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Original Research

ERBB4 gene polymorphisms and the risk of prostate cancer in a sample of Iranian Population

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Abstract: Genetic polymorphisms in *ERBB4* are thought to be associated with cancer susceptibility. In the present study, we aimed to assess the impact of *ERBB4* rs12052398 T>C, rs13393577 A>G, rs13424871 A>T, rs16847082 A>G and rs6147150 (12-bp I/D) polymorphisms on risk of prostate cancer (PCa) in a sample of Iranian population. In a case-control study, we enrolled 169 patients with pathologically confirmed PCa and 182 subjects with benign prostatic hyperplasia (BPH). No significant association was found among *ERBB4* polymorphisms and risk of PCa. Subjects carrying TT/AA/AA/AG/ID, TC/AA/AA/AA/II, TT/AA/AT/AA/II and TT/AA/AT/AG/ID genotypes are associated with a decreased risk of PCa. Our findings suggest that haplotypes CAAAI and TAAAD (rs12052398, rs13393577, rs13424871, rs16847082 and rs6147150I) of the *ERBB4* polymorphisms are associated with a significantly lower risk of PCa. Further studies with a larger sample sizes and diverse ethnicities are necessary to verify our findings.

Key words: Prostate cancer, ERBB4, polymorphism.

Introduction

Prostate cancer (PCa) is a common cancer that occurs in the prostate epithelial cells (1). In 2016, a total of 180,890 new cases of Pca and 26,120 deaths from the disease are expected to occur in the United States (2). In Iran, the incidence rate of PCa is approximately 9.6 per 100,000 (3, 4), which is similar to the Asia-Pacific region, but it is considerably lower than the rest of the world (32.8 per 100,000) (5). Although there are several unanswered questions regarding PCa etiology, it has been proposed that both genetic and environmental factors have played an important role in pathogenesis of the disease for many years (6-9).

The EGFR (epidermal growth factor receptor) family, which is implicated in the development and normal growth of several organs, consists of four receptor tyrosine kinases: EGFR (HER1/ ErbB1), ErbB2 (HER2/ neu), ErbB-3 (HER3) and ErbB4 (HER4) (10, 11). They are widely expressed in epithelial, mesenchymal and neuronal tissue and activate a series of complex cellular signal transduction pathways that mediate diverse cellular functions including cell proliferation, differentiation, motility and survival (12-16).

ERBB4, a member of the EGFR subfamily of receptor tyrosine kinases, is mapped to chromosome 2q33.3-q34. It contains 28 exons that code for a 1308 amino acid protein. Accumulating evidence indicates that ErbB4 plays critical roles in the development and prognosis of different tumors (13, 15) and genetic variants of *ERBB4* are involved in the risk of developing many cancers including breast cancer, hepatocellular carcinoma (HCC) and colorectal cancer (CRC) (17-20). In the present study, we aimed to examine the impact of *ERBB4* rs12052398 T>C, rs13393577 A>G, rs13424871 A>T, rs16847082 A>G and rs6147150 (12-bp I/D) polymorphisms on the risk of developing PCa in a sample of Iranian population.

Materials and Methods

Patients

The current case-control study included 169 unrelated men with histopathologically confirmed prostate adenocarcinoma and 182 age-matched unrelated men with benign prostatic hyperplasia (BPH) with no history of any type of cancer. The study design and enrolment procedure have been previously described (21, 22). All the subjects were enrolled from the Shahid Labbafinejad Medical Center at the Shahid Beheshti University of Medical Sciences, Tehran, Iran. Ethics approval for recruitment was obtained from the local Ethics Committee of the Zahedan University of Medical Sciences, and written informed consent was obtained from all subjects (patients and controls). Blood samples were collected in EDTA-containing tubes and genomic DNA where was extracted using the salting-out method, as

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described previously (23).

Genotyping

Genotyping of *ERBB4* rs12052398 T>C, rs13393577 A>G, rs13424871 A>T and rs16847082 A>G gene polymorphisms was determined using the PCR-RFLP method. Genotyping of the 12-bp insertion/deletion (I/D) in the *ERBB4* gene was performed using PCR. The primers are listed in Table 1. PCR was performed using a commercially available Prime Taq premix (Genetbio, South Korea), according to the manufacturer's recommended protocol. In each 0.20-ml reaction, 1 µl of genomic DNA (approximately 100 ng/ml), 1 µl of each primer, 10 µl of 2X Prime Taq Premix and 7 µl ddH_2O were added. The PCR conditions were set as follows: 95°C for 5 min, 30 cycles of 95°C for 30s, annealing at the appropriate temperature (Table 1) for 30s, and 72°C for 30 s and a final extension step of 72°C for 10 min. PCR product (10 µl) was then digested using the appropriate restriction enzyme (Table 1), electrophoresed on 2.5% agarose gels containing 0.5 µg/ml ethidium bromide and observed under UV light.

Statistical analysis

Statistical analysis was performed using the SPSS 18 statistical software. Data were analyzed using an independent sample *t*-test and the χ^2 test. Association between *ERBB4* polymorphisms and PCa were calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. Haplotype analysis was performed using SNPStats software (24). A p-value < 0.05 was considered statistically significant.

Results

The study group consists of 169 PCa patients with an average age of 61.36±6.60 years and 182 BPH with a mean age of 62.51±7.67 years. There was no significant difference in age between the groups (p=0.135).

The genotypes and allele frequencies of *ERBB4* rs12052398 T>C, rs13393577 A>G, rs13424871 A>T, rs16847082 A>G and rs6147150 (12-bp I/D) polymorphisms in PCa and control subjects are shown in Table 2. Our findings indicate that *ERBB4* variants were not associated with PCa in any inheritance models tested (co-dominant, dominant and recessive).

We found that subjects carrying the TT/AA/AA/AG/ID, TC/AA/AA/AA/II, TT/AA/AT/AA/II and TT/AA/AT/AG/ID genotypes had a decreased the risk of developing PCa compared to those with the rs12052398TT/rs13393577AA/rs13424871AA/rs16847082AA/rs6147150II genotypes (Table 3). Haplotype analysis is shown in Table 4. The haplotypes C/A/A/A/I and T/A/A/A/D were associated with a decreased risk of developing PCa (OR=0.39, 95%CI=0.20-0.74, p=0.007, and OR=0.37, 95%CI=0.15-0.91, p=0.031, respectively) compared to rs12052398T/rs13393577A/rs13424871A/rs16847082A/rs6147150I genotypes. There was no significant association between ERBB4 variants and clinicopathological characteristics (Table 5).

Discussion

The ErbB family of receptor tyrosine kinases (ErbB1, ErbB2, ErbB-3 and ErbB4) mediates cellular responses to growth factors through their intracellular domain and interacts with downstream signaling pathways, which are important for development, differentiation and proliferation (12-16). ERBB4 is a single transmembrane receptor tyrosine kinase (25).

Several reports show that the importance of ErbB4 dysregulation, which is possibly involved in tumorigenesis (15, 26, 27). ErbB4 expression in ependymoma is high and it is associated with a low patient survival rate (27). Overexpression of ErbB4 in a non-small cell lung cancer (NSCLC) cell line resulted in increased cell proliferation (26). In addition, genetic components in ErbB4 also play a vital role in the pathogenesis of cancer (17, 18).

In the current study, we investigated the impact of *ERBB4* rs12052398 C>T, rs13393577 G>A, rs13424871 A>T, rs16847082 A>G, and the 12-bp insertion/deletion (I/D) polymorphisms on the risk of developing PCa in a sample of Iranian population.

Because all of the ERBB members are important in tumor cell survival and proliferation, genetic polymorphisms of these proteins might contribute to the cancer risk. However, we found no significant association between *ERBB4* variants and the risk of develo-

Table 1. The primers used for detection of *ERBB4* polymorphisms using PCR-RFLP methods.

ERBB4 polymorphism	Primer sequence (5′->3′)	Restriction Enzyme	Annealing Temperature (°C)	Fragment (bp)
rs12052398 T>C				
Forward	ATGCCCTTTGAGACTTCGTACA	AdeI	68	T allele, 460; C allele, 309+152
Reverse	AGAGTGGGGAAGAAAGAGACATC			
rs13393577 A>G				
Forward	AAAGGCCATCCCTCAAGGTGATAGCACC	MSPI	65	A allele, 172; G allele, 144+ 28
Reverse	ACCAAATCAAGGATTTTTCACTACTTTG			
rs13424871 A>T				
Forward	TGATTGTTGAACCCTATGGACA	BseGI	59	A allele, 267; T allele, 188+ 79
Reverse	GAAAGCATACTAGAAATGG			
rs16847082 A>G				
Forward	CCGTAACATTGTTCCTTTGGGTG	MboII	65	A allele, 379; G allele, 243+136
Reverse	ATACACAACAAAAACTCCCCTGC			
rs6147150 (12bp I/D)				
Forward	TCACCCAACTTTGTAGATTATACCT	-	62	I allele, 116; D allele, 104
Reverse	AGGCCATCTTTCCTCACCTG			

Table 2. Genotype and allele frequencies of *ERBB4* polymorphisms in prostate cancer (PCa) and control patients.

ERBB4 polymorphism	Prostate Cancer Patients n (%)	Control Patients n (%)	OR (95%CI)	P-value
rs12052398 T>C				
Codominant				
TT	89 (52.7)	87 (47.8)	1.00	-
TC	72 (42.6)	91 (50.0)	0.77 (0.50-1.18)	0.276
CC	8 (4.7)	4 (2.2)	1.96 (0.57-6.73)	0.375
Dominant				
TT	89 (52.7)	87 (47.8)	1.00	-
TC+CC	80 (47.3)	93 (52.2)	0.84 (0.55-1.28)	0.454
Recessive				
TT+TC	161 (95.3)	178 (97.8)	1.00	-
CC	8 (4.7)	4 (2.2)	2.21 (0.65-7.49)	0.245
Allele				
T	250 (74.0)	265 (72.8)	1.00	-
C	88 (26.0)	99 (27.2)	0.94 (0.67-1.32)	0.733
rs13393577 A>G				
AA	153 (90.5)	169 (92.9)	1.00	-
AG	16 (9.5)	13 (7.1)	1.38 (0.63-3.04)	0.431
GG	0 (0.0)	0 (0.0)	-	-
Allele				
A	322 (95.3)	351 (96.4)	1.00	-
G	16 (4.7)	13 (3.6)	1.32 (0.64-2.73)	0.467
rs13424871 A>T				
Codominant				
AA	130 (76.9)	133 (76.0)	1.00	-
AT	37 (21.9)	37 (21.1)	1.02 (0.61-1.71)	0.918
TT	2 (1.2)	5 (2.9)	0.41 (0.08-2.15)	0.449
Dominant				
AA	130 (76.9)	133 (76.0)	1.00	-
AT+TT	39 (23.1)	42 (24.0)	0.95 (0.58-1.56)	0.899
Recessive				
AA+AT	167 (98.8)	170 (97.1)	1.00	-
TT	2 (1.2)	5 (2.9)	0.41 (0.78-2.13)	0.407
Allele				
A	297 (88.8)	303 (86.6)	1.00	-
T	41 (11.2)	47 (13.4)	0.89 (0.57-1.39)	0.648
rs16847082 A>G				
Codominant				
AA	112 (66.3)	111 (61.0)	1.00	-
AG	51 (30.2)	62 (34.1)	0.82 (0.52-1.28)	0.419
GG	6 (3.5)	9 (4.9)	0.66 (0.23-1.92)	0.594
Dominant				
AA	112 (66.3)	111 (61.0)	1.00	-
AG+GG	57 (33.7)	71 (39.0)	0.79 (0.51-1.23)	0.319
Recessive				
AA+AG	163 (96.5)	168 (95.1)	1.00	-
GG	6 (3.5)	9 (4.9)	0.69 (0.24-1.97)	0.600
Allele				
A	275 (81.4)	284 (78.0)	1.00	-
G	63 (18.6)	80 (22.0)	0.81 (0.56-1.18)	0.303
rs6147150 (12bp I/D)				
Codominant				
II	103 (60.9)	100 (54.9)	1.00	-
ID	61 (36.1)	79 (43.4)	0.75 (0.49-1.16)	0.226
DD	5 (3.0)	3 (1.7)	1.62 (0.38-6.95)	0.722
Dominant				
II	103 (60.9)	100 (54.9)	1.00	-
ID+DD	66 (39.1)	82 (45.1)	0.78 (0.52-1.20)	0.280
Recessive				
II+ID	164 (97)	179 (98.3)	1.00	-
DD	5 (3.0)	3 (1.7)	1.82 (0.43-7.73)	0.489
Allele				
I	267 (79.0)	279 (76.4)	1.00	-
D	71 (21.0)	85 (23.3)	0.87 (0.61-1.25)	0.469

ping PCa in our population. Recently, a genome-wide association study (GWAS) identified the ERBB4 gene as a PCa susceptibility gene (28). GWAS in Korean women showed that the rs13393577 variant of the *ERBB4* gene is breast cancer (BC) susceptibility variant (17). Rokavec et al. (18) found that the -782G>T (rs62626348) variant of the *ERBB4* gene is associated with BC and CRC risk. They found that other variants of *ERBB4* including (-718 C>T, -815 A>T, -609 G>A, -267 C>G) were not associated with cancer risk. The 12-bp I/D polymorphism (rs6147150) in the 3'UTR of ERBB4 increased the risk of developing CRC and HCC in a Chinese population (19, 20). The D/D variant may interrupt the binding site in 3'UTR for some microRNAs and lead to their up-regulation in tumor tissues (20). Qu et al. (29) reported that the *ERBB4* rs1595066 variant is significantly associated with reduced esophageal squamous cell carcinoma (ESCC) risk (29). They analyzed haplotypes of rs1595066 and rs16845990, and found that rs1595066A/rs16845990C and rs1595066A/rs16845990T haplotypes reduce the risk of ESCC, while rs1595066G/rs16845990C and rs1595066G/rs16845990T haplotypes increase ESCC risk.

Rokavec et al. (18) found that the -782G>T (rs62626348) variant of the ERBB4 gene is associated with BC and CRC risk. They found that other variants of *ERBB4* (-718 C>T, -815 A>T, -609 G>A, -267 C>G) were not associated with cancer risk. Kurppa et al. (30) investigated the frequency and prognostic significance of two *ERBB4* promoter region polymorphisms, -782G>T (rs62626348) and -815A>T (rs62626347). They found that the rs62626347 variant was signifi-

Table 3. Effect of *ERBB4* gene polymorphisms interactions on prostate cancer (PCa) risk.

rs12052398	rs13393577	rs13424871	rs16847082	rs6147150	PCa Patients n (%)	Control Patients n (%)	OR (95%CI)	P-value
TT	AA	AA	AA	II	27 (16.0)	14 (7.7)	1.00	-
TT	AA	AA	AA	ID	14 (8.3)	19 (10.4)	0.38 (0.15 - 0.98)	0.060
TT	AA	AA	AG	II	12 (7.1)	4 (2.2)	1.56 (0.42 - 5.73)	0.752
TT	AA	AA	AG	ID	5 (3.0)	12 (6.6)	0.22 (0.06 - 0.74)	0.019
TC	AA	AA	AA	II	22 (13.0)	31 (17.0)	0.37 (0.16 - 0.87)	0.023
TC	AA	AA	AA	ID	14 (8.3)	15 (8.2)	0.48 (0.18 - 1.28)	0.218
TC	AA	AA	AG	II	7 (4.1)	10 (5.5)	0.36 (0.11 - 1.16)	0.142
TC	AA	AA	AG	ID	5 (3.0)	9 (4.9)	0.29 (0.08-1.03)	0.064
TC	AA	AT	AG	II	3 (1.8)	6 (3.3)	0.26 (0.06-1.12)	0.130
TT	AA	AT	AA	ID	7 (4.1)	0 (0.0)	7.91 (0.42-147.6)	0.089
TT	AA	AT	AA	II	7 (4.1)	0 (0.0)	7.91 (0.42-147.6)	0.089
TT	AA	AT	AA	II	0 (0.0)	8 (4.4)	0.03 (0.002-5.77)	0.0007
TT	AA	AT	AG	ID	0 (0.0)	4 (2.2)	0.06 (0.003-1.66)	0.021
TC	AA	AT	AG	ID	1 (0.6)	4 (2.2)	0.13 (0.01-1.27)	0.069
TC	AA	AT	AA	ID	1 (0.6)	4 (2.2)	0.13 (0.01-1.27)	0.069
TT	AA	AA	AG	DD	1 (0.6)	1 (0.5)	-	-
TT	AG	AA	AA	II	2 (1.2)	3 (1.6)	-	-
TT	AG	AA	AG	II	1 (0.6)	0 (0.0)	-	-
TT	AG	AA	AG	ID	2 (1.2)	2 (1.1)	-	-
TT	AA	AA	GG	II	0 (0.0)	2 (1.1)	-	-
TT	AA	AA	GG	ID	0 (0.0)	2 (1.1)	-	-
TC	AA	AA	AA	DD	3 (1.8)	0 (0.0)		
TC	AA	AA	GG	II	1 (0.6)	1 (0.5)	-	-
TC	AA	AA	GG	ID	1 (0.6)	1 (0.5)	-	-
TC	AG	AA	AA	II	4 (2.4)	3 (1.6)		
TC	AG	AA	AG	ID	1 (0.6)	0 (0.0)	-	-
TC	GG	AA	AG	ID	1 (0.6)	0 (0.0)	-	-
TC	AG	AA	AA	ID	0 (0.0)	2 (1.1)	-	-
TC	AG	AA	AG	II	0 (0.0)	1 (0.5)	-	-
CC	AA	AA	AA	II	2 (1.2)	3 (1.6)	-	-
CC	AA	AA	AA	ID	2 (1.2)	0 (0.0)	-	-
CC	AA	AA	AG	II	1 (0.6)	0 (0.0)	-	-
CC	AA	AA	GG	II	1 (0.6)	0 (0.0)	-	-
CC	AG	AA	AG	II	1 (0.6)	0 (0.0)	-	-
CC	AA	AA	AG	ID	0 (0.0)	1 (0.6)	-	-
TT	AA	AT	AA	DD	1 (0.6)	0 (0.0)	-	-
TT	AA	AT	AG	II	3 (1.8)	0 (0.0)	-	-
TT	AA	AT	AG	ID	2 (1.2)	0 (0.0)	-	-
TT	AA	AT	GG	ID	2 (1.2)	0 (0.0)	-	-
TT	AA	AT	GG	ID	2 (1.2)	0 (0.0)	-	-
TT	AG	AT	AA	ID	1 (0.6)	0 (0.0)	-	-
TT	AG	AT	AG	II	1 (0.6)	0 (0.0)	-	-
TT	AA	AT	AG	ID	0 (0.0)	1 (0.6)	-	-
TT	AA	AT	AA	ID	0 (0.0)	3 (1.6)	-	-
TT	AA	AT	AA	DD	0 (0.0)	1 (0.5)	-	-
TT	AA	AT	AA	II	0 (0.0)	3 (1.6)	-	-
TT	AA	AT	GG	II	0 (0.0)	1 (0.5)	-	-
TT	AA	AT	GG	ID	0 (0.0)	1 (0.5)	-	-
TT	AG	AT	AG	II	0 (0.0)	2 (1.1)	-	-
TC	AG	AT	AG	II	1 (0.6)	0 (0.0)	-	-
TC	AA	AT	AA	II	3 (1.8)	0 (0.0)	-	-
TC	AA	AT	AA	ID	2 (1.2)	0 (0.0)	-	-
TC	AA	AT	GG	ID	1 (0.6)	0 (0.0)	-	-
TC	AA	AT	GG	II	0 (0.0)	1 (0.6)	-	-
CC	AA	AT	AA	II	1 (0.6)	0 (0.0)	-	-
TT	AA	TT	AA	II	1 (0.6)	2 (1.1)	-	-
TT	AA	TT	AG	II	0 (0.0)	1 (0.5)	-	-
TT	AA	TT	AG	ID	0 (0.0)	1 (0.5)	-	-
TC	AG	TT	AA	II	1 (0.6)	0 (0.0)	-	-
TC	AA	TT	AA	II	0 (0.0)	1 (0.5)	-	-
TC	AA	TT	AA	ID	0 (0.0)	1 (0.5)	-	-

cantly associated with poor survival (HR=2.86 [95% CI 1.15–6.67], P=0.017), and that variant rs62626348 was associated with well-differentiated cancer (P=0.019). Ma et al. (31) evaluated the *ERBB4* variants in cervical cancer and found that the 11892696 and 16847082 variants of ERBB4 increased susceptibility, while 12052398, 13424871, 1978873 and 16847416 polymorphisms were not associated with the disease.

The discrepancy in the results may be caused by differences in the populations studied, study design, genetic background of the participants and the environmental background. There are also some limitations in our study that might affect the results including: i) the sample size of our study is relatively small; ii) we did not determine gene-environment interactions; and iii) the effect of *ERBB4* variants on the survival of patients with PCa has not been determined.

In conclusion, this study is the first report that has

Table 4. Haplotype association of ERBB4 variants with prostate cancer (PCa) risk.

rs12052398	rs13393577	rs13424871	rs16847082	rs6147150	PCa Patients	Control Patients	OR (95%CI)	P-value
T	A	A	A	I	0.4382	0.3049	1.00	-
C	A	A	A	I	0.1308	0.2230	0.39 (0.20 - 0.74)	0.005
T	A	A	A	D	0.0804	0.1362	0.37 (0.15 - 0.91)	0.031
T	A	A	G	I	0.0614	0.0765	0.60 (0.25 - 1.49)	0.280
T	A	T	A	I	0.0545	0.0674	0.54 (0.22 - 1.30)	0.170
T	A	A	G	D	0.0327	0.0551	0.26 (0.06 - 1.11)	0.069
C	A	A	G	I	0.0409	0.0207	1.29 (0.29 - 5.67)	0.740
T	A	T	G	I	0.0142	0.0313	0.20 (0.02 - 2.63)	0.220
T	A	T	A	D	0.0025	0.0075	1.68 (0.21 - 13.14)	0.620
T	G	A	A	I	0.0101	0.0194	0.39 (0.06 - 2.41)	0.310
C	G	A	A	I	0.0177	0.0041	1.17 (0.18 - 7.59)	0.870
C	A	A	G	D	0.0091	0.0076	0.59 (0.00 - 267.66)	0.870
C	A	A	A	D	0.0447	0.000	-	-

Table 5. Association of *ERBB4* polymorphisms with clinicopathologic parameters in prostate cancer (PCa) patients.

Factors	rs12052398		p	rs13393577		p	rs13424871		p	rs16847082		p	rs6147150		p
	TT	TC+CC		AA	AG+GG		AA	AT+TT		AA	AG+GG		II	ID+DD	
Age at diagnosis Y, n			0.234			0.846			0.427			0.475			0.163
≤65	60	61		109	12		95	26		78	43		78	43	
>65	29	19		44	4		35	13		34	14		25	23	
Stage			0.506			0.348			0.191			0.417			0.339
pT1	3	5		6	2		4	4		7	1		6	2	
pT2a	12	15		23	4		21	6		19	8		14	13	
pT2b	7	4		11	0		6	5		8	3		4	7	
pT2c	45	31		71	5		61	15		48	28		51	25	
pT3a	7	6		11	2		10	3		6	7		8	5	
pT3b	15	19		31	3		28	6		24	10		20	14	
PSA at diagnosis (ng/ml), n			0.607			0.826			0.378			0.687			0.408
≤4	1	0		1	0		1	0		1	0		0	1	
4-10	43	41		75	9		61	23		54	30		50	34	
>10	45	39		77	7		68	16		57	27		53	31	
Gleason score, n			0.286			0.566			0.421			0.674			0.349
≤6	29	28		50	7		43	14		36	21		39	18	
7	43	30		68	5		54	19		48	25		41	32	
>7	17	22		35	4		33	6		28	11		23	16	
Perineural invasion, n			0.874			0.595			0.257			0.503			0.872
Positive	55	51		99	9		85	21		68	38		64	42	
Negative	34	29		56	7		45	18		44	19		42	24	
Surgical margin, n			0.875			0.426			0.456			0.506			0.147
Positive	36	31		59	8		54	13		42	25		36	31	
Negative	53	49		94	8		76	26		70	32		67	35	

evaluated the impact of *ERBB4* variants on susceptibility to PCa in a sample of Iranian population. Our findings did not support an association between *ERBB4* polymorphism and PCa risk. Larger sample sizes with diverse ethnicities are required to confirm our findings.

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