T.C.
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INVESTIGATION OF DNA REPAIR GENE ERCC1 POLYMORPHISM AND GLIOMA SUSCEPTIBILITY AMONG TURKISH POPULATION.

MASTER OF SCIENCE THESIS

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APPROVAL

This thesis has been deemed by the jury in accordance with the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by Administrative Board of Institute with decision dated 04.11.2021 and numbered 2021/11-03.

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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.

12.10.2021

Dalmar Omar Abdi

DEDICATION

To my mother….

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12.10.2021

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LIST OF ABBREVIATIONS AND SYMBOLS

BER : Base excision repair

CNS : Central nervous system

CSF : Cerebrospinal fluid

CST : Charge swith

95% Cl : 95% confidence interval

DNA : Deoxyribonucleic Acid

EDTA : Ethylenediaminetetracetic acid

ERCC1: Excision repair cross-complimenting group 1

GBM : glioblastoma multiform

HR : Homologous recombination

mRNA : messenger RNA

ul : microliter

mg : milligram

NER : Nucleotide excision repair

NHEJ : Non homologous end joining

nM : nanomolar

OD : optical density

OR : Odd ratio

PCR : Polymerase Chain Reaction

PNS : Peripheral nervous system

PTEN : Phosphotase and tensin homolog

RNA : Ribonucleic acid

RT-PCR : Real time PCR

SD : Standard deviation

SNPs : single nucleotide polymorphisms

TP53 : Protein 53

UV : Ultraviolet

XPF : Xeroderma pigmentosum group F

WHO: world Health Organization

ABSTRACT

Dalmar Omar Abdi, investigation of DNA repair gene ERCC1 polymorphism and glioma susceptibility among Turkish population. Yeditepe university, Institute of Health Science, Department of Molecular Medicine, MSc Thesis. Istanbul,2021.

Glioma, which account 26% of CNS cancer and 80% of malignant primary brain, involve histologically many different types of tumors that have several distinct etiologies. Since ERCC1 is a crucial protein in NER mechanism and affects instability of the genome, ERCC1 variation might influence humain tumors. Therefore, in this learning we studied the connection between ERCC1 SNP rs11615 and glioma with 40 controls and 34 cases groups. We performed RT-PCR for genotyping analysis and statistics analysis to compare the significance differences between the groups. According to our results, in controls groups AA genotype 27% (10 out 40), AG genotype 43,2% (16 out 40) and GG genotype 29,7% (11 out 40) were found. AA genotype 27,3%, (n=9), AG genotype 36,4% (n=12) and GG genotype 36,4% (n=12) were distributed in the glioma patients. No significance difference was observed between groups with p value, 0.802. Further studies with big amounts of patients are required to investigate exact association.

Key words: glioma, ERCC1, polymorphism

ÖZET

Dalmar Omar Abdi, Türk popülasyonunda DNA onarım geni ERCC1 polimorfizmi ve glioma duyarlılığının araştırılması. Yeditepe Üniversitesi, Sağlık Bilimleri Enstitüsü, Moleküler Tıp Anabilim Dalı, Yüksek Lisans Tezi. İstanbul, 2021.

MSS (merkezi sinir sistemi) tümörlerinin %26'sını ve primer beyin tümörlerinin %80'ini oluşturan glioma, histolojik olarak birçok farklı etiyolojiye sahip birçok farklı tümör tipini içerir. Eksizyon onarımı çapraz tamamlama grubu 1 (ERCC1), nükleotid şeytan çıkarma onarım (NER) yolunda çok önemli bir protein olduğundan ve genomun kararsızlığını etkilediğinden, ERCC1 varyasyonu insan tümörlerini etkileyebilir. Bu nedenle, bu çalışmada ERCC1 SNP rs11615 ile glioma arasındaki ilişkiyi 40 kontrol ve 34 vaka grubu ile araştırdık. Gruplar arasındaki anlamlılık farklılıklarını karşılaştırmak için genotipleme analizi ve istatistik analizi için RT-PCR gerçekleştirdik. Sonuçlarımıza göre kontrol gruplarında AA genotipi %27 (40 üzerinden 10), AG genotipi %43,2 (40 üzerinden 16) ve GG genotipi %29,7 (40 üzerinden 11) bulundu. Glioma hastalarında AA genotipi %27,3, (n=9), AG genotipi %36,4 (n=12) ve GG genotipi %36,4 (n=12) dağıldı. p değeri 0.802 olan gruplar arasında anlamlı bir fark gözlenmedi. Kesin ilişkiyi araştırmak için büyük örneklemli daha ileri çalışmalara ihtiyaç vardır.

Anahtar kelimeler: glioma, ERCC1, polimorfizm

1.INTRODUCTION AND PURPOSE

Glioma is the most abundant tumors in malignant primary brain tumors which affects 7 out of 100.000 patients each year in the world. Although the massive efforts and the avancement of the knowledge about glioma mechanism, it remains incurable. The average of the rate survival is around 12-15 months for the patients with glioblastoma (highest grade of glioma) (29). As reported by World Health Organization (WHO), glioma was grouped into grade I to IV according to their histological features. Cancer grades are used to rate how much the cancer cells are like normal cells to predict the prognosis of the cancer and treatment (18,19). Astrocytoma, anaplastic astrocytoma, glioblastoma multiform (GBM) which known as astrocytic tumors, oligodendrogliomas, ependymomas and mixed glioma (rare carcinoma) are the subgroups of gliomas (1,8). When using WHO grades some pathological factors are considered such as, nuclear atypical, mitotic activity, vascular proliferation, necrosis, clinical course and therapeutic conclusion. GBM is made up of 45% of central nervous system (CNS) cancers, 80% of primary brain carcinoma and 54% of all gliomas. People aboved 45 years are the most diagnosed and men are more affected than women.

Glioma is a fatal disease due to its heterogeneous, complexity and aggressive nature and because of many drugs that are unable to traverse the barrier of the blood-brain (1,2). Even with the development in treatment, the gliomas etiology are still poor, but some research have confirmed ionizing radiation as environmental risk factor, and also genetic factors such as genetic variations in the risk of glioma. The polymorphisme make us more susceptible to some diseases and more resistance to others (such as heart attack, stroke,cancer,diabetes etc). Studying showed that the genes with single nucleotide polymorphisms (SNPs) are related to glioma risk such as DNA repair genes polymorphisms (2,3). Ioning radiation can harm DNA in various ways, including DNA damage caused by oxidation, the breaks in DNA single- and double-strand chains, cross-links of DNA–DNA or DNA–protein (10). since DNA damage cause glioma, DNA repair genes are needed to prevent the propagation of error, to maintain stability of genome and stop cancer development. Therefore, if there are polymorphism in genes of the DNA repair, the quantity or the function of their protein can be change and also multiple the

susceptibility of diseases or cancer (5). One of this genes in the DNA repair mechanism is excision repair cross-complementing group1 (ERCC1).

ERCC1 are an important products in nucleotide excision repair (NER). ERCC1 with XPC forms a heterodimer. As a unit, they recruited to incise the damage DNA strand 5' at the site of DNA lesions, in the process of repair excision by nucleotide excision and it located in putative glioma suppression region on chromosome 19q13.3. The variation in ERCC1 can change the activity of NER process , thus lead to the risk of human tumors.

Several previous studied in western countries have already investigated ERCC1 polymorphism in the risk of glioma, but the results of these studies are consistent. It might be different terms of gender, age, race, ethnicity, and even geographical region. Therefore, we performed a study patients with glioma (cases groups) and without glioma (controls groups) in Yeditepe university hospital to evaluate the relationship between polymorphisms in ERCC1 gene and the risk of adult glioma among turkish population because the evidences from turkish populations are still lacking.

2.LITERATURE REVIEW

2.1 Brain Tumors

To understand how a part of the brain is damaged by the diseases, we must know first how a normal and health brain functions.

2.1.1 Brain Anatomy

Central nervous system (CNS) and the peripheral nervous system (PNS) are the nervous system that responsible of sending, receiving, and interpreting information from all parts of the body. It responds to external stimulation and also monitors and coordination the functions of internal organs. Brain along with the spine cord form the central nervous system (CNS). Brain is protected by layers called meninges and located on the cranium of the skull (11). It is a complicated organ with several function units. Brain coordinates and regulates all functions and others organ in our bodies. The brain is the seat of consciousness, intelligence and language. Since the brain is a complex organ that several parts work together, it is hard to tell which parts is responsible for certain activity of the brain. Forebrain, cerebellum, and brain stem are the principal regions of the brain. The cerebrum is the most frontal part of the brain because it is 83% of the total mass of the brain and is seperated into two hemisphere and it is responsable for complex aspects of consciousness.The thalamus and hypothalamus, that are in charge of controling, the emotion, pain, body temperature and stress, are parts of the diencephalon. Cerebellum called little brain is second largest part and is 11% of the mass brain. It is responsible of correlating muscle mouvement, keeping posture and balance. While, the brain stem is the central part that connect the spinal cord to the cerebrum and cerebellum. It responsable for various automatic function such as: respirations, heart race, body warmth, wake, sleep etc. The spinal cord relay messages between the brain and the body. Cerebrospinal fluid (CSF), a colorless fluid is responsible of protecting CNS and helping the head to absorb the shocks. It is on change of regulation and maintenance the chemicals environment of the CNS functions (12). The anatomy of the brain is illustrated in the Figure 2.1.1-1.

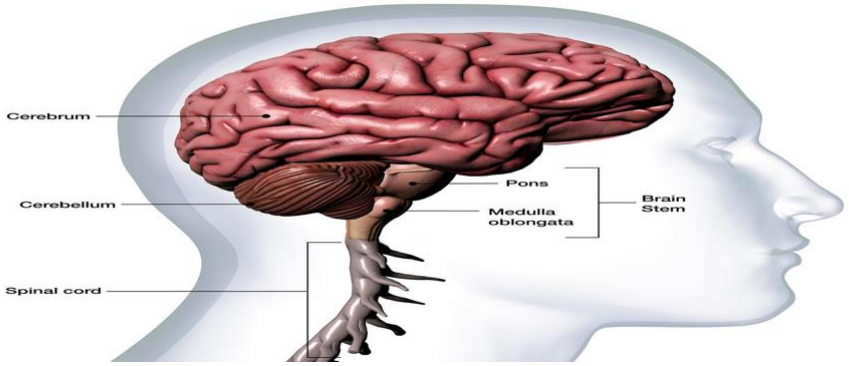

Figure 1.2.1.1: Structure of the brain **(13).**

Cells are the building blocks of the body. Billions of cells build the brain and spinal cord, which is more than one type. The two main cells are nerve cells (neurons) and glial cells. Nerve cells, the longest and the eldest cells in the body work as the signals cells of the nervous system. It main function is the communication between nervous system and body by relying electro-chemical signal.

Although neuron contain similar basic form, there are different in their size and shapes. All the animal have same organelles as well as nerve cells. However, neuron have four different parts, the cell body (soma), the axon, its presynaptic terminal and the dendrites. The structure of neuron is illustrated in the below figure 2.2.1-1.

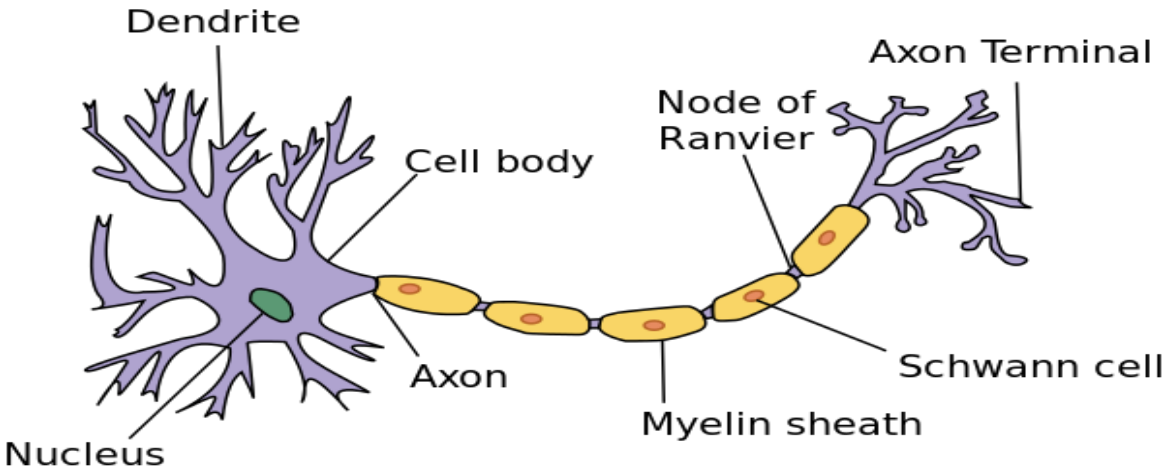

Fıgure 2.2.1.1: Nerve Cell (10)

Glial cells provide support and protection to the nerve cells. There are many more glial cells than neurons. There are various type of glial cells, each one have their own function.

Astrocytes, oligodendrocytes, ependymal cells and microglia are the four types of the glial cells. The structure of the glial cells are seen in the figure 3.2.1-1. Astrocytes are star shapes cells and the main structural cells which support neuron. Their main functions are to maintain the proper balance of chemicals in the brain. Protoplasmic that are in the grey matter of the brain and the spinal cord and the fibrous that are in the white matter are the deux groups of astrocytes. Oligodendrocytes are much smaller than astrocytes. Their main job are to make a fatty membrane called myelin, which, surrounds the axon. It might be located in grey matter and white matter of the central nervous system. The cells that found in the peripheral nervous system are called Schwann cells. The cells which, found in the surface of the ventricular system of the brain and the spinal cord's central canal are called ependymal cells. Its role is to help making cerebrospinal fluid. Phagocytic cells are called microglial which, work when there are an injury, infection or disease. Microglial are derive from the bloodborne macrophages.

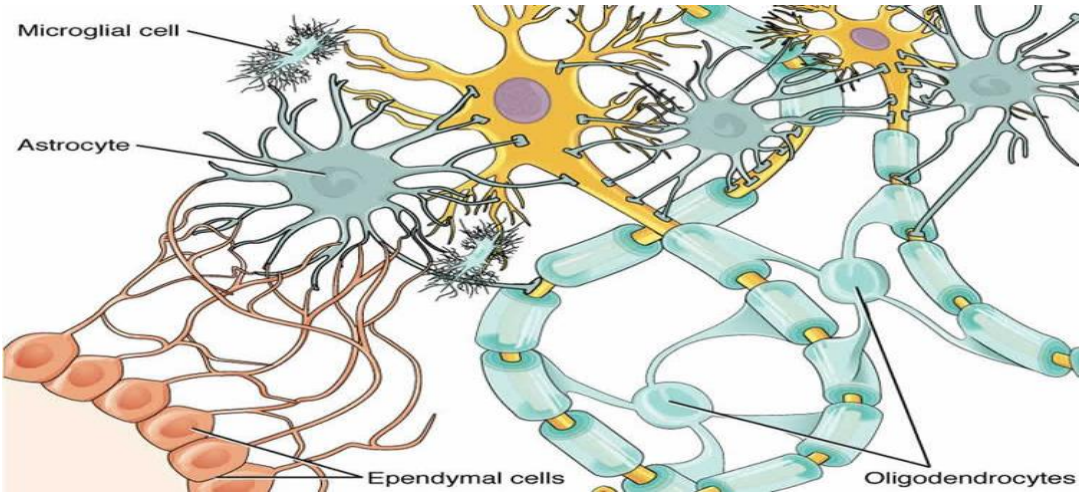

Figure 3.2.1.1: Structure of Glial Cells **(12)**

2.1.2 Brain Tumors

Brain tumors are abnormal growth of cells in the brain parenchyma, brain membrane, nerves, or others tissus in the brain. They affect around 200,000 people worldwide per year. Brain tumors are around 1.4% of all cancer which, lead to 40% of deaths in adults from 2012-2016 and are the leading cause of deaths in children. As reported by statistics 2014 data of the ministry of health of Turkish that 7739 patients were affect with brain tumors between 2008and 2012. (T.C. Ministry of Heath,2014).

More than 120 different type of brain tumors exist but they are grouped into primary brain tumors and secondary brain tumors (30).

Primary malignant brain tumors are tumors that begin in the central nervous system (CNS) and can be benign tumors, which are slow- growing cells or malignant tumors that usually grow quickly and can be life threatening. They rarely spread to others part of the body.

Secondary brain tumors begin in other part of the body then spread to the brain, it is called metastasis. One example for this cancer are breast cancer and lung cancer. However, secondary brain tumors are more commun than primary brain tumors. The type of the brain can be seen in figure 4.2.1-2.

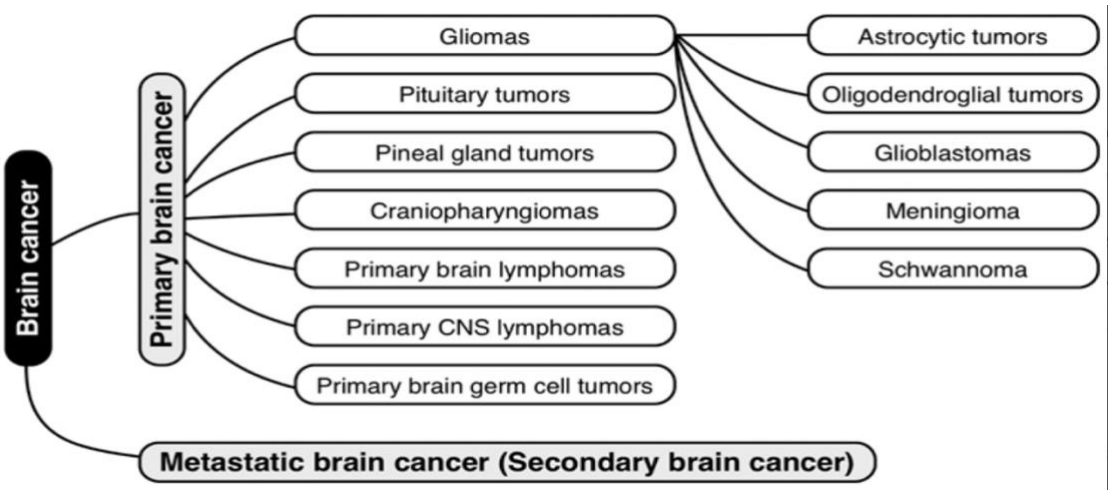

Figure 4.2.1.2: Type and Subunıt of the brain tumors (13)

2.1.3 Classifications of Central Nervous System Tumors

Central nervous system (CNS) malignant tumors are specified being deathly disease by its high rate of the morbidity and mortality. Therefore, CNS tumors should be classified and graded according to the location, behaviour and histopathological features. The importance is to find a classification that help the estimation of the prognosis and evaluation of the treatment and the response of the kind of treatment because the classification was made several times and have been rearranged with the avancement of the technology. There really are not a reliable classification to choose better diagnosed and appropriate treatments. However, CNS tumors classification chose by everyone is World Heath Organization (WHO) classification.

WHO classification of CNS tumors have modify in 2016. The past few years, the molecular pathogenosis of carcinogens in both common and rare cancer were better understood which lead to classified it. Therefore, in the 2007 classification the phenotypic feature was used, so astrocytic and oligodendroglial tumors were classified no matter if it has clinically similarities or differences characteristics. In contrast the 2016 WHO classification used phenotypic as well as genotypic features. They classified astrocytic and oligodendroglial tumors in one groups and there were non diffuse stages others astrocytic tumors grouped in different groups.

2.1.4 Oview of Glioma

Gliomas are tumors that derived by the glial cells and constitute most commun tumors in central nervous system. They affect approximately, 6.6 per 100000 person each year (22).

Gliomas are named according to their arise cell, microscopic appearance and genes variations (1,8). Astrocytomas, oligodendrogliomas, ependymomas, and mixed glioma are the main types of gliomas. Astrocyomas , are widespread brain tumors. According to World Health Organization (WHO) classification of brain tumors, astrocytomas are four group of malignancies. Grade I are considered benign, which are noticed in children. Pilocytic astrocytomas tumors can be removed by operation. Grade II are low level and invade brain tissue by growing slowly. The survival ratio various between 4-5 months according to the case. These gliomas are treated by diffuse

astrocytomas X surgical resection, chemotherapy, and radiotherapy. Malignant gliomas such as anaplastic oligodendrogliomas, anaplastic astrocytomas, glioblastomas and high-grade glioma include both grade III and IV gliomas.

Glioblastoma (GBM) or grade IV gliomas, account more than 60% of the brain tumors in adults. Patient with GBM, have survival rate of 14-15 month. Glioblastoma occur as a result of genetic alteration. Therefore, molecular genetic tools are used to improve patients prognosis and therapy. For example, the patients who have TERT mutation, the scientist developed different chemotherapy and radiotherapy for this patient. Classification of gliomas and WHO grade can seen in table 1-2.1.4.

Table 1-2.1.4: Classification of gliomas and WHO grades (15).

Grade / Type	WHO grade I	WHO grade II	WHO grade III	WHO grade IV
	Circumscript	Diffuse		
		Low-grade	High-grade	
Astrocytoma	Pilocytic astrocytoma	Low-grade astrocytoma	Anaplastic astrocytoma	Glioblastoma
Oligodendroglioma		Low-grade oligodendro-glioma	Anaplastic oligodendro-glioma	
Oligo-astrocytoma		Low-grade oligo-astrocytoma	Anaplastic oligo-astrocytoma	

Patients with tumors are mostly diagnosed by using imaging techniques such as nuclear magnetic resonance imaging and computer tomography. However, this technology screening examination are very expensive, to use as a routine diagnostics tool. Surgery and biopsy for the histopathogical examination are needed when glioma is suspected. Recent evidence showed that biomarkers could be another method for diagnostic of glioma instead of this expensive screening techniques. Some molecules such as tensin homolog (PTEN), phosphotase, and protein 53 (TP53) were found deregulated in glioma. this biomarkers are not enough to procession in glioma, so new biomolecules in serum, blood, and tissues sample are need to improved.

Glioblastoma, the most occurring primary brain tumor in adult is an aggressive cancinos. The causes are unknown in large proportion of cases. Many studies shown, ioning radiation is an established environmental cause of brain tumors. However, exposure to large amount of ioning radiation is limited. Therefore, it didn't explain many new cases of GBM. In epidemiological studies, occupational and lifestyle factors are unassociated with brain tumors. A small proportion of cases are caused by family history of glioma and genetic syndrome including , neurofibramatosis type I and II, Li-Fraumeni, Gorlin's and Tucor syndrome. Looking for other environmental and genetic risk factors for glioblastoma patient remain crucial.

Many factors lead to development of glioma and one of them is the single polymorphisms (SNPs) which, are in the genetic variation factors. Other than environmental agents, and the genetic syndromes, polymorphism in various genes were reported to be a crucial factors in the susceptibility of glioma. Several evidence showed that ERCC1 polymorphism a DNA repair genes lead to risk of glioma.

2.2 ERCC1 in Glioma

DNA repair machinery conserve and stabilize genomic information. Numerous endogenous and exogenous agents endanger the DNA. Therefore, thousands of DNA lesions are in each cell. Unrepaired DNA cause apoptosis and unregulated cell growth that lead to cancer. Different DNA repair mechanisms are involved depending on the type of damage.

Base excision repair (BER), multi-step process responsible of recognizing and removing damaged bases from the genome. BER, repaire the damage which is produced by methylation or oxidation. Nucleotide excision repair (NER), general process that cell use to correct UV-induced lesions, intra-strand cross-links and bulky adducts. Direct repair is another pathway responsable of reverse rather than excise DNA damage. Double strand breaks can occur due to ioning radiation or products of cellular processes resulting in hydrolysis, oxidation or methylation of DNA. Non homologous end joining (NHEJ) and homologous recombination (HR) repaire this kind of damage (47). DNA repair pathway is illustrated in **figure 5.2-2.**

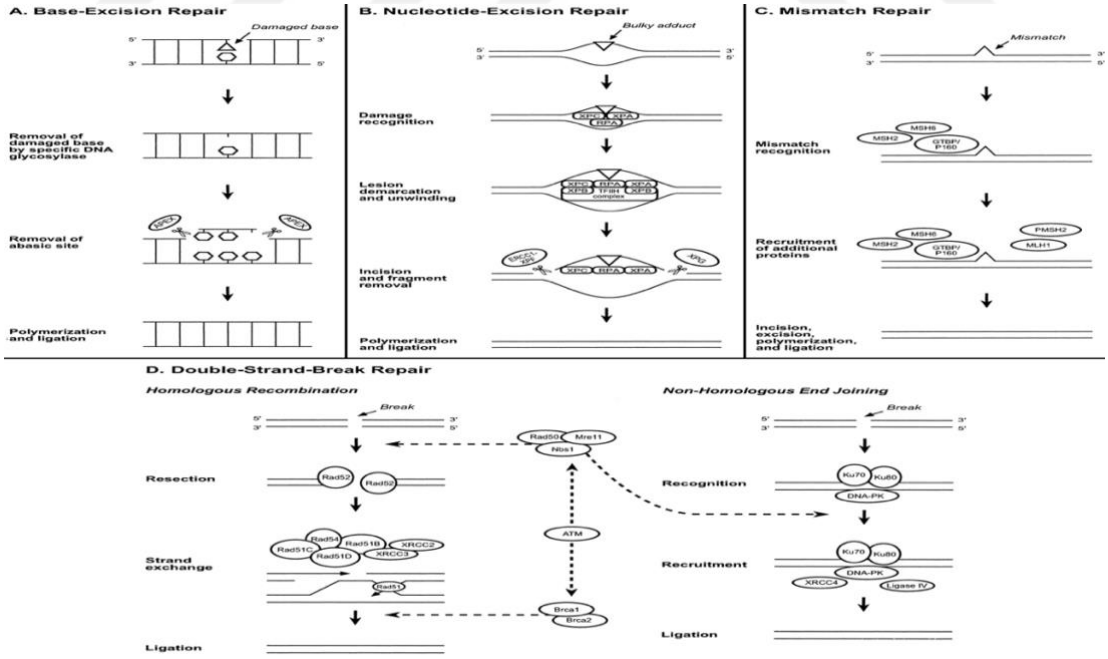

Figure 5.2.2: DNA repair pathway (48).

DNA repair pathway is a complex process involving cascade of proteins and genetic variation of DNA repair genes lead to changing this proteins, activity and quantity. Most genetic variation widely study is single polymorphism nucleotide (SNP) because of their increasing the risk of cancer in particular. Excision repair cross-complimenting group 1 (ERCC1), DNA binding subunit involving repair may related to glioma susceptibility.

Excision repair cross-complimenting group 1 (ERCC1) gene comprise 10 exons, located on chromosome 19q 13,2-13,3 (figure 6.2-2).

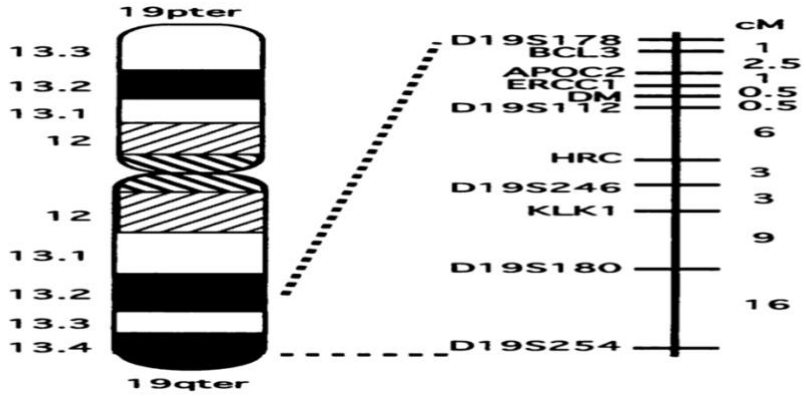

Figure 6.2.2: Location of ERCC1

ERCC1 is a component in NER system that presides over the genome integrity. Nucleotide excision repair, a sophisticated process recognized many types of DNA damage spot. ERCC1 with XPF form an heterodimer, is indispensabe fair cleavage of the DNA strand 5'at the DNA lesions.

Ercc1 mRNA overexpression conduct to drug resistant to cisplatin in glioma patient and genetic polymorphismes may have an impact on the expression of Ercc1.
Two Ercc1 variants are widely studies, C118T variant, T to C transversion in codan 18 lead to a substitution of asparagine for aspartate and the other is C8092A, leading to lysine to glutamine substitution. There are evidence of C118T was related to Ercc1 mRNA level and protein expression and C8092A may influence Ercc1 mRNA stability (24).
The role of ERCC1 polymorphism have investigated in glioma susceptibility.

ERCC1 variant, C118T was shown to influence on the prognosis of glioma. Polymorphism in Ercc1 C8092A may be low-penetrance glioma risk genes. However, variation of glioma rates according to demographic factors, geo-political boundaries, and genotypes distribution of Ercc1 polymorphism variation among different population, the

results can be inconclusive. Thus, we performed this study to determine the association between Ercc1 polymorphism and glioma susceptibility among Turkish population.

3.MATERIALS AND METHODS

3.1 Study Population

This study included 34 patients diagnosed with glioblastoma and 40 controls groups between the age 11-85 year old. All of the participants were selected from yeditepe university hospital. The case group composed of patients who diagnosed by the department of neurosurgery and controls group consist of healthy individuals without glioblastoma. These study was approved by ethics committee of Yeditepe university (ethics committee decision no: 1757).

3.2 Laboratory Materials

3.2.1 Materials Used in the DNA Isolation

The peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA) at +4°C, EDTA tubes are used because of preventing bloods clotting. The DNA extraction was performed using an iPrep Purification Instrument (Invitrogen, Life Technologies; Thermo Fisher Scientific Inc., Waltham, MA, USA), with 350 µl of peripheral blood and an iPrep Pure link gDNA blood isolation kit (Invitrogen, Life Technologies; USA) according to the manufacturer's instructions.

3.2.2 Devices Used in this Study

The equipment used in this study are : iPrep Purification Instrument (Invitrogen, Life Technologies; Thermo Fisher scientific Inc, Waltham, MA, USA), nanodrop 2000 (Thermofisher Scientific inc), real-time Pcr (light cycle 480 II instrument, Roche Diagnotics, fast real time 7500, applied Biosystems), 7500 fast real-time PCR instrument, +4°C and -20°C refrigerator (Haier), ultra-pure water (pure lab option Q,EICT), Vortex (V.I. Plus Biosan) and pipette Kit (Thermo-Fisher Scientific inc.).

3.3 Laboratory Methods

3.3.1 DNA Extraction from the Blood

The blood samples for both controls and cases groups were collected into EDTA tubes with 5ml volume and stored at +4°C. An iPrep Purification Instrument (invitrogen) was used to perform the DNA extraction within 30 min and iPrep™ PureLink® gDNA Blood Kit allows isolation of genomic DNA from fresh blood sample. 350 µl peripheral blood might be extracted by this automatic system. These robots use magnetic bead technology to process 12 blood sample per run. Prepackaged reagents in cartridges was used to set-up variation and allow for simple process validation. The prepackaged reagents was agitated for few seconds to mixe magnetic beads in the reagents well.

IPrep robotic isolation equipment take advantage of ChargeSwitch® (CST®), powerful and automated in DNA purification. ChargeSwitch® allows rapid and efficient purification of DNA from a small volumes of blood sample. This technology use a magnetic bead-based technology to isolate amounts of genomic DNA from sample. This method is based on a unique principle unlike silica-based purification methods, use aqueous buffers . Magnetic bead charge switch due to the pH of the surrounding buffer to facilitate nucleic acid purification. In the condition of low pH, the DNA blackbone because of its negative charge binds to the positive charged of the CST® beads. Proteins and other particules are not bound and are washed away in an aqueous wash buffer. DNA is eluted by neutralizing the charge of ChargeSwitch beads by a pH of 8.5 using a low salt elution buffer. Purified DNA pass instantly into the elution buffer, and is ready for use in the next step.

3.3.2 DNA Measurement

In this study purify DNA was measured by Nanodrop 2000 (Thermofisher scientific inc), who able to measure the purity and the concentrations of nucleic acid. Nucleic acids absorp UV light around a wavelength and less at 260nm. Nanodrop doesn't request any cuvettes or capillaries unlike the other classical spectrophotometer. Both proportions OD260/OD280 and OD260/OD230 of DNA concentration was measured by this instrument. The amount of DNA used in this study was 1.5µl. The DNA was placed

directly onto an optical measurement surface and then it's closed to complet the measurement. Once the measurement was done the surface wiped with a tissue for the safety of the measurement. Every 10 measurement the surface was cleaned by distilled water. The results was detected by the computer and we were able to see DNA purity as well as DNA concentration. The ratio of OD260/OD280 is between 1.7-1.9 for Genotyping.

3.3.3 Genotyping Analysis

Genotyping analysis was performed by Real-Time PCR (light cycle 480 II instrument, Roche Diagnotics, fast real time 7500, applied Biosystems), a powerful technologies in molecular biology.

Real-time PCR use fluorescence dyes of probes to detect the single nucleotide polymorphism (SNP). This method allows Genotyping by reading the fluorescence radiation. In this study we used two type of TaqMan probes, FAM dye and VIC dye. TaqMan probes are covalently joined the amplified region of the DNA template. Taq polymerase hydrolyzed the probes, and increase fluorescent signals. Therefore real time PCR instruments can monitor and record this increase in fluorescent and with its two wavelengths for allele-specific, wildtype and mutant alleles can be detected.

Real Time RT-PCR is used for the detection of polymorphism in the ERCC1 gene by the amplicafication of the target sequences in ERCC1 gene. The choice of the target gene depends on the primers. DNA amplification of ERCC1 variant was performed separately with predesigned reverse 5'GAGCTCACCTGAGGAACAGG3' and forward 5'GTGCGAGGAGGCAGGAGGTGTGGG3' primers. Genotyping a region of the target gene, ERCC1 rs11615 was analyzed. Then allele discrimination was illustrated in the 7500 fast Real-time PCR. ERCC1 primers is illustrated in table 2.3.3.3.

Table 2-3.3.3: ERCC1 primers (26)

SNP	Primers (5'-3')	restriction endonuclease	Length (by)
ERCC1 rs11615	FP:GTGCGAGGAGGCAGGAGGTGTGGG RP: GAGCTCACCTGAGGAACAGG	BsrD1	239

To genotype the polymorphisms of ERCC1 rs11615, Real time RT-PCR procedure was used. First step to PCR amplification is the preparation of the reaction mixture. In this step, a premixed concentrated solution called master mix are used. This solution consists of buffer, reverse transcriptase enzyme, nucleotide, primers (forward and reverse), Taqman probe and DNA polymerase and to complete DNA template is added in this mixture. The reaction mixture can be seen in table 3-3.33

Table 3-3.3.3: The reaction mixture

The Product used	Quantity (µl)
Master mix	5
TaqMan Genotyping Assay	0,2
DNAse,RNAse free water	3,75
Template DNA	1

The reaction mixture is loaded into the RT-PCR plate which, contain 96 well, allowing the analysis of several sample at the time. The plate is placed in the RT-PCR machine that is essentially a thermal cycler. In a real-time PCR reaction, three major steps make up each cycle and the reactions (dénaturation and elongation) are tend to run for 40 cycles. Real-time PCR condition arranged of waiting 10 minute at 95°C, then denatured at 92°C for 15 second and elongated for 1min at 60°C for each cycle. PCR condition are illustrated in table 4-3.3-3.

Table 4-3.3.3: RT-PCR conditions

	Waiting	Denaturation	Elongation
Temperature	95°C	92°C	60°C
Time	10 minute	15 seconds	1 minute

3.3.4 Statistical Analysis

The statistical analyses was performed using IBM SPSS Statistics version 23(IB Corp, Armonk, NY, USA).

The significance of differences between the groups was examined with the Student's t-test, and the comparisons of the demographic information were evaluated the chi squared and Fisher's exact tests. The statistical significance level was denoted as $p<0.05$

4.RESULTS

4.1. Demographic Characteristics Results of Study Patients

The demographics characteristics of working groups are summarized in Table 5-4.4.1.

The number of cases groups was 34 of which 24 were males and 10 were females, and controls groups was 40, 28 were males and 12 were females.

Chi-square test and independent student t test was used to compare between the gender and the age of two groups.

According to the table above (table 5.4.4.1) a significant different was found in age with p=0.025 and no statistical significance in gender with p=0.956.

Table 5-4.4.1: Demographic characteristic of cases and controls groups

	Controls groups n=40		**Cases groups n=34**		**P value**
Gender	Male 53.68% n=28	Female 54.5% n=12	Male 46,2% n=24	Female 45.5% n=10	0.956 NS**
Age (years) **Mean±SD**	40.59 ± 11.80		49.82 ± 19.62		0.025 S*

*S= significant different (p<0.05), **SN= non significant (p>0.05).
X± SD = Mean ±Standard Deviation, n= number of sample.
The demographics characteristics of the groups were analyzed by chi-square test and the double independent sample student t-test.

Table 6-4.4.1: Cases groups clinal data

Tumor location	Number (n) and percentage value (%)
Brain stem	5.9% n=2
Neocortex	35.3% n=12
Multicentric	11.8% n=4
cerebellar	2.9% n=1
Thalamic	8.8% n=3
Limbic	29.4% n=10
Corpus callosum	5.9% n=2
Total	100% n=34

n= number of sample. %= percentage values based on sample group in total

Table 6.4.1.1, showed that the tumors are mostly located in the limbic and the neocortex.

4.2 Real-Time PCR Result

Type of Allele for control and cases groups was read by the 7500 Fast real time device.

Allele discrimination plot is illustrated in figure 7.4.1.

Fluorescence dyes of probes allow the reading and interpretation of the fluorescence radiation. The blue color is FAM and the green color is VIC.

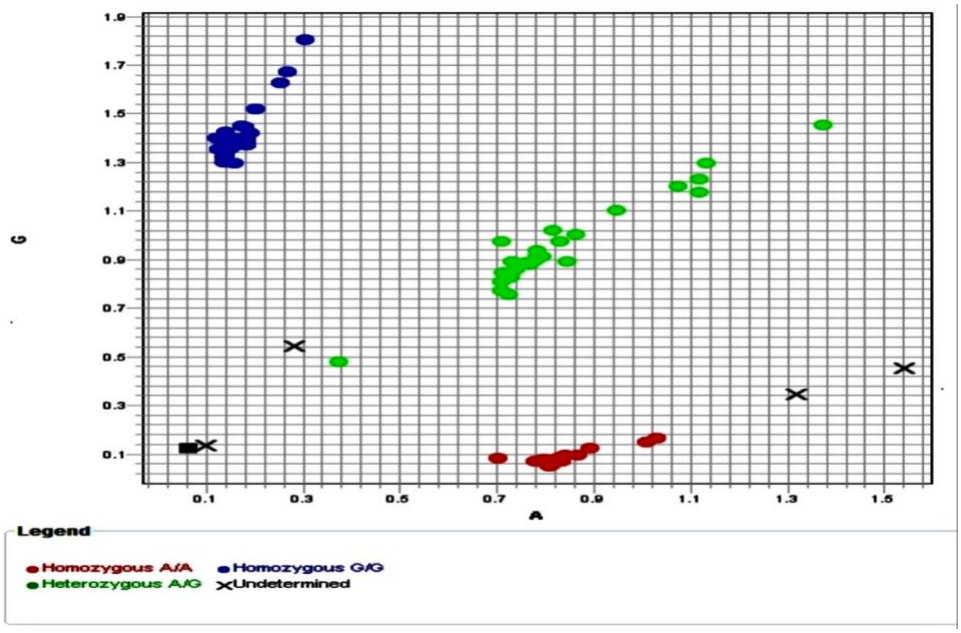

Figure 7.4.2: Allele Discrimination plot

AA is homozygous mutant type, AG is heterozygous and GG is the wild homozygous type.

Figure 8.4.2 illustre G allele dicrimination plot. The yellow line is the threshold value (0.02544).

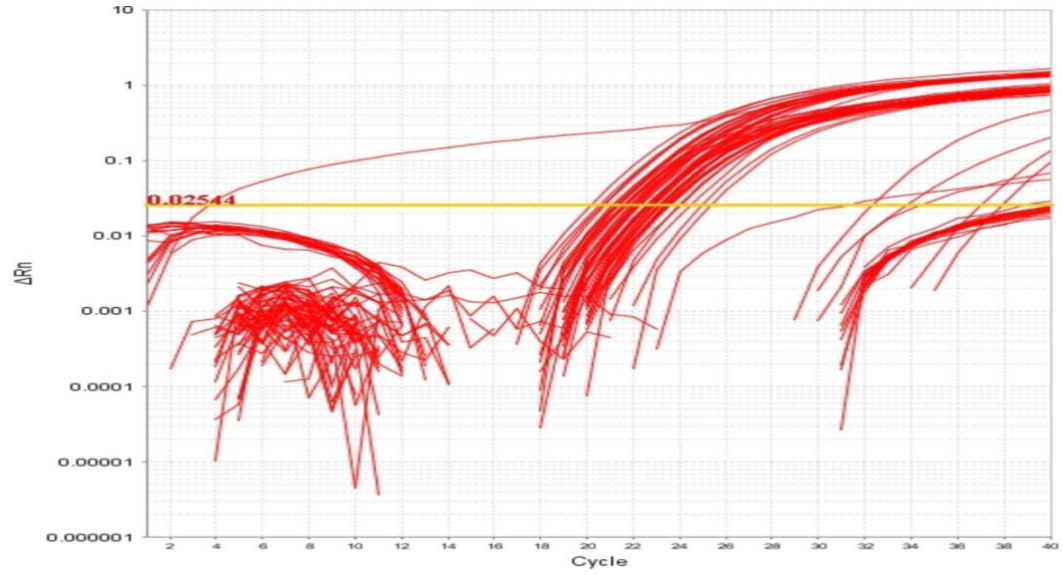

Figure 8.4.2: Allele G amplification plot

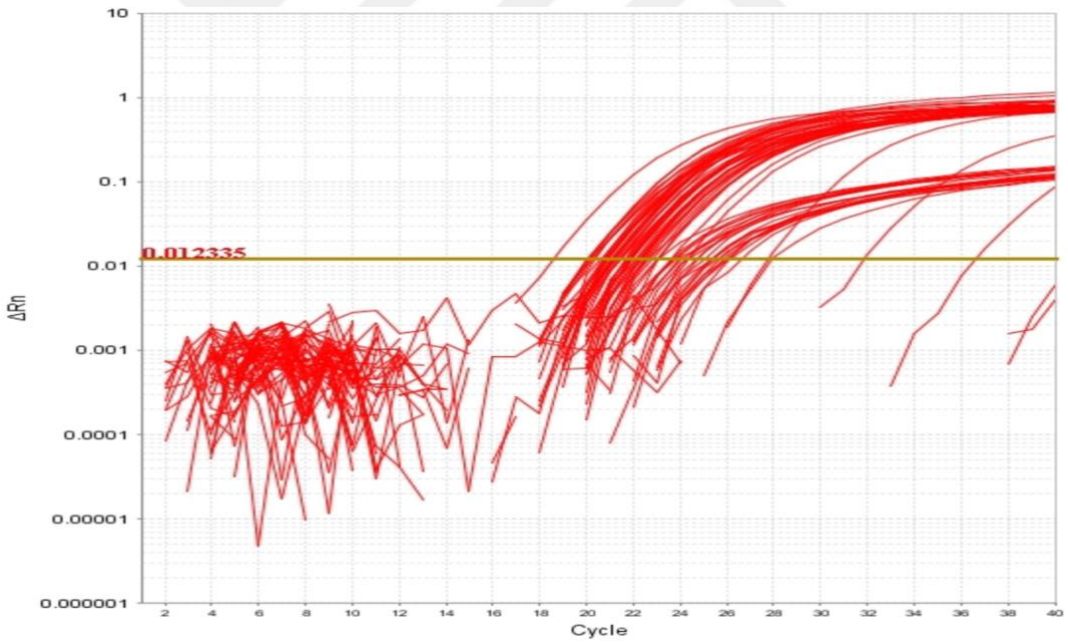

Figure 9.4.3: Allele A discrimination plot

The figure 9.4.3 above illustre A allele dicrimination plot and the yellow line is the threshold value (0.012335)

4.3 Genotyping Analysis of Study Groups

ERCC1 polymorphism in both patient with glioma and without glioma was performed in this experiment. The genotypes (AA, AG, GG) and alleles (A,G) distribution of ERCC1 for patients and controls groups are presented in table 7.4.4.3
To analyse the signifiant different between health and patients groups the fisher exact test was used. The Genotypes frequency of homozygous mutant (AA), heterozygous mutant (AG) and homozygous wild (GG) in case groups are 27.3%, 36.4% and 36.4%. In the control groups are 27%, 43.2% and 29.7% table 7.4.4.3. No significant statistical between the two groups was shown. The P values of the homozygous mutant genotype is 0.982, the homozygous wild genotype is 0.555 and the heterozygous wild genotype is 0.558.

Table 7-4.4.3: Ercc1 Genotypes and alleles distribution of working groups

Genotypes	Controls groups n=40	Cases groups n=34	P value	P value
AA	27% n=10	27,3% n=9	0.982** NS	
AG	43.2% n=16	36.4% n=12	0.558 ** NS	0.802** NS
GG	29.7% n=11	36.4% n=12	0.555** NS	
Alleles				
A	n=36	n=31	0.088** NS	
G	n=38	n=35	0.137** NS	

*S= significant different ($p<0.05$), **NS= non significant ($p>0.05$). X± SD = Mean ±Standard Deviation, n= number of sample. The difference between patients were analyzed by exact fisher tesgytoiu

According to the table 8.4.3.3, Ercc1 genotypes were compared and we found that homozygous mutant AA frequency was 27% in controls groups and 27,3% in cases, heterozygous mutant AG was 43.2% in controls and 36.4% in cases, and homozygous wild GG was 29.7% in controls and 36.4% in cases. No significant different shown between the two groups for each genotypes, homozygous mutant, homozygous wild and heterozygous mutant

Among cases groups, thirty one had Allele A and thirty five had allele G. In controls groups, thirty six had Allele A and thirty eight had allele G. According to this results the Ercc1 alleles distribution are a bit similar and no significance difference was found between groups which p values of Allele A is 0.088 and Allele G is 0.137.

Table 8-4.4.3: Eccr1 Genotypes and alleles frequency comparison

Genotypes	Controls	Case	P value	Odd ratio	Confidence interval %
AA	27% n=10	27,3% n=9	0.082** NS	0.988	0.344- 2.837
AG	43.2% n=16	36.4% n=12	0.558** NS	1.333	0.509- 3.490
GG	29.7% n=11	36.4% n=12	0.555** NS	0.740	0.272- 2.013
Alleles					
A	n=36	n=31	0.088** NS	2.511	0.941- 6.700
G	n=38	n=35	0.137** NS	2.250	0.829- 6.104

*S= significant different ($p<0.05$), **NS= non significant ($p>0.05$). X± SD = Mean ±Standard Deviation, n= number of sample. The difference between patients were analyzed by the chi-square

In this study, Ercc1 genotypes and alleles were evaluated and no significant association between the studying subjects were found (p>0.05). In general, according to our analysis, there are no significant different between ERCC1 rs11615 and glioma susceptibility under five genetics models, homozygous GG 0.558 (OR: 1.333 95%Cl 0.509-3.490) and homozygous AA 0.082(OR: 0.988 95% Cl 0.509-3.490), heterozygous mutant AG 0.558 (OR=1.333, 95%Cl 0.509-3.490), Alleles A 0.088 (OR=2.511, 95% Cl 0.941-6.700) and G 0.137 (OR=2.250,95%Cl 0.829-6.104).

5.DISCUSSION AND CONCLUSION

Central nervous system (CNS) consists 2% of all cancer. However, its rates of morbidity and mortality are not accord. Studying showed that in children malignant CNS cancers are the main source of death, and the third cause of mortality in people within the age 15-34 years, but people above 45 years are the most diagnosed by CNS tumors.

Glioblastoma (GBM), the most occurring CNS tumors and a deadly cancinos. The survival rare GBM is 14-15 month. Although it has various treatments such as, radiotherapy, chemotherapy and the application of operation treatment, it has weak prognosis. GBM is known with a great invasive character that allow it to escape focal and operation treatment and its ability of resistance to the radiotherapy and chemotherapy treatment. It occur due to several genetic variation (18).

DNA repair mechanism, a key of maintaining the stability of genome, which include numous pathways. Nucleotide exorcism repair play a main role in the DNA repair process. ERCC1 gene is one of the factor in the NER pathway and product a protein that bind with XPF and cleave in the incision 5' of damage DNA.

ERCC1 has 10 exons and many variations of genes was shown, which may affect cancer therapies response, prognosis as well as rate of survival. Studying demonstrated that in mice, cells with mutation from Ercc1-deficient lead to NER pathway deficiency and high mutation rate as well as increase level of instability of the genome (25).

NER pathways play crucial role in bulky DNA lesion and the damage of the UV. However, other mechanisms of the damage of DNA which is related to gliomogenesis can be need NER pathway. Other research showed that DNA damage caused by low N-nitrosodiethylamine concentration were repaired by NER mechanisms and base excision repair. Therefore, NER pathway repair the DNA damage by oxidative in the bacteria. Bases of DNA lesions are detached NER pathway by presenting nicks 5'-3' to an a basic site in vitro.

ERCC1 works as biomarkers in efficacy of the treatment or rate survival of some cancer in the protein, genomic and transcription level. Research indicated that excessive expression of ERCC1 RNA messenger lead to resistance of the drugs (ciplatin) in glioma.

As far as we know, this study is the first to investigate the association between ERCC1 polymorphism and risk of glioma in Turkish population. Therefore, we did not

notice a significant different in ERCC1 rs11615 in glioma susceptibility. Our studying are similar to the other past finding that focused on ERCC1 variants. Case-Controls studying conducted of ERCC1 and ERCC2 with risk of glioma have shown no relationship in Ercc1 rs11615 (32). And another meta-analysis done with Asian and Caucasian failed to prove association between ERCC1 rs11615 and glioma patients (31).

Some limitations were observed in this study. The numbers of both groups were comparatively small that reduced the statical power for determined the significant different between the subjects. Evidently, more researches with large size of sample are needed. In addition, since ERCC1 interact with XPF and XPD, those other repair gene should be considered. In consequently, further studies on variants in ERCC1 and other DNA repair are still required.

In conclusion,

As the first study in Turkish population which investigated the relationship between ERCC1 gene and the risk of glioma, this finding did not prove statistically significant between ERCC1 rs11615 gene and glioma susceptibility. Future studies in Turkish population are still necessitate.

6.REFERENCES

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7.APPENDICES

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	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.
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	Elapsed Time	00:00:00,02

Group Statistics

	groupe	N	Mean	Std. Deviation	Std. Error Mean
SMEAN(age)	case	34	49,8235	19,62137	3,36504
	controle	40	41,5447	10,69916	1,69169

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means		
		F	Sig.	t	df	
SMEAN(age)	Equal variances assumed	16,318	,000	2,298	72	
	Equal variances not assumed			2,198	49,133	

Independent Samples Test

		t-test for Equality of Means			
		Sig. (2-tailed)	Mean Difference	Std. Error Difference	
SMEAN(age)	Equal variances assumed	,024	8,27883	3,60211	
	Equal variances not assumed	,033	8,27883	3,76633	

Independent Samples Test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
SMEAN(age)	Equal variances assumed	1,09816	15,45951
	Equal variances not assumed	,71062	15,84705

```
DATASET ACTIVATE DataSet1.
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  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI RISK
  /CELLS=COUNT EXPECTED ROW COLUMN TOTAL
  /COUNT ROUND CELL .
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Crosstabs

Notes

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	N of Rows in Working Data File	74
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=gender BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT EXPECTED ROW COLUMN TOTAL /COUNT ROUND CELL.

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Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
gender * groupe	74	100,0%	0	0,0%	74	100,0%

gender * groupe Crosstabulation

			groupe		
			case	controle	Total
gender	female	Count	10	12	22
		Expected Count	10,1	11,9	22,0
		% within gender	45,5%	54,5%	100,0%
		% within groupe	29,4%	30,0%	29,7%
		% of Total	13,5%	16,2%	29,7%
	male	Count	24	28	52
		Expected Count	23,9	28,1	52,0
		% within gender	46,2%	53,8%	100,0%
		% within groupe	70,6%	70,0%	70,3%
		% of Total	32,4%	37,8%	70,3%
Total		Count	34	40	74
		Expected Count	34,0	40,0	74,0
		% within gender	45,9%	54,1%	100,0%
		% within groupe	100,0%	100,0%	100,0%
		% of Total	45,9%	54,1%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,003[a]	1	,956		

Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,003	1	,956		
Fisher's Exact Test				1,000	,580
Linear-by-Linear Association	,003	1	,956		
N of Valid Cases	74				

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 10,11.
b. Computed only for a 2x2 table

Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	-,006	,956
	Cramer's V	,006	,956
N of Valid Cases		74	

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for gender (female / male)	,972	,357	2,645
For cohort groupe = case	,985	,572	1,696
For cohort groupe = controle	1,013	,641	1,600
N of Valid Cases	74		

```
CROSS-TABS
  /TABLES=genotype BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:43:12
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	74
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 BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,05
	Elapsed Time	00:00:00,62
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00,04

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotype * groupe	70	94,6%	4	5,4%	74	100,0%

genotype * groupe Crosstabulation

			groupe		
			case	controle	Total
genotype	AA	Count	9	10	19
		% within genotype	47,4%	52,6%	100,0%
		% within groupe	27,3%	27,0%	27,1%
		% of Total	12,9%	14,3%	27,1%
	AG	Count	12	16	28
		% within genotype	42,9%	57,1%	100,0%
		% within groupe	36,4%	43,2%	40,0%
		% of Total	17,1%	22,9%	40,0%
	GG	Count	12	11	23

	% within genotype	52,2%	47,8%	100,0%
	% within groupe	36,4%	29,7%	32,9%
	% of Total	17,1%	15,7%	32,9%
Total	Count	33	37	70
	% within genotype	47,1%	52,9%	100,0%
	% within groupe	100,0%	100,0%	100,0%
	% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,440[a]	2	,802	,792	
Likelihood Ratio	,441	2	,802	,792	
Fisher's Exact Test	,485			,792	
Linear-by-Linear Association	,118[b]	1	,732	,760	,425
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,115
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,96.

b. The standardized statistic is -,343.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	,079	,802	,792
	Cramer's V	,079	,802	,792

N of Valid Cases	70		

Risk Estimate

	Value
Odds Ratio for genotype (AA / AG)	[a]

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

```
CROSSTABS
  /TABLES=AlleleA BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:56:47
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
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	Split File	<none>
	N of Rows in Working Data File	74
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=AlleleA BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,03

	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00,01

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
AlleleA * groupe	70	94,6%	4	5,4%	74	100,0%

AlleleA * groupe Crosstabulation

			groupe		
			case	controle	Total
AlleleA	A-	Count	17	11	28
		% within AlleleA	60,7%	39,3%	100,0%
		% within groupe	51,5%	29,7%	40,0%
		% of Total	24,3%	15,7%	40,0%
	A+	Count	16	26	42
		% within AlleleA	38,1%	61,9%	100,0%
		% within groupe	48,5%	70,3%	60,0%
		% of Total	22,9%	37,1%	60,0%
Total		Count	33	37	70
		% within AlleleA	47,1%	52,9%	100,0%
		% within groupe	100,0%	100,0%	100,0%
		% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3,449[a]	1	,063	,088	,053

Continuity Correction[b]	2,601	1	,107		
Likelihood Ratio	3,471	1	,062	,088	,053
Fisher's Exact Test				,088	,053
Linear-by-Linear Association	3,400[c]	1	,065	,088	,053
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Continuity Correction[b]	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,036
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 13,20.

b. Computed only for a 2x2 table

c. The standardized statistic is 1,844.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,222	,117	1,877
Ordinal by Ordinal	Spearman Correlation	,222	,117	1,877
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance	Exact Significance
Interval by Interval	Pearson's R	,065[c]	,088
Ordinal by Ordinal	Spearman Correlation	,065[c]	,088
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 AlleleA (A- / A+)	2,511	,941	6,700
For cohort groupe = case	1,594	,979	2,594
For cohort groupe = controle	,635	,378	1,065
N of Valid Cases	70		

```
CROSSTABS
  /TABLES=AlleleG BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:59:40
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	74
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=AlleleG BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,02
	Elapsed Time	00:00:00,04
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00,03

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
AlleleG * groupe	70	94,6%	4	5,4%	74	100,0%

AlleleG * groupe Crosstabulation

		groupe		
		case	controle	Total
AlleleG G-	Count	15	10	25
	% within AlleleG	60,0%	40,0%	100,0%
	% within groupe	45,5%	27,0%	35,7%
	% of Total	21,4%	14,3%	35,7%

G+	Count	18	27	45
	% within AlleleG	40,0%	60,0%	100,0%
	% within groupe	54,5%	73,0%	64,3%
	% of Total	25,7%	38,6%	64,3%
Total	Count	33	37	70
	% within AlleleG	47,1%	52,9%	100,0%
	% within groupe	100,0%	100,0%	100,0%
	% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	2,580[a]	1	,108	,137	,087
Continuity Correction[b]	1,840	1	,175		
Likelihood Ratio	2,590	1	,108	,137	,087
Fisher's Exact Test				,137	,087
Linear-by-Linear Association	2,543[c]	1	,111	,137	,087
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Continuity Correction[b]	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,056
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 11,79.

b. Computed only for a 2x2 table

c. The standardized statistic is 1,595.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Interval by Interval	Pearson's R	,192	,117	1,613
Ordinal by Ordinal	Spearman Correlation	,192	,117	1,613
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance	Exact Significance
Interval by Interval	Pearson's R	,111[c]	,137
Ordinal by Ordinal	Spearman Correlation	,111[c]	,137
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 AlleleG (G- / G+)	2,250	,829	6,104
For cohort groupe = case	1,500	,928	2,424
For cohort groupe = controle	,667	,390	1,140
N of Valid Cases	70		

```
CROSSTABS
  /TABLES=genotypeAA BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI CORR RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:46:15
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	74
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=genotypeAA BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI CORR RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,03
	Elapsed Time	00:00:00,12

Dimensions Requested	2
Cells Available	524245
Time for Exact Statistics	0:00:00,03

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotypeAA * groupe	70	94,6%	4	5,4%	74	100,0%

genotypeAA * groupe Crosstabulation

			groupe		
			case	controle	Total
genotypeAA	AA-	Count	24	27	51
		% within genotypeAA	47,1%	52,9%	100,0%
		% within groupe	72,7%	73,0%	72,9%
		% of Total	34,3%	38,6%	72,9%
	AA+	Count	9	10	19
		% within genotypeAA	47,4%	52,6%	100,0%
		% within groupe	27,3%	27,0%	27,1%
		% of Total	12,9%	14,3%	27,1%
Total		Count	33	37	70
		% within genotypeAA	47,1%	52,9%	100,0%
		% within groupe	100,0%	100,0%	100,0%
		% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,001[a]	1	,982	1,000	,596
Continuity Correction[b]	,000	1	1,000		
Likelihood Ratio	,001	1	,982	1,000	,596

Fisher's Exact Test				1,000	,596
Linear-by-Linear Association	,001[c]	1	,982	1,000	,596
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Continuity Correction[b]	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,212
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 8,96.

b. Computed only for a 2x2 table

c. The standardized statistic is -,023.

Symmetric Measures

		Value	Asymptotic Standard Error[a]	Approximate T[b]
Nominal by Nominal	Phi	-,003		
	Cramer's V	,003		
Interval by Interval	Pearson's R	-,003	,120	-,023
Ordinal by Ordinal	Spearman Correlation	-,003	,120	-,023
N of Valid Cases		70		

Symmetric Measures

		Approximate Significance	Exact Significance
Nominal by Nominal	Phi	,982	1,000
	Cramer's V	,982	1,000
Interval by Interval	Pearson's R	,982[c]	1,000
Ordinal by Ordinal	Spearman Correlation	,982[c]	1,000
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 genotypeAA (AA- / AA+)	,988	,344	2,837
For cohort groupe = case	,993	,570	1,733
For cohort groupe = controle	1,006	,611	1,657
N of Valid Cases	70		

```
CROSSTABS
  /TABLES=genotypeAG BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:51:02
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	74
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=genotypeAG BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,03
	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00,01

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotypeAG * groupe	70	94,6%	4	5,4%	74	100,0%

genotypeAG * groupe Crosstabulation

			groupe		
			case	controle	Total
genotypeAG	AG-	Count	21	21	42
		% within genotypeAG	50,0%	50,0%	100,0%
		% within groupe	63,6%	56,8%	60,0%
		% of Total	30,0%	30,0%	60,0%
	AG+	Count	12	16	28
		% within genotypeAG	42,9%	57,1%	100,0%
		% within groupe	36,4%	43,2%	40,0%
		% of Total	17,1%	22,9%	40,0%
Total		Count	33	37	70

	% within genotypeAG	47,1%	52,9%	100,0%
	% within groupe	100,0%	100,0%	100,0%
	% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,344[a]	1	,558	,629	,367
Continuity Correction[b]	,117	1	,732		
Likelihood Ratio	,345	1	,557	,629	,367
Fisher's Exact Test				,629	,367
Linear-by-Linear Association	,339[c]	1	,560	,629	,367
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Continuity Correction[b]	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,163
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 13,20.

b. Computed only for a 2x2 table

c. The standardized statistic is ,582.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	,070	,558	,629
	Cramer's V	,070	,558	,629
N of Valid Cases		70		

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for genotypeAG (AG- / AG+)	1,333	,509	3,490
For cohort groupe = case	1,167	,691	1,970
For cohort groupe = controle	,875	,563	1,360
N of Valid Cases	70		

```
CROSSTABS
  /TABLES=genotypeGG BY groupe
  /FORMAT=AVALUE TABLES
  /STATISTICS=CHISQ PHI RISK
  /CELLS=COUNT ROW COLUMN TOTAL
  /COUNT ROUND CELL
  /METHOD=EXACT TIMER(5).
```

Crosstabs

Notes

Output Created		26-JUL-2021 15:53:09
Comments		
Input	Data	C:\Users\seda.gulec\Desktop\dalmar spss.sav
	Active Dataset	DataSet1
	Filter	<none>
	Weight	<none>
	Split File	<none>
	N of Rows in Working Data File	74
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=genotypeGG BY groupe /FORMAT=AVALUE TABLES /STATISTICS=CHISQ PHI RISK /CELLS=COUNT ROW COLUMN TOTAL /COUNT ROUND CELL /METHOD=EXACT TIMER(5).
Resources	Processor Time	00:00:00,03

	Elapsed Time	00:00:00,03
	Dimensions Requested	2
	Cells Available	524245
	Time for Exact Statistics	0:00:00,01

Case Processing Summary

	Cases					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
genotypeGG * groupe	70	94,6%	4	5,4%	74	100,0%

genotypeGG * groupe Crosstabulation

			groupe		
			case	controle	Total
genotypeGG	GG-	Count	21	26	47
		% within genotypeGG	44,7%	55,3%	100,0%
		% within groupe	63,6%	70,3%	67,1%
		% of Total	30,0%	37,1%	67,1%
	GG+	Count	12	11	23
		% within genotypeGG	52,2%	47,8%	100,0%
		% within groupe	36,4%	29,7%	32,9%
		% of Total	17,1%	15,7%	32,9%
Total		Count	33	37	70
		% within genotypeGG	47,1%	52,9%	100,0%
		% within groupe	100,0%	100,0%	100,0%
		% of Total	47,1%	52,9%	100,0%

Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	,348[a]	1	,555	,616	,368

Continuity Correction[b]	,112	1	,738		
Likelihood Ratio	,348	1	,555	,616	,368
Fisher's Exact Test				,616	,368
Linear-by-Linear Association	,343[c]	1	,558	,616	,368
N of Valid Cases	70				

Chi-Square Tests

	Point Probability
Pearson Chi-Square	
Continuity Correction[b]	
Likelihood Ratio	
Fisher's Exact Test	
Linear-by-Linear Association	,169
N of Valid Cases	

a. 0 cells (,0%) have expected count less than 5. The minimum expected count is 10,84.

b. Computed only for a 2x2 table

c. The standardized statistic is -,586.

Symmetric Measures

		Value	Approximate Significance	Exact Significance
Nominal by Nominal	Phi	-,071	,555	,616
	Cramer's V	,071	,555	,616
N of Valid Cases		70		

Risk Estimate

	Value	95% Confidence Interval Lower	Upper
Odds Ratio for genotypeGG (GG- / GG+)	,740	,272	2,013
For cohort groupe = case	,856	,517	1,418
For cohort groupe = controle	1,157	,703	1,904
N of Valid Cases	70		

T.C. YEDİTEPE ÜNİVERSİTESİ

Savı : 37068608-6100-15- 2134 22/04/2021

Konu: Klinik Araştırmalar
Etik Kurul Başvurusu hk.

İlgili Makama (Dalmar Omar Abdı)

Yeditepe Üniversitesi Moleküler Tıp AD. Prof. Dr. Turgay İşbir'in proje koordinatörü olduğu,

Beyin ve Sinir Cerrahisi AD. Prof. Dr. Ahmet Hilmi Kaya ve Kadm Hastalıkları ve Doğum

AD. Prof. Dr. Erkut Attar'm sorumlu araştırmacı olduğu "Türk Popülasyonunda DNA Onarım Geni ERCCI Polimorfizmleri ve Glioma Duyarlılığının Araştırılması" isimli araştırma projesine ait Klinik Araştırmalar Etik Kurulu (KAEK) Başvuru Dosyası (2161) kayıtlı Numaralı KAEK Başvuru Dosyası, Yeditepe Üniversitesi Klinik Araştırmalar Etik Kurulu tarafından 21.04.2021 tarihli toplantıda incelenmiştir.

Kurul tarafından yapılan inceleme sonucu, yukarıdaki isim belirtilen çalışmanın yapılmasının etik ve bilimsel açıdan uygun olduğuna karar verilmiştir (KAEK Karar No: 1430)

Prof. Dr. Hasan AYDIN

Yeditepe Universitesi
Klinik Araştırmalar Etik Kurul Başkan Yrd.