T.C.
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GENETIC VARIATION IN THE *EGFR* GENE AND THE RELATION WITH GLIOMA IN TURKISH POPULATION

MASTER OF SCIENCE THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

Gözde ÖZCAN

DEDICATION

To me in the future…

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May the science be with you!

Gözde ÖZCAN

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LIST OF SYMBOLS AND ABBREVIATIONS

cDNA : Complementary DNA

CNS : Central Nervous System

CT : Computed Tomography

DNA : Deoxyribonucleic Acid

dsDNA : Double-Stranded DNA

ssDNA :Single-Stranded DNA

EGFR : Epidermal Growth Factor Receptor

FGF : Fibroblast growth factor

GBM : Glioblastoma Multiforme

IDH1 : Cytosolic $NADP^+$ related isocitrate dehydrogenase

kD : Kilodalton

µl : Microliter

mg : Milligram

MGMT : O-6-methylguanine-DNA methytransferase gene

miRNA : Micro RNA

mRNA : Messenger RNA

ng : Nanogram

nM : Nanomolar

OD : Optical Density

ORF : Open reading Frame

PCR : Polymerase Chain Reaction

PDGF : Platelet-derived growth factor

PDGFR : PDGF receptor

PTEN : Phosphatase and tensin homolog

pg : picogram

RNA : Ribonucleic Acid

rpm : Rounds per minute

RT-PCR : Real-Time Polymerase Chain Reaction

SD : Standard deviation

siRNA : Small Interfering RNA

snRNA :Small Nuclear RNA

SNP : Single Nucleotide Polymorphism

TP53 : Protein 53

UV : Ultraviolet

WHO : World Health Organization

ABSTRACT

Özcan, G. Genetic Variation in the EGFR Gene and the Relation with Glioma in Turkish Population. Yeditepe University, Institute of Health Science, Department of Molecular Medicine. MSc Thesis. Istanbul, 2020.

While glioma constitutes approximately 30% of brain and central nervous system carcinomas, it covers 80% of all brain carcinomas. [1]. The gene that encodes 170 KD transmembrane receptor tyrosine kinase, located on chromosome 7p12-13 and enounced on the facial of epithelial cells, is the EGFR gene. In most prior works, single nucleotide polymorphisms (SNPs) within the EGFR gene are evaluated to correlate cancer risks like carcinoma, carcinoma, prostatic adenocarcinoma, and esophageal cancer. How EGFR contributes to glioma susceptibility variants in the gene and in order to examine that, in a case-control study from Turkey (35 cases, 36 controls) genotyping both groups determined by real-time PCR and was carried out using statistical analysis SPSS data. According to our results, CC genotype (homozygous wild type) 19 (52.8%), CT genotype (heterozygous type) 16 (44.4%), TT genotype (homozygous variant type) 1 (2.8%) were found in the control group. Genotype distribution in the patient group was 8 (23.5%), 22 (64.7%) and 4 (11.8%), respectively. Significant relationship was found between the patient and control groups compared to genotypes ($p = 0.028$). This study can provide a new approach to the clinical treatment of glioma patients.

Key Words: Glioma, *EGFR* Gene, Variation

ÖZET

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Glioma, tüm beyin ve merkezi sinir sistemi tümörlerinin yaklaşık % 30'unu ve tüm malign beyin tümörlerinin % 80'ini oluşturur [1]. EGFR geni, 7p12-13 kromozomu üzerinde bulunur ve epitelyal hücrelerin yüzeyi üzerinde eksprese edilen 170 kD'lik bir transmembran reseptör tirozin kinazı kodlar. Daha önce yapılan birkaç çalışma, EGFR genindeki tek nükleotid polimorfizmlerini (SNP), akciğer kanseri, meme kanseri, prostat kanseri ve özofagus kanseri gibi kanser riski ile ilişkilendirmek için değerlendirmiştir. EGFR genindeki varyantların glioma duyarlılığına katkıda bulunup bulunmadığını ve bunun nasıl olduğunu incelemek için, Türkiye'den bir vaka kontrol çalışmasında (35 vaka, 36 kontrol) her iki grubun genotiplemesi gerçek zamanlı PZR ile belirlenmiş ve verilerin istatistiksel analizi SPSS programı kullanılarak gerçekleştirilmiştir. Sonuçlarımıza göre, kontrol grubunda CC genotipi (homozigot yabani tip) 19 (% 52.8), CT genotipi (heterozigot tip) 16 (% 44.4), TT genotipi (homozigot varyant tip) 1 (% 2.8) bulunmuştur. Hasta grubunda genotip dağılımı sırasıyla 8 (% 23.5), 22 (% 64.7) ve 4 (%11.8) olarak tespit edilmiştir. Hasta ve kontrol grubu arasında genotiplerle karşılaştırıldığında anlamlı bir ilişki bulunmuştur ($p = 0.028$). Bu çalışma, glioma hastalarının klinik tedavisi için yeni bir yaklaşım sağlayabilir.

Anahtar Kelimeler: Glioma, *EGFR* Geni, Varyasyon

1. INTRODUCTION AND PURPOSE

Primary brain tumor, which accounts for 80% of malignant tumors, is identified in about 5-6 patients each year out of every 100,000 people [2]. Astrocytomas and oligodendrogliomas, found in ependymomas, are in the subclass of gliomas, the most widespread group of primary brain carcinomas. As reported by the World Health Organization (WHO), brain carcinomas have been sub-categorized into grade III/IV carcinomas, like anaplastic astrocytoma, anaplastic oligodendroglioma, anaplastic ependymomas with anaplastic oligoastrocytoma and glioblastoma multiforme (GBM). There are a number of characters under consideration when assigning a WHO degree. These are some pathological characters like nuclear atypical, mitotic activity, vascular proliferation, necrosis, proliferative potential, clinical course, and therapeutic conclusion. Its prevalence in the United States is predicted to be about 3: 100,000, but the diagnosed cases per year are more than 10,000. GBM consists of 45.2 % of malignant central nervous system (CNS) tumors, 80% of all primary malignant CNS tumors, and about 54.4% of whole malignant gliomas. Sixty-four is corresponding to the age average of study, and males are in a more dangerous situation rather than the females, and the ratio of it is 1.5 times more likely, as whites are two times more likely than blacks [3]. In the last 20 years, mostly advanced radiological diagnosis has increased incidence, especially in the elderly [4]. Grade III carcinomas and GBM are classified in terms of treatment and treated similarly.

All other patients with glioma are known as sporadic cases. A crucial way of the development of gliomas is due to two main causes. The first is the sequential accumulation of genetic changes in malignant transformation, while the other is the aberrant organizing of growth factor signaling pathways.[5] A small sub-category (about 5%) of cases with gliomas were found to be related with some inherited syndromes. [6].

The development of gliomas can be grouped into two separate titles. One of these is the single nucleotide polymorphisms (SNPs) that are among genetic factors. The other is cooperation among environmental elements like lifestyle habits and comorbidities [7]. For this reason, epidemiological works announce mutations in different SNPs as sensibility elements for gliomas. [8,9]. Last human genome studies demonstrate that heightened peril has been observed in a person with variants in genes in order to the epidermal growth factor receptor (EGFR).

In spite of this, there are mechanisms in gliomagenesis that need to be clarified. Actually, the development and improvement of diverse carcinoma are seen in angiogenesis. This is a critical physiological process [12]. Further, the EGFR signaling pathway subscribes to many biological courses; for example, these include cell cycle progression, metastasis, and angiogenesis, which leads to tumor development [13].

In this case, the target of this study is to appraise for the first time the relationship of gene polymorphisms of rs1468727 on gliomas in the Turkish population.

2. LITERATURE REVIEW

2.1. The Structure of the Brain

The brain, a member of the central nervous system along with the spinal cord, is the management center of the neural conduction. There is a network of neurons between CNS and all other organs and tissues. It is estimated that there is an average of one hundred billion neurons in an adult. The brain keeps all movements and changes in the body under control, thanks to this communication network. Due to its existing functions and imp-ortance in the body, the brain has various protection mechanisms different from other organs. The skull, which has a strong bone tissue, is protected against impacts by wrapping the brain from the outside—the brain membranes located under the skull and surrounding the brain act as a buffer. In addition, all CNS members are filled with a special fluid called Cerebrospinal fluid (CSF) [14]. Structure of brain can be seen in Figure 2.1-1.

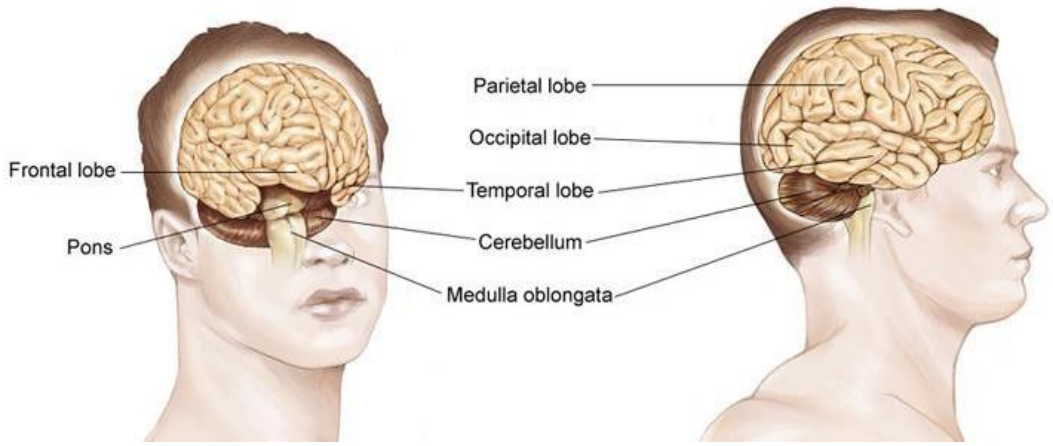

Figure 2.1- 1 The Structure of the Brain [15]

When nervous system cells are evaluated histologically, they are divided into two groups as neuron and neuroglial cells. Neurons acting as functional units of the nervous system are responsible for all neural transmission. In neurons, consisting of four parts:

the cell body (corpus), axon, dendrites, and synaptic ends, the part where the nerve conduction is detected is dendrite, and the part where the conduction is provided is the axons. Cell body; cell fluid consists of the cell nucleus and cell membrane. The main task of the cell nucleus is to determine the shape of the cell. The structure of the neuron can be seen in Figure 2.1-2.

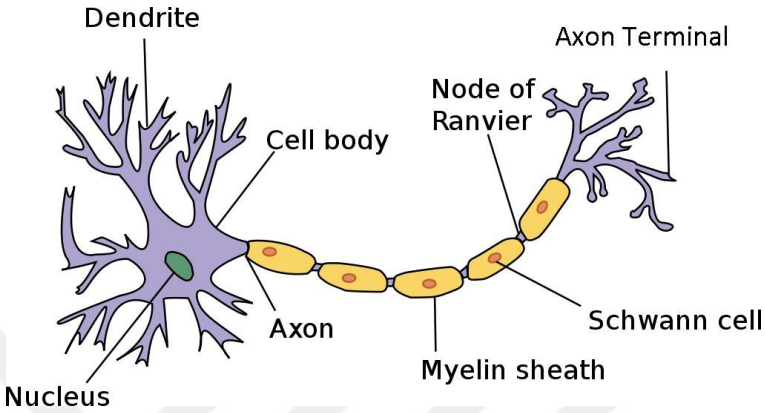

Figure 2.1- 2 The structure of the neuron [16]

Neuroglia plays the role of auxiliary elements of the nervous system. A large part of the human brain consists of neuroglia cells. Neuroglia cells can be very different types considering both their functions and structures. These cells surround a neuron. Astrocytes, oligodendrocytes, ependymal cells, Schwann cells, radial glial cells, and microglial cells belong to the group of neuroglia cells. Each cell responds to the different needs of CNS in terms of shape and function. Four types of glial cells appear in the central nervous system can be seen in Figure2.1-3.

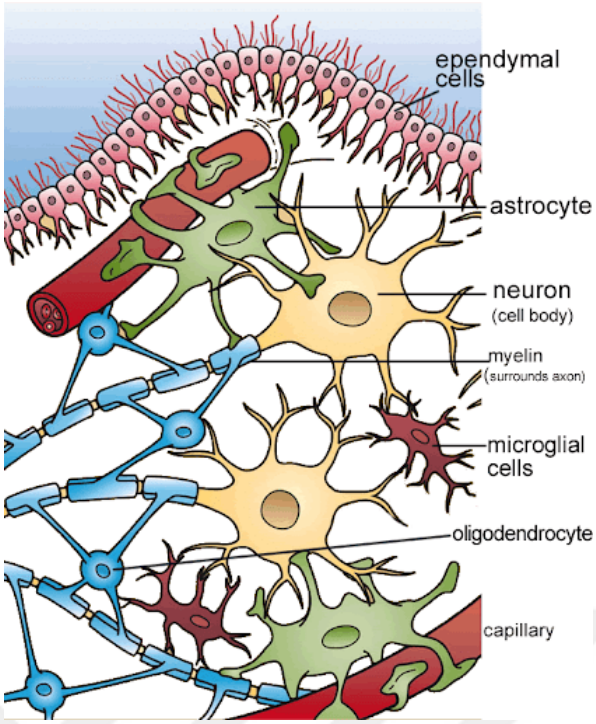

Figure 2.1- 3 Four types of glial cells appear in the central nervous system. Light pink ones are ependymal cells, and green ones are astrocytes, dark red ones are microglial cells, and light blue ones are oligodendrocytes [17].

The most common glial cell type in CNS is astrocytes. The functions of these glial cells, whose structural shapes are star-shaped, can be considered as providing nutrients to neurons, providing structural support, and establishing ion balance. Another important task of astrocytes is to provide ENT integrity by supporting endothelial cells. Oligodendrocytes take the task of forming the myelin sheath by wrapping the axon part of MSS 9 neurons. With the myelin sheath, the speed of neural conduction is increased. Schwann cells perform the same task for the peripheral nervous system (PNS) neurons [18]. The main task of the ependymal cells is to form the ependymal, a thin membrane that surrounds the ventricular system of the brain, and the spinal cord. Radial glial cells are cells that take place a significant part in the improvement of CNS in the embryonic period. The role of microglia is to eliminate some unnecessary substances and dead neurons and protect CNS by playing macrophage in pathogenic situations [19].

2.1.1. Central Nervous System Tumors

To assign the biological attitude of a tumor, histologically needs to be classified and graded. CNS tumors
The main points in the classification are the location, behavior, and histopathological features of the tumor. A consistent and accepted classification in neurooncology in terms of both estimating prognosis and evaluating treatment options and response to treatment is important. For this reason, tumor classification has been made many times since the beginning of the twentieth century, and these classifications have been rearranged in the light of new findings with the developing technology. Today, it is not possible to talk about a reliable classification that allows choosing the correct diagnosis and the most appropriate treatment method. However, within the existing classifications, the classification of CNS tumors that have been accepted by everyone is provided by WHO [20].

It is the classification made in 2016. Recent studies have revealed the genetic basis of carcinogenesis in common or rare brain tumors, and such an approach has contributed to the classification of brain tumors. In the classification of WHO's 2016 CNS tumors, updates were made in the 2007 classification, taking into account the phenotypic features of tumors as well as their genotypic features. In the 2007 classification, all astrocytic and oligodendroglial tumors were classified, regardless of whether astrocytic tumors have clinically similar or different characteristics. In the classification of 2016, all astrocytic and oligodendroglial tumors, an exception of WHO stage I, were grouped in one group, and non-diffuse stage I-II-III other astrocytic tumors were grouped separately [21].

2.1.2. Brain Tumors

Brain tumors are originating from the brain parenchyma, brain membranes, nerves, or other tissues in the head. About 200,000 people worldwide every year affected by a malignant brain tumor [22]. Brain tumors are approximately 1.4% of all cancer types and constitute 2.7% of cancers resulting in death and are very frequent solid tumors in children [23]. According to the Ministry of Health in Turkey Cancer Statistics 2014 data; Between 2008 and 2012, it was reported that 7739 patients were diagnosed with brain tumors, and the dispersion of age-standardized effect ratio of brain tumors by gender was

4.1 per hundred thousand in women and 5.2 for men in 2014. (T. C. Ministry of Health, 2014)

Primary brain carcinomas begin in the brain. Primary brain tumors are classified as low-level or high-level. Low-level carcinoma can usually grow gradually; however, it is likely to develop into high-level carcinoma. High-level carcinoma can grow faster than a low-grade tumor. Secondary brain tumors, named metastasis, are most common in adults than primary brain carcinomas.

A cancerous tumor that begins elsewhere in the body as like the chest, lung, or colon, and then spreads to the brain is named a secondary brain tumor. The condition in which cancer outstretch to the meninges, and cerebrospinal fluid (CSF) is named leptomeningeal metastases, or neoplastic meningitis. This case is common in people with leukemia, lymphoma, mel-anoma, breast cancer, or lung cancer. Types of brain tumors can be seen in Figure 2.2.1-1 and Subunits of Brain Tumors can be seen in Figure 2.2.1-2

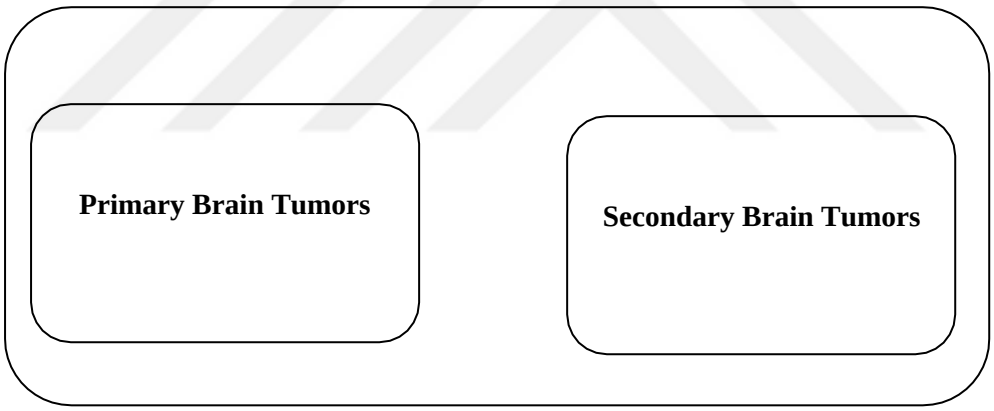

Figure 2.2.1- 1 Types of Brain Tumors [25]

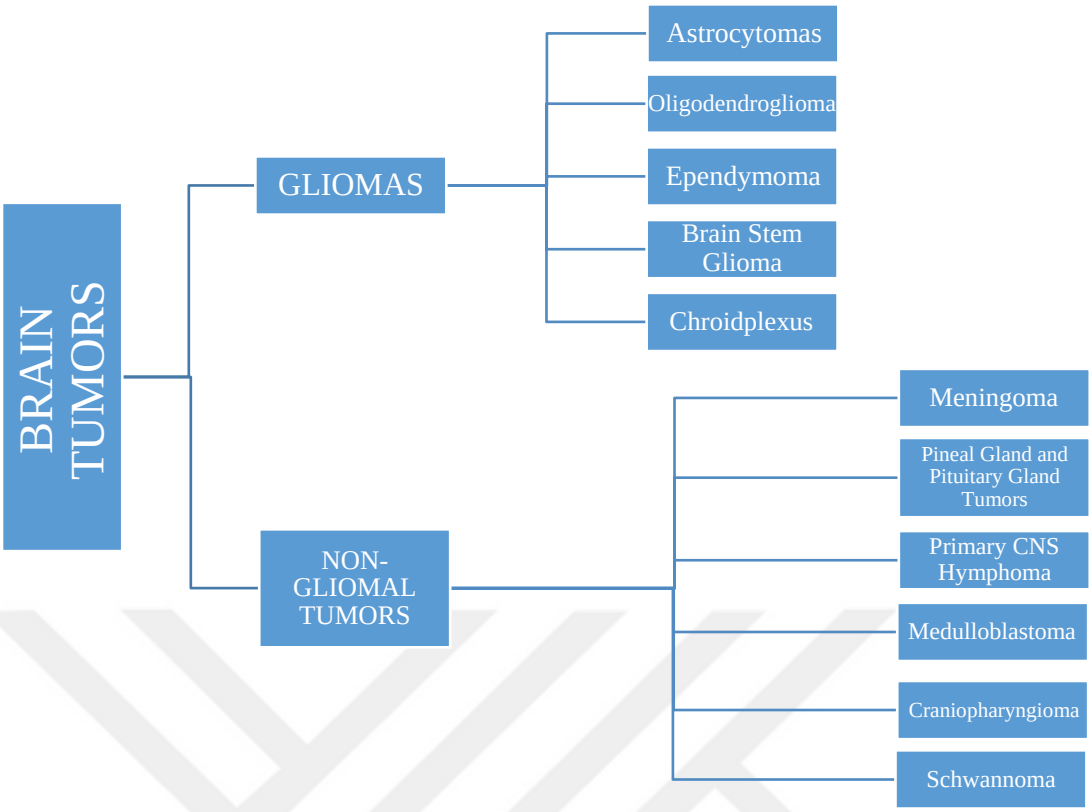

Figure 2.2.1- 2 Subunits of Brain Tumors [25].

Primary brain tumor has several kinds. But some are not given an accurate form, for removal of the carcinoma is difficult due to its location.

2.2.2. Overview of the Glioblastoma Multiform

The definition of "glioma " means" glue " in Greek, and this tumor originates from brain glial, or supporting tissue.

Gliomas account for the plurality of primary brain tumors, with a yearly frequency of 5/100, 000 person [24]. Group of gliomas is done according to more than one measure. These are markers of origin kind of cell, histopathological view, location, and origin. Astrocytomas, choroid plexus tumors, ependymomas, oligodendrogliomas, and oligoastrocytomas are in the class of gliomas [25]. Astrocytomas are a very widespread sort of brain carcinoma. According to the World Health Organization (WHO), astrocytic cancers are four classes of malignancies [26]. Grade I, pilocytic astrocytomas tumors are

known as benign are often noticed in youthful grownups and kids. Operation is chiefly used as a therapy choice. Grade II is low-level or common astrocytomas that attack brain construction by growing slowly. The survival ratio varies according to the case, but the average is 4-5 years. There are some methods used to treat Grade II gliomas. These are low-level or common astrocytomas low-level or diffuse astrocytomas X surgical resection, chemotherapy, and radiotherapy. Grade III, Grade IV, anaplastic oligodendrogliomas, and anaplastic astrocytomas, glioblastomas are labeled as flagitious or high-level gliomas [25]. Grade IV (GBM) is the very prevalent class with a rate of 3' in 100,000 diagnosed each year as a type of malignant primary glioma [27]. The survival rate in this disease is one year on average [28]. In just 3-5 % of Grade IV cases, more than 3-year endurance is notified. Spite of molecular and clinical elements affecting endurance ratio is not well identified, the relationship among longer endurance and promoter methylation of O-6-methylguanine-DNA methyltransferase (MGMT) gene or IDH1 mutation have been noticed lately [29]. Classification of primary brain tumors and subtypes of can be seen in Figure 2.2.2-1.

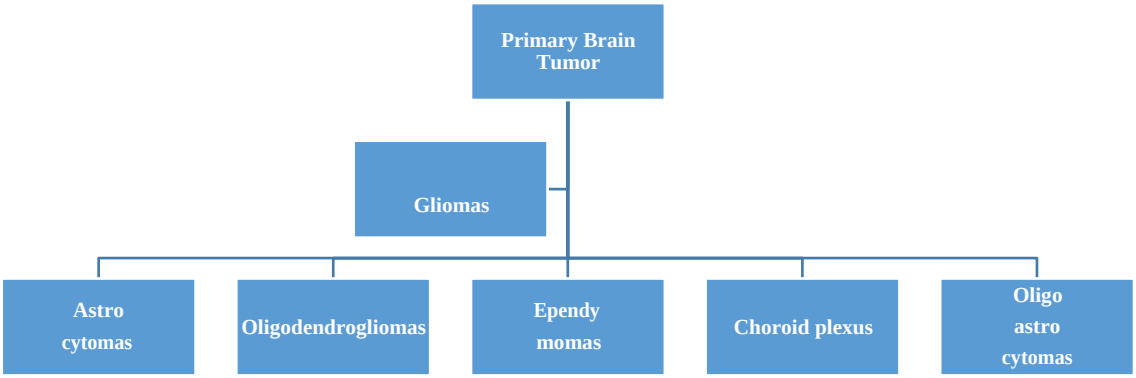

Figure 2.2.2 - 1 Classification of primary brain tumors and subtypes of glioma [25].

Imageological examinations such as nuclear magnetic resonance imaging and computer tomography are diagnostic screening instruments for carcinoma patients. Owing to the expensive high technology screening examinations, unfortunately, they

could not be able to use as a routine diagnostic tool. Histopathological examination through microsurgical resection or stereotactic biopsy is another alternative for the diagnosis of glioma. Nevertheless, it might be hindered from extensive practice by its invasive nature. Recent publications put forward that molecular biomarkers could be account for candidate diagnostic markers instead of high-cost screening tools in glioma diagnosis. For instance, molecules like tensin homolog (PTEN), phosphatase, and protein 53(TP53) were deregulated in glioma procession, but for further applications in cancer diagnosis, new methods need to be improved for detection of biomolecules in serum, blood and tissue samples [30]. Therefore, there is an urgent need for a novel, highly efficient, and noninvasive biomarker for glioma diagnosis [31].

GBMs are recognized to account for a percentage of 60-70 of whole gliomas [24], and the way they improve from Degree II to Degree III astrocytomas [32] is classified kind of primary de novo carcinomas or secondary GBMs made. Great deal properties of GBM indicate Complex chromosome anomaly, widespread gliding along brain parenchyma, powerful angiogenesis, violent strength to apoptosis, pseudopalisading necrosis, and genomic uncertainty [33]. Two main characteristics are used to distinguish GBM from low-grade gliomas; these are vascular hyperproliferation and necrosis. Also, the changing view of primary and secondary GBMs are distinct. The markers of secondary glioblastomas are Fibroblast growth factor (FGF), TP53 mutation, PDGF receptor (PDGFR), and Platelet-derived growth factor (PDGF) overexpression, a deficit of RB and- Phosphatase and tensin homolog. On the other hand, the markers of primary glioblastomas are known as the CT in of role of alteration in Epidermal Growth Factor Receptor (EGFR), the deficit of PTEN, and Cyclin-dependent kinase 2a/p16 (CDHN2A/p16) [34][35]. Genetic pathway in the evolution of primary and secondary glioblastoma can be seen in Figure 2.2.2-1.

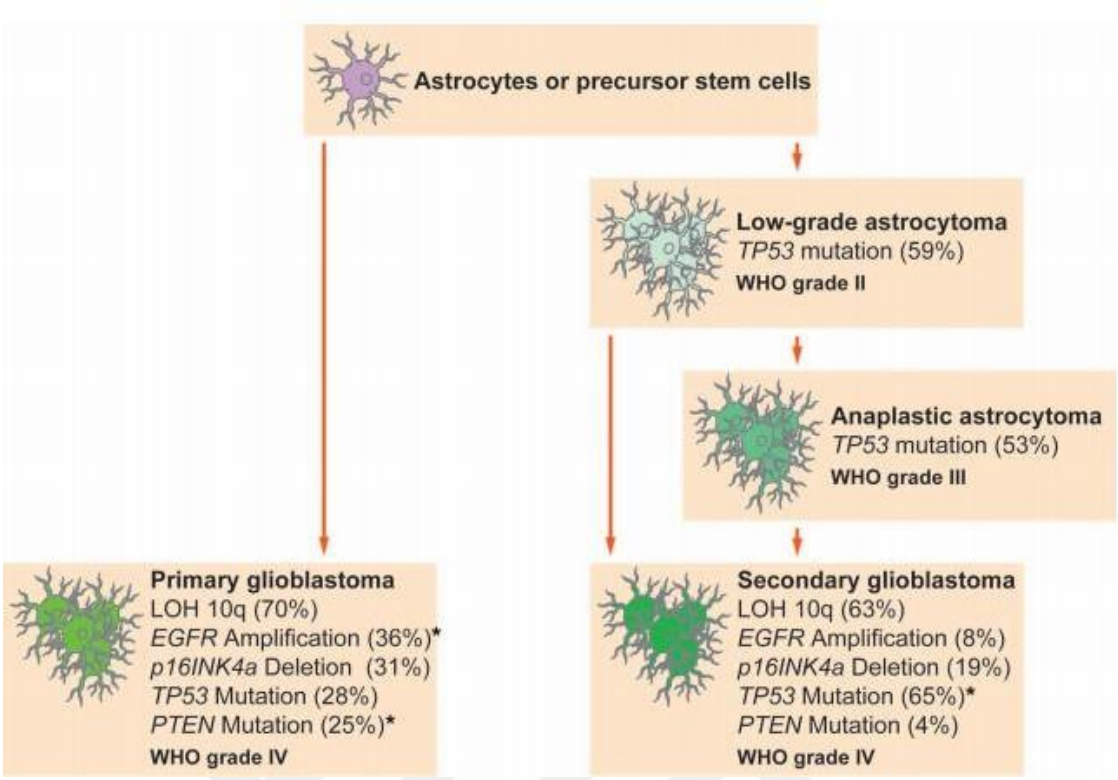

Figure 2.2.2- 1 Genetic pathway in the evolution of primary and secondary glioblastoma [26].

There are several factors that the development of gliomas is linked to. One of these is the single nucleotide polymorphisms (SNPs) that are among the genetic factors. Besides, cooperation among environmental agents like behavior pattern and comorbidities are also linked to glioma development. Therefore, mutations in various SNPs report in epidemiological studies as susceptibility factors to gliomas. In recent human genome studies, the expanded danger has been observed in genes with an epidermal growth factor receptor (EGFR) variant. However, the need to clarify the mechanisms involved in gliomagenesis has increased. As a matter of fact, angiogenesis is a physiological action that promotes the enhancement and improvement of a variety of carcinomas. Furthermore, the EGFR signaling route takes part in a vital purpose in the progression of plenty of biological processes, including co-tumor improvement, metastasis, cell cycle development, and angiogenesis.

2.2. Epidermal Growth Factor Receptor

The gene that encodes 170 KD transmembrane receptor tyrosine kinase, located on chromosome 7p12-13 and the face of epithelial cells explains, is the EGFR gene [36]. It also has the distinction of being the earliest receptor explored and sequenced to have tyrosine kinase activity. Chromosomal location of EFGR Gene can be seen in Figure 2.3-1.

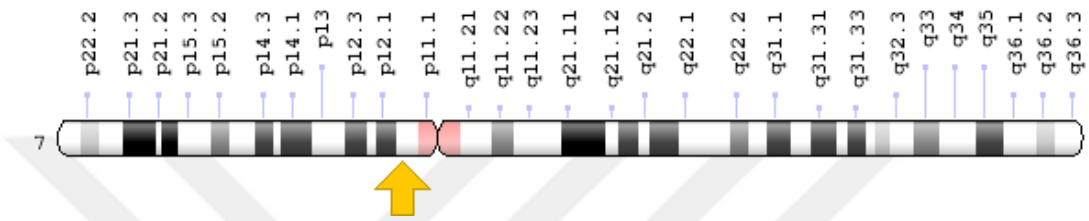

Figure 2.3- 1 Chromosomal location of EFGR Gene [36].

The epidermal growth factor receptor (EGFR) gene ensures directives for creating a receptor protein has entitled the epidermal growth factor receptor, which spread the cell membrane so that one exit of the protein relics within the mobile and the against end throw from the outer substructure of the cellular. This localization permits the receptor to connect to different proteins, which named as ligands, out of doors the cell, and to acquire alerts that assist the cellular reply to the circumference. Ligands and receptors match key-lock relations collectively. The Epidermal growth problem receptor has the ability to bind to at least one of seven species. The connection of a ligand to the epidermal growth factor receptor permits the receptor to connect to different close by the epidermal growth factor receptor protein (dimerize), stimulating (switching on) the receptor complex. Consequently, signaling pathways in mobile are initiated, which cell mobile increase and separation (multiplication) and cellular survival.

Previous studies have shown that activation of the EGFR signaling pathway leads to a lot of biological operations. All these biological processes of tumor progression, which is related to cell survival, proliferation, apoptosis, differentiation, cell cycle progression, invasion, metastasis, and angiogenesis facility [37, 38]. EGFR signaling is started by ligand linking to the outside of the cell ligand-linking site [39]. The

extracellular binding site begins receptor dimerization and tyrosine auto-phosphorylation, resulting in receptor activation. Amplification and/or overexpression of EGFR have been determined in about fifty percent of gliomas, which are malignancy[40] crosschecked to almost ten to twenty-six percent of anaplastic astrocytomas [41]. EGFR amplification was found to be related to substandard results in glioblastoma cases [42]. Nevertheless, the effect of polymorphisms in EGFR, contributing to glioma progression on pathogenesis and biological mechanisms, is not fully clear.

Yet, prior partnership works have shown that genetic variations in EGFR are related to glioma threat. There are a lot of works focusing on the influence of the changes in glioma cases predicts. It has been thought that SNPs in EGFR may affect the predictions of glioma patients. To check the claim, an SNP was scanned in EGFR, and their relationship among these SNPs and the prediction of glioma cases in the Turkish population was evaluated. Signaling due to EGFR kinase can be seen in Figure 2.3-2.

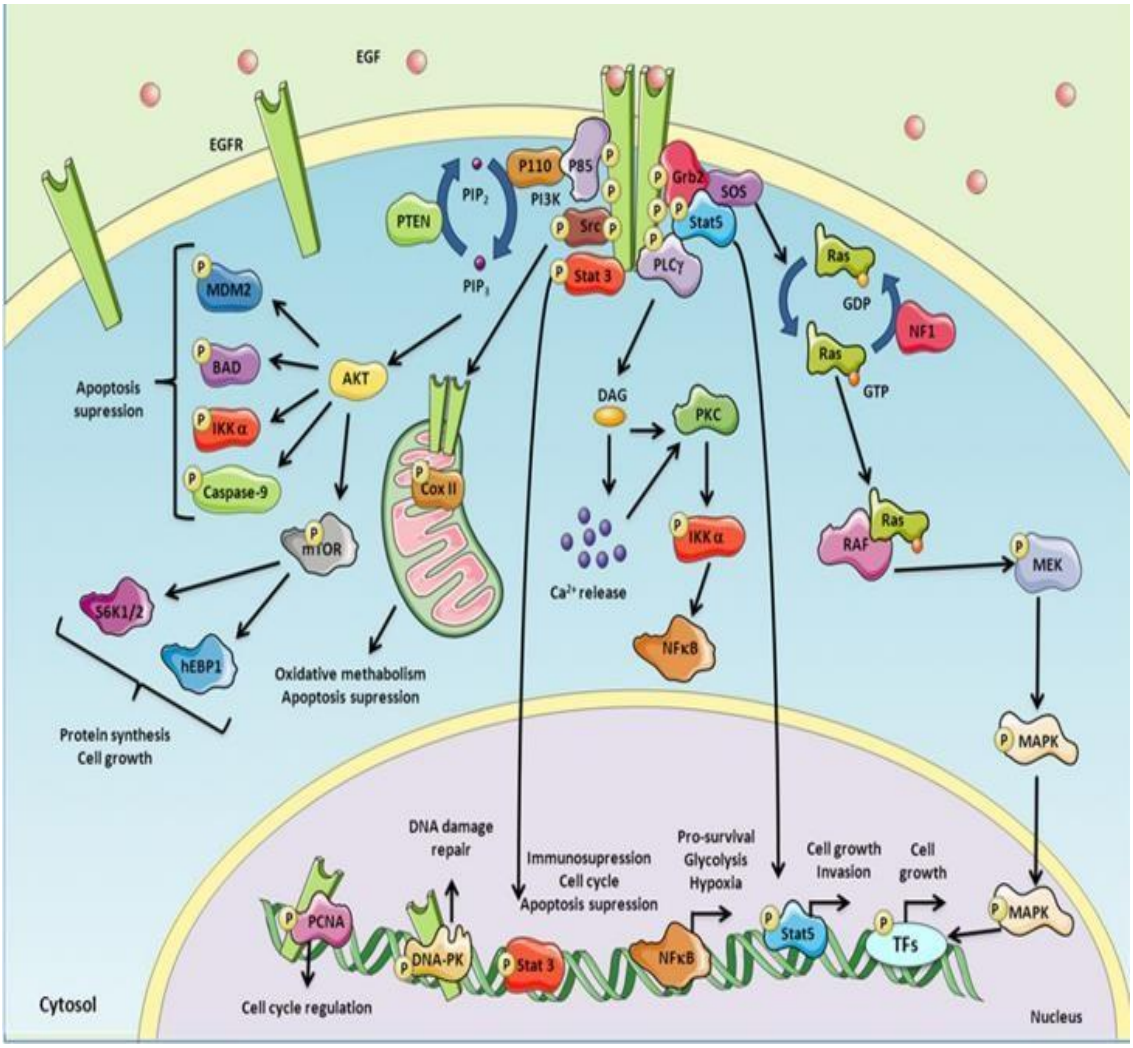

Figure 2.3- 2 Signaling due to EGFR kinase [43].

2.2.1. EGFR Expression in Gliomas

The Epidermal growth factor receptor (EGFR) is an extramarital glycoprotein and belongs to the tyrosine kinase superfamily receptor. Gliomas are carcinomas that originate from glial cells, showing a variety of aggression hingeing on diploma and stage. Lots of EGFR gene modifications have been observed in glial carcinomas, particularly glioblastoma, which hosts amplification, deletion, and single nucleotide polymorphisms (SNPs). Glioblastomas are mentioned like a distinct presence because they have immoderate correlations with EGFR mutants and are mentioned by using the survival and remedy response of the latter on this glioma subgroup.[44]

Since EGFR is a powerful oncogene, it is not surprising that neoplasms develop ways to increase the activity of this gene. EGFR is expressed to be altered in various methods by overexpression, amplification, deletion mutants, and others at different

degrees of astrocytoma, especially glioblastoma (GBM). Proliferation, survival, angiogenesis, and infestation are encouraged by EGFR changes. Hence, the changes may have an effect on gliomagenesis, but the clinical significance of prognosis, diagnosis and treatment is controversial. GBM has increased due to invasion, proliferation, and resistance to radiotherapy and chemotherapy [45-46].

Recurrent amplification of the EGFR gene, known to have been confirmed in several subsequent studies in GBM, was at first informed in 1985 [47]. It is predicted that the EGFR gene reproduces in 30-40% of GBMs, and about fifty percent overexpress the receptor [48, 49, 50]. While high EGFR mRNA grades are not well understood, they have been observed in fewer carcinoma astrocytomas and oligodendrogliomas without gene multiplication [51]. Examination suggests that underlying the appropriate duty of EGFR in glial cells and other carcinomal facts can be associated with increment transcription of genes. Actually, EGFR magnification is reported in just three percent of anaplastic (level III) astrocytomas [52], which is rare in secondary glioblastoma multiform (just 8%). However, 60% of basic GBMs indicate EGFR overexpression and 40% store EGFR multiplication. EGFR multiplication is uncommon in GBM cases smaller than 35, and the medium-years of cases with such changes is 62 years [53]. From a histopathological point of view, EGFR gene multiplication is un rare in young-cell GBMs (69%) but common in gliosarcomas (0%) and giant-cell GBMs (6%). From a neurological standpoint, EGFR mutations or EGFR amplification to identify the presence of the tumor is a GBM, necrosis and microvascular proliferation even in the absence of a biopsy or at least should be treated as such represents strong evidence that GBM [54]. Epidermal growth factor receptor (EGFR) modifications in gliomas can be seen in Figure 2.3.1-1.

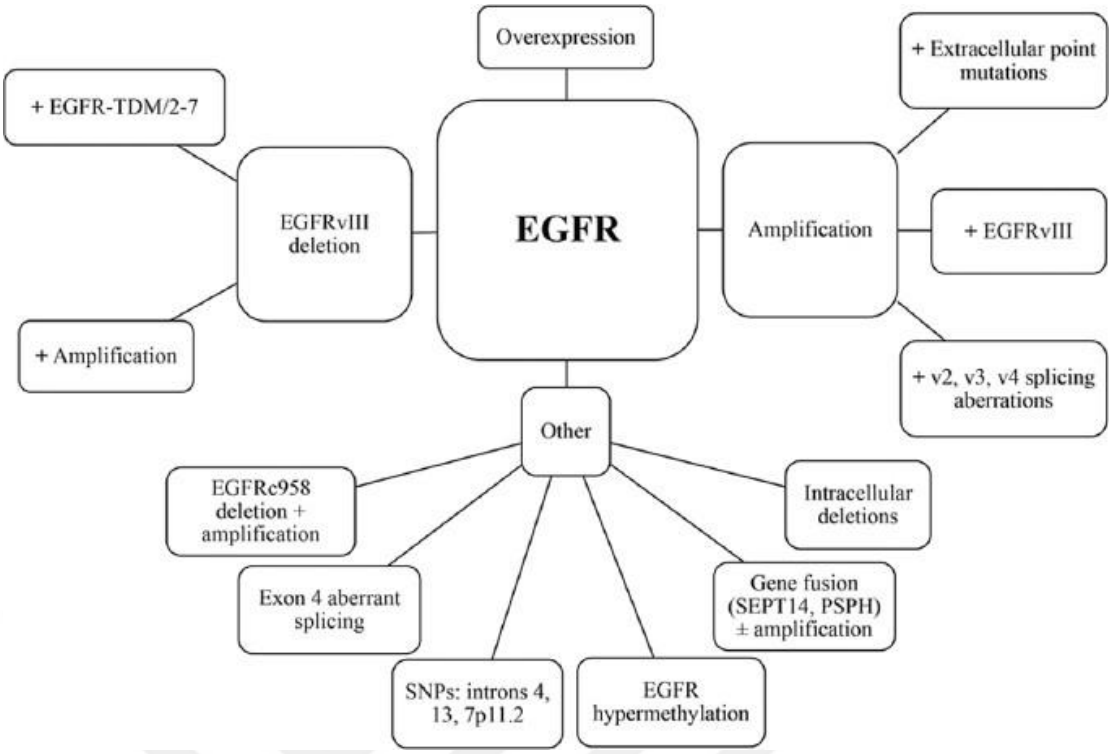

Figure 2.3.1 - 1 Epidermal growth factor receptor (EGFR) modifications in gliomas [55]

3. MATERIALS AND METHODS

3.1. Sample Selection and Definition

As part of the study, patients diagnosed with glioma (n=35) and a control group (n=36) were included. The patient group was composed of patients who were diagnosed by the department of neurosurgery department, and the healthy control group consisted of healthy individuals. Ethical approval was obtained from the Ethics Committee of Yeditepe University for the study (Ethics committee decision no: 1757).

Control Group: The control group consisted of healthy individuals aged 18-85 years who were not diagnosed with glioblastoma following the clinical examination.

Patient Group: The patients were composed of individuals within the Yeditepe University Hospital. They were diagnosed with glioblastoma, and their age range was between 18-85 years old.

3.2. Materials and Devices Used in the Experiment

3.2.1. Materials Used in the DNA Isolation from the Peripheric Blood

The peripheral venous blood samples were set in tubes at +4 °C. The tubes contained Ethylenediaminetetraacetic acid (EDTA), which prevents blood clotting. DNA İsolation Robot (IPrep pure link, Invitrogen, and the Thermo Fisher Scientific Inc) system was used for DNA isolation.

3.2.2. The Equipment Used in the Experiment

DNA Isolation Robot (Iprep Purelink, Invitrogen, Thermo Fisher Scientific Inc), Nanodrop 2000 (Thermofisher Scientific Inc), Real-Time PCR (LIGHT CYCLE 480 II Instrument, Roche Diagnostics, Fast Real Time 7500, Applied Biosystems), 7500 Fast Real-Time PCR Instrument, Plate Centrifuge (Hettich), Centrifuge (Centrifuge 22R-Beckman Coulter), +4 C° Refrigerator (Haier), -20 C° Refrigerator (Haier), Ultra-Pure Water (Pure Lab Option Q,ElCT), Vortex (V.I. Plus Biosan) and a Pipette Kit (Thermo Fisher Scientific Inc.) were used in this experiment.

3.3. Methods

3.3.1. Genomic DNA Isolation from Blood

All venous blood samples of the patient and control groups were taken into the tubes with EDTA in a volume of 5 ml. Blood samples were stored in a refrigerator at + 4 ° C until DNA isolation. DNA isolation was performed by using a robot of iPrep DNA extraction (Invitrogen), and from the blood genomic DNA isolation Kit with iPrep. DNA can be isolated from 350 µl peripheric blood by this system, so 13 blood samples could be studied at the same time. One cartridge is used for each of the samples, and the cartridges are agitated for a period of time to bond the magnetic beads to the DNA efficiently before putting the samples to own cartridge.

The robot of iPrep works according to ChargeSwitch® technology (CST®), as an automated extraction method. A high amount of DNA can be prepared from samples with this method. In this method, amounts of genomic DNA are prepared from samples purely by using paramagnetic particles. These particles are surrounded by a DNA-binding surface. CST® (Charge Switch® Technology) extraction method has a unique principle when compared to the silica-based DNA extraction method. The charge of beads can be changed by the pH of its surrounding buffer. In the event of low pH conditions, the backbone of the DNA is negatively charged, then it binds to the positively charged beads. These charged beads are neutralized by using a low salt buffer that has a higher pH in order to allow for the elution of DNA. Purified nucleic acids pass into the wash buffer, then DNA samples are ready to use.

At the end of the experiment, aqueous DNA samples were obtained and stored at +4 ° C in the refrigerator.

3.3.2. Measurement of DNA Purity

UV absorbance of nucleic acids is measured at 260 nm by UV spectroscopy that called NanoDrop. In this spectrophotometer cuvettes or capillaries are not required. DNA concentrations of both OD260/OD280 and OD260/OD230 proportions are determined by NanoDrop. Thanks to the NanoDrop device, the purity as well as the concentration of nucleic acid molecules such as DNA were able to observed. On the other hand, it has not been shown to distinguish several molecules like RNA, nucleotides, double-stranded DNA (dsDNA) or single-stranded DNA (ssDNA) because this device quantifies the absorbance of nucleic acids [56].

In this experiment, we quantified DNA using the NanoDrop 2000 (Thermofisher Scientific Inc). 1,5 µl of DNA samples were used. The DNA samples before measurement were diluted in the ratio of 1/100. The sample was put into place for measurement by opening the arm then the device's arm was turned off. After each measurement, the surface was cleaned by distilled water, and thus it could be safe for the next measurement.

50 µg / ml of double-stranded DNA at a wavelength of 260 nm is equal to one Optical Density (OD) Unit. The purity of DNA samples was measured by analyzing the OD260 / OD280 ratio. The suitable ratio of OD260 / OD280 is between 1.7-1.9 when performing genotyping [57].

The next formula was used to find the concentration of DNA at 260 nm. Formula of concentration of DNA at 260nm can be seen in Figure 3.3.2-1

$$\text{dsDNA concentration} = 50\ \mu g/mL \times OD260 \times \text{dilution factor}$$

Figure 3.3.2 - 1 Formula of concentration of DNA at 260nm

3.3.3. Real-Time PCR Conditions for EGFR Polymorphism

Genotyping analysis was performed by using the 7500 Fast-Real-Time Polymerase chain reaction (Applied Biosystems) device with Real-Time PCR.

By Real-Time PCR, fluorescence dyes of probes are utilized to determine the single nucleotide polymorphisms (SNPs). It is a system that allows the genotyping by reading the fluorescence radiations. There are two TaqMan probes, one prob is labeled a FAM dye, and the other prob is labeled a VIC dye. The fluorescent dye-bound DNA probes bind to the amplified region. The probes are hydrolyzed by Taq polymerase. Fluorescent signals can be easily detected. The probes with fluorescence dye used in the Real-Time PCR have two different wavelengths for allele-specific, which are wildtype and mutant alleles, detection.

The primer sequence of EGFR is indicated below. This primer was determined according to the sequence of the EGFR gene in human cells, and it was used in this experiment is used. In this method, the region containing this polymorphism was increased by using 5′GATCCAGAAATATTTAGGAGC3′ (Forward) and 5′TTTCATCACCTTGCCTCT3' (Reverse) primers. A region of the gene was generated by genotyping, and EGFR (rs 1468727) polymorphism was analyzed. The focused gene region of genotyping was rs 1468727 for the EGFR gene. Region-specific primer and probe sets are used 'TaqMan Genotyping Assays', and fluorescence dyes of probes are given below. Allelic discrimination has been shown using the software of the 7500 Fast Real-Time PCR tool. EGFR Primers can be seen in Table 3.3.3-1

Table 3.3.3 - 1 EGFR Primers [58]

SNP	***Primers***	***Endonuclease***	***Product length (bp)***	***Annealing Temperature (°C)***
rs1468727	Forward: 5′GATCCAGAAATATTTAGGAGC3′	MaeI	207	56
	Reverse: 5′TTTCATCACCTTGCCTCT3’			

3.3.3.1. Real-Time Protocol

Real-time PCR reagents and the mixture of reactions have been recorded in Table 3.3.3-1. The total volume for each sample was determined by the protocol. The reaction mixture for the Real-Time PCR can be seen in Table 3.3.3.1-1.

Table 3.3.3.1- 1 The reaction mixture for the Real-Time PCR

The Material Used	Quantity
Master Mix	5 µl
TaqMan Genotyping Assay	0.5 µl
DNAse, RNAse Free water	3.5 µl
Template DNA	1 µl

The conditions for Real-Time PCR were arranged by waiting for 10 minutes at 95° C, accomplishing denaturation for 15 seconds at 92° C for each cycle, and also connecting/elongation for 1 minute at 60° C for each cycle. As illustrated in Table 3.2., denaturation and connecting/elongation were completed for 40 cycles. The Real-Time PCR conditions can be seen in Table 3.3.3.1-2.

Table 3.3.3.1- 2 The Real-Time PCR conditions [59]

40 Cycles			
Waiting		**Denaturation**	**Connecting/Elongation**
Temperature	95° C	92° C	60° C
Duration	10 minutes	15 seconds	1 minutes

3.3.4. Statistical Analysis

The student's t-test was utilized to investigate numeric values. The information obtained from genotyping was evaluated using Chi-square and Fisher's Exact Tests via the SPSS 25.0 Program for statistical analysis. Chi-square and Fisher's Exact Tests were utilized for evaluating the distributions of genotypes and alleles among groups. P-values less than 0,05 were considered statistically significant.

4. RESULTS

4.1. Demographic Results of Working Groups

Comprehensive demographic results of 35 GBM patients and 36 healthy controls can be seen in Table 4.1-1.

The control team occurred of 23 male and 13 female, contributors while the patient sample team occurred of 26 male and 9 female contributors (Table 4.1-1).

Table 4.1 - 1 Demographic characteristics of patients

	Control (n=36)	Patient (n=34)	p value
Gender	Male / Female 63.88% / 36.12% (n=23) / (n=13)	Male / Female 73.52% / 26.48% (n=25) / (n=9)	0.385** NS
Age(years): mean±SD	42.75 ± 11.70	48.29 ± 18.46	0.138** NS

* (S)= significantly different (p< 0.05), **NS= not significant (p>0.05).
The difference between the groups was analyzed by the advanced chi-square test (X^2) and the double independent sample student t-test.
n=number of sample, X± SD (mean ± Standard Deviation)

Upon examination of the table above, it can be concluded that no statistical significance between the two groups was observed.

When the age (p=0.138) and gender (p=0.385) data of both groups were examined no statistical significance was found Table 4.1-1.

When tumor locations of GBM cases are examined, it is seen that the tumors are mostly present in the temporal area (35.3%) (Table 4.1-2)

Table 4.1 - 2 Clinical data of GBM patients

Tumor Location	
Temporal n=12 (35.3%)	Thalamus n=4 (11.7%)
Frontal n=4 (11.7%)	Singulat n=3 (8.82%)
Parietal n=5 (14.7%)	Corpus callosum n=1 (2.9%)
Occipital n=2 (5.8%)	Pons n=1 (2.9%)
Cerebellum n=1 (2.9%)	Brainstem n=1 (2.9%)
Total ***n=34*** ***(100%)***	

n=patient number, %= percentage value based on sample group total

4.2. Statistical Evaluation of Real-Time PCR Results

Allele types of each individual within the patient and control groups were determined by the 7500 Fast real-time devices. The obtained allelic discrimination plot from this tool is shown below.

Allelic discriminations were analyzed automatically by the software of the 7500 Fast-Real Time PCR instrument. The readings and interpretations of the fluorescence irradiation are performed by dyes found in the probes. However, some samples could not be discriminated. FAM dye shows a blue color, while VIC dye shows a green color. ROX is a reference color for comparing FAM and VIC dyes. Allelic discrimination was analyzed by examining and interpreting the radiance curves (Figure 4.3-1.).

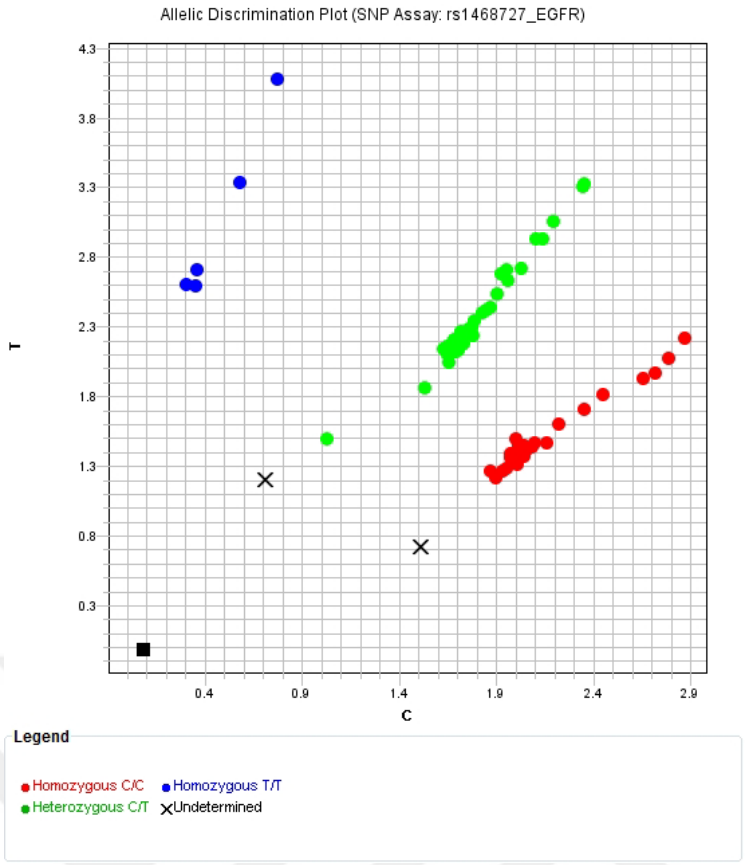

Figure 4.1 - 1 Allele Discrimination Display

CC: Homozygote Wild Type **CT:** Heterozygote **TT:** Homozygote Mutant Type

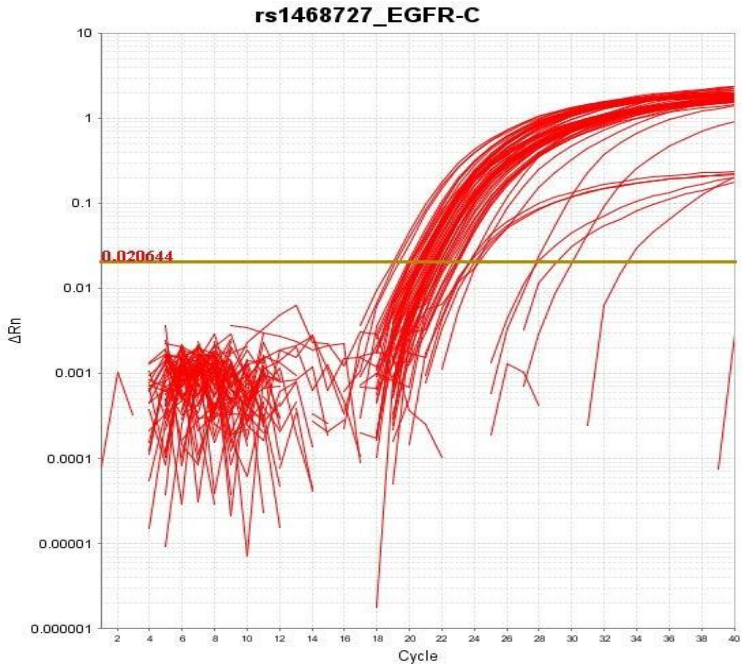

Figure 4.1 - 2 Amplification plot display of Allele C

Figure 4.1-2 shows amplification plots of Allele C. Threshold value (0.0206) is shown as a yellow line.

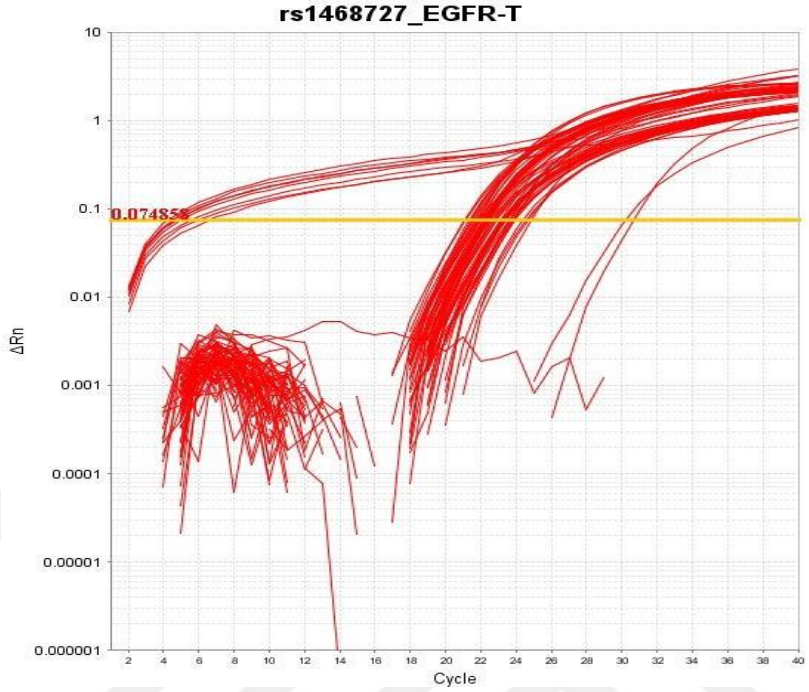

Figure 4.1 - 3 Amplification plot display of Allele T

Figure 4.1-3. shows amplification plots of Allele T. The threshold value (0.0748) is shown as a yellow line.

4.3. Analysis of Genotype and Allele Related to the Patient and Control Groups

In this experiment, EGFR polymorphism in patients with glioma and healthy control groups. This study found statistically significant findings among the two groups (p=0.028).

Table 4.2-2. shows that there is a significant relationship between the patient and control groups. The value of the homozygote mutant genotype is 0.145 that of heterozygote genotype is 0.089, while homozygote wild type genotype's value is 0.005.

According to our findings, homozygote wild type (CC), heterozygote (CT), and homozygote mutant type (TT) rates are calculated respectively as 23.5%, 64.7% and 11.8% in the patient's group. Moreover, homozygote wild type (CC), heterozygote (CT) and homozygote mutant type (TT) rates are determined respectively as 52.8%, 44.4%, and 2.8% in the control group (Table 4.2-2.).

Table 4.2-2 shows that the value of the C allele (55.88%) in the patient group was found to be lower than the control group (75%). Accordingly, the value of the T allele is also not statistically significant distinctly among groups like in the C allele (p=0.145) (Table 4.2-2.).

Table 4.2 - 1 EGFR genotype and allele distributions of patient and control groups

Genotype	**Control Group (n=36)**	**BM Patient Group (n=34)**	***p* value**	**P value**
CC	52.8% (n=19)	23.5% (n=8)	**0.005* S**	**0.028* S**
CT	44.4% (n=16)	64.7% (n=22)	0.089 NS**	
TT	2.8% (n=1)	11.8% (n=4)	0.145 NS	
Allele	**Allele Count (n)**			
C	54	38	0.145 NS	
T	18	30	0.012* S	

* (S)= significantly different (p< 0.05), **NS= not significant (p>0.05).
X± SD (Mean ± Standard Deviation), n= (number of samples)
The difference between the groups was analyzed by the fisher exact test.

When the characteristics of the two groups were evaluated, the frequency of the homozygous wild genotype (CC) was 23.5% in the patient group and 52.8% in the control group. Heterozygous genotype (CT) was 64.7% in the patient group and 44.4% in the control group. Homozygous mutant genotype (TT) was 11.8% in the patient group and 2.8% in the control group. There was significant difference between the patient and control group for each homozygous CC wild type (*p*=0.005), heterozygous CT type

(*p*=0.089), or homozygous TT mutant type (*p*=0.145) (Table 4.2-1).

Thirty-eight alleles were "C" allele, and 30 were "T" allele in the patient group (p=0.145). Fifty-four alleles were "C" allele, and 18 were "T" allele in the control group (p=0.012) (Table 4.2-1).

To sum up, when *EGFR* gene polymorphism is compared in terms of genotype and allele frequencies, not significant relation is obtained among groups (p=0.028) (Table 4.2-1)

Table 4.2 - 2 Comparison of EGFR genotype between patient and control groups

EGFR Genotypes	Control Group (n=36)	Patient Group (n=34)	*p value*	Odd Ratio (OR)	Confidence interval %
CC	52.8% (n=19)	23.5% (n=8)	**0.005* S**	**0.232****	0.081-0.068
CT	44.4% (n=16)	64.7% (n=22)	0.089 NS	2.292	0.875 – 6.0002
TT	2.8% (n=1)	11.8% (n=4)	0.145 NS	4.667	0.494 – 44.051
Alleles Distributions					
C	54 (75%)	38 (55.88 %)	0.145 NS	0.214	0.023 – 2.023
T	18 (25%)	30 (44.12 %)	**0.012 S**	3.632	1.300– 10.151

* (S)= significantly different (p< 0.05), NS= not significant (p>0.05).
**O.R (Odds Ratio), X± SD (Mean ± Standard Deviation), n (number of sample)The difference between the groups was analyzed by the double independent sample student t-test.

Having a CC genotype was considered to have a protective effect (OR= 0.232) than that compared to having CT or TT genotype (p=0.005*) for GBM patients. However, carrying the C allele does not give an advantage for patients. Also, it has been understood by the interpretation of statistical data that homozygous mutant genotype (TT) (O.R: 4.667; p: 0.145) and heterozygous genotype (CT) (O.R: 2.292; p: 0.089) have the risk for disease (Table 4.2-2). Carrying the T allele significantly increases the risk. Even carrying the T allele as heterozygous in the form of CT is a risk factor.

5. DISCUSSION AND CONCLUSION

One of the tumor characteristics is the irregularity of cell growth. The cause of the disorder may be thought to be hereditary or obtained genetic or epigenetic changes in cistron that affect signal transmission or check input inside the cell circulation.

Glioblastoma multiforme (grade IV) is the most widespread and flagitious kind of primer glioma diagnosed in about 3 out of 100,000 cases each twelve months [60]. The survival ratio in GBM cases was thereabout twelve months [61]. Just 3 to 5% of GBM cases have reported survival over three years. The average survival time after diagnosis is ~ 12 months [62-63]. Investigated prognosticators to survive to include multiple elements. These include age, a grade of a role, radiological and histopathological proof of tumor necrosis, tumor form, and scope of abscission. Evidence displaying the impressiveness of particular genetic differences in prognosticating the analysis of Glioblastoma multiforme has been combined or, at best, found out a complicated relation among plural medical and genetic factors. [64-65]. Nonetheless, the impact of commonplace genetic alteration going on the kind of polymorphisms inside the common populace has not been researched like elements that can have practical importance in predicting clinical results in GBM affected people.

The EGFR gene is located on chromosome 7p12-13 to encode 170 kD transmembrane receptor tyrosine kinase enounced on the facial of epithelial cells [66]. It is the early discovered receptor that has additional tyrosine kinase activity and is arrayed. Prior studies of the activating of the EGFR signaling path, all of which are related to carcinoma development cell survival, multiplication, apoptosis, differentiation, cell cycle progression, invasion, metastasis, and lend to many biological processes, including angiogenesis [67,68]. EGFR sign is produced by ligand linking to the extracellular ligand-binding site [69]. The extracellular binding site begins receptor dimerization and tyrosine auto-phosphorylation, consequencing in receptor activation [70]. The amplification and/or overexpression of EGFR differs in GBMs. For example, it occurs in about 50% of malignant gliomas and in about 10 to 26% of anaplastic astrocytomas [71]. EGFR amplification was found to be interrelated with worse conclusions in glioblastoma cases [72]. Despite these results still, the effect of polymorphisms that trigger glioma development in EGFR on pathogenesis and biological mechanisms is not fully clear.

EGFR amplification/overexpression or constituent activation resulting from mutations were found kind of repeated pathological genetic changes in bigger than 40% of entire glioblastomas [73-74]. Animal models of EGFR mutation or overexpression, combined with other known genetic changes found in GBM, indicate young glial cell malignant changing and speeding up of the beginning of cancerous phenotype [75, 76, 77]. Amplification or activating gene changes in EGFR have been analyzed in neoplasms. These include breast adenocarcinoma, malignant melanoma, prostate adenocarcinoma, and lung carcinomas [78-79]. Curiously, both in vitro and in vivo targeted overexpression of growth factor α. EGFR and malignant transformation of mammary duct epithelium, converting the cause, but only in the presence of EGFR has been shown [80].

The conclusions allow us to determine the relationship of SNPs in people with glioblastoma multiforme, in which change of growth factor signaling thanks to the EGFR is common.

In primary brain tumors, receptor tyrosine kinases crucial to ordinary cell multiplication and derivation, which is seen to be major aims of gliomagenesis, are existing. Disorder in receptor tyrosine kinases may be caused by a mutation resulting in gene overexpression, gene amplification, or constituent activation [81, 82, 83].

Difference in Epidermal growth factor (EGF) receptor (EGFR) happens in almost 50% of the entire cases with glioblastoma multiforme (GBM), which is the most offensive among glioma types [81, 82, 84-85]. EGFR changes are common in de novo-emerging GBMs, especially in contrast to those resulting from the improvement of lower-degree astrocytoma that changes in cell cycle checkpoints like p53 and p16 are more widespread [81, 82, 84-85].

For the first time in this patient-control study, the risk related to glioma was identified in the EGFR rs1468727 gene in the Turkish population and the prognosis of this gene in a population of Turkish glioma cases, an SNP association was examined.

Although several investigations have shown genetic changes in the epidermal growth factor receptor (EGFR) gene increase the possibility of GBM, the relation among genetic changes of EGFR and glioma in the Turkish people has not yet been proven.

This study shows the rs1468727 CC genotype was more frequent in control group than in patient group ($p = 0.005$). The study also shows T allele frequency is higher in glioma group than in control group ($p = 0.012$; ratio ratio [or] = 3,632 confidence interval [CI]: 1,300–10.151).

The study reveals the association of SNP in the EGFR gene (rs1468727) with glioma possibility. The T allele in EGFR rs1468727 may increase the possibility of glioma. Heretofore, in a study with the European population, the rs1468727 T allele was discovered to reduce the risk of glioma. On the other hand, results were found in the Chinese population that the rs1468727 C allele decreased the risk of glioma.

This SNP may be related to increased receptor activation, EGFR expression, or stability, which may raise the possibility of carcinoma by supporting cell multiplication.

Consequently, although our data showed that an SNP in EGFR (rs1468727) was related to the prognosis of glioma cases, no significant correlation was studied among the prognosis of SNP and glioma patients in EGFR.

Consequently,

The Association of EGFR SNP rs1468727 with glioma in the Turkish population had not previously been identified, but this study showed that this SNP might have implications in carcinogenesis.

In line with the results obtained, this study showed that it is advantageous to carry CC homozygous in patients. The C allele does not have a protective effect alone, and the p-value is not significant. However, it is advantageous to carry CC in patients, reducing the risk of glioma five times. Considering the results of CT carrying, this risk is large since the OR value is 2.292, but the p-value is meaningless. In other words, even carrying the T allele as CT heterozygous is a risk for glioma, even if TT is not homozygously carried The TT allele increases this risk by 3.6 times. The OR value of the T allele is 3.632.

Also, CC transport has no effect on the tumor location. Besides, carrying TT does not have an effect on location. These results were found as statistically insignificant.

The study shows the EGFR SNP rs1468727 increases the risk of glioma in the Turkish population.

6. REFERENCES

1. Jovčevska I, Kočevar N and Komel R: Glioma and glioblastoma - how much do we (not) know? Mol Clin Oncol 1: 935–941, 2013.
2. Schwartzbaum, J. A., Fisher, J. L., Aldape, K. D., & Wrensch, M. (2006). Epidemiology and molecular pathology of glioma. Nat Clin Pract Neurol 2(9), 494–503.
3. Ostrom, Q. T., Gittleman, H., Farah, H., Ondracek, A., Chen, Y., Wolinsky, Y., et al. 2013,CBTRUS statistical report: Primary brain and central nervous system tumors diagnosed in the United States in 2006–2010. Neuro Oncol 15(Suppl 2), ii1–ii56.
4. Fisher, J. L., Schwartzbaum, J. A., Wresch, M., & Wiemels, J. L. (2007). Epidemiology of brain tumors. Neurol Clin 25, 867–890.
5. Sathornsumetee S, Reardon D a, Desjardins A, Quinn J a, Vredenburgh JJ and Rich JN: Molecularly targeted therapy for malignant glioma. Cancer 110: 13–24, 2007.
6. Bai R-Y, Staedtke V and RiCC ins GJ: Molecular targeting of glioblastoma: Drug discovery and therapies. Trends Mol Med 17: 301–12, 2011.
7. Adamson C: The future role of personalized medicine in the treatment of glioblastoma multiforme. Pharmgenomics Pers Med: 111, 2010.
8. Huse JT and Holland EC: Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. Nat Rev Cancer 10: 319–31, 2010.
9. Iacob G and Dinca EB: Current data and strategy in glioblastoma multiforme. 2: 386–393,2009.
10. Sathornsumetee S, Reardon D a, Desjardins A, Quinn J a, Vredenburgh JJ and Rich JN: Molecularly targeted therapy for malignant glioma. Cancer 110: 13–24, 2007.
11. Bai R-Y, Staedtke V and RiCC ins GJ: Molecular targeting of glioblastoma: Drug discovery and therapies. Trends Mol Med 17: 301–12, 2011.
12. Zhang R, Pang B, Xin T, et al. Plasma miR-221/222 Family as Novel Descriptive and Prognostic Biomarkers for Glioma. Mol Neurobiol. 2016; 53(3): 1452-1460.
13. Smith JS, Tachibana I, Passe SM, et al. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytoma and glioblastoma multiforme. J Natl Cancer Inst. 2001; 93(16): 1246–1256.
14. Brodal, P. (2004). The central nervous system: Structure and function (3rd ed.). Oxford

15. Brain Tumor: Medical Illustrations, January, 2020 https://www.cancer.net/cancer-types/brain-tumor/medical-illustrations
16. Neuron, 13 August 2020, en.wikipedia.org/wiki/File:Neuron-no_labels2.png
17. Glial Cells, 8 July 2020 https://commons.wikimedia.org/wiki/File:Glial_Cell_Types.png
18. Schattenkerk, M.E., Li, A.K., Jeppsson, B.W., Eggink, W.F., Jamieson, C.G., Ross, J.S.,and Malt, R.A. (1980). Cholecystectomy has no influence on frequency ofchemically induced colonic cancer in mice. British Journal of Cancer, 42(5), 791-793
19. Shier, D., Butleri J., Lewis, R. (2012). Hole's Essentials of Human Anatomy & Physiology (11. Edition ed.). New York,: McGraw-Hill.
20. Louis, D.N., Perry, A., Reifenberger, G., von Deimling, A., Figarella-Branger, D.,Cavenee, W.K., Ohgaki, H., Wiestler, O.D., Kleihues, P., and Ellison, D.W. (2016).The 2016 World Health Organization Classification of Tumors of the CentralNervous System: a summary. Acta Neuropathologica, 131(6), 803-820.
21. Hiller, R., Laffer, S., Harwanegg, C., Huber, M., Schmidt, W.M., Twardosz, A., Barletta,B., Becker, W.M., Blaser, K., Breiteneder, H., Chapman, M., Crameri, R., Duchene,M., Ferreira, F., Fiebig, H., Hoffmann-Sommergruber, K., King, T.P., Kleber-Janke,T., Kurup, V.P., Lehrer, S.B., Lidholm, J., Muller, U., Pini, C., Reese, G., Scheiner,O., Scheynius, A., Shen, H.D., Spitzauer, S., Suck, R., Swoboda, I., Thomas, W.,Tinghino, R., Van Hage-Hamsten, M., Virtanen, T., Kraft, D., Muller, M.W., andValenta, R. (2002). Microarrayed allergen molecules: diagnostic gatekeepers forallergy treatment. FASEB Journal, 16(3), 414-416.
22. Kheirollahi, M., Dashti, S., Khalaj, Z., Nazemroaia, F., and Mahzouni, P. (2015). Brain tumors: Special characters for research and banking. Advanced Biomedical Research, 4(4), 1-26. doi: 10.4103/2277-9175.148261
23. Lee, C.H., Jung, K.W., Yoo, H., Park, S., and Lee, S.H. (2010). Epidemiology of primary brain and central nervous system tumors in Korea. Journal of the Korean Neurosurgical Society, 48(2), 145-152.
24. Fomchenko EI and Holland EC: Mouse Models of Brain Tumors and Their Applications in Preclinical Trials. Clin Cancer Res 12: 5288–5297, 2006.
25. Argyriou A a, Antonacopoulou A, Iconomou G and Kalofonos HP: Treatment options for malignant gliomas, emphasizing towards new molecularly targeted therapies. Crit Rev Oncol Hematol 69: 199–210, 2009

26. Pham D, Kris MG, Riely GJ, Sarkaria IS, McDonough T, Chuai S, Venkatraman ES, Miller VA, Ladanyi M, Pao W, Wilson RK, Singh B, Rusch VW. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. Journal of clinical oncology. 2006; 24:1700–1704

27. Fadi S. Saadeh, Rami Mahfouz, Hazem I. Assi. EGFR as a clinical marker in glioblastomas and other gliomas. 2017; 10:5301

28. Li, L, Dutra, A, Pak, E. EGFRvIII expression and PTEN loss synergistically induce chromosomal instability and glial tumors. Neuro-oncol. 2009;11(1):9-21

29. Chakravarti, A, Chakladar, A, Delaney, MA, Latham, DE, Loeffler, JS. The epidermal growth factor receptor pathway mediates resistance to sequential administration of radiation and chemotherapy in primary human glioblastoma cells in a RAS-dependent manner. Cancer Res. 2002;62(15):4307-4315.

30. Libermann TA, Nusbaum HR, Razon N, Kris R, Lax I, Soreq H, Whittle N, Waterfield MD, Ullrich A, Schlessinger J (1985) Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumours of glial origin. Nature 313:144–147

31. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, Hahn WC, Ligon KL, Louis DN, Brennan C et al (2007) Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev 21:2683–2710

32. The Cancer Genome Atlas (TCGA) (2008) Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature 455:1061–1068

33. Riddick G, Fine HA (2011) Integration and analysis of genomescale data from gliomas. Nat Rev Neurol 7:439–450

34. Reifenberger J, Reifenberger G, Ichimura K, Schmidt EE, Wechsler W, Collins VP (1996) Epidermal growth factor receptor expression in oligodendroglial tumors. Am J Pathol 149:29–35

35. von Deimling A, Fimmers R, Schmidt MC, Bender B, Fassbender F, Nagel J, Jahnke R, Kaskel P, Duerr EM, Koopmann J et al (2000) Comprehensive allelotype and genetic analysis of 466 human nervous system tumors. J Neuropathol Exp Neurol 59:544–558

36. Ohgaki H, Kleihues P (2013) The definition of primary and secondary glioblastoma. Clin Cancer Res 19:764–772

37. Heimberger AB, Hlatky R, Suki D, Yang D, Weinberg J, Gilbert M, Sawaya R, Aldape K (2005) Prognostic effect of epidermal growth factor receptor and EGFRvIII in glioblastoma multiforme patients. Clin Cancer Res 11:1462–1466

38. Maire CL, Ligon KL. Molecular pathologic diagnosis of epidermal growth factor receptor. Neuro Oncol. 2014;16(Suppl 8): viii, 1-6.

39. Shinojima N, Tada K, Shiraishi S, et al. Prognostic value of epidermal growth factor receptor in patients with glioblastoma multiforme. Cancer Res. 2003;63(20):6962-6970

40. Waha A, Baumann A, Wolf HK, et al. Lack of prognostic relevance of alterations in the epidermal growth factor receptortransforming growth factor-alpha pathway in human astrocytic gliomas. J Neurosurg. 1996;85(4):634-641.

41. Diedrich U, Lucius J, Baron E, et al. Distribution of epidermal growth factor receptor gene amplification in brain tumours and correlation to prognosis. J Neurol. 1995;242(10): 683-688.

42. Ruano Y, Ribalta T, de Lope AR, et al. Worse outcome in primary glioblastoma multiforme with concurrent epidermal growth factor receptor and p53 alteration. Am J Clin Pathol. 2009;131(2):257-263.

43. Pearson G, Robinson F, Beers GT, Xu BE, Karandikar M, Berman K, Cobb MH (2001) Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr Rev 22:153–183

44. Coulibaly B, Nanni I, Quilichini B, et al. Epidermal growth factor receptor in glioblastomas: correlation between gene copy number and protein expression. Hum Pathol. 2010;41(6):815-823.

45. Wong, A. J., Ruppert, J. M., Bigner, S. H., Grzeschik, C. H., Humphrey, P. A., Bigner, D. S., and Vogelstein, B. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proc. Natl. Acad. Sci. USA, 89: 2965–2969, 1992.

46. Kleihues, P., and Cavenee, W. K., eds. Pathology and Genetics of Tumours of the Central Nervous System (WHO Classification of Tumours), 2nd edition, pp. 1–314. Lyon: IARC, 2000.

47. Kapoor, G. S., and O'Rourke, D. M. Mitogenic signaling cascades in glial tumors. Neurosurgery, 52: 1425–1434, 2003.

48. Libermann, T. A., Nusbaum, H. R., Razon, N., Kris, R., Lax, I., Soreq, H., Wittle, N., Waterfield, M. D., Ullrich, A., and Schlessinger, J. Amplification, enhanced expression and possible rearrangement of EGF receptor gene in primary human brain tumors of glial origin. Nature (Lond.), 313: 144–147, 1985

49. Lacroix, M., Abi-Said, D., Fourney, D. R., Gokaslan, Z. L., Shi, W., DeMonte, F.,Lang, F. F., McCutcheon, I. E., Hassenbusch, S. J., Holland, E., Hess, K., Michael, C., Miller, D., and Sawaya, R. A multivariate analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival. J. Neurosurg., 95: 190–198, 2001.

50. Simmons, M. L., Lamborn, K. R., Takahashi, M., Chen, P., Israel, M. A., Berger, M. S., Godfrey, T., Nigro, J., Prados, M., Chang, S., Barker, F. G., and Aldape, K. Analysis of complex relationships between age, p53, epidermal growth factor receptor, and survival in glioblastoma patients. Cancer Res., 61: 1122–1128, 2001.

51. Barker, F. G., Simmons, M. L., Xhange, S. M., Prados, M. D., Larson, D. A., Sneed, P. K., Wara, W. M., Berger, M. S., Chen, P., Israel, M. A., and Aldape, K. D. EGFR overexpression and radiation response in glioblastoma multiforme. Int. J. Radiation Oncology Biol. Phys., 51: 410–418, 2001

52. Weiss, W. A., Burns, M. J., Hackett, C., Aldape, K., Hill, J. R., Kuriyama, H.,Kuriyama, N., Milshteyn, N., Roberts, T., Wendland, M. F., DePinho, R., and Israel, M. A. Genetic determinants of malignancy in a mouse model for oligodendrogliomas. Cancer Res., 63: 1589–1595, 2003.

53. Holland, E. C., Hively, W. P., DePinho, R. A., and Varmus, H. E. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. Genes Dev., 12: 3675–3685, 1998.

54. Nakayama Y, Yamaguchi H, Einaga N, Esumi M. Pitfalls of DNA Quantification Using DNA-Binding Fluorescent Dyes and Suggested Solutions. PLoS One. 2016; 11(3): e0150528.

55. Li X, et al. Comparison of three common DNA concentration measurement methods. Anal Biochem. 2014;451:18–24.

56. Nakayama Y, Yamaguchi H, Einaga N, Esumi M. Pitfalls of DNA Quantification Using DNA-Binding Fluorescent Dyes and Suggested Solutions. PLoS One. 2016; 11(3): e0150528.

57. Li X, et al. Comparison of three common DNA concentration measurement methods. Anal Biochem. 2014;451:18–24.

58. Basu, A., Murthy, U., Rodeck, U., Herlyn, M., Mattes, L., and Das, M. Presence of tumor-associated antigens in epidermal growth factor receptors from different human carcinomas. Cancer Res., 47: 2531–2536, 1987.

59. Iglehart, J. D., Kraus, M. H., Langton, B. C., Huper, G., Kerns, B. J., and Marks, J. R. Increased erbB-2 gene copies and expression in multiple stages of breast cancer. Cancer Res., 50: 6701–6707, 1990.

60. Kuhn, E. J., Kurnot, R. A., Sesterhenn, I. A., Chang, E. H., and Moul, J. W. Expression of the c-erbB-2 (HER-2/neu) oncoprotein in human prostatic carcinoma. J. Urol., 150: 1427–1433, 1993.

61. Jhappan, C., Stahle, C., Harkins, R. N., Fausto, N., Smith, G. H., and Merlino, G. T. TGF overexpression in transgenic mice induces liver neoplasia and abnormal development of the mammary gland and pancreas. Cell, 61: 1137–1146, 1990.

62. Smith, J. S., Tachibana, I., Passe, S. M., Huntley, B. K., Borell, T. J., Iturria, N., O'Fallon, J. R., Schaefer, P. L., Scheithauer, B. W., James, C. D., Buckner, J. C., and Jenkins, R. B. PTEN mutation, EGFR amplification, and outcome in patients with anaplastic astrocytomas and glioblastoma multiforme. J. Natl. Cancer Inst., 93: 1246–1256, 2001.

63. Nishikawa, R., Ji, X. D., Harmon, R. C., Lazar, C. S., Gill, G. N., Cavenee, W. K., and Huang, H. J. A mutant epidermal growth factor receptor common in human glioma confers enhanced tumorigenicity. Proc. Natl. Acad. Sci. USA, 91: 7727–7731, 1994.

64. Frederick, L., Wang, X. Y., Eley, G., and James, C. D,. Diversity and frequency of epidermal growth factor receptor mutations in human glioblastomas. Cancer Res., 60: 1383–1387, 2000.

65. Andersson U, Schwartzbaum J, Wiklund F, Sjostrom S, Liu Y, et al. (2010) A comprehensive study of the association between the EGFR and ERBB2 genes and glioma risk. Acta Oncol 49: 767–775.

66. Pham D, Kris MG, Riely GJ, Sarkaria IS, McDonough T, Chuai S, Venkatraman ES, Miller VA, Ladanyi M, Pao W, Wilson RK, Singh B, Rusch VW. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. Journal of clinical oncology. 2006; 24:1700–1704.

67. Jami MS, Hemati S, Salehi Z, Tavassoli M. Association between the length of a CA dinucleotide repeat in the EGFR and risk of breast cancer. Cancer investigation. 2008; 26:434–437.

68. Yano S, Kondo K, Yamaguchi M, Richmond G, Hutchison M, Wakeling A, Averbuch S, Wadsworth P. Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition. Anticancer research. 2003; 23:3639–3650.

69. Brandt B, Meyer-Staeckling S, Schmidt H, Agelopoulos K, Buerger H. Mechanisms of egfr gene transcription modulation: relationship to cancer risk and therapy response. Clinical cancer research. 2006; 12:7252–7260.

70. Sjostrom S, Andersson U, Liu Y, Brannstrom T, Broholm H, Johansen C, Collatz-Laier H, Henriksson R, Bondy M, Melin B. Genetic variations in EGF and EGFR and glioblastoma outcome. Neuro-oncology. 2010; 12:815–821.

71. Waha A, Baumann A, Wolf HK, Fimmers R, Neumann J, Kindermann D, Astrahantseff K, Blumcke I, von Deimling A, 63063. Schlegel U. Lack of prognostic relevance of alterations in the epidermal growth factor receptor-transforming growth factor-alpha pathway in human astrocytic gliomas. Journal of neurosurgery. 1996; 85:634–641.

72. Bienkowski M, Piaskowski S, Stoczynska-Fidelus E, Szybka M, Banaszczyk M, Witusik-Perkowska M, Jesien- Lewandowicz E, Jaskolski DJ, Radomiak-Zaluska A, Jesionek-Kupnicka D, Sikorska B, Papierz W, Rieske P, et al. Screening for EGFR amplifications with a novel method and their significance for the outcome of glioblastoma patients. PloS one. 2013; 8:e65444.

73. Nakayama Y, Yamaguchi H, Einaga N, Esumi M. Pitfalls of DNA Quantification Using DNA-Binding Fluorescent Dyes and Suggested Solutions. PLoS One. 2016; 11(3): e0150528.

74. Li X, et al. Comparison of three common DNA concentration measurement methods. Anal Biochem. 2014;451:18–24.

75. Jami MS, Hemati S, Salehi Z, Tavassoli M. Association between the length of a CA dinucleotide repeat in the EGFR and risk of breast cancer. Cancer investigation. 2008; 26:434–437

76. Yano S, Kondo K, Yamaguchi M, Richmond G, Hutchison M, Wakeling A, Averbuch S, Wadsworth P. Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition. Anticancer research. 2003; 23:3639–3650.

77. Brandt B, Meyer-Staeckling S, Schmidt H, Agelopoulos K, Buerger H. Mechanisms of EGFR gene transcription modulation: relationship to cancer risk and therapy response. Clinical cancer research. 2006; 12:7252–7260.

78. Sjostrom S, Andersson U, Liu Y, Brannstrom T, Broholm H, Johansen C, Collatz-Laier H, Henriksson R, Bondy M, Melin B. Genetic variations in EGF and EGFR and glioblastoma outcome. Neuro-oncology. 2010; 12:815–821

79. Waha A, Baumann A, Wolf HK, Fimmers R, Neumann J, Kindermann D, Astrahantseff K, Blumcke I, von Deimling A, Schlegel U. Lack of prognostic relevance of alterations in the epidermal growth factor receptor-transforming growth factor-alpha pathway in human astrocytic gliomas. Journal of neurosurgery. 1996; 85:634–641.

80. Bienkowski M, Piaskowski S, Stoczynska-Fidelus E, Szybka M, Banaszczyk M, Witusik-Perkowska M, Jesien- Lewandowicz E, Jaskolski DJ, Radomiak-Zaluska A, Jesionek-Kupnicka D, Sikorska B, Papierz W, Rieske P, et al. Screening for EGFR amplifications with a novel method and their significance for the outcome of glioblastoma patients. PloS one. 2013; 8:e65444.

81. Ohgaki, Genetic Pathways to Primary and Secondary Glioblastoma

82. Farrell, C. J., & Plotkin, S. R. (2007). Genetic causes of brain tumors: neurofibromatosis, tuberous sclerosis, von Hippel-Lindau and other syndromes. Neurol Clin 25,925–946. Rich JN and Bigner DD: Development of novel targeted therapies in the treatment of malignant glioma. Nat Rev Drug Discov 3: 430–46, 2004.

83. Zhang R, Pang B, Xin T, et al. Plasma miR-221/222 Family as Novel Descriptive and Prognostic Biomarkers for Glioma. Mol Neurobiol. 2016; 53(3): 1452-1460

84. Wen, P. Y., & Kesari, S. (2008). Malignant gliomas in adults. N Engl J Med 359, 492–507

85. Bonavia R, Inda M-M, Cavenee WK and Furnari FB: Heterogeneity maintenance in glioblastoma: a social network. Cancer Res 71: 4055–60, 2011.

7. APPENDICES

```
CROSSTABS
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  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
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Crosstabs

Notes

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Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.
Syntax		CROSSTABS /TABLES=Genotype_rs1468727 CC CT TT C_allel T_allel BY grup /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
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Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Genotype_rs1468727 * grup	70	97,2%	2	2,8%	72	100,0%
CC * grup	70	97,2%	2	2,8%	72	100,0%
CT * grup	70	97,2%	2	2,8%	72	100,0%
TT * grup	70	97,2%	2	2,8%	72	100,0%
C_allel * grup	70	97,2%	2	2,8%	72	100,0%
T_allel * grup	70	97,2%	2	2,8%	72	100,0%

Genotype_rs1468727 * grup

Crosstab

			grup		
			kontrol	hasta	Total
Genotype_rs1468727	CC	Count	19	8	27
		% within Genotype_rs1468727	70,4%	29,6%	100,0%
		% within grup	52,8%	23,5%	38,6%
		% of Total	27,1%	11,4%	38,6%
	CT	Count	16	22	38
		% within Genotype_rs1468727	42,1%	57,9%	100,0%
		% within grup	44,4%	64,7%	54,3%
		% of Total	22,9%	31,4%	54,3%
	TT	Count	1	4	5
		% within Genotype_rs1468727	20,0%	80,0%	100,0%
		% within grup	2,8%	11,8%	7,1%
		% of Total	1,4%	5,7%	7,1%
Total		Count	36	34	70
		% within Genotype_rs1468727	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	7,178[a]	2	,028
Likelihood Ratio	7,436	2	,024
Linear-by-Linear Association	7,031	1	,008
N of Valid Cases	70		

a. 2 cells (33,3%) have expected count less than 5. The minimum expected count is 2,43.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,319	,108	2,778
Ordinal by Ordinal	Spearman Correlation	,320	,110	2,784
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,007[c]
Ordinal by Ordinal	Spearman Correlation	,007[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value
Odds Ratio for Genotype_rs1468727 (CC / CT)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

CC * grup

Crosstab

			grup		
			kontrol	hasta	Total
CC	yok	Count	17	27	44
		% within CC	38,6%	61,4%	100,0%
		% within grup	47,2%	79,4%	62,9%
		% of Total	24,3%	38,6%	62,9%
	var	Count	19	7	26
		% within CC	73,1%	26,9%	100,0%
		% within grup	52,8%	20,6%	37,1%
		% of Total	27,1%	10,0%	37,1%
Total		Count	36	34	70
		% within CC	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7,760[a]	1	,005		
Continuity Correction[b]	6,443	1	,011		
Likelihood Ratio	7,990	1	,005		
Fisher's Exact Test				,007	,005
Linear-by-Linear Association	7,650	1	,006		
N of Valid Cases	70				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 12,63.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	-,333	,110	-2,912
Ordinal by Ordinal	Spearman Correlation	-,333	,110	-2,912
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,005[c]
Ordinal by Ordinal	Spearman Correlation	,005[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for CC (yok / var)	,232	,081	,668
For cohort grup = kontrol	,529	,341	,820
For cohort grup = hasta	2,279	1,160	4,478
N of Valid Cases	70		

CT * grup

Crosstab

			grup kontrol	hasta	Total
CT	yok	Count	20	12	32
		% within CT	62,5%	37,5%	100,0%
		% within grup	55,6%	35,3%	45,7%
		% of Total	28,6%	17,1%	45,7%
	var	Count	16	22	38
		% within CT	42,1%	57,9%	100,0%
		% within grup	44,4%	64,7%	54,3%
		% of Total	22,9%	31,4%	54,3%
Total		Count	36	34	70
		% within CT	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,893[a]	1	,089		
Continuity Correction[b]	2,134	1	,144		
Likelihood Ratio	2,916	1	,088		
Fisher's Exact Test				,100	,072
Linear-by-Linear Association	2,851	1	,091		
N of Valid Cases	70				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 15,54.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,203	,117	1,712
Ordinal by Ordinal	Spearman Correlation	,203	,117	1,712
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,091[c]
Ordinal by Ordinal	Spearman Correlation	,091[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for CT (yok / var)	2,292	,875	6,002
For cohort grup = kontrol	1,484	,938	2,350
For cohort grup = hasta	,648	,384	1,093
N of Valid Cases	70		

TT * grup

Crosstab

			grup kontrol	hasta	Total
TT	yok	Count	35	30	65
		% within TT	53,8%	46,2%	100,0%
		% within grup	97,2%	88,2%	92,9%
		% of Total	50,0%	42,9%	92,9%
	var	Count	1	4	5
		% within TT	20,0%	80,0%	100,0%
		% within grup	2,8%	11,8%	7,1%
		% of Total	1,4%	5,7%	7,1%
Total		Count	36	34	70
		% within TT	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,129[a]	1	,145		

Continuity Correction[b]	,990	1	,320		
Likelihood Ratio	2,255	1	,133		
Fisher's Exact Test				,192	,161
Linear-by-Linear Association	2,099	1	,147		
N of Valid Cases	70				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 2,43.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,174	,104	1,461
Ordinal by Ordinal	Spearman Correlation	,174	,104	1,461
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,149[c]
Ordinal by Ordinal	Spearman Correlation	,149[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for TT (yok / var)	4,667	,494	44,051
For cohort grup = kontrol	2,692	,460	15,766
For cohort grup = hasta	,577	,346	,962
N of Valid Cases	70		

C_allel * grup

Crosstab

			grup kontrol	hasta	Total
C_allel	yok	Count	1	4	5
		% within C_allel	20,0%	80,0%	100,0%
		% within grup	2,8%	11,8%	7,1%
		% of Total	1,4%	5,7%	7,1%
	var	Count	35	30	65
		% within C_allel	53,8%	46,2%	100,0%
		% within grup	97,2%	88,2%	92,9%
		% of Total	50,0%	42,9%	92,9%
Total		Count	36	34	70
		% within C_allel	51,4%	48,6%	100,0%

	% within grup	100,0%	100,0%	100,0%
	% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,129[a]	1	,145		
Continuity Correction[b]	,990	1	,320		
Likelihood Ratio	2,255	1	,133		
Fisher's Exact Test				,192	,161
Linear-by-Linear Association	2,099	1	,147		
N of Valid Cases	70				

a. 2 cells (50,0%) have expected count less than 5. The minimum expected count is 2,43.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	-,174	,104	-1,461
Ordinal by Ordinal	Spearman Correlation	-,174	,104	-1,461
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,149[c]
Ordinal by Ordinal	Spearman Correlation	,149[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for C_allel (yok / var)	,214	,023	2,023
For cohort grup = kontrol	,371	,063	2,175
For cohort grup = hasta	1,733	1,040	2,889
N of Valid Cases	70		

T_allel * grup

Crosstab

			grup kontrol	hasta	Total
T_allel	yok	Count	19	8	27
		% within T_allel	70,4%	29,6%	100,0%

		% within grup	52,8%	23,5%	38,6%
		% of Total	27,1%	11,4%	38,6%
	var	Count	17	26	43
		% within T_allel	39,5%	60,5%	100,0%
		% within grup	47,2%	76,5%	61,4%
		% of Total	24,3%	37,1%	61,4%
Total		Count	36	34	70
		% within T_allel	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6,313[a]	1	,012		
Continuity Correction[b]	5,139	1	,023		
Likelihood Ratio	6,455	1	,011		
Fisher's Exact Test				,015	,011
Linear-by-Linear Association	6,223	1	,013		
N of Valid Cases	70				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 13,11.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,300	,113	2,596
Ordinal by Ordinal	Spearman Correlation	,300	,113	2,596
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,012[c]
Ordinal by Ordinal	Spearman Correlation	,012[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for T_allel (yok / var)	3,632	1,300	10,151
For cohort grup = kontrol	1,780	1,143	2,773

For cohort grup = hasta	,490	,261	,920
N of Valid Cases	70		

```
T-TEST GROUPS=grup(1 0)
  /MISSING=ANALYSIS
  /VARIABLES=yas
  /CRITERIA=CI(.95).
```

T-Test

Notes

Output Created		12-MAR-2020 09:56:56
Comments		
Input	Data	F:\Gözde ÖZCAN Master Tez.sav_11_03_2020.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	72
Missing Value Handling	Definition of Missing	User defined missing values are treated as missing.
	Cases Used	Statistics for each analysis are based on the cases with no missing or out-of-range data for any variable in the analysis.
Syntax		T-TEST GROUPS=grup(1 0) /MISSING=ANALYSIS /VARIABLES=yas /CRITERIA=CI(.95).
Resources	Processor Time	00:00:00,00
	Elapsed Time	00:00:00,06

Group Statistics

	grup	N	Mean	Std. Deviation	Std. Error Mean
yas	hasta	34	48,29	18,466	3,167
	kontrol	36	42,75	11,702	1,950

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	df
yas	Equal variances assumed	8,875	,004	1,509	68
	Equal variances not assumed			1,491	55,281

Independent Samples Test

		t-test for Equality of Means

		Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference Lower
yas	Equal variances assumed	,136	5,544	3,674	-1,786
	Equal variances not assumed	,142	5,544	3,719	-1,909

Independent Samples Test

		t-test for Equality of Means 95% Confidence Interval of the Difference Upper
yas	Equal variances assumed	12,875
	Equal variances not assumed	12,997

```
CROSSTABS
  /TABLES=sex BY grup
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL.
```

Crosstabs

Notes

Output Created		12-MAR-2020 09:57:11
Comments		
Input	Data	F:\Gözde ÖZCAN Master Tez.sav_11_03_2020.sav
	Active Dataset	DataSet3
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	72
Missing Value Handling	Definition of Missing	User-defined missing values are treated as missing.
	Cases Used	Statistics for each table are based on all the cases with valid data in the specified range(s) for all variables in each table.

Syntax		CROSSTABS /TABLES=sex BY grup /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL.
Resources	Processor Time	00:00:00,05
	Elapsed Time	00:00:00,05
	Dimensions Requested	2
	Cells Available	524245

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
sex * grup	70	97,2%	2	2,8%	72	100,0%

sex * grup Crosstabulation

			grup		
			kontrol	hasta	Total
sex	erkek	Count	23	25	48
		% within sex	47,9%	52,1%	100,0%
		% within grup	63,9%	73,5%	68,6%
		% of Total	32,9%	35,7%	68,6%
	kadın	Count	13	9	22
		% within sex	59,1%	40,9%	100,0%
		% within grup	36,1%	26,5%	31,4%
		% of Total	18,6%	12,9%	31,4%
Total		Count	36	34	70
		% within sex	51,4%	48,6%	100,0%
		% within grup	100,0%	100,0%	100,0%
		% of Total	51,4%	48,6%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,754[a]	1	,385		
Continuity Correction[b]	,373	1	,541		

Likelihood Ratio	,758	1	,384		
Fisher's Exact Test				,446	,271
Linear-by-Linear Association	,743	1	,389		
N of Valid Cases	70				

a. 0 cells (0,0%) have expected count less than 5. The minimum expected count is 10,69.

b. Computed only for a 2x2 table

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	-,104	,118	-,861
Ordinal by Ordinal	Spearman Correlation	-,104	,118	-,861
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance
Interval by Interval	Pearson's R	,393[c]
Ordinal by Ordinal	Spearman Correlation	,393[c]
N of Valid Cases		

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

c. Based on normal approximation.

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for sex (erkek / kadın)	,637	,229	1,769
For cohort grup = kontrol	,811	,514	1,279
For cohort grup = hasta	1,273	,719	2,253
N of Valid Cases	70		

8. FORMS

8.1. Ethical Approval

T.C. YEDİTEPE ÜNİVERSİTESİ

Sayı : 37068608-6100-15-1772 **21/11/2019**
Konu: Klinik Araştırmalar
Etik kurul Başvurusu hk.

İlgili Makama (Gözde Özcan)

Yeditepe Üniversitesi Moleküler Tıp Anabilim Dalı Prof. Dr. Turgay İşbir'in sorumlu araştırmacı olduğu **"EGFR Genindeki Genetik Varyasyon ve Türk Populasyonunda Glioma İle İlişkisi"** isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası **(1757)** kayıt Numaralı KAEK Başvuru Dosyası,Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından **20.11.2019** tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isimi belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (**KAEK Karar No:** 1117).

Prof. Dr. Turgay ÇELİK
Yeditepe Üniversitesi
Klinik Araştırmalar Etik Kurulu Başkanı

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