2.75)
T allele	147 (28.5)	251 (48.6)			
C allele	43 (8.3)	75 (14.5)	0.940	1.03 (0.67–1.57)	
−160C>A	*n* = 92	*n* = 169			
CC	31 (34.7)	92 (32.4)		1 (Reference)	1 (Reference)
CA	49 (52.5)	60 (43.7)	0.002	0.43 (0.25–0.74)	0.51 (0.35–0.86)
AA	12 (12.8)	17 (23.9)	0.082	0.49 (0.22–1.12)	0.53 (0.31–1.25)
C allele	111 (21.3)	244 (46.7)			
A allele	73 (14.0)	94 (18.0)	0.006	0.59 (0.42–0.87)	
−347G→GA	*n* = 97	*n* = 165			
GG	74 (76.3)	95 (57.6)		1 (Reference)	1 (Reference)
G/GA	19 (19.6)	54 (32.7)	0.009	2.23 (1.21–4.07)	1.57 (0.87–3.19)
GA/GA	4 (4.1)	16 (9.7)	0.042	3.21 (1.01–9.71)	3.15 (1.04–8.55)
G allele	167 (31.9)	244 (46.6)			
GA allele	27 (5.2)	86 (16.4)	0.001	2.12 (1.24–3.34)	

[a] The χ^2 for HWE of E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA polymorphisms in case and control group is 3.37 and 2.22, 1.17 and 2.27, and 3.23 and 3.75, respectively (all $P > 0.05$)

[b] ORs were adjusted for gender, age (≤30, 31–49, 50–69 and ≥70 years) and smoking status (never and current smokers)

have been reported [17–19, 27]; however, studies on PTC risk have been sparse. This is perhaps the first molecular epidemiologic study evaluating the association of E-cadherin (CHD1) gene polymorphisms with PTC susceptibility in Han Chinese population.

The present study shows that there is no association between the CDH1 +54T>C SNPs and PTCs development. Although a study by Zhang et al. [28] has found an association between +54T>C and esophageal and gastric cancer, other studies were negative [21]. A previous study also has shown that the haplotypes analysis of +54T>C genotype revealed the OR of gastric cancer 1.5 (95% CI 0.7–3.5), but did not reach statistical significance [29]. The reason for the difference can be attributed to differences in polymorphisms studied, genetic background and local environmental factors, and highlights the need for comparative studies between different ethnic groups.

Several molecular epidemiological studies have demonstrated an association between the CDH1 −347G→GA polymorphism and the risk of cancers, including gastric, colorectal, and esophageal cancers [30]. The authors of these studies suggested that the CDH1 −347G→GA polymorphism may be functional, and that the GA-allele could result in transcriptional downregulation of CDH1 and low expression of E-cadherin compared with the G-allele, thereby increasing the risk of cancer. However, recent studies have indicated that some functional polymorphisms may play more important roles in the prognosis of cancer than in its formation [31]. To further investigate the association between the functional CDH1 -347G → GA polymorphism and PTC, we conducted the present case–control study in a Chinese population. We found that the GA-allele increased the risk of PTC compared with the G-allele in this Chinese population ($P = 0.001$, OR = 2.12). Meanwhile, our result showed that GA-allele seems to be a low penetrance tumor susceptibility allele in the development of PTC in Han Chinese population.

Recently, genetic variants of the CDH1 gene in the etiology of several cancers have drawn increasing attention. Growing number of studies have proposed that −160AA in the promoter region of the CDH1 gene was emerging as a tumor susceptibility allele in the development of several kinds of cancer, such as prostate cancer, urothelial cancer, and gastric cancer [32, 33]. Yu et al. [34] genotyped the functional promoter polymorphisms −160C/A (rs16260) among 468 breast cancer cases and 470 controls and found that the SNP were in high-linkage disequilibrium (LD). On the contrary, Lei et al. [35] genotyped the −160C/A (rs16260) SNP among 576 cases and 348 controls, and found no association with breast cancer risk. Interestingly, in our study, −160AA genotype showed a negative role in susceptibility to PTCs

Table 3 Clinicopathological relevance of E-cadherin (CDH1) +54T>C, −160C>A, and −347G→GA polymorphisms in PTC

Parameters	+54T>C		*P* value	−160C>A		*P* value	−347G→GA		*P* value
	TT + TC (%)	CC (%)		CC + CA (%)	AA (%)		GG + G/GA (%)	GA/GA (%)	
Gender									
Male	30 (31.6)	2 (2.1)	0.59	29 (31.5)	3 (3.3)	0.45	31 (32.0)	2 (2.1)	0.49
Female	57 (60.0)	6 (6.3)		51 (55.4)	9 (9.8)		62 (63.9)	2 (2.1)	
Age									
<45 years	36 (37.9)	3 (3.2)	0.83	37 (40.2)	2 (2.2)	0.06	37 (38.1)	2 (2.1)	0.68
≥45 years	51 (35.7)	5 (5.3)		43 (46.7)	10 (10.9)		56 (57.7)	2 (2.1)	
Size									
<20 mm	50 (52.6)	4 (4.2)	0.68	47 (51.1)	4 (4.3)	0.10	48 (49.5)	0 (0)	0.04
≥20 mm	37 (38.9)	4 (4.2)		33 (35.9)	8 (8.7)		45 (46.4)	4 (4.1)	
Type									
CPTC	48 (50.5)	2 (2.1)	0.10	48 (55.2)	4 (4.3)	0.08	49 (50.5)	2 (2.1)	0.92
FVPTC	39 (41.1)	6 (6.3)		32 (34.8)	8 (8.7)		44 (45.4)	2 (2.1)	
Multicentricity									
Present	35 (36.8)	4 (4.2)	0.59	34 (37.0)	5 (5.4)	0.96	40 (41.2)	0 (0)	0.09
Absent	52(54.7)	4 (4.2)		46 (50.0)	7 (7.6)		53 (54.6)	4 (4.1)	
Capsule invasion									
Present	46 (48.4)	3 (3.2)	0.41	45 (48.9)	3 (3.3)	0.04	49 (50.5)	2 (2.1)	0.92
Absent	41 (43.2)	5 (5.3)		35 (38.0)	9 (9.8)		44 (45.4)	2 (2.1)	
T stage									
1 + 2	52 (54.7)	2 (2.1)	0.06	46 (50.0)	8 (8.7)	0.55	54 (55.7)	0 (0)	0.02
3 + 4	35 (36.8)	6 (6.3)		34 (37.0)	4 (4.3)		39 (40.2)	4 (4.1)	
Lymph node metastasis									
Present	45 (47.4)	2 (2.1)	0.15	42 (45.7)	2 (2.2)	0.02	44 (45.4)	2 (2.1)	0.92
Absent	42 (44.2)	6 (6.3)		38 (41.3)	10 (10.9)		49 (50.5)	2 (2.1)	
TNM pathological stage									
Stage 1 and 2	55 (57.9)	5 (5.3)	0.97	45 (48.9)	11 (12.0)	0.02	60 (61.9)	1 (1.0)	0.11
Stage 3 and 4	32 (33.7)	3 (3.2)		35 (38.0)	1 (1.1)		33 (34.0)	3 (3.1)	

CPTC conventional papillary thyroid carcinoma, *FVPTC* follicular variant of papillary thyroid carcinoma

($P = 0.006$, OR = 0.59), and also, it seems to be in association with lower lymph node metastasis rate and pathological stage. However, further investigation with a larger sample size is needed to support our results.

In conclusion, this is the first study of E-cadherin (CHD1) gene polymorphisms and risk of PTC susceptibility from Han Chinese population. These findings imply that a continued research into −347G→GA and −160C/A polymorphisms will be an important source of information on the pathogenesis and prediction of clinical behavior of thyroid cancers. Functional studies are further required to evaluate genotype and phenotype correlation in a large cohort of various ethnicities.

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