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Investigation of FGFR4 (Gly388Arg) Gene Polymorphism in Primary Lung Cancer Patients

Mehmet Ture[1*], Tahsin Yakut[1], Adem Deligonul[2], Mutlu Karkucak[3], Sebnem Ozemri Sag[1], Mustafa Hartavi[4], Erdem Cubukcu[2], Tuna Gulten[1] and Turkkan Evrensel[2]

[1]Department of Medical Genetics, Uludag University, Medical Faculty, Bursa, Turkey
[2]Department of Medical Oncology, Uludag University, Medical Faculty, Bursa, Turkey
[3]Department of Medical Genetics, Sakarya University Education and Research Hospital, Sakarya, Turkey
[4]Department of Internal Medicine, Uludag University, Medical Faculty, Bursa, Turkey

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ABSTRACT Several studies have shown relationships between predisposition to various types of cancer and polymorphisms of the fibroblast growth factor receptor 4 (*FGFR4*) gene. In the present study, researchers investigated the relationship between primary lung cancer and (PLC) *FGFR4* Gly388Arg polymorphism in regard to tendency, histopathologic sub-type, early onset, and metastatic status. The present study included 124 PLC patients and 100 healthy controls. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to identify gene polymorphism of *FGFR4* Gly388Arg. Statistical significance was considered when $p < 0.05$, and a statistically significant difference was not found in FGFR-4 polymorphism between the patient group and control group in regard to tendency, histopathologic sub-type, early onset, and metastatic status ($p > 0.05$). The findings in this study demonstrated that there was no relationship between polymorphism of *FGFR4* Gly388Arg gene and PLC. However, these results should be confirmed in larger studies and in specific histopathological sub-types of PLC.

INTRODUCTION

Primer Lung Cancer (PLC) is the most frequently diagnosed cancer and is the leading cause of cancer related mortality worldwide, accounting for almost 1.4 million deaths annually (Siegel et al.2013). While small cell lung cancer (SCLC) represents 20% of PLC cases, Nonsmall cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer cases, and the most common forms of NSCLC include squamous cell carcinoma, adenocarcinoma, and large cell cancer (LCC) (Chheang and Brown 2013). In SCLC, the survival rate is lower than with other lung cancers and the prognosis is worse. It is not possible to identify the histopathological subtypes in approximately 10% of NSCLC cases (Chheang and Brown 2013).

Many cases have suggested that genetic effects play a role in the development of lung cancer. Although, smoking is the major risk factor, <20% of all smokers develop lung cancer. Additionally, familial risk and variation between different races, regarding lung cancer, support the hypothesis that there is a correlation between lung cancer and genetic factors (Bag et al. 2014). Overall, 5-10% of lung cancer is observed in people under age 50. When compared to older people, variation existed in these patients in terms of gender and histological distribution, as well as genetic predisposition. Studies have suggested that genetic factors may contribute more in the development of lung cancer in patients below age 50 (Bourke et al. 1992; Green et al. 1993; Rosenberger et al. 2008).

Fibroblast Growth Factor Receptors (*FGFR*) are trans-membrane receptors that demonstrate tyrosine kinase activity. Members of the FGFR family play a major role in cell growth, differentiation, migration, angiogenesis and tumorigenesis (Lesca et al. 2014). Expression of *FGFR4* is tissue specific and it occurs in different splice variants. The Gly388Arg polymorphism (rs351855) in the *FGFR4* gene encodes *FGFR4* containing either glycine (Gly) or arginine (Arg) at codon 388 (Marme et al. 2012). Further, compared with the Gly388 variant, the Arg388 variation in the transmembrane domain of the *FGFR4*

[*]Address for correspondence:
Dr. Mehmet Ture
Uludag University Faculty of Medicine
Department of Medical Genetics
16059, Gorukle, Bursa, Turkey
Telephone: +90 224 295 43 98,
Fax: +90 224 295 00 99
E-mail: dr.mehmetture@hotmail.com

protein results in *FGFR4* stabilization, and increases phosphorylation after ligand binding (Fang et al. 2013).

Several previous studies have investigated the correlation between the FGFR gene and cancer; increased expression of *FGFR4* has been shown in breast, pancreatic and renal cell carcinomas (Sasaki et al. 2008; Masaru et al. 2014). Specifically, N535K and V550E mutants are activating mutations in rhabdomyosarcoma (Wesche et al. 2011; Crose et al. 2012). Streit et al. (2004) has shown that in squamous cell carcinomas of the head and neck with poor prognosis, high expression of the *FGFR* gene is related to *FGFR4* Arg388. In a study conducted by Bange et al. (2002), although, no correlation was observed between the development, diagnosis age and tumor stage of breast cancer and the *FGFR4* Gly388Arg polymorphism, a significant correlation was identified between the *FGFR4* Arg388 allele and recurrence in cases where lymph nodes are positive for carcinoma, as was a low survival rate. That report led to consideration that the *FGFR4* Arg388 allele may be a dominant parameter in disease progression. Bange et al. (2002) showed a significantly higher frequency of *FGFR4* Arg388 in colon cancer cases with aggressive progression and bad prognosis. It has been suggested that this polymorphism may be correlated with motility, invasion and resistance to chemotherapy in cancer cells. Thus, due to its oncogenic potential, *FGFR4* is a valid therapeutic target. Particularly, it has been shown that Ponatinib inhibits tumor growth in mouse models expressing mutationally activated *FGFR4* (Li et al. 2013). The Gly388Arg polymorphism in the *FGFR4* gene has also been associated with chemotherapy in some types of cancers. In NSCLC patients, Fang et al. (2013) showed that *FGFR4* 388Arg polymorphism occurred more frequently in the responders to the chemotherapy than in non-responders.

Objectives

In different populations, there may be variation in the relationship between FGFR polymorphisms and cancer (Fang et al. 2013; Katoh and Nakagama 2014). This is the first study investigating the correlation between *FGFR4* Gly388Arg polymorphism and patients with PLC in a Turkish population, up to the literature. The researchers did not find any reports of studies that investigated the association between *FGFR4* Gly388Arg polymorphisms and PLC incidence in patients under 50 years of age. In this study, researchers aimed to investigate the relationship between FGFR-4 Gly388Arg polymorphism and primary lung cancer (PLC) tendency, early onset PLC, histopathological sub-type, or metastatic status in Turkish population.

MATERIAL AND METHODS

Study Subjects

This study was approved by the Research Ethics Committee of Medical School of Uludað University (1st June 2010, 2010-2/3). A total of 100 patients with "primary lung cancer" were included in the study were diagnosed via clinical, histopathological and radiological examinations in the department of Medical Oncology between 2010 and 2011. The control group included 100 volunteers who were not diagnosed with any cancer after clinical examination and laboratory tests.

DNA Isolation and Genotyping of *FGFR4* Gly388Arg

Blood samples from both the patient and control groups were taken in EDTA tubes, and genomic DNA was extracted from whole blood using a DNA isolation kit (Dr. Zeydanlý Life Sciences, Ltd., Turkey) according to the manufacturer's instructions, and samples were stored at -20 °C until PCR was performed. *FGFR4* Gly388Arg gene polymorphisms were identified using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. For the *FGFR4* Gly388Arg polymorphism, forward 5'- GACCGCAGCAGCGCCCGAGGCCAG -3' and reverse 5'- AGAGGGAAGAGGGAGAGCTTCTG -3' primers were used (Ho et al. 2010). To identify the *FGFR4* Gly388Arg gene polymorphism among the PCR products, the BstN I enzyme was used. After digestion with the enzyme, the products were run in a 4% agarose gel and the three possible genotypes of the *FGFR4* were determined as follows: if the 168 bp PCR product from the *FGFR4* gene was cut into three distinct products of 109 bp, 37 bp and 22 bp, then the *FGFR4* allele genotype was identified as Gly / Gly; if the 168 bp PCR product resulted in five distinct prod-

ucts of 109 bp, 80 bp, 37 bp, 29 bp and 22 bp, then the genotype was identified as Arg / Gly; and if the 168 bp PCR product was cut into four distinct products of 80 bp, 37 bp, 29 bp and 22 bp, then the genotype was identified as Arg /Arg.

Statistical Analysis

Analyses related to this study were conducted using the SPSS 13.0 (Chicago, IL.) program. $p<0.05$ was considered to be statistically significant.

RESULTS

The characteristics of the study population are shown in Table 1. The patient group consisted of 124 patients (115 males and 9 females) and the control included 100 healthy volunteers (88 males and 12 females). The average age in the patient group was 59.45±0.94 (mean±SD), and the median age was 59. In the control group, the average age was 58.48±1.42 (mean±SD), and the median age was 59. There was no significant difference between the two groups in terms of age and gender ($p_{age} = 0.568$, $p_{gender}=0.226$).

Table 1: Characteristics and distributions of the FGFR4 Gly388Arg genotypes in the Primary Lung Cancer (PLC) patient and control groups

Variables	*Patient group n=124*	*Control group n=100*	*p-value*
Age (years) (MN±SD)	59.45±0.94	58.48±1.42	>0.05
Gender			
Female	9 (7%)	12 (12%)	>0.05
Male	115 (93%)	88 (88%)	
Genotypes			
Gly388Gly	66 (53%)	48 (48%)	>0.05
Gly388Arg	47 (38%)	46 (46%)	
Arg388Arg	11 (9%)	6 (6%)	
Frequency of Allele			
Gly (%)	72.2	71	>0.05
Arg (%)	27.8	29	
Patients with metastasisn=69 Patients without metastasisn=55			
Gly388Gly	35 (50.7%)	31 (56.4%)	>0.05
Gly388Arg	28 (40.6%)	19 (34.5%)	
Arg388Arg	6 (8.7%)	5 (9.1%)	
Patients under the 50 years N=22 Control group n=100			
Gly388Gly	11 (50%)	48 (48%)	>0.05
Gly388Arg	8 (36.4%	46 (46%)	
Arg388Arg	3 (13.6%)	6 (6%)	

MN: mean; SD: standard deviation

Analysis of the *FGFR4* Gly388Arg polymorphism in the PLC group determined that 66 (53.2%) cases were identified to have the Gly / Gly genotype, 47 (37.9%) cases had the Gly / Arg genotype and 11 (8.9%) cases had the Arg / Arg genotype. In the control group, 48 (48.0%) people were identified to have the Gly / Gly genotype, 46 (46.0%) people had the Gly / Arg genotype and 6 (6.0%) people had the Arg / Arg genotype. In terms of genotype distribution, no statistical significance was identified between the PLC group and the control group ($p = 0.412$) (Table 1). Regarding *FGFR4* Gly388Arg polymorphism, the frequency of the Gly allele was determined to be 72.2% in the patient group and 71% in the control group. Arg allele frequency was determined to be 27.8% in the patient group and 29% in the control group. No statistical significance was identified in terms of the frequency of alleles when the patient and control groups were considered ($p=0.783$) (Table 1).

During diagnosis, 17.7% of patients with PLC (22 cases) were under the age of 50, and 82.3% of patients with PLC (102 cases) were over the age of 50. In the patients under 50 years old, the distribution of Gly / Gly, Gly / Arg, Arg / Arg genotypes was found to be 50.0% (11 cases), 36.4% (8 cases), 13.6% (3 cases), respectively. These were not significantly and statistically different from the control group. Metastasis was detected in 55.6% of patients with PLC (69 cases). In the patients with metastasis, the distribution of Gly / Gly, Gly / Arg, and Arg / Arg genotypes was found to be 50.7% (35 cases), 40.6% (28 cases), and 8.7% (6 cases), respectively. In the patients without metastasis, the distribution of Gly / Gly, Gly / Arg, and Arg / Arg genotypes was found to be 56.4% (31 cases), 34.5% (19 cases), and 9.1% (5 cases), respectively. Similarly, no significant difference was identified between PLC patients who suffered from metastasis and those who did not have metastasis ($p=0.686$, $p=0.403$) (Table 1).

Regarding histopathological diagnosis of PLC cases, 17 cases (13.8%) were identified to have SCLC, while 107 cases (86.2%) were diagnosed with NSCLC. While 41% of NSCLC cases had adenocarcinoma, as well as 36% of SCC and 1% of LCC, the type could not be identified in 22% of the patients. In the patients with SCLC, the distribution of Gly / Gly, Gly / Arg, and Arg /

Arg genotypes was found to be 35% (6 cases), 53% (9 cases), 12% (2 cases), respectively. In the patients with adenocarcinoma, the distribution of Gly / Gly, Gly / Arg, and Arg / Arg genotypes was found to be 50% (22 cases), 39% (17 cases), 11% (5 cases), respectively. In the patients with SCC, the distribution of the Gly / Gly, Gly / Arg, and Arg / Arg genotypes was found to be 66% (25 cases), 26% (10 cases), 8% (3 cases), respectively. When SCLC and NSCLC were compared in terms of *FGFR4* Gly388Arg polymorphism distribution, no statistical significance was identified (p=0.294). Also in SCLC, adenocarcinoma and SCC not statistical significance was identified in terms of distribution of the Gly/ Gly, Gly/ Arg, and Arg/ Arg genotypes (p=0.149, p=0.236, p=0.719, respectively).

DISCUSSION

Lung cancer is one of the most frequently diagnosed cancers and is also considered one of the most frequently lethal malignancies worldwide. Despite the high morbidity and mortality of lung cancer, its etiology remains largely unknown. Genetic and environmental risk factors may both contribute lung cancer carcinogenesis (Tan et al. 2014). *FGFR* genes are proto-oncogenes activated in human cancers as a result of genetic variations, especially SNPs, and genomic alterations, such as gene amplifications, chromosomal translocations, and point mutations (Katoh and Nakagama 2014). In the present study, researchers aimed to investigate the relationship between PLC and gene polymorphisms of *FGFR4* Arg388. A statistically significant difference was not found in *FGFR4* polymorphism between the patient group and control group in regard to tendency, histopathologic sub-type, early onset, and metastatic status (p> 0.05).

Several previous studies have investigated the correlation between *FGFR4* Gly388arg polymorphism and lung cancer development risk, lung cancer phase, survival, histopathological sub-types. Fang et al. (2013) showed that the distribution of the 388Arg genotype was significantly lower in 629 patients in the NSCLC group compared to the distribution in the control group of 729 healthy individuals. In a study conducted by Spinola et al. (2005) with 274 lung cancer patients (adenocarcinoma) and a control group of 401 people, no statistically significant difference was identified between the group with adenocarcinoma and the control group in terms of *FGFR4* Gly388Arg genotype distribution and allele frequency. In a study conducted by Sasaki et al. (2008), patients with adenocarcinoma and those with non-adenocarcinoma lung cancer were compared, and no statistically significant difference was identified between the two patient groups of a total 274 people, in terms of 388Gly and 388Arg frequency. In a study conducted by Matakidou et al. (2007), no correlation was identified between SCLC, NSCLC and adenocarcinoma groups in terms of the *FGFR4* Gly388Arg genotype distribution. In the present study, researchers did not identify any significant correlation between SCLC and NSCLC groups in terms of *FGFR4* Gly388Arg genotype distribution, and researchers also could not identify any statistically significant correlation between adenocarcinoma, SCC and SCLC.

There are various conflicting results published in relation to the correlation of *FGFR4* Gly388Arg polymorphisms and metastatic status of PLC or prognosis. In a study that compared two groups, Fang et al. (2013) showed that the frequency of the 388Arg genotype was significantly lower in 629 patients with Stage III (A+B) or IV NSCLC than in a group of 729 healthy control subjects. In a study of lung cancer patients (274 adenocarcinoma and 401 control group) Spinola et al. (2005) compared two groups that they classified as Stage I and Stage II-IV, in terms of *FGFR4* Gly388Arg polymorphism. Stage II-IV PLC was significantly correlated with Gly/Arg and Arg/Arg genotypes. In contrast, in a study conducted by Matakidou et al. (2007) with patients suffering from lung cancer in English population (SCLC and NSCLC), no correlation was identified between *FGFR4* Gly388Arg polymorphism and disease stage. In a study conducted with an Italian population, Falvella et al. (2009) classified 541 patients with adenocarcinoma into two groups: those in Stage I and those in Stage II-IV. Compared to Stage I, Stage II-IV was statistically and significantly correlated with the *FGFR4* 388arg polymorphism. In another study conducted by Falvella et al. (2009), 84 patients with SCC and 107 patients with adenocarcinoma were investigated in a Norwegian population. No correlation was identified between the *FGFR4* Gly388arg polymorphism and PLC stage. In a meta-analysis including brain, breast, colorectal, head and neck, larynx, lung, melanoma, and prostate cancers, as well as sarcomas, it was revealed that the Arg388 allele carriers showed an increased risk of poor

survival when overall compared with homozygous carriers of the common Gly388 allele, even after adjusting for nodal status (Frullanti et al. 2011). In this study, researchers separated patients as those that had metastases and those did not, and did could not identify any significant correlations between the two groups in terms of *FGFR4* Gly388arg polymorphism.

When the patients suffer from PLC under the age of 50, they are compared with older patients, variations can be observed in histological distribution and genetic predisposition. Several reports have led to the consideration that the genetic component has more contribution in lung cancers observed at or before the age of 50 (Bourke et al. 1992; Green et al. 1993). In a study conducted by Bromen et al. (2000), it was noted that the first degree relatives of lung cancer patients at the age of 50 or earlier had a five times higher risk of developing lung cancer. Li et al. (2004) showed that the risk of developing lung cancer is higher for the siblings of patients with lung cancer at an age below 50 when compared to the risk of development of lung cancer in their children. This is interpreted as evidence supporting recessive heredity in cases of lung cancer developing before the age of 50. Sasaki et al. (2008) reported no correlation between lung cancers at an age of 60 and below and those at an age above 60, in terms of *FGFR4* Gly388Arg polymorphism types. Further, because a genetic tendency was reported for those patients with lung cancer who are 50 years old or younger, in this research, researchers studied the patient groups separately: those that are at the age of 50 and younger and those who are older than 50. The researchers could not identify a correlation between these two groups in terms of Gly388Arg genotype distribution.

CONCLUSION

In conclusion, researchers did not identify a correlation between the cases with PLC and the control group in terms of *FGFR4* Gly388Arg polymorphisms, and researchers also did not identify any significant correlation between SCLC, SCC and adenocarcinoma. Additionally, researchers did not identify a statistically significant difference in PLC cases between those who have metastasis and those who do not in terms of *FGFR4* Gly388Arg genotype distribution. Inconsistencies exist in the literature regarding the relationship of stage, prognosis and histopathologic sub-type of lung cancer and *FGFR4* Gly388Arg polymorphism. Some reports have mentioned a relationship with some histopathologic sub-types, but others did not establish such a correlation with genetic polymorphisms. These inconsistent results may be caused by variations in the histopathological types and distribution of stages among the patients, various gene-gene interactions caused by geographical differences, potential false positive and false negative results in studies and also because of certain unknown factors.

Allele frequency of a gene may show geographical variations. Thus, identifying the allele frequency of *FGFR4* Gly388Arg in the control group will contribute to studies that will be conducted on this gene in Turkish populations. Studies with a higher number of cases and specific histopathological sub-types are necessary to accurately identify the correlation between *FGFR4* Gly388Arg polymorphism and PLC.

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