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EGFR and CTNNB1 Gene Variants in Oral Squamous Cell Carcinoma and Fanconi Anemia Patients

Dinara Nemetova [1] , Selçuk Daşdemir [1] , Bora Başaran [2] , Yavuz Uyar [3] , Tülin Tiraje Celkan [4] , Şahin Öğreden [5],Haydar Murat Yener [6],Tunç Fışgın [7] ,Günter Hafız [8],Mehmet Güven Günver [9] , Arzu Pınar Erdem [10] , Zişan Asal Kılıç [1] , Nevin Yalman [1]

1. Istanbul University, Istanbul Faculty of Medicine, Department of Medical Biology, İstanbul, Turkey
2. Istanbul University, Istanbul Faculty of Medicine, Department of Otorhinolaryngology, İstanbul,
Turkey
3. Prof. Dr. Cemil Taşçıoğlu City Hospital, Department of Otorhinolaryngology, Istanbul, Turkey
4. Istanbul Cerrahpasa University, Cerrahpasa Faculty of Medicine, Department of Pediatric Hematology Oncology, İstanbul, Turkey
5. Bağcılar Training and Research Hospital, Department of Otorhinolaryngology, Istanbul, Turkey
6. Istanbul Cerrahpasa University, Cerrahpasa Faculty of Medicine, Department of Otorhinolaryngology, İstanbul, Turkey
7. Altınbaş University Faculty of Medicine, Department of Pediatric Hematology Oncology BMT Unite, İstanbul, Turkey
8. VKV American Hospital, Department of Otorhinolaryngology, İstanbul, Turkey
9. Istanbul University, Istanbul Faculty of Medicine, Department of Bioistatistics, İstanbul, Turkey
10. Istanbul University, Faculty of Dentistry, Pedodontics Department, İstanbul, Turkey
Dinara Nemetova: dinemetova@gmail.com; Selcuk Dasdemir: selcuk.dasdemir@istanbul.edu.tr;
Bora Başaran: borabas@yahoo.com; Yavuz Uyar: uyaryavuz.yu@gmail.com; Tülin Tiraje Celkan: tirajecelkan@yahoo.com; Şahin Öğreden: drsahinogredenr@gmail.com; Haydar Murat Yener: hmuratyener@gmail.com; Tunç Fışgın: tunc.fisgin@medicalpark.com.tr; Günter Hafız: gunterhafiz66@gmail.com; Mehmet Güven Günver: guven.gunver@istanbul.edu.tr; Arzu Pınar Erdem: aperdem@gmail.com, Zişan Asal Kılıç: zisanasal@gmail.com; Nevin Yalman: nevinyalman@gmail.com;
Corresponding Author: Department of Medical Biology, Istanbul Faculty of Medicine, Istanbul University Esnaf Hospital, Fatih 34116, İstanbul, Turkey. zisanasal@gmail.com; +905336263127

Abstract

Oral squamous cell carcinoma (OSCC) is the most common epithelial malignancy in the oral cavity. The risk of the development of OSCC is high in Fanconi anemia (FA) patients owing to the DNA repair deficiency in somatic cells. EGFR, and CTNNB1 genes are suggested to be effective in development of OSCC. EGFR has been reported to have higher expression in OSCC, there are limited studies of EGFR and CTNNB1 gene polymorphisms in the development of OSCC. Therefore, we aimed to investigate the potential connection between EGFR rs845561 and CTNNB1 rs3864004 gene polymorphisms, and OSCC and FA. We performed polymerase chain reaction (PCR)/sanger sequencing for detection of the variations in these regions. EGFR rs845561, and CTNNB1 rs3864004

gene variants were compared between OSCC patients, and controls, no significant difference was detected (*P*>0.05). The EGFR rs845561 C allele frequency was lower in OSCC patients who had lymh node metastasis (*P*= 0.001), a significant association was detected between the EGFR rs845561 T allele frequency, and tumor perineural invasion (*P*= 0.05) in OSCC patients. CTNNB1 rs3864004 A allele frequency was associated with increased tumor invasion in OSCC patients (*P*= 0.01).
The CTNNB1 rs3864004 G/G genotype was higher in FA patients compared with the levels in the control group however, the A allele frequency was found lower (p:0.049). EGFR rs845561 gene variant showed no statistically significant difference between FA and control groups (p>0.05). EGFR T allele frequency was detected higher in FA patients who develop OSCC (p:0.03). The EGFR rs845561, and CTNNB1 rs3864004 gene variants may be suggested to contribute to the development of OSCC however, further studies are required.

Keywords: Oral squamous cell carcinoma, Fanconi Anemia, Epidermal Growth Factor Gene, CTNNB1, Polymorphism

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant neoplasm originating from the multilayer squamous epithelium of the skin, and mucosas of the oral cavity. Higher than 95% of oral tumors developing in the head, and neck region are described as OSCC [1]. OSCC is among the most fatal eight cancer types worldwide[2]. The TNM staging (T-tumor dimension, N-regional lymp node dimension, and count, and M-distant metastasis) was reported as the most important clinical prognostic factor, and was an identifier in treatment in OSCC[3]. Patients with stage I tumor generally have the survival rate of 90%, however patients with stage II tumors have a survival rate of 70%. Advanced stage tumors (III/IV, etc.) have worser prognosis[3,4]. Many genetic, and environmental risk factors have effects in the development of OSCC. Main risk factors in OSCC development may be reported as smoking, alcohol consumption, poor mouth hygiene, and FA[1]. Some genetic diseases increase the risk of OSCC which include Li Fraumeni Syndrome, Plummer-Vinson Syndrome, Fanconi Anemia, dyskeratosis congenita, Xeroderma pigmentosum, and discoid lupus erythematosus[5,6]. OSCC has a tendency to emerge in earlier ages in individuals with such genetic diseases(even with no carcinogen exposure)[7].

Fanconi Anemia (FA) is an autosomal recessive or X dependently heredited genetic, rare disease characterized with bone marrow deficiency, various congenital physical abnormalities, and chromosomal instability. The clinical features identified in FA patients are the various physical abnormalities, bone marrow deficiency, and increased malignity risk. The most commonly detected solid tumors in FA patients are the mouth and esophageal squamous cell cancers[8]. FA patients have a higher OSCC incidence of 500-700 fold compared with the general population [9,10].

Epidermal growth factor receptor (EGFR or HER1) is a tyrosine kinase receptor from ErbB family, and is an important regulator of the cell proliferation[11]. The overexpression of EGFR was detected in epithelial originated tumors, in head and neck cancers, lung, kidney, ovarian, breast, prostate, bladder, and colorectal carcinomas[12]. EGFR was highly expressed in 80% of head and neck SCCs [13]. The higher expression of EGFR is detected in 70-90% of cases in OSCC[14]. Intronic SNP's at EGFR (rs2075110, rs12535536, rs845561, rs6970262 and rs1253871) are associated with head and neck cancers[15].

Cadherin associated protein beta-1 gene (CTNNB1) encodes the beta-catenin(β-catenin) protein which is an important signal molecule for the cell, and between the cells. It is a transcriptional co-regulator, and functions as an adaptor protein for cell adhesion[16]. The somatic mutations in the CTNNB1 gene are associated with Wilms tumor, sporadic medulloblastoma, hepatocellular carcinoma, hepatoblastoma, colorectal, lung, breast, ovarian, endometrial, thyroid, and prostate cancers[17]. Some

studies reported the association of the rs386400 gene variant with the hepatocellular carcinoma risk in the promoter region of CTNNB1 gene. This gene variant was suggested to be effective in the development of OSCC [18,18].

Aim

We compared the distribution of the EGFR rs845561, and CTNNB1 rs3864004 gene variants in OSCC, and FA patients with the distribution in healthy controls in our study.

Material and Methods

Study Design

The present study was conducted in the Department of Medical Biology in Istanbul University Istanbul Faculty of Medicine between 2019, and 2020. A total of 29 patients (mean age±s.d.: 52.6±11.72 y) who were followed up in the Otorhinolaryngology Department of Istanbul Faculty of Medicine, and Cerrahpaşa Faculty of Medicine, and were diagnosed with OSCC in accordance with the TNM staging system of the American Joint Committee on Cancer staging (2002) were included in the study as the OSCC patient group. 33 patients diagnosed with FA (mean age±s.d.: 21.03±5.96y) aged over 12 years who were followed up in the Department of Pediatrics and Diseases, Pediatric Hematology Oncology in Istanbul Cerrahpaşa Faculty of Medicine., Istanbul University Dentistry Pedodonty department, and Altınbaş University Faculty of Medicine Pediatric Hematology Oncology Department were included as the Fanconi anemia group. 40 volunteered individuals (mean age±s.d.: 42.12±5.96y) with no diagnosis of any diseases were included in the control group. The Ethics board approval was granted from Istanbul University Clinical Research Ethics Board with the date 09.08.2019, and no: 2019/13, and the signed consents of all patients and healthy volunteers were taken after they were informed about the study.

Polymorphism Analysis

The genomic DNA was extracted from the complete blood using the Roche DNA purification kit(Roche Diagnostics GmbH, Mannheim, Germany). Polymerase chain reaction(PCR)/Sanger sequencing analysis was performed for detection of the variations in these regions. Polymerase chain reaction was performed in the beginning for identifying the polymorphic regions using the appropriate primers (Table 1). Then, the Sanger sequencing reaction was performed. The products obtained after the sequence PCR reaction were purified using the sephadex mixture. The PCR products purified with sephadex were inserted on ABI 310 Genetic Analyzer device for capillary electrophoresis procedure, and DNA sequence analysis was performed. The DNA sequence analysis results obtained after the capillary electrophoresis was ended in the sequence device were converted to chromatogram data using the GeneMapper Software (Thermo Fisher Sci.) program (Figure 1, and Figure 2).

Ethics

The present research was screened and approved by the Clinical Ethics Committee of Istanbul Faculty of Medicine.

Statistical analysis

The statistical analysis of the data was performed using the Statistical Package for the Social Science (SPSS 21.0) program in the study. The T test was used for measuring the quantitative, and continuous variables (Independent Samples T-TEST). One-way variant analysis (ANOVA) test was used for measuring the age, and three or more independent variables of the data. The Pearson's chi square test was used in the genotype, and allele frequency distribution of gene variants in all groups and in the

comparison of the clinical and various variables. The SPSS was used as the measuring program. The obtained results were evaluated as significant for percentage values, and on $p \leq 0.05$ level.

Results

The controls, and patients were matched for age, and sex. Table 2 demonstrates the features of the patients and control groups.

Table 3 summarizes the genotypes, and alleles of EGFR and the distribution of the CTNNB1 gene polymorphisms in OSCC patients, and in healthy controls.

Distribution of the EGFR rs845561, and CTNNB1 rs3864004 genotypes was appropriate to the Hardy-Weinberg balance between controls (P= 0.46, P:0.12, respectively), OSCC (P= 0.29, P=0.35 respectively) and FA cases (P=0.17, P=0.34 respectively).

No significant result was detected in the comparison of the genotype, and allele results of EGFR rs845561, and CTNNB1 rs3864004 gene variants between OSCC patients, and the controls (P> 0.05).

Table 4 summarizes the distribution of the genotypes and alleles of EGFR, and CTNNB1 gene polymorphisms in FA patients, and healthy controls.

The comparison of the EGFR rs845561 gene variant of FA, and control group showed no statistically significant difference(P> 0,05). The CTNNB1 rs3864004 G/G genotype was higher, and the A allele frequency was lower in the FA patients compared with the levels in the control group (P= 0.049).

The below results were obtained after the subgroup analyses of the patients were performed:

A significant association was detected between the EGFR rs845561 T allele frequency, and tumor perineural invasion in OSCC patients (n:18) (P= 0.05). The EGFR rs845561 C allele frequency was found lower in OSCC patients who had lymph node metastasis (n:11) (P= 0.001).

The EGFR 845561 T allele frequency was found higher in 7 FA patients who developed cancer (P< 0.03).

A statistically significant association was found between CTNNB1 rs3864004 A allele frequency, and increased tumor invasion in OSCC patients (P=0.01).

Discussion

The SNPs in the genes associated with cell proliferation, signal transduction, cell cycle, and control, DNA repair, and apoptosis are known to be associated with increased sensitivity to mouth cancer. Chou et al., reported the association of AURKA rs2064863 SNP with the OSCC sensitivity, and advance stage of the tumor[19]. Yang et al. found that the RETN rs3745367, rs7408174, rs1862513, and rs3219175 gene variants were associated with OSCC risk, and patients with G allele frequency had the tendency to develop tumor in a larger size[20]. Liu et al. reported that PTEN rs9651495 gene variant showed a positive correlation with the CC genotype, and OSCC[21]. Researchers suggested in a study conducted in the Turkish population that MTHFR A1298C variant might be effective in the prognosis of OSCC[22].

The deregulation of the growth factors in OSCC develops with the increased expression, and autocrine stimulation. The overexpression of EGFR in oral keratinocyte cell membranes was shown to be associated with the increase of the tumor size, and recurrence risk, decrease of the radiation sensitivity, and with the poor prognosis rate of the disease[23,24]. EGFR polymorphisms were shown to be associated with the risk of developing esophageal, lung, and kidney cancers[26,25]. EGFR gene variants (such as EGFR 181946C> T, 8227 G> A) in lung cancers were associated with the higher tendency[26]. EGFR rs730437, and rs1468727 SNPs were associated with glioma tendency. The EGFR intronic

rs12535536, rs2075110, rs1253871, rs845561, and rs6970262 SNPs were found associated with the head, and neck cancers[15].

CTNNB1 polymorphisms have been found to be associated with breast, colorectal and prostate cancers. The genetic polymorphisms in CTNNB1 genes were found associated with the hepatocellular risk and progression in China population in the conducted studies. Researchers observed that the polymorphisms in CTNNB1 gene might regulate the β-catenin expression with upper activation(upregulation), and might result with the β-catenin accumulation[27]. CTNNB1 gene polymorphisms were shown to be possibly associated with the development of HCC in HBV associated HCC patients, and with the overall survival. Polymorphisms in the CTNNB1 gene have been reported to be associated with susceptibility and prognosis in gastric cancers.[141].

To the best of our knowledge, this is the first case-control study investigating the possible association between the EGFR rs845561, and CTNNB1 rs3864004 gene polymorphisms in OSCC patients. In addition, these polymorphisms were studied in FA patients which is an additional factor for OSCC disease risk.

EGFR rs845561 gene variant is located at EGFR gene intron 20' region (55185015.position of the chromosome 7), and has the C/T allele frequency[28]. rs845561 variant has a potential role of regulation in EGFR expression. Some studies reported the association of rs845561 polymorphism in different cancers. The head-neck SCC risk in tobacco users and rs845561 variant C allele were associated in a study[15]. The SNP here may increase the risk of the development of OSCC, and FA by affecting the EGFR protein function, and causing changes in signal transmission. However, we found no significant difference between the EGFR genotypes, and allele frequencies between the OSCC, FA patient, and controls in the present study.

Moreover, we first time demonstrated the positive association of CTNNB1 gene variants with FA risk. Although CTNNB1 rs3864004 G/G genotype was higher in FA patients compared with the control group in our study, the A allele frequency was found lower (p:0.049). CTNNB1 rs3864004 gene variant is located in the promoter region of the gene[28]. Despite the lack of functional studies performed on this variant, this variant may result with the increased signal production by changing the obtained protein amount by affecting the gene expression owing to its location. This possible mechanism partially explains the CTNNB1 rs3864004 polymorphism, and FA sensitivity, the functional dimensions are required to be explained in physiological conditions.

Conclusion

Our results suggest that CTNNB1 genetic variants might be a risk factor for FA. However, there is a need for more research for understanding the exact mechanisms increasing the FA risk of CTNNB1 rs3864004 polymorphism. There is a need for more number of studies with wider sample groups for clarifying the role of EGFR, and CTNNB1 genes in the development of OSCC, and FA.

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Conflicts of interest

There are no conflicts of interest.

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Figures

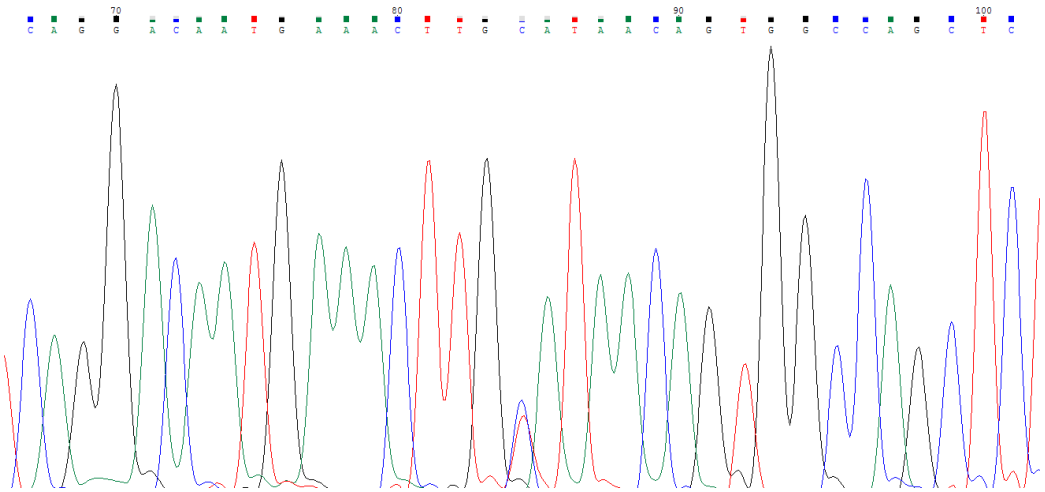

Figure 1: The Sanger chromatogram image of the heterozygous (CT) genotype EGFR rs845561 gene of the OSCC case

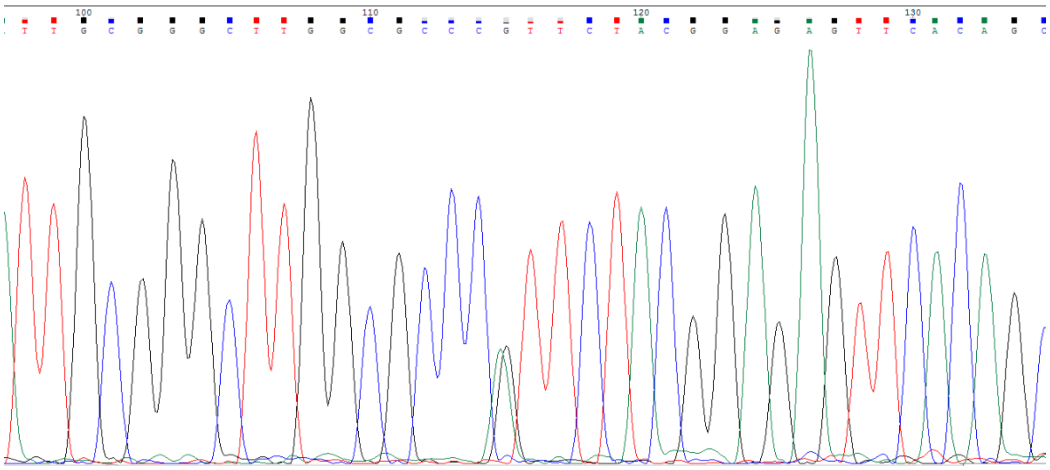

Figure 2: The Sanger chromatogram image of the heterozygous (GA) genotype CTNNB1 rs3864004 gene variant of the OSCC case

Tables

Table 1. The PCR Primer Sequences of the EGFR rs845561 (C/T), and CTNNB1 rs3864004 (G/A) Polymorphisms

Genes	***Primers(forward and reverse)***
EGFR rs845561 (C/T)	5’ -CTCCTGGCATCCTCAAAATGGG- 3’, 5’ -ACTGGTCTCATATGGATTCCTCCCA -3’
CTNNB1 rs3864004 (G/A)	5’ -TGATACCTAGTGACAAGTGGAACCAG- 3’, 5’ -TGTCCCCACTCACGAAGGCT- 3’

Table 2. The features of the OSCC patients, FA patients, and healthy control group

	OSCC	**FA**	**Controls**	**P value**
Number. n	*29*	*33*	*40*	
Sex; n (male/female)	*18/11*	*5/18*	*14/26*	*0.049*
Age. year: Mean±S.D.	*52.6±11.72*	21.03±5.96	42.12±5.96	0.101
Interval	*30-71*	*14-32*	*25-64*	

Table 3. Distribution of EGFR rs845561 (C/T), and CTNNB1 rs3864004 (A/G) gene polymorphisms in OSCC patients, and healthy control group

	Controls n (%)	**OSCC patients(%)**	**P value**
EGFR rs845561			
C/C	5 (12.8)	3 (10.7)	*0.54*
C/T	15 (38.5)	*14* (50)	*0.32*
T/T	19 (48.7)	11 (39.3)	*0.31*
C	0.32	0.36	0.32
T	*0.68*	0.*64*	*0.54*
CTNNB1 rs3864004			
G/G	14 (35.0)	10 (34.5)	*0.93*
G/A	*23 (57.5)*	16 (55.1)	*0.43*
A/A	*3 (7.5)*	3 (10.4)	*0.54*
G	*0.64*	0.62	*0.56*
A	*0.33*	0.38	*0.68*

Table 4. Distribution of EGFR rs845561 (C/T), and CTNNB1 rs3864004 (A/G) gene polymorphisms in FA patients, and healthy control group

	Controls n (%)	**FA patients (%)**	**P value**
EGFR rs845561			
C/C	5 (12.8)	5 (10.7)	*0.72*
C/T	15 (38.5)	*10* (50)	*0.80*
T/T	19 (48.7)	15 (39.3)	*0.79*
C	0.32	0.36	0.79
T	*0.68*	0.*64*	*0.72*
CTNNB1 rs3864004			
G/G	14 (35.0)	17 (53.1)	***0.049***
G/A	*23 (57.5)*	14 (43.8)	*0.24*
A/A	*3 (7.5)*	1 (3.1)	*0.56*
G	*0.64*	0.75	*0.54*
A	*0.33*	0.25	***0.049***