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Association of EGFR Gene Polymorphism with Glioma Susceptibility in Turkish Population

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ABSTRACT

Objective: Gliomas are devastating adult brain tumors of unknown etiology, occupying 8 out of 10 primary brain tumors. Epidermal growth factor receptor (EGFR) as a tyrosine kinase family member is encoded by the EGFR gene located in chromosome 7p12-13. Various studies have identified numerous SNPs, including those in the EGFR gene, as linked to gliomas. The objective of the investigation was to determine whether the genotype and allele frequencies of the EGFR may have a role in glioma susceptibility.

Materials and Methods: To examine the association of EGFR SNP rs1468727 with glioma susceptibility in a case-control study from Türkiye (34 cases, 36 controls), genotyping and statistical analyses were performed by using real-time-polymerase chain reaction (RT-PCR) and SPSS version 25.0, respectively.

Results: A significant relationship was found between the study groups EGFR SNP rs1468727 genotypes (p = 0.028). The CC genotype frequency was significantly greater in the control group compared to the glioma group (p=0.005). When compared with the control group, the frequency of mutant type T allele carriers was significantly higher in glioma patients (p=0.012).

Conclusion: As a result of the preliminary findings, having the mutant T allele may increase risk by 3.36 times, whereas having the ancestral homozygote CC genotype lowers the risk for glioma in Turkish population.

Keywords: Glioma, EGFR gene, variation, SNP

INTRODUCTION

Gliomas, which account for 3 out of 10 of all brain tumors and 8 out of 10 malignant brain tumors, are adult tumors that develop from the neuroglia, the brain's support cells (1). They encompass malignant brain tumor groups of varying degrees, in which genetic factors affect the development and progression of the disease, and patients have a very short life expectancy after diagnosis (1- 3).

Most gliomas are the result of inherited genetic variations intertwined with environmental factors. Due to this, the roots of the evolution of gliomas can be grouped under two major headings. One is genetic factors like single nucleotide polymorphisms (SNPs), and another is cooperation among environmental elements like lifestyle habits and comorbidities (2,4). Epidemiological studies have identified numerous SNPs, including those in the epidermal growth factor receptor (EGFR) gene, as linked to gliomas (3,5,6).

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One of the ErbB receptor family members, EGFR, is responsible for inducing the proliferation to the survival of different cell types as a product of chromosome 7's short arm, plays a role in the pathogenesis of various types of cancer such as nearly half of the malignant gliomas (6,7). Further, the EGFR pathway joins into cellular processes through a progression of the cell cycle to angiogenesis, which also prepares the basis for tumorigenesis (6). The external ligand-binding region of the EGFR is activated when a ligand binds to it. This causes the receptor to dimerize and becomes tyrosine auto-phosphorylated, which activates the receptor (8). Multifarious biological processes, from somatic mutations to gene amplification, underlie the changes in EGFR gene expression. Among gliomas, both high expression and amplification of the EGFR gene are distinctive for primary glioblastoma (9). Also, alterations in the expression of this gene are known as a causative factor for resistance to treatments such as radiation and chemotherapy. Due to this feature, anti-EGFR therapies have become a treatment strategy. However, treatment strategies are aiming to rule over EGFR activation seem to be interrupted in the glioblastoma subtype (10,11). It is essential to comprehend how variations in EGFR's structure affect the development and progression of glioma types since EGFR plays a significant role in the molecular biology of gliomas. This knowledge is necessary to create effective treatment strategies.

Numerous SNPs have been reported in different genes in gliomas (4). A few of these reported SNPs (rs1052576, rs55705857, and rs17577) have been investigated to find if they are associated with gliomas, in studies performed in the Turkish population (12-15). According to human genome studies, EGFR gene SNPs may both be protective against glioma and offer a risk for it. Various populations have been studied for EGFR SNPs including rs1468727, rs730437, rs2252586, rs11979158, and rs11506105 (6). On the other hand, no studies have been performed to determine whether EGFR SNPs are linked to gliomas in the Turkish population. Although several studies have demonstrated the linkage between EGFR SNPs and gliomas, it is unclear which EGFR SNPs can serve as glioma biomarkers.

Considering the previously stated information, understanding EGFR, which contributes to tumor development by participating in processes such as metastasis , and by alternations due to different genetic and/or environmental factors, will enable the nature of gliomas to be clarified. In addition, due to the highly heterogeneous character of gliomas, examining whether it shows a population-based difference in its origin demonstrates a path for new treatment approaches. With this knowledge, this was the first Turkish population-based investigation that aimed to present a different perspective on the molecular biology of glioma by appraising the relationship between EGFR SNP rs1468727 and glioma.

MATERIALS AND METHODS

Study Population

Blood samples for this case-control study were obtained from glioma patients aged 18-85 (n = 34) and healthy individuals (n = 36) selected by the Department of Neurosurgery of Yeditepe University Hospital (Istanbul, Turkiye). This study was carried out in conformity with the principles of the Declaration of Helsinki and was approved by the Yeditepe University Faculty of Medicine's ethical committee with decision number 1757.

Genetic Analyses

The DNA of the study groups was extracted from peripheral blood samples that were kept in 5ml EDTA-coated tubes at +4°C until the analysis. An iPrep DNA extraction robot and Invitrogen iPrep Pure Link gDNA Blood Kit (Invitrogen, Life Technologies, Carlsbad, CA, USA) were utilized to perform the DNA isolation. With the help of NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA), DNA samples were measured.

The real-time-polymerase chain reaction (RT-PCR) instrument 7500 Fast (Applied Biosystems, Foster City, CA, USA) was used. This system and TaqMan Assay were used in genotyping for EGFR SNP rs1468727. F: 5'GATCCAGAAATTTAGGAGC3' and R: 5' TTTCATCACCTTGCCTCT3' were specifically designed primers for the EGFR gene (rs1468727) polymorphism (Applied Biosystems, Foster City, CA, USA).

The phases of RT-PCR after holding the stage at 95°C for ten minutes were as follows: denaturation 40 cycles at 92°C for fifteen seconds, binding/elongation step 40 cycles at 60°C for one minute.

Statistical Analyses

All data were analyzed using SPSS version 25.0. Student's t-test was utilized to evaluate the difference between healthy individuals, patients with glioma. χ^2 and Fisher's exact tests were used to analyze the EGFR SNP rs1468727 in the study population. p values that were less than 0.05 were considered significant.

RESULTS

Demographic Profile of the Study Population

In this study, genotyping in blood samples was completed in seventy individuals. Of the thirty-four glioma cases, 9 (26.48%) were female, 25 (73.52%) were male. The control group consisted of 13 females (36.12%) and 23 males (63.88%) (Table 1). The mean age (p = 0.138) and gender (p = 0.385) of the patients with glioma and healthy controls were not significant.

Genotyping

The allelic and genotypic information of the study groups are provided in Table 2. The frequencies of CC:CT:TT genotypes were 19:16:1 in healthy controls and 8:22:4 in glioma patients. As a result of our analyses, the frequency of the EGFR SNP rs1468727 was significantly different between the patients with glioma and healthy controls (p = 0.028). The genotype

distribution p values were as follows: the CC homozygous wild type (p = 0.005), CT heterozygous (p = 0.089), and TT homozygous mutant (p = 0.145).

Table 1. Demographic characteristics of patients and control groups.

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

The CC genotype was observed at lower frequency (p = 0.005, odds ratio (OR) = 0.232, 95% confidence intervals (CI) = 0.081-0.068), and T allele was found to be higher (p = 0.012, OR = 3.632, 95% CI = 1.300-10.151) in the patients with glioma. In both EGFR CT and TT genotype samples, there were no significant differences between the study groups (p = 0.089 and p = 0.145, respectively). Also, the EGFR ancestral C allele frequency was not statistically significant (p = 0.145). These results can be interpreted such that carrying T allele variant increased the glioma risk (p = 0.012, OR = 3.632, 95% CI = 1.300-10.151), and carrying CC homozygote genotype decreased the disease risk (Table 2).

DISCUSSION

Despite the current acceleration of genome research, particularly in polymorphism studies, there is still a great deal of uncertainty regarding the genetic background of glioma susceptibility. Therefore, large-scale patient-control studies to be carried out in different populations are of great importance in clarifying this molecular issue.

As a gene that encodes a transmembrane receptor tyrosine kinase and is localized on chromosome 7p12-13, EGFR is known to be involved in cell processes that are at the core of cancer research (16). Prior studies on activation of the EGFR signaling and genetic changes in its genes have reported the relationship of these changes with carcinoma development (17, 18). Especially for glioblastoma, changes in EGFR expression are more important and associated with poor outcomes (19). Amplification of EGFR caused by genetic alterations was discovered to be a type of pathological genetic change in more than 40% of gliomas (20, 21).

Molecularly targeted studies are necessary for the well-rounded treatment of cancer. Genetic investigations have been performed on human gene polymorphisms such as the EGFR SNPs in different populations and different types of cancers to identify their potential role in the diseases. Therefore, we investigated the SNP rs1468727 of the EGFR gene in a Turkish population. This SNP may be linked to factors that lead to cancer progression by allowing cells to grow and by causing changes in the activation of receptors, expression, or stability of EGFR.

The results of this study showed a link between EGFR SNP rs1468727 and glioma. In patients within the glioma group, the CC genotype was significantly lower; however, the T allele was found to possibly contribute to glioma development. Population studies have determined a link between glioma development and EGFR SNPs; a Chinese population-based study reveals the association of EGFR SNP rs1468727 with susceptibility to glioma. Li et al. have shown that the CC genotype may increase the risk of glioma (6). Furthermore,

Table 2. Genotype and allele frequencies between patients with glioma and the healthy controls.

Polymorphism	Glioma % (n)	Control % (n)	p value	Odds Ratio	95% CI
EGFR (rs1468727)	n=34	n=36	**0.028***		
CC	23.5 (8)	52.8 (19)	**0.005***	0.232	0.081-0.068
CT	64.7 (22)	44.4 (16)	0.089	2.292	0.875-6.0002
TT	11.8 (4)	2.8 (1)	0.145	4.667	0.494-44.051
	Allelic count	Allelic count			
C	55.88 (38)	75 (54)	0.145	0.214	0.023-2.023
T	44.12 (30)	25 (18)	**0.012***	3.632	1.300-10.151

*statistically significant
n: number of observation, OR: odds ratio, CI: confidence interval.

they explained that the reason for such a result is that SNP rs11468727 may increase cell proliferation by causing a change in EGFR activation (6). Also, Hou et al., obtained the same results to confirm other studies on the Han Chinese Population; the CC of this SNP was pointed out as a risk marker (22). Other studies in Chinese populations showed that the C allele (23) and the CC genotype (24) elevated the disease risk, which was consistent with other results obtained in the aforementioned studies. Also, Yu et al. stated that rs1468727 is a genetic factor that increases the risk of glioma in the Asian population (25). In addition to these results, the EGFR SNP rs1468727 TT genotype was reported as protective from glioma (26).

On the other hand, Andersson et al. and Baek et al. have investigated the effect of several SNPs in European and Korean populations with glioma. They could not validate that EGFR SNP rs1468727 affects glioma development and/or progression in both populations (27, 28).

According to the literature review, this case-control study is the first investigation to address the EGFR SNP rs1468727 as a glioma-related indication in the Turkish population. Our study revealed that EGFR gene polymorphism rs1468727 could affect glioma development. The outcomes showed that carrying EGFR SNP rs1468727 homozygous wild-type may be a risk-reducing factor ($p = 0.005$), whilst carrying the T allele may increase the risk for glioma ($p = 0.012$). These observations can be interpreted such that an intronic SNP rs1468727 may interfere with EGFR expression and have a role in glioma genetics.

As expected, different results could be reported due to variables such as ethnic differences and increasing sample sizes. Since there is a significant relationship between EGFR SNP rs1468727 and glioma, it seems to be an important gene region for elucidating the molecular mechanism of the disease. In addition, because it is a polymorphism in the intronic region, which gene regions it interacts with is another issue that needs to be investigated.

Limitations

The small sample size was a major limitation of the study. Future investigations with a larger sample size would be helpful to confirm these preliminary findings.

CONCLUSION

Consequently, this study is the first to suggest that EGFR SNP rs1468727 is associated with glioma in the Turkish population. While carrying the CC genotype appears to be a protective factor, the T allele might be a genetic marker for the risk of glioma.

Ethics Committee Approval: All procedures performed in studies involving human participants were made under the ethical standards of the 1975 Declaration of Helsinki guidelines and its later amendments. The research on humans study protocol was approved by the Yeditepe University Medical Faculty Ethics Committee (file no: 21.11.2019/1757).
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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Ostrom, QT, Francis SS, Barnholtz-Sloan JS. Epidemiology of brain and other CNS tumors. Curr Neurol Neurosci Rep 2021; 21(12): 68. [CrossRef]
2. Mayer O, Bugis J, Kozlova D, Leemann A, Mansur S, Peerutin I, et al. Cytoskeletal protein palladin in adult gliomas predicts disease incidence, progression, and prognosis. Cancers 2022; 14(20): 5130. [CrossRef]
3. Finch A, Solomou G, Wykes V, Pohl U, Bardella C, Watts C. Advances in research of adult gliomas. Int J Mol Sci 2021; 22(2): 924. [CrossRef]
4. Molinaro AM, Taylor JW, Wiencke JK, Wrensch MR. Genetic and molecular epidemiology of adult diffuse glioma. Nat Rev Neurol 2019; 15(7): 405-17. [CrossRef]
5. Kinnersley B, Houlston RS, Bondy ML. Genome-wide association studies in glioma. Cancer Epidemiol Biomarkers Prev 2018; 27(4): 418-28. [CrossRef]
6. Li B, Zhao W, Li J, Yan M, Xie Z, Zhu Y, et al. Effect of epidermal growth factor receptor gene polymorphisms on prognosis in glioma patients. Oncotarget 2016; 7(39): 63054-64. [CrossRef]
7. Arienti C, Pignatta S and Tesei A. Epidermal growth factor receptor family and its role in gastric cancer. Front Oncol 2019; 9: 1308. [CrossRef]
8. Hajdu T, Váradi T, Rebenku I, Kovács T, Szöllösi J, Nagy P. Comprehensive model for epidermal growth factor receptor ligand binding involving conformational states of the extracellular and the kinase domains. Front Cell Dev Biol 2020; 8: 776. [CrossRef]
9. Hatanpaa KJ, Burma S, Zhao D, Habib AA. Epidermal growth factor receptor in glioma: signal transduction, neuropathology, imaging, and radioresistance. Neoplasia 2010; 12(9): 675-84. [CrossRef]
10. Taylor TE, Furnari FB, Cavenee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. Curr Cancer Drug Targets 2012; 12(3): 197-209. [CrossRef]
11. Lee A, Arasaratnam M, Chan DLH, Khasraw M, Howell VM, Wheeler H. Anti-epidermal growth factor receptor therapy for glioblastoma in adults. Cochrane Database Syst Rev 2020; 5(5): CD013238. [CrossRef]
12. Oktay Y. Ulgen E, Can O, Akyerli C, Yuksel S, Erdemgil Y, et al. IDH-mutant glioma specific association of rs55705857 located at 8q24.21 involves MYC deregulation. Sci Rep 2016; 6: 27569. [CrossRef]
13. Ozden M, Katar S, Hanimoglu H, Ulu MO, Isler C, Baran O, et al. Polymorphisms in the matrix metalloproteinase-9 promoters and susceptibility to glial tumors in Turkey. Turk Neurosurg 2017; 27(5): 690-5. [CrossRef]
14. Ozdogan S, Kafadar A, Gulec Yılmaz S, Tımırcı-Kahraman O, Gormus U, Isbır T. Role of caspase-9 gene Ex5+32 G>A (rs1052576) variant in susceptibility to primary brain tumors. Anticancer Research 2017; 37 (9): 4997-5000. [CrossRef]

15. Billur D, Akdeniz FT, Güleç Yılmaz S, Barut Z, Yaltırık CK, İsbir T. Caspase-9 rs1052576 polymorphism is not associated with glioblastoma in Turkish patients. Experimed 2022; 12(3): 108-12. [CrossRef]
16. Pham D, Kris MG, Riely GJ, Sarkaria IS, McDonough T, Chuai S, et al. Use of cigarette-smoking history to estimate the likelihood of mutations in epidermal growth factor receptor gene exons 19 and 21 in lung adenocarcinomas. J Clin Oncol 2006; 24: 1700–04. [CrossRef]
17. 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 Invest 2008; 26: 434–7. [CrossRef]
18. Yano S, Kondo K, Yamaguchi M, Richmond G, Hutchison M, Wakeling A, et al. Distribution and function of EGFR in human tissue and the effect of EGFR tyrosine kinase inhibition. Anticancer Res 2003; 23: 3639–50.
19. Bienkowski M, Piaskowski S, Stoczynska-Fidelus E, Szybka M, Banaszczyk M,Witusik-Perkowska M, et al. Screening for EGFR amplifications with a novel method and their significance for the outcome of glioblastoma patients. PloS one 2013; 8: e65444. [CrossRef]
20. Sjostrom S, Andersson U, Liu Y, Brannstrom T, Broholm H, Johansen C, et al. Genetic variations in EGF and EGFR and glioblastoma outcome. Neuro Oncol 2010; 12: 815–21. [CrossRef]
21. Waha A, Baumann A, Wolf HK, Fimmers R, Neumann J, Kindermann D, et al. Lack of prognostic relevance of alterations in the epidermal growth factor receptor-transforming growth factor-alpha pathway in human astrocytic gliomas. J Neurosurgery 1996; 85: 634–41. [CrossRef]
22. Hou WG, Ai WB, Bai XG, Dong HL, Li Z, Zhang YQ, Xiong LZ. Genetic variation in the EGFR gene and the risk of glioma in a Chinese Han population. PLoS One 2012; 7(5): e37531. [CrossRef]
23. Yan M, Li J, He N, Shi X, Du S, Li B, Jin T. A case-control study of the association between the EGFR gene and glioma risk in a Chinese Han population. Oncotarget 2017; 7; 8(35): 59823-30. [CrossRef]
24. Wang X, Zhang H, Wang D, Li X. Association of genetic polymorphisms of EGFR with glioma in a Chinese population. Genet Test Mol Biomarkers 2015; 19(1): 59-62. [CrossRef]
25. Yu, X, Sun NR, Jang HT, Guo SW, Lian MX. Associations between EGFR gene polymorphisms and susceptibility to glioma: a systematic review and meta-analysis from GWAS and case-control studies. Oncotarget 2017; 8(49), 86877–85. [CrossRef]
26. Liu HB, Peng YP, Dou CW, Su XL, Gao NK, et al. Comprehensive study on associations between nine SNPs and glioma risk. Asian Pac J Cancer Prev 2012; 13: 4905–08. [CrossRef]
27. Andersson U, Schwartzbaum J, Wiklund F, Sjöström S, Liu Y, Tsavachidis S, et al. A comprehensive study of the association between the EGFR and ERBB2 genes and glioma risk. Acta Oncol 2010; 49:767–75. [CrossRef]
28. Baek IK, Cheong HS, Namgoong S, Kim JH, Kang SG, Yoon SJ, et al. Two independent variants of epidermal growth factor receptor associated with risk of glioma in a Korean population. Sci Rep 2022; 12(1): 19014. [CrossRef]