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TCF7L2, CASC8, and GREM1 polymorphism and colorectal cancer in south-eastern Romanian population

Anca Florentina Mitroi, MD, PhD[a,b], Nicoleta Leopa, MD, PhD[b,c,*], Eugen Dumitru, MD, PhD[b,d], Andrei Dumitru, MD[b,d], Cristina Tocia, MD, PhD[b,d], Ioana Popescu, MD[d], Adrian Mitroi, MD, PhD[b], Răzvan Cătălin Popescu, MD, PhD[b,c]

Abstract

Colorectal cancer (CRC) is a heterogeneous disease with an increasing trend and with multiple epigenetic alterations and different molecular features, a major cause of mortality and morbidity. The Wnt/β-Catenin pathway is involved in multiple aspects of cell dynamics, architecture of developing gastrointestinal tissues, and intestinal tissue homeostasis in adults, but its aberrant activity plays an important role in every aspect of colorectal carcinogenesis. The aim of our study was to investigate the association of the TCF7L2 rs7903146, CASC8 rs6983267, and Gremlin1 (GREM1) rs16969681 polymorphism in patients with CRC without other pathologies. A case-control study conducted on 31 patients diagnosed with CRC and 30 healthy controls age and sex-matched with the patients. Real time PCR was used to determine the genotypes of rs7903146, rs698267, rs1696981. We observed no association between rs6983267 and rs16969681 polymorphism and risk of CRC and low association between TCF7L2, rs7903146, polymorphism and risk of CRC. The recessive model of the TCF7L2 rs7903146 had an OR of 1.6 (95% CI 0.058–4.414, $P < .05$) which means that TT genotype increased the risk and possibility of development of CRC. Our study did not confirm a significant association between TCF7L2 rs7903146, CASC8 rs6983267, and GREM1 rs16969681 with CRC, but emphasizes the possibility of existence of a high risk of CRC development in patients with TT genotype of rs7903146.

Abbreviations: BMI = body mass index, CRC = colorectal cancer, GREM1 = Gremlin1, SNP = single nucleotide polymorphisms, TCF7L2 = transcription factor 7-like 2, TNM = tumor-node-metastasis.

Keywords: CASC8 rs6983267, colorectal cancer, genes, GREM1 rs16969681, TCF7L2 rs7903146

1. Introduction

Colorectal cancer (CRC) is the third most common cancer and the second cause of cancer deaths worldwide,[1,2] which originates from colon epithelium.[3] CRC is initiated by mutations in tumor suppressor genes and oncogenes, and the accumulation of multiple mutations leads to a selective growth advantage for transformed epithelial cells that is modulated by epigenetic changes.[4,5] These tumor-promoting lesions interfere with the regulated activity of the Wnt/β-Catenin pathway and thereby affect proliferation, migration, invasion, and tumor initiation capacity of CRC cells. A major molecular pathway is Wnt signaling activation of the transcription factor β-catenin to promote expression of cell proliferation genes.[6] Aberrant Wnt/β-Catenin pathway activity plays a crucial role in virtually every aspect of colorectal carcinogenesis.[7]

In the healthy gut, these Wnt/β-Catenin pathway functions are executed exclusively via transcription factor 7-like 2 (TCF7L2) gene.[8,9] The TCF7L2 (10q25.2), one of these genes, encodes a transcription factor member of the Wnt signaling pathway.[10] Although biologically plausible, few studies have examined associations between polymorphisms of the TCF7L2 gene and CRC.[8,11,12] Duval et al (1999; 2000) characterized the genomic structure of TCF7L2 in CRC cell lines and demonstrate that long C-terminal end may mediate transcriptional repression.[13,14] Findings by Folsom and colleagues using data from the Atherosclerosis Risk in Communities Study suggest that an association exists.[15] The rs7903146 T allele in TCF7L2 is the strongest genome-wide association studies signal for diabetes risk in different populations across the world and it is associated with insulin synthesis, processing, secretion and action mechanisms.[16]

Genome-wide association studies have revealed that some single nucleotide polymorphisms (SNP) at 8q24, such as rs6983267, located in Cancer Susceptibility Candidate 8 Noncoding, might be effective in susceptibility to various cancers in different

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[a] Department of Pathology, Emergency Hospital of Constanţa, Romania, [b] Ovidius University, Faculty of Medicine and Pharmacy Constanta, Romania, [c] Department of General Surgery, Emergency Hospital of Constanţa, Romania, [d] Department of Gastroenterology, Emergency Hospital of Constanţa, Romania.

**Correspondence: Nicoleta Leopa, Ovidius University, Faculty of Medicine and Pharmacy Constanta, 900470, Romania (e-mail: gherghe_nicoleta02@yahoo.com).*

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populations[17,18] and also are strongly associated with CRC and the risk allele gave out a 1 to 3 times risk increases.[16] A number of studies have shown that variations in the TCF7L2 gene, considerably affects risk of type 2 diabetes.[12,19,20] A rare germline duplication upstream of the bone morphogenetic protein antagonist Gremlin1 (GREM1) causes a Mendelian-dominant predisposition to CRC; there are studies that confirm that a common GREM1 polymorphism, rs16969681, is also associated with CRC susceptibility, conferring ~20% differential risk in the general population.[21] Epidemiological studies suggest a link between type 2 diabetes and CRC.[22,23] We evaluated the association of the rs7903146, rs6983267and rs16969681 polymorphism with CRC in patients without other pathologies.

2. Methods

2.1. Study group

A case-control study was conducted among South-Eastern Romanian adult patients (n = 31) with a diagnosis of CRC between September 2020 and 2021. The patients had positive colonoscopic results for malignancy, histologically confirmed as CRC and were recruited from Emergency Hospital of Constanta. Unrelated subjects (controls) (n = 30) were recruited in the same period as the cases from the same hospital and were judged to be in good health according to their colorectal screening examination and medical history. Controls were frequency matched to cases by sex and age. Patients with familial adenomatous polyposis, hereditary nonpolyposis CRC, inflammatory bowel disease or any cancer personal history were excluded from the study. Both colon cancer patients and controls were excluded if they had diabetes or high blood pressure, or if they were under any treatment course.

Cancer lesions were treated appropriately by open or laparoscopic surgery. For each case, the localization and size of the tumor and the pathological stage were recorded. Tumor staging were based on World Health Organisation criteria and the tumor-node-metastasis (TNM) system. According to tumor localization, samples were classified as "right-sided" (localized in the cecum or in the ascending or transverse colon), "left-sided" (set in the descendant or sigmoid colon) and in the rectum. Demographic and clinical data included age, gender, alcohol consumption status (according to National Institute on Alcohol Abuse and Alcoholism[24]), smoking status (according to Center for Disease Control and Prevention[25]), the body weight, height, and blood pressure of participants were recorded, and the body mass index (BMI) was calculated.

2.2. Genotyping

Genomic deoxyribonucleic acid was extracted from paraffin embedded tissue using GeneJET Genomic deoxyribonucleic acid Purification Kit (ThermoScientific), according to manufacturer protocol. SNPs polymorphisms of the TCF7L2 (rs7903146, C/T), cancer susceptibility 21 (rs6983267, G/T), and GREM1 (rs1696981, C/T) were identified using a real-time PCR method based on the TaqMan® Genotyping Master Mix (Applied Biosystems) and 20× SNP Genotyping Assay (Applied Biosystems), using a 7500 Fast Real-Time Systems (Applied Biosystems) according to manufacturer procedure. Allelic discrimination was made with the help of 7500 Fast Real-Time PCR software, version 2.3.

2.3. Statistical analysis

Descriptive statistics were used to describe the profile of study participants. Quantitative variables were described using the mean and standard deviation. Meanwhile, qualitative variables were summarized as frequencies and percentages. Hardy-Weinberg equilibrium was determined using GeneCalc software at the level of significance 0.05. The association between disease status and the genetic variants were tested by Pearson's Chi-square test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used in calculating the corresponding χ^2 distribution test. Multivariate logistic regression analysis was used for association analyses with adjustments for age and BMI. Comparisons of clinical parameters of different genotypes among patients with CRC and healthy controls were assessed by 1-way analysis of variance and the least significant difference test. A P value < .05 was considered statistically significant. The SPSS statistical software package for Windows version 28.0 (IBM, Armonk, NY) was used for all statistical analyses.

2.4. Ethical consideration

The study was carried out in accordance with the Declaration of Helsinki on experimentation with human subjects and was approved by the Local Ethics Commission for the Approval of Clinical and Research Developmental Studies (No 5/2020).

3. Results

3.1. General characteristics of study objects

Patients with CRC were matched with control participants by age (within 5 years) and date of diagnosis (within 3 month). The mean age was 66.1 years for CRC patients and 63.9 years for controls. Table 1 summarizes selected characteristics of patients and controls. There were no significant differences in mean age, sex, or the numbers of current smokers, habitual alcohol drinkers, habitual vegetable consumers and habitual exercisers between the 2 groups. Moreover, there was no significant difference with respect to systolic blood pressure and diastolic blood pressure between the 2 groups. However, BMI were higher in patients with CRC than in controls (P = .049).

3.2. Genotype distribution of rs7903146, rs6983267 and rs1696981 in case and control groups

The genotypes distribution in control groups for rs7903146, rs6983267 and rs16969681 were consistent to Hardy Weinberg law at the level of significance 0.05. Genotypic and allelic distributions of the rs7903146, rs6983267, rs16969681 polymorphism in patients with CRC and controls are summarized in Table 2. For rs7903146, the frequencies of CC, CT, and TT genotypes were 48.4, 41.9, and 9.7% in patients with CRC and 60, 36.7, and 3.3% in controls. Comparing with the TT genotype, the CT and CC genotypes demonstrated no significant association with the risk of CRC. There was also no significant correlation with the risk of CRC in dominant models, but a significant correlation was found for recessive models (P = .036).

For rs6983267 SNP, the most frequent genotype was GT (54.8%) in CRC patients and was 53.3% in controls. On the other hand, the TT genotype frequency was significantly higher in CRC cases (22.6%) than in controls (16.7%). The frequencies of CC, CT, and TT genotypes for rs16969681 were 71%, 25.8%, and 1% in CRC patients and 63.3%, 23.3%, and 13.3% in control subjects. For rs6983267 and rs16969681 gene, there was no significant correlation with the risk of CRC in either dominant or recessive models.

When focusing on individuals with BMI ≥ 25 kg/m², for rs7903146 gene, was found to significantly increase the risk of CRC development in individuals with CC + CT genotypes (OR = 2.684, 95% CI 0.254–28.311, P = .037), while for rs6983267 and rs16969681 genes, no significant correlation with the risk of CRC was found (Table 3).

We observed a significant tendency toward higher stage colorectal adenocarcinomas and depth of invasion, based on the

Table 1

Characteristics of patients with CRC and controls.

Variable	Patients with CRC (n = 31)	Controls (n = 30)	*P* value
Age (yrs), mean ± SD	66.1 ± 11.69	63.9 ± 11.99	.583*
Sex			.252**
Male	19 (61.3)	14 (46.7)	
Female	12 (38.7)	16 (53.3)	
Smoking	7 (22.6)	7 (23.3)	.944**
Habitual alcohol use	2 (6.5)	7 (23.3)	.117**
Habitual vegetable consumer	5 (16.1)	9 (30%)	.430**
Habitual exercise	4 (12.9)	12 (40%)	.836**
SBP, mm Hg	146.12 ± 29.33	122.48 ± 11.26	.098*
DBP, mm Hg	71.44 ± 12.16	69.88 ± 0.77	.114*
BMI, kg/m²	28.61 ± 4.66	26.31 ± 5.23	.049**

Number of cases, with percentages in parenthesis.

BMI = body mass index, CRC = colorectal cancer, DBP = diastolic blood pressure, SBP = systolic blood pressure, SD = standard deviation.

**P* value evaluated by analysis of variance.

***P* value evaluated by X^2 test.

Table 2

Multivariate analysis of association between rs7903146, rs6983267, rs16969681 and risk of CRC.

	Variable	Patients with CRC (n = 31)	Controls (n = 30)	OR [95% CI]	*P* value
rs7903146	Genotype				
	TT	3 (9.7)	1 (3.3)	-	-
	CT	13 (41.9)	11 (36.7)	(Reference)	-
	CC	15 (48.4)	18 (60)	1.418 [0.494–4.075]	.516
	Dominant				
	TT	3 (9.7)	1 (3.3)	(Reference)	-
	CC + CT	28 (90.3)	29 (96.7)	3.107 [0.305–31.680]	.317
	Recessive				
	TT + CT	16 (51.6)	12 (40)	(Reference)	-
	CC	15 (48.4)	18 (60)	1.600 [0.0.580–4.414]	*.036*
rs6983267	Genotype				
	TT	7 (22.6)	5 (16.7)	-	-
	GT	17 (54.8)	16 (53.3)	(Reference)	-
	GG	7 (22.6)	9 (30)	1.366 [0.411–4.539]	.610
	Dominant				
	TT	7 (22.6)	5 (16.7)	(Reference)	-
		24 (77.4)	25 (83.3)	1.458 [0.407–5.230]	.561
	GG + GT				
	Recessive				
	TT + GT	24 (77.4)	21 (70)	(Reference)	-
	GG	7 (22.6)	9 (30)	1.469 [0.466–4.633]	.510
rs16969681	Genotype				
	TT	1 (3.2)	4 (13.3)	-	-
	CT	8 (25.8)	7 (23.3)	(Reference)	-
	CC	22 (71)	19 (63.3)	0.987 [0.302–3.230]	.983
	Dominant				
	TT	1 (3.2)	4 (13.3)	(Reference)	-
	CC + CT	30 (96.8)	26 (86.6)	0.217 [0.023–2.063]	.150
	Recessive				
	TT + CT	9 (29)	11 (39.6)	(Reference)	-
	CC	22 (71)	19 (63.3)	0.707 [0.241–2.068]	.525

Variables are expressed as number of cases, with percentages in parenthesis. Values in italics indicate statistical significance ($P < .050$).

CI = confidence interval, CRC = colorectal cancer, OR = odds ratio.

T and N factor of the TNM system, in patients with CRC with the rs7903146 and rs16969681, CT genotype (Table 4). For rs6983267, based on T and N factor for TNM system, patients with higher stage of CRC have a higher percent GT genotype. Comparative analysis between colon cancer and rectal cancer in terms of SNPs is not statistically significant in the study.

4. Discussion

CRC is a multigenic disease in which single SNP may only have a modest independent effect, and multiple SNPs may provide a more accurate representation of the risk. The present study explored the interaction of rs7903146, rs6983267, rs16969681 SNPs in 30 controls and 31 cases of CRC. Although a single SNP may have a moderate effect on the development of cancer, several SNPs together can exert a significant influence. Single SNPs (except one, rs7903146) were not associated with CRC risk, underlining the importance of integrating SNP information across genes in a pathway.

TCF7L2 is a gene that can increase the risk of type 2 diabetes.[12,26,27] The function of rs7903146 is under investigation. Surely, it may be another mutation in linkage disequilibrium

Table 3

Multivariate analysis of association between rs7903146, rs6983267, rs16969681 and risk of CRC stratified by BMI.

Genotype	BMI (kg/m²)	CRC (n = 31)	Controls (n = 30)	OR [95% CI]	*P* value
rs7903146					
TT	<25	0	0	-	-
CT + CC	<25	9 (29)	12 (40)	-	NS
TT	≥25	3 (9.7)	1 (3.3)	(Reference)	-
CT + CC	≥25	19 (61.3)	17 (56.7)	2.684 [0.254–28.311]	*.037*
rs6983267					
TT	<25	2 (6.5)	2 (6.6)	(Reference)	-
GT + GG	<25	7 (22.6)	10 (33.4)	1.429 [0.161–12.701]	.748
TT	≥25	5 (16.1)	3 (10)	(Reference)	-
GT + GG	≥25	17 (54.8)	15 (50)	1.471 [0.300–7.218]	.634
rs16969681					
TT	<25	0	2 (6.6)	(Reference)	-
CT + CC	<25	9 (29)	10 (33.4)	1200 [0.932–1.546]	.198
TT	≥25	1 (3.2)	2 (6.6)	(Reference)	-
CT + CC	≥25	21 (67.8)	16 (53.4)	0.381 [0.032–4.581]	.433

Variables are expressed as number of cases, with percentages in parenthesis. Values in italics indicate statistical significance ($P < .050$).

BMI = body mass index, CI = confidence interval, CRC = colorectal cancer, NS = nonsignificant, OR = odds ratio.

with this SNP that affects gene function. Anyway, a causal link between TCF7L2 variation and CRC seems biologically plausible. The TCF7L2 gene, has a central role in the Wnt/β-catenin signaling pathway, which is strongly implicated in colon cancer etiology.[8,11] Findings by Folsom and colleagues using data from the Atherosclerosis Risk in Communities Study suggest that an association between colon cancer and TCF7L2 exists.[28] In their study, the TT genotype of the rs7903146 TCF7L2 gene was associated with a > 2-fold increased risk of colon cancer (hazard rate ratio, 2.15; 95% CI, 1.27–3.64). Our finding was that variation in TCF7L2 SNPs, particularly rs7903146, was low associated with incidence of CRC.

The SNPs rs6983267 and rs16969681 has been investigated by many groups; some researchers examined those association with cancer and a few others studied, those relationship with CRC.[17,18,21,29] In this study, we observed no association between rs6983267 and rs16969681 polymorphism and risk of CRC, with results similar to those in the literature.[17] Findings by Karimi and colleagues suggests that there were no remarkable associations between rs6983267 and susceptibility to esophageal and colon cancers.[17,30,31] Other authors have described that rs6983267 significantly increased the risk of CRC[32–35] and given these differences in outcomes, studies in this regard are needed.

ORs and CIs were calculated for the dominant and recessive inheritance model for each polymorphism. The dominant model is presumed to demonstrate whether the presence of minor allele (T), as a risk allele, increase the risk for CRC, while the recessive model establish the necessity of the presence of 2 copy of the T allele, the TT genotype, in order to increased the CRC risk. Our study revealed that the recessive model of the TCF7L2 rs7903146 had an OR (95% CI) of 1.6 (0.058–4.414). These results may reveal that the TT genotype of rs7903146 increased the risk and possibility of development of CRC.

There are studies that attribute a more specific association between colon cancer alone and TCF7L2 than for grouped CRC.[28] Although we had too few cases of colon cancer to analyze separately, the association with TCF7L2 was not more specific than that of groped CRC.

Obesity is a risk factor for cancer in general and CRC in particular.[36] The association between TCF7L2 and obesity has been a topic of research over time, and the association between the 2 does not appear to be causal related.[37] In this study, only a modest association with rs7903146 CC + CT genotypes was found in patients with BMI ≥ 25 kg/m². Evaluating the SNPs rs6983267 and rs16969681 with BMI, in our study, did not reveal a significant interaction. The results of our study were similar to studies in the literature.

A significant tendency toward higher stage colorectal adenocarcinomas and depth of invasion was seen in CT heterozygotes for rs7903146 and rs16969681 genes, respective GT heterozygotes for rs6983267 gene; indicating maybe that the CT/GT genotype could be a CRC biomarker correlating with stage progression.

4.1. Study limitations

Although the number of subjects was small in this study limiting the statistical power, the finding of this study should be considered. Despite this limitation, the cases and controls were matched by age, sex, smoking status and comorbidities. Nonetheless, most of the findings were similar with those observed from some other populations. Therefore, further research should be conducted to verify this conclusion.

5. Conclusions

The rs7903146 TCF7L2 polymorphism has a low association with development of CRC, and between rs6983267, rs16969681 and CRC, no association was identified in our study. However, our study emphasizes the possibility of existence of a high risk of CRC development in patients with TT genotype of rs7903146. A strategy utilizing biomarkers to stratify patients into appropriate screening programs can potentially prevent CRC and in this regard, future studies should approach epistatic relationships from the SNP level.

Author contributions

Conceptualization: Anca Florentina Mitroi, Nicoleta Leopa, Răzvan Cătălin Popescu.
Data curation: Anca Florentina Mitroi, Nicoleta Leopa, Andrei Dumitru, Cristina Tocia, Răzvan Cătălin Popescu.
Formal analysis: Nicoleta Leopa, Eugen Dumitru, Andrei Dumitru, Cristina Tocia, Ioana Popescu, Adrian Mitroi.
Investigation: Anca Florentina Mitroi, Nicoleta Leopa, Eugen Dumitru, Andrei Dumitru, Cristina Tocia, Ioana Popescu, Adrian Mitroi, Răzvan Cătălin Popescu.
Methodology: Anca Florentina Mitroi, Nicoleta Leopa, Eugen Dumitru, Ioana Popescu, Adrian Mitroi, Răzvan Cătălin Popescu.
Project administration: Răzvan Cătălin Popescu.
Supervision: Eugen Dumitru, Răzvan Cătălin Popescu.
Validation: Nicoleta Leopa, Eugen Dumitru.
Visualization: Eugen Dumitru.
Writing – original draft: Anca Florentina Mitroi, Nicoleta Leopa.

Table 4

Relationship between rs7903146, rs6983267, rs16969681 variants and clinicopathologic features in patients with CRC.

		Genotype - rs7903146			
Variable/category	**n (%)**	**CC**	**CT**	**TT**	***P* value**
Tumor site					.667
Right-sided	8 (25.8)	4 (12.9)	3 (9.7)	1 (3.2)	
Left-sided	9 (29)	4 (12.9)	5 (16.1)	0	
Rectum	14 (45.2)	7 (22.6)	5 (16.1)	2 (6.5)	
TNM (T)					.058
1–2	11 (35.5)	7 (22.6)	3 (9.7)	1 (3.2)	
3–4	20 (64.5)	8 (25.8)	10 (32.2)	2 (6.5)	
TNM (N)					.511
N0	12 (38.7)	6 (19.3)	4 (12.9)	2 (6.5)	
N+	19 (61.3)	9 (29)	9 (29)	1 (3.2)	
TNM (M)					.886
M0	29 (93.5)	14 (45.2)	12 (38.7)	3 (9.7)	
M+	2 (6.5)	1 (3.2)	1 (3.2)	0	
Tumor stage					.511
1–2	2 (38.7)	6 (19.3)	4 (12.9)	2 (6.5)	
3–4	19 (61.3)	9 (29)	9 (29)	1 (3.2)	
			Genotype - rs6983267		
		GG	GT	TT	
Tumor site					.767
Right	8 (25.8)	3 (9.7)	3 (9.7)	2 (6.5)	
Left	9 (29)	1 (3.2)	7 (22.6)	1 (3.2)	
Rectum	14 (45.2)	3 (9.7)	7 (22.6)	4 (12.9)	
TNM (T)					.076
1–2	11 (35.5)	2 (6.5)	4 (12.9)	5 (16.1)	
3–4	20 (64.5)	5 (16.1)	13 (41.9)	2 (6.5)	
TNM (N)					.433
N0	12 (38.7)	4 (12.9)	5 (16.1)	3 (9.7)	
N+	19 (61.3)	3 (9.7)	12 (38.7)	4 (12.9)	
TNM (M)					.548
M0	29 (93.5)	7 (22.6)	16 (51.6)	6 (19.3)	
M+	2 (6.5)	0	1 (3.2)	1 (3.2)	
Tumor stage					.433
1–2	12 (38.7)	4 (12.9)	5 (16.1)	3 (9.7)	
3–4	19 (61.3)	3 (9.7)	12 (38.7)	4 (12.9)	
			Genotype - rs16969681		
		CC	CT	TT	
Tumor site					.638
Right	8 (25.8)	5 (16.1)	2 (6.5)	1 (3.2)	
Left	9 (29)	7 (22.6)	2 (6.5)	0	
Rectum	14 (45.2)	10 (32.2)	4 (12.9)	0	
TNM (T)					.544
1–2	11 (35.5)	9 (29)	2 (6.5)	0	
3–4	20 (64.5)	13 (41.9)	6 (19.3)	1 (3.2)	
TNM (N)					.711
N0	12 (38.7)	9 (29)	3 (9.7)	0	
N+	19 (61.3)	13 (41.9)	5 (16.1)	1 (3.2)	
TNM (M)					.146
M0	29 (93.5)	22 (70.9)	6 (19.3)	1 (3.2)	
M+	2 (6.5)	0	2 (6.5)	0	
Tumor stage					.711
1–2	12 (38.7)	9 (29)	3 (9.7)	0	
3–4	19 (61.3)	13 (41.9)	5 (16.1)	1 (3.2)	

M = metastasis, N = node, TNM = T-tumor.

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