Turk Neurosurg 31(4):587-593, 2021

Original Investigation

DOI: 10.5137/1019-5149.JTN.33347-20.2

Received: 30.11.2020
Accepted: 09.12.2020

Published Online: 25.05.2021

Association of MTHFR, MTRR and RAD54L Gene Variations with Meningioma and Correlation with Tumor's Histopathological Characteristics on Turkish Cohort

Timucin AVSAR[1,2,3], Rashid MOHIYUDDIN[2,3], Seyma CALIS[3,4], Ozlem YAPICIER[5], Turker KILIC[2,3,6]

[1]Bahcesehir University, School of Medicine, Department of Medical Biology, Istanbul, Turkey
[2]Bahcesehir University, Health Sciences Institute, Neuroscience Program, Istanbul, Turkey
[3]Bahcesehir University, Health Sciences Institute, Neuroscience Laboratory, Istanbul, Turkey
[4]Istanbul Technical University, Engineering and Technology, Graduate School of Science, Molecular Biology, Genetics and Biotechnology Graduate Program, Istanbul, Turkey
[5]Bahcesehir University, School of Medicine, Department of Neurosurgery, Istanbul, Turkey
[6]Bahcesehir University, School of Medicine, Department of Pathology, Istanbul, Turkey

Corresponding author: Timucin AVSAR ✉ timucin.avsar@med.bau.edu.tr

ABSTRACT

AIM: To elucidate the association of the MTHFR, MTRR, and RAD54L gene variations with meningioma in Turkish cohort.

MATERIAL and METHODS: DNAs were isolated from 87 retrospective meningioma samples. The MTHFR, MTRR, and RAD54L gene hotspot regions were amplified with specific primers via polymerase chain reaction (PCR), and next-generation sequencing (NGS) was performed. All the detected variations and single-nucleotide polymorphisms (SNPs) were listed and compared with healthy control frequencies in different genomic databases. The histopathological characteristics of meningiomas and genomic variations were compared. Pearson's chi-squared test was used to detect the statistical differences of SNPs, and correlation analysis was conducted.

RESULTS: rs1801131, rs1801133, and rs4846051 on MTHFR, rs1801394 on MTRR, and rs1048771 on RAD54L gene frequencies were found to be significantly altered in the overall cohort of 87 patients with meningioma. The frequency of rs18011031 is 0.09 in the meningioma cohort, which is significantly correlated with WHO tumor grades ($p = 0.038$). The frequency of rs18011033 is 0.29 in the meningioma cohort, which is significantly correlated with WHO tumor grades ($p = 0.045$). Furthermore, the frequency of rs4846051 is 0.18 in the meningioma cohort, which is significantly correlated with WHO tumor grades ($p = 0.023$) and also with low Ki67 proliferation index ($p = 0.00455$). The frequency of rs1801394 is 0.15 and significantly associated with high Ki67 proliferation index in the meningioma cohort ($p = 0.0144$). The frequency of rs1048771 is 0.09 in the meningioma cohort and is significantly associated with the non-necrotic histopathological form of the tumor ($p = 0.05$).

CONCLUSION: We reported a significant association between the genetic alterations of folate metabolism (MTHFR, MTRR) and DNA repair mechanism (RAD54L) genes with the histopathological characteristics of meningioma. Five significant SNPs on these genes and four significant correlations of SNPs with histopathological characteristics were identified. This is a preliminary promising study conducted to establish the genetic marker analysis for meningioma diagnosis and prognosis for folate metabolism and DNA repair genes in Turkish cohort.

KEYWORDS: Meningioma, Histopathology, Genetic markers, Folate metabolism genes, DNA repair genes

ABBREVIATIONS: MTHFR: Methylenetetrahydrofolate reductase, **MTRR:** 5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase, **RAD54L:** DNA repair and recombination protein RAD54-like, **NF2:** Neurofibromatosis 2, **MADH2:** Mothers against decapentaplegic homolog 2, **MADH4:** Mothers against decapentaplegic homolog 4, **APM-1:** Adipose most abundant gene transcript 1, **DCC:** Deleted in Colorectal Carcinoma, **SMO:** Smoothened, frizzled class receptor, **TRAF7:** TNF Receptor Associated Factor 7, **KLF4:** Kruppel Like Factor 4, **AKT1:** AKT Serine/Threonine Kinase 1, **ANOVA:** Analysis of variance

Timucin AVSAR : 0000-0001-8841-4811
Rashid MOHIYUDDIN : 0000-0002-3100-5117
Seyma CALIS : 0000-0003-2959-5055
Ozlem YAPICIER : 0000-0001-6977-8927
Turker KILIC : 0000-0001-5982-9700

INTRODUCTION

Meningiomas are the most common non-glial primary tumors of the central nervous system. Furthermore, it is the most common extra-axial neoplasm that accounts for approximately 37.1% of all intracranial tumors (12). Meningiomas arise from the arachnoid cap cells and are mostly benign; however, slow growth has been observed. The discovery of meningiomas is difficult, and if left untreated, they can cause severe disability and chronic illness to the patient, depending on their exact location (4).

Meningiomas classified as WHO grade I are benign; WHO grade II, intermediate or atypical; and WHO grade III, anaplastic. Anaplastic meningiomas exhibit a more aggressive biological behavior and tend to invade the brain as well as infiltrate and affect the bones, muscles, dura, or venous sinuses (3).

The treatment protocol for meningioma is designed very carefully by the surgeon, taking into consideration numerous factors. The most important factors that contribute to the prediction of the respective treatment for meningioma include the appropriate location of the tumor; awareness of the nature of the tumor, whether it is benign or malignant; and the feasibility of potential treatment options and health preferences by the clinicians (1). About 70%–80% of meningiomas are suitable for a surgical resection. While the recurrence rate of grade I meningioma is as low as 5%–10%, those of grade II and III meningiomas are 50% and 80%, respectively, upon total resection in a 5-year period (12).

To detect potentially pathogenic meningiomas at early onset and accurately define their histopathological grades, genetic markers must be considered. Many studies that emphasize the role of genetics in meningioma have been conducted. Gene variations, which are known to be driver mutations that contribute to the development of meningiomas, have been reported, such as focal mutations and intrinsic aberrations of the NF2 gene (6); alterations of *MADH2*, *MADH4*, *APM-1*, and *DCC* genes (5); and mutations of *SMO*, *TRAF7*, *KLF4*, and *AKT1* genes (9).

Further advancements in meningioma research, which are significantly promising, will certainly affect the molecular taxonomy of meningioma. However, a significant number of meningiomas still remain to be unidentifiable on the basis of oncogenic driver variations. Therefore, new potential biological markers related to certain pathways need to be determined. For instance, in an attempt to define markers such as folate metabolism genes, which have certain polymorphisms and are believed to be involved with various human cancers, studies have been conducted on different cohorts of meningioma patients (2,7,8). Similarly, DNA repair genes and their mutations are found to be associated with tumor development (22).

Here, we investigated the association of the *MTHFR*, *MTRR*, and *RAD54L* gene variations with meningioma in Turkish patients because we believe that particular variations of these genes are correlated with the pathological characteristics of meningioma. In a cohort consisting of 87 patients, 5 particular single-nucleotide polymorphism (SNPs) were found to be significantly associated with meningioma and its histopathological characteristics.

MATERIAL and METHODS

Samples and Patient's Information Collection

A total of 87 meningioma samples were retrospectively obtained from Bahcesehir University, School of Medicine, Brain Tumor Tissue Biobank. All the patients previously underwent operation at the neurosurgery department, and pathological specimens were processed at the neuropathology department. The pathology reports of patients with meningioma were reviewed after obtaining approval from our Institutional Research Ethics Committee (2018-16/03). Sample selection was performed based only on the pathology results. The pathological data and clinical notes collected for each of the patient included the following information: age, gender, pathology, histopathological subtype, recurrence, WHO grade, and Ki67 score, with level of necrosis.

DNA Isolation and Library Preparation for Sequencing

For DNA isolation, tumor tissue samples were obtained from tumor biobank and washed with cold phosphate-buffered saline. About 10–20 mg of the tissue was used for crude DNA extraction. The alkaline lysis protocol was followed. Briefly, the tumor sample was first incubated in an alkaline solution (1 mM Na2EDTA, 25 mM NaOH, pH 12) for 7–8 min at 95 °C. Then, an equal volume of a neutralization buffer (40 mM Tris–HCl buffer) was added. Subsequently, a supernatant for quick centrifugation was used or stored at –20°C until use.

PCR amplification was performed for library preparation prior to sequencing. The mutation hotspot regions of the *MTHFR*, *MTRR*, and *RAD54L* genes were amplified by using specific primers as provided in Supplementary Table I. Genomic DNA (50 ng) was used for PCR. All PCR products were subjected to agarose gel electrophoresis and were cleaned up and all PCR product amplicons were mixed prior to library preparation for next-generation sequencing (NGS).

Next-Generation Sequencing (NGS)

A total of 96 samples were prepared for NGS using the DNA Flex Library Prep Kit (Illumina Inc., San Diego, USA) according to the manufacturer's protocol. Briefly, PCR amplicons larger than 150 bp were pooled and tagmented. Amplicons were diluted to 500 ng DNA amplicon concentration and assessed *via* Qubit high sensitivity assay (Thermo Fisher Scientific). Amplicon enriched samples were sequenced with paired end reaction MiSeq sequencing platform (Illumina Inc., San Diego, USA).

Fastq files were obtained and processed using freely available online tools. NGS QC Toolkit v2.3.3 was used to determine the base quality. Low-quality bases ($Q < 30$) were trimmed using FASTX-Toolkit 0.0.13. The BWA-MEM algorithm was adopted to align the reads on the hg19 human reference genome. Following GATK recalibration, indel alignment, and duplication removals, BAM files were obtained, which were screened for mutations using the Genome Browser software (Golden Helix, MT, USA).

Statistical Analysis

SNP data converted into tabular form for further assessment for each mutant SNP with its respective demographic and pathological parameters. GraphPad Prism8 (GraphPad Software, San Diego, USA) was utilized to assess the findings on statistical basis. The results for the meningioma cohort were compared with different exome databases containing healthy people frequencies of selected variations. Exome Aggregation Consortium (ExAC), the Genome Aggregation Database (gnomAD), and 1000 Genomes Project database were used to obtain major allele frequency of each genomic variations for the investigated variations.

Pearson's chi-squared test was employed to determine statistical differences in categorical variables. Multiple comparisons of the continuous variables were performed using the Tukey–Kramer method. A p-value less than 0.05 was considered statistically significant, and the statistical results for each of the SNP against each parameter of the histopathological data that we collected (including meningioma types, meningioma subtypes, meningioma's WHO grade, Ki67 proliferation index scores, necrosis level, and recurrence of each patient) were analyzed.

Table I: Demographic and Pathologic Characteristics of Meningioma Patients (n=87)

Characteristic	Value
Age (Mean ± SD) (years)	55.3 ± 14.7
Gender	
Male	34
Female	53
Pathology	
Typical	60
Atypical	23
Anaplastic	4
Histopathological Subtype	
Meningothelial	57
Non-meningothelial	30
Grade	
I	60
II	23
III	4
Ki-67 index (% Mean ± SD)	4.2 ± 3.6
Negative or Ki-67<10	77
Ki-67>10	10
Necrosis	
Negative	78
Positive	9
Recurrence	
Negative	80
Positive	7

RESULTS

Total of 87 patients' meningioma tissues have been evaluated. The cohort data is presented in Table I. The median age of the cohort was 55.3 years old, with a standard deviation of 14.7 years and male to female ratio of 1:1.56. Among the tumors, 61 were grade I (benign), 23 were grade II, and 4 were grade III. The findings of the pathological assessment were as follows: 60 typical, 23 atypical, and 4 anaplastic meningiomas; 57 of them were meningothelial, and 30 were non-meningothelial. Average of 87 patients' Ki67 proliferation index was 4.2% ± 3.6. All the samples were classified into two groups: high Ki67-expressing tumor group (>10) (n=77), and negative/low Ki67-expressing tumor group (<10) (n=10). Of the 87 tumors, 8 demonstrated necrotic activities. A total of 80 patients demonstrated no recurrence in the 5-year follow-up, and 7 patients experienced tumor recurrence.

Five different SNPs have been evaluated in the meningioma cohort. rs1801131, rs1801133, and rs4846051 on *MTHFR*, rs1801394 on *MTRR*, and rs1048771 on *RAD54L* gene frequencies were found to be significantly altered in the overall cohort or significantly correlated with the histopathological characteristics of the tumors in our meningioma cohort when compared with the healthy subject frequencies obtained from different databases (Table II).

The identified SNPs were analyzed according to their correlation with six histopathological criteria: type of meningioma, respective histopathological subtypes, WHO grade, proliferation index, level of necrosis, and disease recurrence. Chi-squared test, Fisher's exact test, and ANOVA test were used for correlation analysis.

rs18011031 is a missense variation causing to p.Glu470Ala (g.16685A>C) amino acid conversion on the *MTHFR* gene. The frequency of rs18011031 is 0.09 in the meningioma cohort. It is significantly less than the frequencies in the ExAC (0.29), gnomAD (0.28), and 1000 Genomes Project (0.25) databases ($p<0.01$). Moreover, it is significantly correlated with the WHO tumor grades ($p=0.038$) (Figure 1A). rs1801133 is missense variation that causing to p.Ala263Val (g.14783C>T) amino acid conversion on the *MTHFR* gene. The frequency of rs18011033 is 0.29 in the meningioma cohort. Although it is not significantly different from those of the ExAC (0.30), gnomAD (0.31), and 1000 Genomes Project (0.25) databases, the rs18011033 variation significantly correlated with the WHO tumor grades ($p=0.045$). Grade I tumors have more variations compared with grades II and III (Figure 1B). rs4846051 is a synonymous variation on the *MTHFR* gene. The frequency of rs4846051 is 0.18 in the meningioma cohort. It is significantly higher than

Table II: Genomic Variations on MTHFR, MTRR and RAD54L Genes. Variation Frequencies were Compared within Various Databases. Bold Lines Indicates Statistical Significance

Gene	Variation on DNA	rs number	Variation on protein	Meningioma Cohort Frequency	ExAC Frequency	gnomAD Frequency	1000 Genomes Frequency
MTHFR	**g.16685A>C**	**rs1801131**	**p.Glu470Ala**	**0.09**	**0.29**	**0.28**	**0.25**
MTHFR	g.14783C>T	rs1801133	p.Ala263Val	0.26	0.30	0.31	0.25
MTHFR	**g.16704C>T**	**rs4846051**	**p.Phe476=**	**0.18**	**0.03**	**0.02**	**0.10**
MTRR	**g.6757A>G**	**rs1801394**	**p.Ile22Met**	**0.15**	**0.47**	**0.46**	**0.36**
RAD54L	g.35534C>T	rs1048771	p.Ala730=	0.09	N/A	0.10	0.19

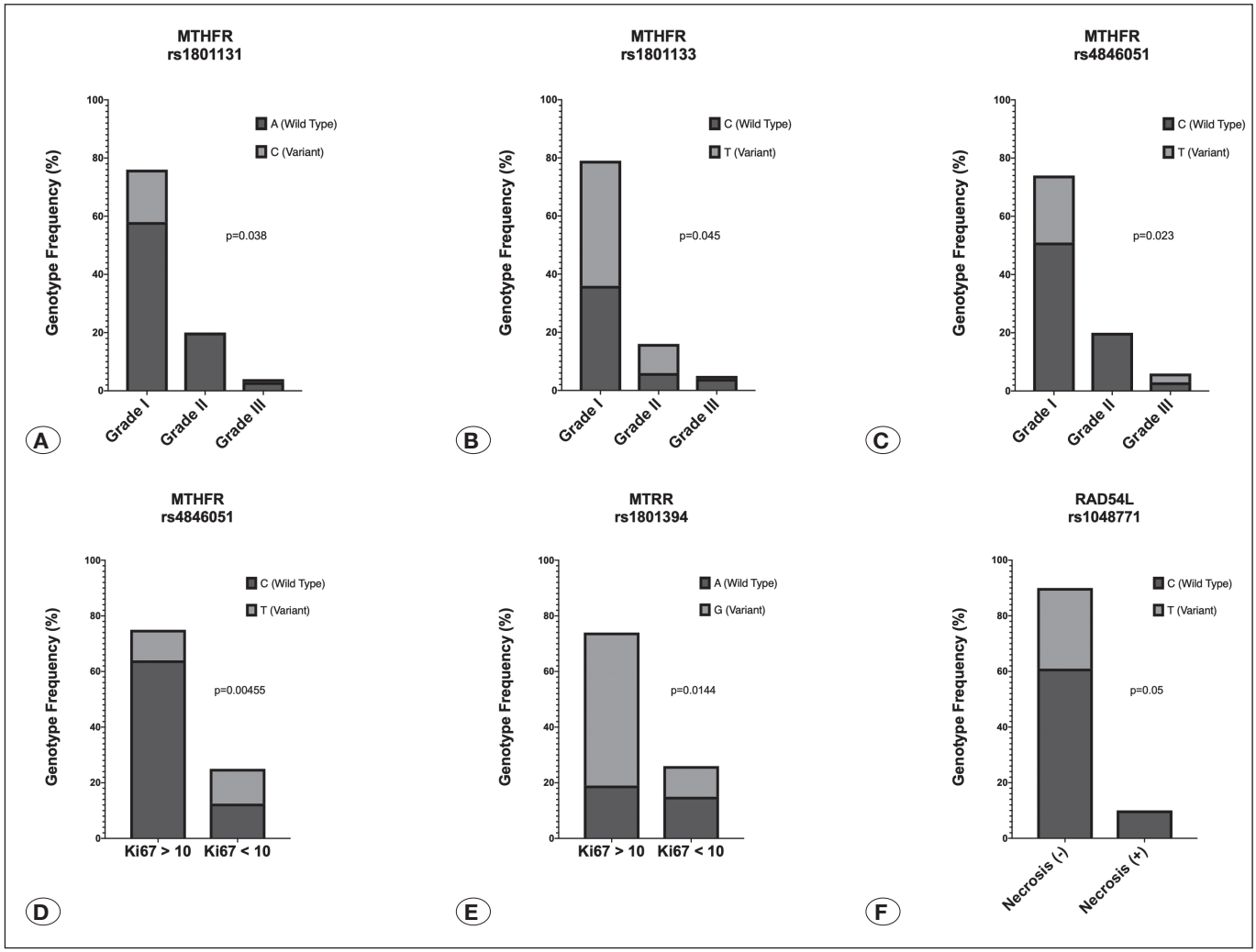

Figure 1: SNPs associated with tumor histopathological characteristics. **A)** MTHFR gene rs1801131 variation, C variant significantly increased in WHO grade I, **B)** MTHFR gene rs1801133 variation, T variant significantly increased in WHO grade I and II, **C)** MTHFR gene rs4846051 variation, T variant significantly increased in WHO grade I, **D)** MTHFR gene rs4846051 variation, T variant significantly increased in Ki67<10 group, **E)** MTRR gene rs1801394 variation, G variant significantly increased in Ki67>10 group, **F)** RAD54L gene rs41048771 variation, T variant significantly increased in non-necrotic meningioma group.

those of the ExAC (0.03), gnomAD (0.02), and 1000 Genomes Project (0.10) databases (p<0.01). Moreover, it is significantly correlated with the WHO tumor grades and accumulated more on grade I tumors (p=0.023) (Figure 1C). rs4846051 variation was also found to be significantly associated with low Ki67 proliferation index (p=0.00455) (Figure 1D). rs1801394 is a missense variation on the *MTRR* gene, causing to p.Ile22Met (g.6757A>G) amino acid conversion. The frequency of rs1801394 is 0.15 in the meningioma cohort. It is significantly less than those of the ExAC (0.47), gnomAD (0.46), and 1000 Genomes Project (0.36) databases (p<0.01). Moreover, it is significantly associated with high Ki67 proliferation index on the meningioma cohort (Figure 1E) (p=0.0144). Lastly, the rs1048771 variation on the *RAD54L* gene is synonymous variation causing no amino acid change. The frequency of rs1048771 is 0.09 in the meningioma cohort. Although it is not significantly different from those of the gnomAD (0.10) and 1000 Genomes Project (0.19) databases, the rs1048771 variation is significantly associated with the non-necrotic histopathological form of tumor (p=0.05) (Figure 1F).

DISCUSSION

In this study, we investigated the association of the folate mechanism- and DNA repair mechanism-associated genes in Turkish meningioma cohort. To the best of our knowledge, this is the first study to investigate the Turkish meningioma population using an extended approach, including not only single-point variations on a gene but also all hotspot variation regions of the aforementioned genes as well as their association with histopathological characteristics. The recent advancements in meningioma research revealed some of the very important connections between the genetic profiles and histopathological features. The proper diagnosis requires objective approach and parameters rather than subjective guidelines. The objective markers have always been proven to be useful for patient assessment, which enables the design of a good treatment model. The results of this study are quite promising and can be considered for a larger cohort of studies of a variety of genes involved in meningiomas. The risk of meningioma and its association with the genes *MTHFR*, *MTRR*, and *RAD54L* have been investigated by numerous studies.

MTHFR is a gene coding for methylene tetrahydrofolate reductase enzyme that is plays a significant role in folate vitamin metabolism. Moreover, it is important for the multistep process that converts the amino acid homocysteine to another amino acid, namely, methionine. It is a central enzyme in cellular metabolism. Numerous studies suggest the association of *MTHFR* polymorphisms with the risk of meningioma. In our study, among several single-nucleotide variations within the *MTHFR* gene, three SNPs were significantly altered in our cohort. rs1801131 (g.16685A>C, p.Glu470Ala), frequency of C allele (minor allele) in our cohort is 0.09. rs1801133 (g.14783C>T, p.Ala263Val), frequency of T allele (minor allele) in our cohort is 0.26 and rs4846051 (g.16704C>T, p.Phe476=) frequency of T allele (minor allele) in our cohort is 0.18. All of them were significantly altered compared with those of healthy controls reported in large databases. Moreover, they are significantly associated with the WHO tumor grades as mentioned above. Kumawat et al. conducted a study on Indian cohort, and the A1298C (rs1801131) SNP was found to be a significant risk factor of meningioma (11). They reported reduced C allele frequency in their 76 meningioma samples and concluded that rs1801131 C allele increases homocysteine level and plays a protective role (17). Zhang et al. conducted a case–control study by analyzing 600 meningioma and 600 control samples from Chinese Han population. They employed the genotyping method for selected variations. In addition, they revealed no association of rs1801131 but a significant association with C677T (rs1801133) T allele with meningioma occurrence as well as an increased risk of meningioma development. Further, they revealed no association between the WHO tumor grades and SNPs (24). Contrary to the study by Zhang et al., in our study, a significant association of rs1801131, rs1801133, and rs4846051 SNPs of the *MTHFR* gene and WHO tumor grades was observed. This indicates a minor allele occurrence in WHO grade I tumors (Figure 1A-C). Bethke et al. screened British meningioma cohort for rs1801131 and rs1801133 SNPs and revealed a significant association of both SNPs with increased risk of minor allele occurrence (2). To the best of our knowledge, there is only one study that analyzed the C677T SNP of the *MTHFR* gene in Turkish meningioma cohort, namely, the study by Kafadar et al. They reported no association in 74 meningioma patients (10). As far as we know, the rs4846051 (Phe476=) variation of *MTHFR* was not previously reported for meningioma, and Kafadar et al. reported for the first time a significant association with the occurrence of meningioma and MTHFR gene variation. rs4846051 was also found to be significantly associated with the Ki67 score, which is a proliferation marker for tumors cells. The G variant is more frequent in the group with a Ki67 score below 10 (Figure 1D). In spite of the established effect of the *MTHFR* gene variations, there are conflicting results in different cohorts. However, our study reported a significant risk of the three SNPs listed above and association with histopathological characteristics.

The *MTRR* gene codes for enzyme called methionine synthase reductase which plays a role in amino acid methionine biosynthesis. It is another key enzyme in amino acid metabolism; therefore, variations on the *MTRR* gene are extensively analyzed in several tumors, including meningioma (2,18,23). Kumawat et al. analyzed the *MTRR* gene exon 2 A66G (rs1801394) variation on their Indian cohort and reported a reduced risk association of G allele with meningioma along with the *MTHFR* gene rs1801131 variation (11). Zhang et al. (2013) and Bethke et al. (2008) have yielded contrary results and reported no association of WHO tumor grades or other histopathological characteristics with the G allele SNP of *MTRR*, as a risk factor for adult meningioma in Chinese Han ethnicity and British populations, respectively (2,24). In the meta-analysis of the A66G polymorphism in 2,236 cases and 2,248 controls conducted by Chen D et al., a significant association between *MTRR* A66G (rs1801394) and *MTHFR* A1298C (rs1801131) was observed, which contribute to the genetic susceptibility to meningioma (7). There is no other SNP reported on the *MTRR* gene for meningioma. There

are limited studies reporting on the *MTRR* gene variations and meningioma association, and none of them reported a correlation between histopathological characteristics and SNPs. In our study, we revealed that the rs1801394 SNP G minor allele significantly increased in the meningioma cohort compared with the controls in different databases (Table II). Furthermore, we reported that Ki67 score above 10 is significantly associated with rs1801394 variation (Figure 1E).

RAD54L gene codes for DNA repair and recombination protein RAD54-like. Moreover, it plays a significant role in homolog recombination for the repair of double-stranded DNA breaks, single-stranded DNA breaks, and collapsed replication forks (14,20,21). The *RAD54L* gene has been previously proposed as a potential onco-suppressor, and its mutations/variations can be linked to the development of breast cancer (19) and colon carcinoma and lymphoma (15). *RAD54L* variations were also proposed as risk factors for meningioma; thus, Leone et al. investigated the rs1048771 2290C/T variation in 70 meningioma and 287 control samples in Spain and Ecuador cohorts. They suggested a significant association between minor T allele and meningioma development (13). A study by Mendiola et al. analyzed 29 meningioma patients and revealed that most of the sporadic tumors exhibited allelic loss in 1p32, which bears the *RAD54L* gene (16). In our study, we found that the rs1048771 variation significantly increased in the meningioma cohort compared with healthy controls in the databases (Table II). Further, it is found to be significantly associated with the non-necrotic form of tumor. The T allele is only found in non-necrotic meningioma (Figure 1F).

CONCLUSION

In this retrospective study, we aimed to determine whether there is an association between the genetic alterations of folate metabolism (*MTHFR* and *MTRR* genes) as well as DNA repair mechanism genes (*RAD54L*) and the histopathological characteristics of meningioma. We found five significant single-nucleotide polymorphisms (SNPs) on three genes and four significant correlations of SNPs with the tumor grades, Ki67 scores, and necrotic characteristics of tumor in Turkish meningioma cohort. To validate our results, other large-scale studies should to be conducted in order to add new genetic parameters to the diagnosis and prognosis of meningioma.

REFERENCES

1. Apra C, Peyre M, Kalamarides M: Current treatment options for meningioma. Expert Rev Neurother 18:241-249, 2018
2. Bethke L, Webb E, Murray A, Schoemaker M, Feychting M, Lönn S, Ahlbom A, Malmer B, Henriksson R, Auvinen A, Kiuru A, Salminen T, Johansen C, Christensen HC, Muir K, McKinney P, Hepworth S, Dimitropoulou P, Lophatananon A, Swerdlow A, Houlston R: Functional polymorphisms in folate metabolism genes influence the risk of meningioma and glioma. Cancer Epidemiol Biomarkers Prev 17:1195-1202, 2008
3. Brassesco MS, Valera ET, Neder L, Castro-Gamero AM, de Oliveira FM, Santos AC, Scrideli CA, Oliveira RS, Machado HR, Tone LG: Childhood radiation-associated atypical meningioma with novel complex rearrangements involving chromosomes 1 and 12. Neuropathology 29:585-590, 2009
4. Buerki RA, Horbinski CM, Kruser T, Horowitz PM, James CD, Lukas RV: An overview of meningiomas. Future Oncol 14:2161-2177, 2018
5. Büschges R, Ichimura K, Weber RG, Reifenberger G, Collins VP: Allelic gain and amplification on the long arm of chromosome 17 in anaplastic meningiomas. Brain Pathol 12:145-153, 2002
6. Cai DX, Banerjee R, Scheithauer BW, Lohse CM, Kleinschmidt-Demasters BK, Perry A: Chromosome 1p and 14q FISH analysis in clinicopathologic subsets of meningioma: Diagnostic and prognostic implications. J Neuropathol Exp Neurol 60:628-636, 2001
7. Chen D, Dong J, Huang Y, Gao F, Yang X, Gong X, Lv X, Chu C, Wu Y, Zheng Y: Folate metabolism genetic polymorphisms and meningioma and glioma susceptibility in adults. Oncotarget 8:57265-57277, 2017
8. Choi SW, Mason JB: Folate and carcinogenesis: An integrated scheme. J Nutr 130:129-132, 2000
9. Clark VE, Harmancı AS, Bai H, Youngblood MW, Lee TI, Baranoski JF, Ercan-Sencicek AG, Abraham BJ, Weintraub AS, Hnisz D, Simon M, Krischek B, Erson-Omay EZ, Henegariu O, Carrión-Grant G, Mishra-Gorur K, Durán D, Goldmann JE, Schramm J, Goldbrunner R, Piepmeier JM, Vortmeyer AO, Günel JM, Bilgüvar K, Yasuno K, Young RA, Günel M: Recurrent somatic mutations in POLR2A define a distinct subset of meningiomas. Nat Genet 48:1253-1259, 2016
10. Kafadar AM, Yilmaz H, Kafadar D, Ergen A, Zeybek U, Bozkurt N, Kuday C, Isbir T: C677T gene polymorphism of methylenetetrahydrofolate reductase (MTHFR) in meningiomas and high-grade gliomas. Anticancer Res 26:2445-2449, 2006
11. Kumawat R, Gowda SH, Debnath E, Rashid S, Niwas R, Gupta Y, Upadaya AD, Suri A, Chandra PS, Gupta DK, Lakshmy R, Sarkar C, Sinha S, Chosdol K: Association of single nucleotide polymorphisms (SNPs) in genes encoding for folate metabolising enzymes with glioma and meningioma in indian population. Asian Pac J Cancer Prev 19:3415-3425, 2018
12. Lee YS, Lee YS: Molecular characteristics of meningiomas. J Pathol Transl Med 54:45-63, 2020
13. Leone PE, Mendiola M, Alonso J, Paz-y-Miño C, Pestaña A: Implications of a RAD54L polymorphism (2290C/T) in human meningiomas as a risk factor and/or a genetic marker. BMC Cancer 3:6, 2003
14. Machida S, Takaku M, Ikura M, Sun J, Suzuki H, Kobayashi W, Kinomura A, Osakabe A, Tachiwana H, Horikoshi Y, Fukuto A, Matsuda R, Ura K, Tashiro S, Ikura T, Kurumizaka H: Nap1 stimulates homologous recombination by RAD51 and RAD54 in higher-ordered chromatin containing histone H1. Scientific Reports 4:4863, 2014
15. Matsuda M, Miyagawa K, Takahashi M, Fukuda T, Kataoka T, Asahara T, Inui H, Watatani M, Yasutomi M, Kamada N, Dohi K, Kamiya K: Mutations in the RAD54 recombination gene in primary cancers. Oncogene 18:3427-3430, 1999

16. Mendiola M, Bello MJ, Alonso J, Leone PE, Vaquero J, Sarasa JL, Kusak ME, De Campos JM, Pestaña A, Rey JA: Search for mutations of the hRAD54 gene in sporadic meningiomas with deletion at 1p32. Molecular Carcinogenesis 24:300-304, 1999
17. Olteanu H, Munson T, Banerjee R: Differences in the efficiency of reductive activation of methionine synthase and exogenous electron acceptors between the common polymorphic variants of human methionine synthase reductase. Biochemistry 41:13378-13385, 2002
18. Pardini B, Kumar R, Naccarati A, Prasad RB, Forsti A, Polakova V, Vodickova L, Novotny J, Hemminki K, Vodicka P: MTHFR and MTRR genotype and haplotype analysis and colorectal cancer susceptibility in a case-control study from the Czech Republic. Mutat Res 721:74-80, 2011
19. Rasio D, Murakumo Y, Robbins D, Roth T, Silver A, Negrini M, Schmidt C, Burczak J, Fishel R, Croce CM: Characterization of the human homologue of RAD54: A gene located on chromosome 1p32 at a region of high loss of heterozygosity in breast tumors. Cancer Res 57:2378-2383, 1997
20. Spies J, Waizenegger A, Barton O, Sürder M, Wright WD, Heyer WD, Löbrich M: Nek1 regulates Rad54 to orchestrate homologous recombination and replication fork stability. Mol Cell 62:903-917, 2016
21. Swagemakers SM, Essers J, de Wit J, Hoeijmakers JH, Kanaar R: The human RAD54 recombinational DNA repair protein is a double-stranded DNA-dependent ATPase. J Biol Chem 273:28292-28297, 1998
22. Torgovnick A, Schumacher B: DNA repair mechanisms in cancer development and therapy. Front Genet 6:157, 2015
23. Weiner AS, Boyarskikh UA, Voronina EN, Selezneva IA, Sinkina TV, Lazarev AF, Petrova VD, Filipenko ML: Polymorphisms in the folate-metabolizing genes MTR, MTRR, and CBS and breast cancer risk. Cancer Epidemiol 36:e95-e100, 2012
24. Zhang J, Zhou YW, Shi HP, Wang YZ, Li GL, Yu HT, Xie XY: 5,10-Methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTRR), and methionine synthase reductase (MTR) gene polymorphisms and adult meningioma risk. J Neurooncol 115:233-239, 2013

Supplementary Table I: Primer Sequences of MTHFR, MTRR and RAD54 Genes

Gene	Primer name	Sequence (5' to 3')
MTHFR	*MTHFR* gen-seq-1_Forward	CCTGATTTGCTTGGCTGCTC
	MTHFR gen-seq-1_Reverse	ACTCAGCGAACTCAGCACTC
MTHFR	*MTHFR* gen-seq-2_Forward	CTTGTGGTTGACCTGGGAGG
	MTHFR gen-seq-2_Reverse	TTTGCCTCCCTAAGCCCTTC
MTRR	*MTRR* gen-seq_Forward	AGGCTCATTTGAGATTAGTGCTGA
	MTRR gen-seq_Reverse	CTGTCATTTACAGTTAAGGAGTTGTTAC
RAD54L	*RAD54* gen-seq_Forward	CACTTCTCTCTGGGCGAGTT
	RAD54 gen-seq_Reverse	TTCTTCCCTGCTGGGCTTAC