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Associations of LncRNA *H19* Polymorphisms at MicroRNA Binding Sites with Glioma Susceptibility and Prognosis

**Running title:** *H19* polymorphisms and glioma

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Abstract

Glioma is the most common tumor of the central nervous system; variation in susceptibility and prognosis worldwide suggests that there is molecular and genetic difference among individuals. The *H19* gene plays a dual role in carcinogenesis. In this study, associations between *H19* polymorphisms and susceptibility as well as prognosis in glioma were evaluated. In total, 605 patients with glioma and 1,300 cancer-free subjects were enrolled in the study. Individuals with the rs3741219 A>G allele were less likely to develop glioma (RR = 0.54, 95% CI: 0.45-0.63, \( P < 0.001 \)). Whereas rs217727 G>A and rs2839698 G>A genotypes were not associated with glioma risk. The associations between *H19* polymorphisms and prognosis were assessed, including overall survival and progression-free survival. Three focused *H19* polymorphisms did not show a significant effect on survival. Further analysis based on false positive report probability had validated this significant results. In the haplotype analysis, individuals with the G<sub>rs217727</sub>A<sub>rs2839698</sub>G<sub>rs3741219</sub> haplotype were less likely to develop glioma (OR = 0.33, 95% CI: 0.23-0.46, \( P = 0.02 \)). Overall, carriers of the rs3741219 AG or GG genotype of *H19* have a decreased susceptibility to glioma, but polymorphisms in this gene are not related to prognosis.

**Keywords:** *H19*, single nucleotide polymorphisms, glioma, susceptibility, prognosis
Introduction

Glioma, which arises from glial or precursor cells, is one of the most common and highly fatal brain tumors.\(^1\)\(^2\) According to the biological and clinical characteristics, glioma is classified into four WHO grades (I to IV).\(^3\) Despite substantial advances in multimodal treatments, including surgery, radiotherapy, and chemotherapy, the overall survival (OS) of patients with glioma remains poor,\(^4\) and vary by race or ethnicity.\(^5\) The risk factors of glioma include allergies/atopic disease, genetic factors, and ionizing radiation et al.\(^6\)\(^7\)

Glioma is a typical example of disease, for which molecular and genetic diagnosis affect patient treatment.\(^8\) Long non-coding RNAs (lncRNAs) play crucial roles in glioma occurrence and development, including tumorigenesis, angiogenesis, and migration.\(^9\)\(^10\) Single nucleotide polymorphisms (SNPs) contribute to altering the binding of transcription factors, RNA splicers, and gene promoters, thereby regulating gene function.\(^11\)

\(H19\), a paternally inherited gene located on chromosome 11p15.5, and is tightly linked to the insulin-like growth factor 2 (IGF2) gene.\(^12\) The imprinted gene \(H19\) does not encode any protein, but encodes a capped, spliced, polyadenylated, and oncofetal 2.7-kb RNA that is down-regulated postnatally.\(^11\) Genome-wide association studies have identified inherited risk factors as a feature of brain cancer genetics, and have indicated that SNPs are usually present in patients with glioma.\(^13\) Effect of \(H19\) on carcinogenesis is controversial. Jiang et al. found that \(H19\) promotes the invasion and tumorigenicity of glioblastoma cells and could be a therapeutic target for
glioblastoma. The rs2071095 in H19 is linked with the risk of breast cancer. The rs2839698 might predict the risk and prognosis of hepatocellular cancer. In addition, the rs3024270 GG genotype might increase neuroblastoma risk in female Chinese children. In contrast, the rs2839698 TC genotype of H19 significantly decreases the risk of bladder cancer. Another six-center case-control study stated that none of three SNPs (rs2839698 G>A, rs3024270 C>G, rs217727 G>A) were relevant to the neuroblastoma susceptibility.

However, the association between H19 SNPs and glioma has not been examined to date. Hence, this hypothesis-driven case-control study aimed to investigate the associations between three SNPs (rs217727 G>A, rs2839698 G>A, and rs3741219 A>G) in H19 and glioma susceptibility and prognosis.

**Results**

**Characteristics of study subjects**

All 605 patients with glioma (270 females and 335 males) included in this study were of Han Chinese ethnicity. The survival time for patients ranged from 2 to 44 months, with a median survival time of 11 months. In addition, the clinical characteristics included gender, age, WHO grade, history of surgery, radiotherapy, and chemotherapy (Table S1). Patients were divided into two groups according to WHO grade: 382 patients (63.1%) with grades I–II, and 223 patients (36.9%) with grades III–IV. A total of 416 patients (68.8%) underwent gross total resection (GTR), and 189 patients (31.2%) underwent subtotal resection (STR) or near total resection.
(NTR). Except for 60 patients, all subjects received radiotherapy. Among these patients, 162 patients (26.8%) underwent conformal radiotherapy and 383 patients (63.3%) underwent gamma knife therapy. In total, 250 patients (41.3%) received 355 chemotherapy (124 patients received platinum-based agents, 52 patients received temozolomide, and 74 patients received nimustine), and 355 patients did not receive any chemotherapy. The age and sex distributions in the case and control groups were balanced \((P = 0.688\) and \(P = 0.534\)). Furthermore, there was no statistically significant difference in the average age between the case (40.71 ± 18.28 years) and control groups (41.68 ± 13.54 years) \((P = 0.195)\).

**Association between H19 polymorphisms and glioma susceptibility**

Table 1 presents the genotypes and allele frequencies of \(H19\) in the two groups and their associations with glioma susceptibility, adjusted for sex and age. The genotype frequency distributions of the three polymorphisms conformed to the Hardy–Weinberg equilibrium (HWE) \((rs217727, P = 0.80; rs2839698, P = 0.06; rs3741219, P = 0.096)\).

We applied six genetic models to investigate the association between \(H19\) polymorphisms and glioma risk. All of the inheritance models indicated that \(rs217727\) and \(rs2839698\) were not associated with glioma susceptibility (Table 1). However, all inheritance models revealed that \(rs3741219\) A>G was significantly associated with a decreased risk of glioma, except for the recessive model (Heterozygote: GA vs. AA, odds ratio [OR] = 0.31, 95% confidence interval [95% CI] = 0.24-0.39, \(P < 0.001\); Homozygote: GG vs. AA, OR = 0.68, 95% CI = 0.49-0.94, \(P = 0.02\); Dominant:
GA+GG vs. AA, OR = 0.38, 95% CI = 0.31-0.47, P < 0.001; Overdominant: GA vs. AA+GG, OR = 0.32, 95% CI = 0.25-0.41, P < 0.001; Allele: A vs. G, OR = 0.54, 95% CI = 0.45-0.63, P < 0.001).

**Associations between H19 gene polymorphisms and clinical characteristics**

We further analyzed the associations between clinical features in patients with glioma and *H19* polymorphisms, stratified by age, gender, tumor sites, and WHO Grade (Table 2). This analysis revealed that the GA/AA and AA genotypes of *H19* rs217727 in patients aged ≥ 40 years were less frequent than the GG genotype in patients aged < 40 years (GA+AA vs. GG: OR = 0.70, 95% CI = 0.50-0.96, P = 0.03; AA vs. GG: OR = 0.67, 95% CI = 0.47-0.94, P = 0.02). For rs2839698 and rs3741219, no significant association between polymorphisms and clinical characteristics was observed.

**False Positive Report Probability (FPRP) Results**

We preset 0.2 as the FPRP threshold. As shown in Table S2, at the prior probability of 0.01, all of the significant findings for the *H19* rs3741219 A>G polymorphism (GA versus AA, GG versus AA, GA+GG versus AA, GA versus AA+GG) remained noteworthy. Moreover, the association with the *H19* rs3741219 allele variation (A>G) was also noteworthy, with a statistical power of 0.709 and the FPRP value of 0.001.

**Haplotype analysis**

As shown in Table 3, we conducted a haplotype analysis to evaluate the joint action of *H19* three SNPs. The G<sub>rs217727</sub>A<sub>rs2839698</sub>G<sub>rs3741219</sub> haplotype was associated with a reduced risk of glioma, compared with the wild-type haplotype G.
rs217727G\textsuperscript{rs2839698}A\textsuperscript{rs3741219} (OR = 0.33, 95% CI = 0.23-0.46, \(P = 0.02\)).

**Association of H19 polymorphisms with glioma prognosis**

We investigated the association between the three SNPs as well as other potential factors and glioma prognosis by univariate Cox analyses. The subgroup analysis was stratified by age, sex, surgery, WHO grade, and history of chemotherapy and radiotherapy. By univariate Cox analyses, no significant association was observed between three \(HI9\) polymorphisms and glioma prognosis (OS and PFS). However, three factors including age, chemotherapy and surgery, were identified as independent prognostic factors of glioma (Table 4-5).

Further, we performed a multivariate Cox analysis of the three factors identified above. As presented in Figures 1-2, patients with glioma aged over 40 years showed worse OS and PFS (OS: hazard ratio [HR] = 1.21, 95% CI = 1.02-1.44, \(P = 0.029\); PFS: HR = 1.21, 95% CI: 1.02-1.43, \(P = 0.03\)) compared with those of younger patients. In addition, compared with patients who underwent STR or NTR, those who underwent GTR showed better OS and PFS (OS: HR = 0.62, 95% CI = 0.51-0.75, \(P < 0.001\); PFS: HR = 0.61, 95% CI = 0.51-0.74, \(P < 0.001\)). Furthermore, there was a sizeable difference in prognosis depending on various chemotherapy regimens. Compared with patients who received no chemotherapy as a reference, patients treated with temozolomide or nimustine presented improved OS (temozolomide: HR = 0.36, 95% CI = 0.24-0.52, \(P < 0.001\); nimustine: HR = 0.74, 95% CI = 0.56-0.97, \(P = 0.030\)). As for PFS, patients who received temozolomide treatment had a longer PFS (HR = 0.38, 95% CI = 0.26-0.55, \(P < 0.001\)), whereas patients who received platinum
or nimustine-based treatment did not show significantly better therapeutic effects.

In the Kaplan-Meier and log-rank analyses (Figure 3), the three \( H19 \) variants showed no association with glioma prognosis (OS: rs217727, \( P = 0.52 \); rs2839698, \( P = 0.99 \); rs3741219, \( P = 0.80 \); PFS: rs217727, \( P = 0.41 \); rs2839698, \( P = 0.88 \); rs3741219, \( P = 0.85 \)).

**Discussion**

Although the function of \( H19 \) has been investigated in the past few years, its exact role in carcinogenesis is controversial. Association between \( H19 \) polymorphisms and glioma occurrence or prognosis has never been reported. In our analysis, the role of \( H19 \) polymorphisms in the regulation of glioma carcinogenesis was found to be complex. The initiation and development of glioma are influenced by both genetic and external factors. In this hospital-based study, the rs3741219 allele variation of \( H19 \) was associated with a decreased risk of glioma occurrence. In addition, the mutant G \( \text{rs217727A}\text{rs2839698G}\text{rs3741219} \) haplotype of \( H19 \) could substantially reduce the risk of glioma, mirroring that rs3741219 A>G variant might play a beneficial role in glioma prevention.

The \( H19 \) gene, which encodes a 2.3 kb lincRNA, is essential in embryonic development. Additionally, its expression is generally decreased after birth, with expression restricted to the cardiac and skeletal muscles. \( H19 \) is an imprinted gene comprising five exons. Genomic imprinting is a gamete-specific inherited modification that determines allele-specific expression in somatic cells. In addition, loss of imprinting (LOI) in the gene might result in the development of some tumors,
but not ubiquitously. However, Uyeno et al. suggested that LOI of \( IGF2 \) but not \( H19 \) is related to glioma development. Another study has indicated that c-Myc combines with the conserved E-boxes near the imprinted control region of \( H19 \), inducing histone acetylation and transcriptional initiation, and increasing the expression of \( H19 \). Structure determines function, and SNPs can affect promoter activity, mRNA stability, and translation efficiency, all of which, in turn, influence gene expression.

In addition, the mechanisms of \( H19 \) gene in cancer including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, genomic instability and mutation, tumor-promoting inflammation, deregulating cellular energetics, avoiding immune destruction. The precise mechanisms deserves further investigation.

The rs217727 polymorphism is located in exon 5 of the \( H19 \) gene. Rs217727 is related to a significantly increased risk of non-small cell lung cancer, urothelial cell carcinoma, bladder cancer, breast cancer, oral squamous cell carcinoma, osteosarcoma, and gastric cancer. By contrast, another study has suggested that rs217727 polymorphism is related to a low risk of breast cancer. It is also been reported that rs217727 C>T shows no association with the occurrence of breast cancer, lung cancer, and bladder cancer. These results provide no consensus on the promoting or protective effect of rs217727 on cancer susceptibility. However, we found that the frequencies of GA+AA and AA genotypes of \( H19 \) rs217727 in patients aged over 40 years were lower than those of the GG genotype in patients aged < 40
years, indicating that rs217727 might play various roles in the development of glioma depending on age.

In the present study, both \textit{H19} rs217727 and rs2839698 showed no significant association with glioma susceptibility. Previous studies have reported that \textit{H19} rs2839698 is not associated with the risk of developing non-small cell lung cancer,\textsuperscript{23} oral squamous cell carcinoma,\textsuperscript{27} and breast cancer.\textsuperscript{32} Another study revealed that heterozygotes of \textit{H19} rs2839698 are more susceptible to hepatocellular carcinoma\textsuperscript{33} and breast cancer.\textsuperscript{26} Rs2839698 is located within the 3\textquotesingle untranslated region of the \textit{H19} gene. A study in the Netherlands indicated that the rs2839698 polymorphism is associated with a decreased risk of bladder cancer,\textsuperscript{31} which might be explained by variation in the exon. Exonic regions are related to the conserved secondary structure of the transcript or its binding affinity with interacting elements.

In our analysis, \textit{H19} rs3741219 was associated with a reduced risk of glioma. The rs3741219 A/G polymorphism is located within the 1st exon of \textit{H19}. Mutant alleles or structural variation in genes are presumed to be important, whereas variants that drive cancers are not unique. Divergent variants in the same gene could produce tumors with different characteristics.\textsuperscript{8} The specific region of \textit{H19}, that harbors rs3741219 is encodes an antisense transcript named the \textit{H19} opposite tumor suppressor (\textit{HOTS}),\textsuperscript{34} which is antisense to the \textit{H19} transcript and is conserved in primates. The overexpression of \textit{HOTS}, which is localized within the nucleus and nucleolus, could inhibit the growth of some tumors. The \textit{H19} locus could encode a tumor suppressor protein.\textsuperscript{35} However, there is no evidence for the coexpression of \textit{HOTS} and \textit{H19} in
vivo. This complexity of inheritance and translation may partially explain the differences between associations of H19 SNPs with distinct types of cancers.\textsuperscript{35}

Furthermore, according to Cox regression analysis, age, surgery type, and chemotherapy were independent prognostic risk factors for glioma. However, our data indicated that these three H19 gene polymorphisms were not associated with glioma prognosis. A previous study has reported that rs2839698 is related to a poor prognosis in hepatocellular cancer.\textsuperscript{16} However, post-operative patients with gastric adenocarcinoma harboring the H19 rs2839698 GA genotype had an improved prognosis. Further studies of the association between H19 gene polymorphisms and cancer prognosis are necessary to verify the results.

Using LncRNASNP2 (http://bioinfo.life.hust.edu.cn/lncRNASNP/), we found that the H19 rs217727 G>A SNP may affect miRNA-lncRNA interactions, resulting in gains and losses miRNA target sites, forming hsa-miR-4804-5p and hsa-miR-8071, and destroying hsa-miR-3960 and hsa-miR-8071 binding sites on H19. The rs3741219 A>G polymorphism causes the gain of miRNA target sites (including hsa-miR-3187-5p, hsa-miR-1285-3p, hsa-miR-6860, and hsa-miR-612), as well as miRNA target losses (including hsa-miR-4486, hsa-miR-24-1-5p, and hsa-miR-566). A similar genotype-phenotype association was also observed for rs3741219 A>G. This indicated that the H19 rs3741219 A>G SNP creates hsa-miR-1539, hsa-miR-3193, and hsa-miR-146b-3p and damages hsa-miR-1914-5p, hsa-miR-6811-3p and hsa-miR-6514-3p miRNA binding sites. The specific mechanisms underlying these effects require further investigations.
This study investigated the effect of $H19$ gene polymorphisms on susceptibility and prognosis of glioma. However, additional studies are needed to verify these results and to address several limitations of our study. The effect of environmental factors on glioma risk might be underestimated, owing to a lack of exposure information. In addition, heterogeneity among individuals in various factors is inevitable. All participants were from northwestern China and were of Han ethnicity, resulting in selection bias. Despite these limitations, our study included the largest sample of patients with glioma to date. And to our knowledge, this is the first study focused on the association of $H19$ gene polymorphisms with glioma susceptibility and prognosis.

Taken together, the present data suggest that rs2839698 G>A in the $H19$ gene is associated with a decreased glioma risk. Additionally, patients carrying the G\textsuperscript{rs217727}A\textsuperscript{rs2839698}G\textsuperscript{rs3741219} haplotype were less prone to develop glioma. Our results mirror the complexity of $H19$ functions in glioma initiation and development.

**Materials and Methods**

**Study population**

In total, 1905 participants of Han Chinese ethnicity (605 patients with glioma and 1,300 controls) were consecutively enrolled in the Department of Neurosurgery at Tangdu Hospital, the second affiliated hospital of the Fourth Military Medical University (Xi’an, China), from September 2010 to May 2014. All patients were pathologically diagnosed with glioma. Further, the patients were not treated with chemotherapy or radiotherapy before surgery. Healthy controls, without a history of
malignancies and underlying disease, were recruited among participants who underwent routine examinations in the same period and were matched with the glioma cases in terms of sex and age. The basic characteristics of the participants including age, gender, ethnicity, WHO grade, surgery, chemotherapy strategy, and radiotherapy were collected from medical records or self-administered questionnaires. Furthermore, a monthly follow-up was conducted by telephone and outpatient interviews. All patients provided written informed consent before participation. With respect to prognosis, the outcome indicators of death and disease progression were considered to explore the association between H19 polymorphisms and OS as well as PFS. Patients with glioma included in this study were followed up for 44 months. This study was approved by the Institutional Review Board of the First Affiliated Hospital of Zhejiang University in Zhejiang Province (Hangzhou, China).

**SNP selection and genotyping**

The NCBI dbSNP database and the online tool software SNPinfo were used to select candidate SNPs. Three widely researched SNPs (rs217727 G>A, rs2839698 G>A, and rs3741219 A>G) in H19 gene were investigated. Peripheral blood was drawn from the participants, and stored at -80°C in EDTA tubes for DNA extraction and genotyping. Genomic DNA was extracted using the Universal Genomic DNA Extraction Kit (TaKaRa, Kyoto, Japan). DNA concentrations were measured by spectrophotometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). Sequenom Mass ARRAY Assay Design 3.0 (Sequenom, Inc., San Diego, CA, USA) was used for designing the Multiplexed SNP Mass EXTEND
assay. Genotyping of \textit{H19} polymorphisms was performed using the Sequenom Mass ARRAY RS1000. Sequenom Typer 4.0 was used for data analysis. Investigators were blind to the case-control group information for samples. All participants were genotyped successfully. The primers for the three SNPs are listed in Table S3.

\textbf{Haplotype analysis}

A haplotype analysis was conducted using SHEsis (http://analysis.bio-x.cn/SHEsisMain.htm)\textsuperscript{37}. There is a lowest frequency threshold for haplotype analysis, any number in [0, 1) could be accepted. Haplotype with frequency less than this number will not be considered in analysis, and the default value is usually 0.03. Haplotypes with frequencies of less than 0.03 were not analyzed.

\textbf{Statistical analysis}

\textit{R} software (version 3.5.1) was used for statistical analyses, as described in our previous studies.\textsuperscript{36, 38–40} HWE was assessed by the goodness-of-fit $\chi^2$ test. Chi-square test or \textit{t}-test were used to compare the distributions of genotype frequencies between the case and control groups. A logistic regression analysis was conducted to evaluate the association between SNPs and glioma risk. ORs and their 95\% CIs were calculated. To assess prognostic effects, univariate and multivariate Cox analyses were conducted, and HRs with 95\% CIs were calculated. Stratified analyses were also conducted by age, gender, surgery, WHO grade, and history of chemotherapy and radiotherapy. Moreover, the FPRP analysis was performed to verify the significant results.\textsuperscript{41, 42} All statistical tests used were two-sided, with a significance threshold of $P < 0.05$. 
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Author contributions

All authors read, critically reviewed, and approved the final manuscript. ZJD and JL designed the research; Y.J.D, Y.Z and J.Y collected the data. L.H.Z and S.Y performed the statistical analysis; Y.W and P.X provided methodological support/advice; L.J.L, N.L, and D.Z conducted the experiments; Y.J.D wrote the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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### Table 1. Genotype Frequencies of H19 Polymorphisms in Cases and Controls

| Model          | Genotype | Control (n, %) | Case (n, %) | OR<sup>a</sup> (95% CI) | P-value<sup>a</sup> |
|----------------|----------|----------------|-------------|--------------------------|---------------------|
| rs217727       |          |                |             |                          |                     |
| Co-dominant    | GG       | 557(42.8%)     | 254 (42.0%) | 1.00 (reference)         |                     |
|                | Heterozygote | GA     | 591(45.5%)     | 278 (45.9%) | 1.03(0.84-1.27)         | 0.77                |
|                | Homozygote  | AA     | 152(11.7%)     | 73 (12.1%)  | 1.05(0.77-1.44)         | 0.75                |
|                | Dominant   | GG     | 557(42.8%)     | 254 (42.0%) | 1.00 (reference)         |                     |
|                |           | GA+AA | 743(57.2%)     | 351 (58.0%) | 1.04(0.85-1.26)         | 0.72                |
|                | Recessive  | GG+GA | 1148(88.3%)    | 532 (87.9%) | 1.00 (reference)         |                     |
|                |           | AA     | 152(11.7%)     | 73 (12.1%)  | 1.04(0.77-1.40)         | 0.81                |
|                | Overdominant | GG+AA | 709(54.5%)     | 327 (54.1%) | 1.00 (reference)         |                     |
|                |           | GA     | 591(45.5%)     | 278 (45.9%) | 1.02(0.84-1.24)         | 0.84                |
| Allele         | G         | 1705(65.6%)    | 786 (65.0%)   | 1.00 (reference)         |                     |
|                | A         | 895(34.4%)     | 424 (35.0%)   | 1.03(0.89-1.19)         | 0.71                |
### rs2839698  
**HWE: P = 0.06**

|                  | Co-dominant |               |               |               |               |
|------------------|-------------|---------------|---------------|---------------|---------------|
|                  | GG          | 675(51.9%)    | 311(51.3%)    | 1.00 (reference) |               |
| Heterozygote     | GA          | 504(38.8%)    | 240(39.7%)    | 1.03(0.84-1.27) | 0.75          |
| Homozygote       | AA          | 121(9.3%)     | 54(9.0%)      | 0.97(0.68-1.37) | 0.86          |
| **Dominant**     | GG          | 675(51.9%)    | 311(51.3%)    | 1.00 (reference) |               |
|                  | GA+AA       | 625(48.1%)    | 294(48.7%)    | 1.02(0.84-1.24) | 0.83          |
| **Recessive**    | GG+GA       | 1179(90.7%)   | 551(91.0%)    | 1.00 (reference) |               |
|                  | AA          | 121(9.3%)     | 54(9.0%)      | 0.96(0.68-1.34) | 0.79          |
| **Overdominant** | GG+AA       | 796(61.2%)    | 365(60.3%)    | 1.00 (reference) |               |
|                  | GA          | 504(38.8%)    | 240(39.7%)    | 1.04(0.85-1.27) | 0.71          |
| **Allele**       | G           | 1854(71.3%)   | 862(71.2%)    | 1.00 (reference) |               |
|                  | A           | 746(28.7%)    | 348(28.8%)    | 1.00(0.86-1.17) | 0.97          |

### rs3741219  
**HWE:P = 0.096**

|                  | Co-dominant |               |               |               |               |
|------------------|-------------|---------------|---------------|---------------|---------------|
|                  | AA          | 651(50.1%)    | 439(72.56%)   | 1.00 (reference) |               |
| Heterozygote     | GA          | 520(40.0%)    | 107(17.69%)   | 0.31(0.24-0.39) | <0.0001*      |
| Homozygote       | GG          | 129(9.9%)     | 59(9.75%)     | 0.68(0.49-0.94) | 0.02*         |
| **Dominant**     | AA          | 651(50.1%)    | 439(72.56%)   | 1.00 (reference) |               |
|                  | GA+GG       | 649(49.9%)    | 166(27.44%)   | 0.38(0.31-0.47) | <0.0001*      |
| **Recessive**    | AA+GA       | 1171(90.1%)   | 546(90.25%)   | 1.00 (reference) |               |
|                  | GG          | 129(9.9%)     | 59(9.75%)     | 0.98(0.71-1.36) | 0.91          |
| **Overdominant** | AA+GG       | 780(60.0%)    | 498(82.31%)   | 1.00 (reference) |               |
|                  | GA          | 520(40.0%)    | 107(17.69%)   | 0.32(0.25-0.41) | <0.0001*      |
| Allele | %   | %    | OR  | CI       | P      |
|--------|-----|------|-----|----------|--------|
| A      | 1822(70.1%) | 985(81.40%) | 1.00 (reference) | |
| G      | 778(29.9%)  | 225(18.60%) | 0.54 (0.45-0.63)  | <0.0001* |

*P ≤0.05 indicates statistical significance. OR = 1 is the reference compared with other genotypes.

* adjusted for age and sex.

HWE: Hardy–Weinberg Equilibrium; OR: odds ratio; CI: confidence interval.
Table 2. The Associations between $H19$ gene Polymorphisms and Clinical Characteristics of Glioma Patients

| Characteristics | Genotype distributions |  |
|-----------------|------------------------|--|---|
|                 | AA                     | Aa | aa | Aa + aa |
| **rs217727**    |                        |    |    |         |
| Age             |                        |    |    |         |
| <40/≥40         | 99/155                 | 136/142 | 32/41 | 168/183 |
| OR (95%CI)      | Ref.                   | 0.67 (0.47-0.94) | 0.82 (0.48-1.39) | 0.70 (0.50-0.96) |
| $P$-value*      | 0.021$^*$              | 0.456 | 0.03$^*$ |
| Sex             |                        |    |    |         |
| Male/ Female    | 138/116                | 155/123 | 42/31 | 197/154 |
| OR (95%CI)      | Ref.                   | 0.94 (0.67-1.33) | 0.88 (0.52-1.48) | 0.93 (0.67-1.29) |
| $P$-value*      | 0.741                  | 0.628 | 0.661 |
| WHO Grade       |                        |    |    |         |
| I+II/ III+IV    | 155/99                 | 184/94 | 43/30 | 227/124 |
| OR (95%CI)      | Ref.                   | 0.80 (0.56-1.14) | 1.09 (0.64-1.85) | 0.86 (0.61-1.19) |
| $P$-value*      | 0.216                  | 0.744 | 0.359 |
| **rs2839698**   |                        |    |    |         |
| Age             |                        |    |    |         |
| <40/≥40         | 130/181                | 118/122 | 18/36 | 136/158 |
| OR (95%CI)      | Ref.                   | 0.75 (0.53-1.05) | 1.44 (0.80-2.71) | 0.84 (0.61-1.16) |
|                    |       |       |       |       |
|--------------------|-------|-------|-------|-------|
| $P$-value*          | 0.091 | 0.237 | 0.285 |

**Sex**

|                |       |       |       |       |
|-----------------|-------|-------|-------|-------|
| Male/ Female    | 171/140 | 134/106 | 31/23 | 165/129 |
| OR (95%CI)      | Ref. | 0.96 (0.68-1.35) | 0.90 (0.50-1.61) | 0.95 (0.69-1.31) |
| $P$-value*      | 0.816 | 0.726 | 0.751 |

**WHO Grade**

|                 |       |       |       |       |
|------------------|-------|-------|-------|-------|
| I+II/ III+IV     | 202/109 | 152/88 | 28/26 | 180/114 |
| OR (95%CI)       | Ref. | 1.07 (0.75-1.52) | 1.71 (0.95-3.07) | 1.17 (0.84-1.63) |
| $P$-value*       | 0.715 | 0.070 | 0.358 |

**rs3741219**

|                |       |       |       |       |
|-----------------|-------|-------|-------|-------|
| Age             |       |       |       |       |
| <40/≥40         | 197/242 | 46/61 | 24/35 | 70/96 |
| OR (95%CI)      | Ref. | 1.08 (0.71-1.66) | 1.19 (0.69-2.08) | 1.12 (0.78-1.60) |
| $P$-value*      | 0.725 | 0.543 | 0.550 |

**Sex**

|                |       |       |       |       |
|-----------------|-------|-------|-------|-------|
| Male/ Female    | 237/202 | 60/47 | 38/21 | 98/68 |
| OR (95%CI)      | Ref. | 0.92 (0.60-1.40) | 0.65 (0.36-1.13) | 0.81 (0.57-1.17) |
| $P$-value*      | 0.697 | 0.133 | 0.265 |

**WHO Grade**

|                 |       |       |       |       |
|------------------|-------|-------|-------|-------|
| I+II/ III+IV     | 281/158 | 62/45 | 39/20 | 101/65 |
| OR (95%CI)       | Ref. | 1.29 (0.84-1.98) | 0.91 (0.51-1.60) | 1.44 (0.79-1.65) |
| $P$-value*       | 0.245 | 0.753 | 0.472 |
*Univariate logistic regression analysis for the distributions of genotype frequencies. $P \leq 0.05$ indicates statistical significance.

A, wild allele; a, variant allele. OR: odds ratio; CI: confidence interval; Ref.: reference; WHO: World Health Organization.
Table 3. The Haplotype Analysis of Three *H19* Gene Polymorphisms (rs217727 G> A, rs2839698 G> A, and rs3741219 A> G)

| Haplotypes                  | Case (%) | Control (%) | OR (95%CI)    | P-value* |
|-----------------------------|----------|-------------|---------------|----------|
| G rs217727 G rs2839698 A rs3741219 | 189(31.3) | 463(35.58)  | Ref.         |          |
| A rs217727 A rs2839698 A rs3741219 | 3(0.4)    | 0(0.00)    | NA            | NA       |
| A rs217727 A rs2839698 G rs3741219 | 1(0.1)    | 0(0.00)    | NA            | NA       |
| A rs217727 G rs2839698 A rs3741219 | 178(29.5) | 445(34.22)  | 0.98(0.77-1.25) | 0.87     |
| A rs217727 G rs2839698 G rs3741219 | 30(5)     | 2(0.16)    | NA            | NA       |
| G rs217727 A rs2839698 A rs3741219 | 122(20.2) | 2(0.18)    | NA            | NA       |
| G rs217727 A rs2839698 G rs3741219 | 49(8.1)   | 369(28.42)  | 0.33(0.23-0.46) | <0.0001* |
| G rs217727 G rs2839698 G rs3741219 | 33(5.4)   | 19(1.44)   | NA            | NA       |

*P ≤0.05 indicates statistical significance.

OR: odds ratio; CI: confidence interval; Ref.: reference; NA, not applicable.
Table 4. Analysis of *H19* Gene Polymorphisms and Clinical Features in Glioma Patient Overall Survival.

| Characteristics | Patients (n) | Events (n) | Rate (%) | Univariate Analysis |
|-----------------|--------------|------------|----------|--------------------|
|                 |              |            |          | HR (95% CI)        | P-Value* |
| **Age**         |              |            |          |                    |         |
| <40 years       | 267          | 229        | 85.77    | Ref.               | Ref.    |
| >=40 years      | 338          | 310        | 91.72    | 1.20 (1.01-1.42)   | 0.039*  |
| **Sex**         |              |            |          |                    |         |
| male            | 335          | 297        | 88.66    | Ref.               | Ref.    |
| female          | 270          | 242        | 89.63    | 1.08 (0.91-1.28)   | 0.355   |
| **WHO Grade**   |              |            |          |                    |         |
| I-II            | 382          | 336        | 87.96    | Ref.               | Ref.    |
| III-IV          | 223          | 206        | 92.38    | 1.18 (0.98-1.40)   | 0.063   |
| **Surgery**     |              |            |          |                    |         |
| STR & NTR       | 189          | 186        | 98.41    | Ref.               | Ref.    |
| GTR             | 416          | 353        | 84.86    | 0.59 (0.49-0.71)   | <0.001* |
| **Chemotherapy**|              |            |          |                    |         |
| No              | 355          | 333        | 93.80    | Ref                | Ref     |
| Platinum        | 124          | 112        | 90.32    | 0.84 (0.68-1.04)   | 0.116   |
| Temozolomide    | 52           | 30         | 57.69    | 0.32 (0.22-0.48)   | <0.001* |
| Nimustine       | 74           | 64         | 86.49    | 0.645 (0.49-0.85)  | 0.001*  |
| **Radiotherapy**|              |            |          |                    |         |
| No | 60 | 49 | 81.67 | Ref. | Ref. |
|----|----|----|--------|------|------|
| Conformal radiotherapy | 162 | 133 | 82.10 | 1.08 (0.77-1.50) | 0.622 |
| Gamma knife | 383 | 357 | 93.21 | 1.17 (0.86-1.58) | 0.303 |

rs217727

| GG | 254 | 226 | 88.98 | Ref. | Ref. |
|----|----|----|--------|------|------|
| GA | 278 | 249 | 89.57 | 1.09 (0.83-1.44) | 0.527 |
| AA | 73  | 64  | 87.67 | 1.14 (0.86-1.50) | 0.357 |

rs2839698

| GG | 311 | 281 | 90.35 | Ref. | Ref. |
|----|----|----|--------|------|------|
| GA | 240 | 211 | 87.92 | 1.00 (0.73-1.36) | 0.974 |
| AA | 54  | 47  | 87.04 | 0.97 (0.71-1.34) | 0.869 |

rs3741219

| AA | 439 | 391 | 89.07 | Ref. | Ref. |
|----|----|----|--------|------|------|
| GA | 107 | 94  | 87.85 | 1.05 (0.84-1.31) | 0.693 |
| GG | 59  | 54  | 91.53 | 1.09 (0.82-1.45) | 0.564 |

*Cox’s proportional hazard regression analysis for univariate analysis.  *P* ≤0.05 indicates statistical significance.

OS: overall survival; HR: hazard ratio; CI: confidence interval; STR: subtotal resection; NTR: near total resection; GTR: gross total resection; Ref.: reference.
Table 5. Analysis of *H19* Gene Polymorphisms and Clinical Features in Glioma Patient Progression-free Survival.

| Characteristics | Patients (n) | Events (n) | Rate (%) | Univariate Analysis |
|-----------------|-------------|------------|----------|---------------------|
|                 |             |            |          | **HR (95% CI)**     | **P-Value** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **1.19(1.00-1.40)** | **0.047** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **1.1(0.93-1.30)** | **0.263** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **1.15(0.97-1.36)** | **0.116** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **0.58(0.48-0.69)** | **<0.001** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **0.99(0.80-1.22)** | **0.916** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **0.35(0.24-0.50)** | **<0.001** |
| **Univariate Analysis** | **Ref.** | **Ref.** | **0.73(0.56-0.96)** | **0.022** |

**Age**

- <40 years: 267 patients, 239 events, 89.51%
- >=40 years: 338 patients, 324 events, 95.86%, HR (95% CI) = 1.19 (1.00-1.40), P-Value = 0.047*

**Sex**

- Male: 335 patients, 310 events, 92.54%
- Female: 270 patients, 253 events, 93.70%, HR (95% CI) = 1.10 (0.93-1.30), P-Value = 0.263

**WHO Grade**

- I-II: 382 patients, 353 events, 92.41%
- III-IV: 223 patients, 210 events, 94.17%, HR (95% CI) = 1.15 (0.97-1.36), P-Value = 0.116

**Surgery**

- STR & NTR: 189 patients, 183 events, 96.83%
- GTR: 416 patients, 380 events, 91.35%, HR (95% CI) = 0.58 (0.48-0.69), P-Value = <0.001*

**Chemotherapy**

- No: 355 patients, 351 events, 98.87%
- Platinum: 124 patients, 116 events, 93.55%, HR (95% CI) = 0.99 (0.80-1.22), P-Value = 0.916
- Temozolomide: 52 patients, 32 events, 61.54%, HR (95% CI) = 0.35 (0.24-0.50), P-Value = <0.001*
- Nimustine: 74 patients, 64 events, 86.49%, HR (95% CI) = 0.73 (0.56-0.96), P-Value = 0.022*

**Radiotherapy**
|      | No  | 55  | 91.67 | Ref. | Ref. |
|------|-----|-----|-------|------|------|
| Conformal | 162 | 137 | 84.57 | 1.13(0.83-1.56) | 0.436 |
| Gamma knife | 383 | 371 | 96.87 | 1.21(0.91-1.60) | 0.199 |

**rs217727**

|      | GG  | 234 | 92.13 | Ref. | Ref. |
|------|-----|-----|-------|------|------|
| GA   | 278 | 261 | 93.88 | 1.00(0.76-1.30) | 0.973 |
| AA   | 73  | 68  | 93.15 | 1.11(0.85-1.46) | 0.429 |

**rs2839698**

|      | GG  | 294 | 94.53 | Ref. | Ref. |
|------|-----|-----|-------|------|------|
| GA   | 240 | 219 | 91.25 | 1.04(0.77-1.40) | 0.797 |
| AA   | 54  | 50  | 92.59 | 0.98(0.72-1.34) | 0.906 |

**rs3741219**

|      | AA  | 408 | 92.94 | Ref. | Ref. |
|------|-----|-----|-------|------|------|
| GA   | 107 | 101 | 94.39 | 1.01(0.81-1.25) | 0.940 |
| GG   | 59  | 54  | 91.53 | 1.08(0.81-1.44) | 0.595 |

*Cox’s proportional hazard regression analysis for univariate analysis. *P* ≤0.05 indicates statistical significance.

PFS: progression-free survival; HR: hazard ratio; CI: confidence interval; STR: subtotal resection; NTR: near total resection; GTR: gross total resection; Ref.: reference.
**Figure legends**

**Figure 1.** Forest Plots of Multivariate Cox Regression Analysis for Overall Survival

Figure legends: STR, subtotal resection; NTR, near-total resection; GTR, gross total resection.

**Figure 2.** Forest Plots of Multivariate Cox Regression Analysis for Progression-free Survival

Figure legends: STR, subtotal resection; NTR, near-total resection; GTR, gross total resection.

**Figure 3.** Kaplan-Meier Analysis of OS and PFS in Three Polymorphisms of H19 Gene

Figure legends: (A) OS of rs217727; (B) OS of rs2839698; (C) OS of rs3741219; (D) PFS of rs217727 ;(E) PFS of rs2839698; (F) PFS of rs3741219. OS, Overall Survival; PFS, Progression-free Survival.
| Age          | Hazard ratio | 95% CI |       |
|--------------|--------------|--------|-------|
| <40 years    | 2.21         | 1.02 - 4.32 | 0.03 *|
| ≥40 years    |              |        |       |

| Surgery      | Hazard ratio | 95% CI |       |
|--------------|--------------|--------|-------|
| STR & NTR    | 0.81         | 0.57 - 1.14 | <0.001***|
| CTM          |              |        |       |

| Chemotherapy | Hazard ratio | 95% CI |       |
|--------------|--------------|--------|-------|
| No           | 0.59         | 0.36 - 0.95 | 0.05  |
| Platinum     | 0.98         | 0.70 - 1.34 |       |
| Teniposide   | 0.36         | 0.16 - 0.71 | <0.001***|
| Nitroxine    | 0.83         | 0.63 - 1.09 | 0.169 |

# Events: 583; Global p-value (Log-Rank): 3.2055e-14
AIC: 6211.96; Concordance Index: 0.57
