Effects of the \textit{MAML2} genetic variants in glioma susceptibility and prognosis

Ming Zhang\textsuperscript{1}, Yonglin Zhao\textsuperscript{2}, Junjie Zhao\textsuperscript{3}, Tingqin Huang\textsuperscript{1}, Xiaoye Guo\textsuperscript{3}, Xudong Ma\textsuperscript{3} and Yuan Wu\textsuperscript{4}

\textsuperscript{1}Department of Neurosurgery, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China; \textsuperscript{2}Department of Oncology, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China; \textsuperscript{3}Department of Neurosurgery, First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China; \textsuperscript{4}Department of Critical Care Medicine, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi 710004, China

\textbf{Correspondence:} Yuan Wu (yiyi7229@163.com)

\textbf{Background:} Abnormal expression of the mastermind-like transcriptional co-activator 2 (\textit{MAML2}) gene is oncogenic in several human cancers, including glioma. However, the relevance of \textit{MAML2} variants with glioma remains unknown. We aimed to investigate the role of \textit{MAML2} polymorphisms in glioma risk and prognosis among the Chinese Han population. \textbf{Methods:} Seven \textit{MAML2} single-nucleotide polymorphisms (SNPs) were genotyped using Agena MassARRAY system among 575 patients with glioma and 500 age- and gender-matched healthy controls. Logistic regression was used to estimate the association between \textit{MAML2} polymorphisms and glioma risk by calculating odds ratios (ORs) and 95\% confidence intervals (CI). Kaplan–Meier survival analysis and univariate, multivariate Cox proportional hazard regression analyses for hazard ratios (HRs) and 95\% CIs were performed to evaluate the contribution of \textit{MAML2} polymorphisms to glioma prognosis. \textbf{Results:} \textit{MAML2} rs7938889 and rs485842 polymorphisms were associated with the reduced risk of glioma (OR = 0.69, \textit{P} = 0.023; and OR = 0.81, \textit{P} = 0.032, respectively). Rs7115578 polymorphism had a lower susceptibility to glioma in males (OR = 0.68, \textit{P} = 0.034), while rs4598633 variant with a higher risk in females (OR = 1.66, \textit{P} = 0.016). Additionally, rs7115578 AG genotype represented a poorer prognosis of glioma (HR = 1.24, \textit{P} = 0.033) and astrocytoma (log-rank \textit{P} = 0.037, HR = 1.31, \textit{P} = 0.036). Furthermore, rs11021499 polymorphism had lower overall survival (OS) and progression-free survival (PFS) in patients with low-grade glioma. \textbf{Conclusion:} We provided some novel data suggesting \textit{MAML2} polymorphisms might contribute to glioma risk and prognosis. Future studies are warranted to validate these findings and characterize mechanisms underlying these associations.

\textbf{Introduction:} Glioma is one of the common types of primary central nervous system (CNS) tumors, accounting for 30\% of all CNS tumors, almost 80\% of which are considered malignant, and are responsible for the majority of deaths from primary brain tumors [1]. In 2015, the incidence and mortality of glioma in China were approximately 101600 and 61000, respectively, with a ratio of 3:2 for men and women [2]. The incidence of glioma in general increases with age, from 0.9 in children to 12.1 in the elderly [3]. Patients with glioma usually have poor survival rates and unfavorable prognosis. The etiology of glioma remains poorly understood and is attributed to different genetic or environmental factors [4]. Recently, a vast number of studies have reported that genetic factors contribute to the development of glioma, which revealed single-nucleotide polymorphisms (SNPs) in cancer-related genes were associated with glioma susceptibility and prognosis [5–7].

Mastermind-like transcriptional co-activator 2 (\textit{MAML2}) is a member of the mastermind-like family of proteins, which is a co-activator of the oncogenic NOTCH signaling pathway [8]. NOTCH signaling activation has been demonstrated to be involved in carcinogenesis, which plays a critical role in cell
proliferation, metastasis and epithelial–mesenchymal transition in many diverse solid tumors including glioma [9,10]. Several studies have demonstrated MAML2 abnormal expression in various cancers, such as mucocutaneous carcinoma, hidradenoma and breast cancer [11–13]. These studies have suggested that MAML2 might be involved in the tumorigenesis and progression of cancers. Based on microarray data of glioma, MAML2 as a novel gene related to glioma was identified [14]. Additionally, epidemiological studies have confirmed that polymorphisms in MAML2, a NOTCH pathway gene, were related to cancer susceptibility and prognosis [15,16]. However, no previous study has investigated the contribution of MAML2 variants to glioma risk and prognosis.

In this hospital-based case–control study, we aimed to investigate the association of MAML2 polymorphisms with the susceptibility of glioma, and the role of these polymorphisms in the prognosis of glioma patients among the Chinese Han population.

Materials and methods

Study subjects

This case–control study recruited 575 glioma patients and 500 cancer-free controls from the Second Affiliated Hospital of Xi’an Jiaotong University. All participants were genetically unrelated ethnic Han Chinese. Patients with primary glioma were newly diagnosed and histopathologically confirmed. The blood samples were collected before radiotherapy and chemotherapy therapies or surgery. The patients who had history of cancer and serious systemic diseases (leukemia, diabetes, cardiovascular and cerebrovascular diseases and genetic diseases) or other diseases were excluded. All the patients were followed up every 3 months. During the follow-up period, overall survival (OS) and progression-free survival (PFS) were measured from diagnosis to death or the last follow-up. The age and gender-matched healthy tumor-free volunteers were recruited from annual checkup visitors of the same hospitals as control subjects. The controls were selected from the skull MRI with a negative diagnosis for glioma, without other cancers or chronic diseases and no diseases related to brain and CNS. Demographic and clinical pathological data were collected through interviewers’ administered questionnaires and/or medical records. The institutional ethics committees of the Second Affiliated Hospital of Xi’an Jiaotong University approved the procedures followed in the present study. All procedures involving human participants were in accordance with the Helsinki Declaration. Signed informed consent was obtained from all individuals who participated in the study.

SNPs genotyping

Genomic DNA was extracted from EDTA anticoagulated peripheral blood samples from each subject using a Qiagen DNA Isolation Kit (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer’s instructions, and stored at −20°C until additional analysis. MAML2 mRNA expression analysis in glioma was performed using GEPIA (http://gepia.cancer-pku.cn/) datasets. Seven MAML2 SNPs (rs7107785, rs479825, rs7938889, rs11021499, rs7115578, rs4598633 and rs485842) were selected as candidate SNPs for genotyping in the current study. These SNPs were selected based on a minor allele frequency (MAF) of >5% in Chinese populations and with a pairwise $r^2 \geq 0.80$, from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the 1000 Genomes Project data (http://www.internationalgenome.org/). To evaluate the potential function of the selected SNPs, we conducted in silico analysis using HaploReg v4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and SNPinfo Web Server (https://snpinfo.nih.gov/). MAML2 SNPs genotyping was performed Agena MassARRAY system (Agena, San Diego, CA, U.S.A.) in double-blinded [17,18]. The primers used in amplification and single base extension were shown in Supplementary Table S1, which was designed by the MassARRAY Assay Design 3.0 software. For quality control, the call rate of genotyping >95%, and approximately 10% of the samples were randomly selected for repeated analysis, of which the reproducibility was 100%.

Statistical analyses

All analyses were performed with the SPSS 18.0 (SPSS Institute, Chicago, IL, U.S.A.) and the PLINK 2.1.7 software. Baseline characteristics were presented as mean ± standard deviation (SD) for continuous data and as number (percentages) for categorical parameters. Variables were compared between the cases and controls using the Chi-squared test for gender and the independent samples t test for age. Hardy–Weinberg equilibrium (HWE) was evaluated by Pearson’s $\chi^2$ test comparing the expected and observed genotype frequencies of MAML2 SNPs in the control group. Differences in allele and genotype frequencies between glioma patients and cancer-free controls were also tested with $\chi^2$ test. The major allele was considered to be the reference allele. To determine the association between genotypes of MAML2 SNPs and glioma risk, logistic regression analysis was performed to compute odds ratios (ORs) and 95% confidence intervals (CIs), with the adjustment of age and gender. Multiple genetic models (allele, genotype, dominant,
Table 1 Characteristics of patients with glioma and controls

| Characteristics          | Cases (n=575) | Controls (n=500) | P     |
|--------------------------|--------------|-----------------|-------|
| Age, years               | Mean ± SD (year) 40.53 ± 13.90 | 40.46 ± 18.08 | 0.942  |
| Gender                   | Male 320 (55.7%) | 279 (55.8%) | 0.968  |
|                         | Female 255 (44.3%) | 221 (44.2%) |       |
| WHO grade                | I–II 369 (64.2%) | 206 (35.8%) |       |
|                         | III–IV 206 (35.8%) | 206 (35.8%) |       |
| Classification           | Astrocytoma 448 (77.9%) | 392 (68.2%) |       |
|                         | Others 127 (22.1%) | 127 (22.1%) |       |
| Surgical method          | STR and NTR 183 (31.8%) | 183 (31.8%) |       |
|                         | GTR 392 (68.2%) | 392 (68.2%) |       |
| Radiotherapy             | No 56 (9.7%) | 56 (9.7%) |       |
|                         | Conformal radiotherapy 155 (27.0%) | 155 (27.0%) |       |
|                         | γ knife 364 (63.3%) | 364 (63.3%) |       |
| Chemotherapy             | No 341 (58.8%) | 341 (58.8%) |       |
|                         | Yes 237 (41.2%) | 237 (41.2%) |       |
| Survival condition       | Survival 40 (7.0%) | 40 (7.0%) |       |
|                         | Lost 21 (3.6%) | 21 (3.6%) |       |
|                         | Death 514 (89.4%) | 514 (89.4%) |       |

Abbreviations: GTR, gross-total resection; NTR, near-total resection; WHO, World Health Organization.

1P-values were calculated by independent samples t test.
2P-values were calculated by Chi-square tests.

recessive and log-additive) were estimated by PLINK software. Kaplan–Meier survival analysis with the log-rank test was used to evaluate the relationship between clinical or genomic factors (MAML2 polymorphisms) and glioma prognosis. Hazard ratios (HRs) and 95% CIs were calculated from univariate and multivariate Cox proportional hazard regression analyses to evaluate the correlation between MAML2 polymorphisms and glioma prognosis. All P-values were two-sided, and a P-value <0.05 was considered statistically significant.

Results

Subject characteristics

Demographic and clinical characteristics of glioma patients and controls are shown in Table 1. The patients included 320 males and 255 females with a mean age of 40.53 ± 13.90 years, and the controls included 279 males and 221 females with a mean age of 40.46 ± 18.08 years. No significant difference was observed in age and gender distribution between the two groups (P=0.942 and P=0.968, respectively). Among all cases, 369 (64.2%) were in grades I–II and 206 (35.8%) in III–IV and 448 (77.9%) with astrocytoma.

The details of MAML2 SNPs

Detailed information about the seven selected SNPs is displayed in Supplementary Table S2. Genotype distribution of all SNPs among controls was in agreement with HWE (P>0.05). In silico analysis using HaploReg v4.1 and SNPinfo Web Server, the function of the selected SNPs was successfully predicted to have biological functions (Supplementary Table S2). Furthermore, we extracted MAML2 expression data between glioma patients and healthy controls from GEPIA database. Supplementary Figure S1 showed that there were significant differences of MAML2 expression in glioblastoma multiforme and brain lower grade glioma compared with normal tissue (P<0.01). Moreover, the expression of MAML2 was particularly associated with the prognosis of lower grade glioma (log-rank P=0.0094, Supplementary Figure S2).

Association between MAML2 SNPs and glioma risk

Among the seven MAML2 SNPs, two SNPs (rs7938889 and rs485842) were found to be significantly related to the risk of glioma by an adjustment for age and gender. The genotype and allele frequencies distribution of rs7938889 and rs485842 and their association with glioma risk are shown in Table 2. Subjects with rs7938889 TT genotype were associated with a reduced risk of glioma (TT vs. CC, OR = 0.69, 95%: 0.48–0.99, P=0.043; and TT vs. CC-CT, OR = 0.69, 95%: 0.50–0.95, P=0.023). Rs485842 polymorphism also displayed a lower risk of developing glioma under allele
Table 2 Relationships between *MAML2* polymorphisms and the risk of glioma and astrocytoma

| SNP ID | Model   | >Genotype | >Control (n) | Glioma | Astrocytoma |
|--------|---------|-----------|-------------|--------|-------------|
|        |         |           |             |        |             |
| rs7938889 | Allele | C         | 671         | 1      | 532         | 1           |
|         | T       | 449       | 471         | 0.86 (0.73–1.02) | 0.088 | 356         | 0.82 (0.68–0.99) | 0.035 |
|         | CC      | 150       | 183         | 1      | 148         | 1           |
|         | CT      | 251       | 305         | 1.00 (0.76–1.31) | 0.980 | 236         | 0.95 (0.71–1.26) | 0.714 |
|         | TT      | 99        | 83          | 0.69 (0.48–0.99) | 0.043 | 60          | 0.61 (0.41–0.90) | 0.013 |
|         | Dominant | CC       | 150       | 183         | 1      | 148         | 1           |
|         |         | CT-TT     | 350       | 388         | 0.91 (0.70–1.18) | 0.473 | 296         | 0.85 (0.65–1.21) | 0.252 |
|         |         | CC-CT     | 401       | 488         | 1      | 384         | 1           |
|         |         | TT        | 99        | 83          | 0.69 (0.50–0.95) | 0.023 | 60          | 0.63 (0.44–0.89) | 0.009 |
|         | Log-additive | –     | –          | –              | 0.85 (0.71–1.02) | 0.080 | –          | 0.81 (0.67–0.98) | 0.027 |
| rs485842 | Allele | C         | 714         | 868         | 1      | 668         | 1           |
|         | T       | 286       | 282         | 0.81 (0.67–0.98) | 0.032 | 228         | 0.85 (0.70–1.04) | 0.123 |
|         | CC      | 258       | 326         | 1      | 250         | 1           |
|         | CT      | 198       | 216         | 0.86 (0.67–1.11) | 0.253 | 168         | 0.88 (0.67–1.15) | 0.347 |
|         | TT      | 44        | 33          | 0.59 (0.37–0.96) | 0.033 | 30          | 0.69 (0.42–1.13) | 0.138 |
|         | Dominant | CC       | 258       | 326         | 1      | 250         | 1           |
|         |         | CT-TT     | 242       | 249         | 0.81 (0.64–1.04) | 0.094 | 198         | 0.84 (0.65–1.09) | 0.192 |
|         |         | CC-CT     | 456       | 542         | 1      | 418         | 1           |
|         |         | TT        | 44        | 33          | 0.63 (0.39–1.01) | 0.064 | 30          | 0.72 (0.45–1.18) | 0.192 |
|         | Log-additive | –     | –          | –              | 0.81 (0.67–0.98) | 0.033 | –          | 0.85 (0.69–1.04) | 0.116 |

P-values were calculated by logistic regression analysis with adjustments for age and gender. P < 0.05 means the data are statistically significant. Bold means the data are statistically significant.

(OR = 0.81, 95%: 0.67–0.98, P = 0.032), homozygote (OR = 0.59, 95%: 0.37–0.96, P = 0.033) and additive (OR = 0.81, 95%: 0.67–0.98, P = 0.033) models. Additionally, rs7938889 variant had a relationship with decreasing astrocytoma risk under multiple genetic model (allele, OR = 0.82, P = 0.035; homozygote, OR = 0.61, P = 0.013; recessive, OR = 0.63, P = 0.009; and additive, OR = 0.81, P = 0.027). No statistically significant associations were observed between *MAML2* rs7107785, rs479825, rs11021499, rs7115578 and rs4598633 variants and the risk of glioma.

We also conducted stratification analyses by age and gender (Table 3), and revealed that the effect of both rs7938889 and rs485842 on glioma risk remained significant. The association between glioma risk and rs7938889 genotypes was more profound in the individuals at age < 40 years (T vs C, OR = 0.75, P = 0.020; TT vs CC, OR = 0.56, P = 0.031) and males (TT vs CC, OR = 0.59, P = 0.032), while rs485842 variant in the subjects at age > 40 years (T vs C, OR = 0.66, P = 0.003; TT vs CC, OR = 0.31, P = 0.0005). Furthermore, we also found that rs7115578 polymorphism had a lower susceptibility to glioma in males (AG-GG vs AA, OR = 0.68, P = 0.034), while rs4598633 variant was associated with a higher risk for glioma in females (CT vs CC, OR = 1.66, P = 0.016; CT-TT vs CC, OR = 1.49, P = 0.043). We further stratified by the glioma grade, and there was a lower prevalence of rs7115578-GG carriers in the high-grade subgroup (WHO III+IV) than in the low-grade subgroup (WHO I+II) (16.99 vs 24.39%) with a marginal P-value in recessive model (OR = 0.64, 95% CI: 0.41–1.00, P = 0.048, Supplementary Table S3), which indicated insufficient evidence for claiming an association and needed further verification.

**Prognostic value of MAML2 SNPs in glioma patients**

Of the 575 patients, 514 patients had complete follow-up data. We evaluated the impact of clinical factors on patients’ survival by Log-rank tests and univariate analysis, as shown in Supplementary Table S4 and Figure S3. The extent of resection (gross-total resection) was associated with the OS (log-rank P < 0.001, HR = 0.63, P < 0.001) and PFS (log-rank P < 0.001, HR = 0.59, P < 0.001) mortality hazards, respectively. Moreover, chemotherapy was a protective factor in all glioma patients (OS: log-rank P < 0.001, HR = 0.67, P < 0.001; PFS: log-rank P = 0.012, HR = 0.81, P = 0.025).

We investigated the association between *MAML2* polymorphisms and the prognosis of glioma patients using Log-rank tests and univariate Cox regression analysis (Table 4 and Figure 1). *MAML2* rs7115578 polymorphism
Table 3  Relationships of MAML2 polymorphisms with glioma risk stratified by age and gender

| SNP ID     | Model | Genotype | Age | Case | Control | OR (95% CI) | P    | Case | Control | OR (95% CI) | P    |
|------------|-------|----------|-----|------|---------|-------------|------|------|---------|-------------|------|
| rs7938889  | Allele| C        | 330 | 264  | 1       | 0.99 (0.77–1.26) | 0.913   | 341  | 287  | 0.75 (0.59–0.96) | 0.020  |
|            |       | T        | 254 | 206  | 1       | 1.44 (0.96–2.14) | 0.075   | 139  | 145  | 0.65 (0.44–0.96) | 0.031  |
|            | Genotype | CC       | 82  | 79   | 1       | 0.83 (0.50–1.38) | 0.473   | 39   | 49   | 0.56 (0.33–0.95) | 0.031  |
|            | Dominant | CC       | 82  | 79   | 1       | 1.24 (0.85–1.80) | 0.265   | 178  | 194  | 0.63 (0.43–0.91) | 0.014  |
|            | Recessive | CC-CT   | 210 | 156  | 1       | 0.75 (0.59–1.04) | 0.073   | 39   | 49   | 0.73 (0.46–1.17) | 0.189  |
|            | Log-additive | –       | –   | –    | –       | 0.97 (0.75–1.25) | 0.796   | –    | –    | 0.73 (0.56–0.94) | 0.016  |
| rs485842   | Allele| C        | 458 | 325  | 1       | 0.66 (0.50–0.86) | 0.003   | 148  | 141  | 1.00 (0.76–1.30) | 0.976  |
|            |       | T        | 134 | 145  | 1       | 0.85 (0.58–1.23) | 0.378   | 139  | 145  | 0.65 (0.44–1.33) | 0.031  |
|            | Genotype | CC       | 177 | 121  | 1       | 0.84 (0.59–1.18) | 0.030   | 177  | 154  | 1.01 (0.65–1.5)  | 0.945   |
|            | Dominant | CC       | 177 | 121  | 1       | 0.70 (0.49–0.99) | 0.043   | 130  | 128  | 0.97 (0.69–1.37) | 0.866   |
|            | Recessive | CC-CT   | 281 | 204  | 1       | 0.63 (0.43–2.89) | 0.005   | 261  | 252  | 1.35 (0.63–2.99) | 0.445   |
|            | Log-additive | –       | –   | –    | –       | 0.66 (0.50–0.86) | 0.002   | –    | –    | 1.03 (0.77–1.37) | 0.844   |
| Gender     |       | Male     |     | Female |         |             |        |      |         |             |        |
| rs7938889  | Allele| C        | 382 | 305  | 1       | 0.8 (0.64–1.01) | 0.059   | 217  | 196  | 0.94 (0.73–1.22) | 0.651   |
|            |       | T        | 254 | 253  | 1       | 0.85 (0.58–1.23) | 0.323   | 149  | 137  | 0.93 (0.65–1.33) | 0.682   |
|            | Genotype | CC       | 107 | 83   | 1       | 0.82 (0.56–1.19) | 0.297   | 141  | 112  | 1.08 (0.71–1.62) | 0.716   |
|            | Dominant | CC       | 107 | 83   | 1       | 0.84 (0.59–1.18) | 0.032   | 76   | 67   | 0.84 (0.49–1.45) | 0.53    |
| rs7115578  | Allele| A        | 365 | 289  | 1       | 0.81 (0.64–1.02) | 0.069   | 248  | 197  | 1.18 (0.91–1.52) | 0.211   |
|            |       | G        | 275 | 269  | 1       | 0.68 (0.47–1.00) | 0.048   | 124  | 107  | 1.16 (0.76–1.77) | 0.495   |
|            | Genotype | AA       | 108 | 72   | 1       | 0.62 (0.43–1.07) | 0.097   | 62   | 45   | 1.38 (0.83–2.29) | 0.217   |
|            | Dominant | AA       | 108 | 72   | 1       | 0.86 (0.58–1.27) | 0.477   | 62   | 45   | 1.26 (0.81–1.94) | 0.302   |
| rs4598633  | Allele| C        | 348 | 295  | 1       | 0.93 (0.74–1.17) | 0.562   | 225  | 183  | 1.13 (0.87–1.46) | 0.370   |
|            |       | T        | 290 | 263  | 1       | 0.97 (0.74–1.26) | 0.562   | 171  | 143  | 1.11 (0.86–1.38) | 0.277   |
|            | Genotype | CC       | 96  | 74   | 1       | 0.82 (0.56–1.19) | 0.297   | 141  | 97   | 1.66 (1.1–2.50)  | 0.016   |
|            | Dominant | CC       | 96  | 74   | 1       | 0.89 (0.56–1.42) | 0.624   | 42   | 43   | 1.11 (0.66–1.9)  | 0.690   |
| rs4598633  | Recessive | CC-CT   | 223 | 205  | 1       | 0.84 (0.59–1.20) | 0.334   | 183  | 140  | 1.49 (1.01–2.20) | 0.043   |
|            | Log-additive | –       | –   | –    | –       | 0.93 (0.74–1.18) | 0.557   | –    | –    | 1.13 (0.87–1.47) | 0.364   |

P-values were calculated by logistic regression analysis with adjustments for age and gender. 

P<0.05 means the data are statistically significant. 

Bold means the data are statistically significant.

© 2019 The Author(s). This is an open access article published by Portland Press Limited on behalf of the Biochemical Society and distributed under the Creative Commons Attribution License 4.0 (CC BY).
Table 4 Univariate analysis of the association between MAML2 polymorphisms and glioma patient OS and PFS

| SNP ID   | Genotype | OS SR (1-/3-year) | HR (95% CI) | P  | FFS SR (1-/3-year) | HR (95% CI) | P  |
|----------|----------|-------------------|-------------|----|--------------------|-------------|----|
| rs7115578 | AA       | 0.052             | 0.369/0.113 | 1  | 0.073              | 0.210/0.117 | 1  |
|          | AG       | 0.276/0.071       | 1.24 (1.02–1.52) | 0.033 | 0.160/0.075       | 1.22 (1.00–1.50) | 0.051 |
|          | GG       | 0.336/0.111       | 1.07 (0.84–1.37) | 0.595 | 0.185/-            | 1.06 (0.89–1.35) | 0.661 |
| Astrocytoma rs7115578 | AA | 0.037             | 0.395/0.055 | 1  | 0.093              | 0.206/0.072 | 1  |
|          | AG       | 0.236/0.065       | 1.31 (1.02–1.69) | 0.036 | 0.145/0.069       | 1.25 (0.97–1.61) | 0.085 |
|          | GG       | 0.362/0.112       | 1.02 (0.75–1.38) | 0.909 | 0.200/0.129       | 1.01 (0.74–1.37) | 0.971 |
| Low-grade glioma (I–II) rs11021499 | GG | 0.046             | 0.406/0.127 | 1  | 0.024              | 0.245/0.142 | 1  |
|          | GA       | 0.274/0.076       | 1.30 (1.00–1.68) | 0.047 | 0.147/-            | 1.33 (1.03–1.72) | 0.032 |
|          | AA       | 0.345/-           | 1.02 (0.75–1.40) | 0.885 | 0.214/-            | 1.02 (0.75–1.40) | 0.883 |

Abbreviation: SR, survival rate.
Log-rank P-values were calculated using the Chi-Square test.
P < 0.05 indicates statistical significance.

Figure 1. Kaplan–Meier survival curves for MAML2 polymorphism and glioma prognosis
Kaplan–Meier survival curves for PFS based on MAML2 rs7115578 in astrocytoma (A) and for OS and PFS based on MAML2 rs11021499 polymorphism in low-grade glioma (B,C).

was only one that affected the prognosis of glioma in the overall. Compared with the AA carriers of rs7115578, the AG genotype carriage represented a poorer prognosis of glioma (HR = 1.24, 95% CI: 1.02–1.52, P = 0.033). In astrocytoma, rs7115578 polymorphism also was a predictor for unfavorable prognosis (OS: log-rank P = 0.037, HR = 1.31, 95% CI: 1.02–1.69, P = 0.036). We next divided all patients into two groups (low- and high-grade gliomas) according to WHO grade. In patients with low-grade glioma, Kaplan–Meier analyses revealed that rs11021499-GA genotype had lower OS (log-rank P = 0.046, HR = 1.30, 95% CI: 1.00–1.68, P = 0.047) and PFS (log-rank P = 0.024, HR = 1.33, 95% CI: 1.03–1.72, P = 0.032) than CC genotype.

We next interrogated the association between MAML2 SNPs and PFS or OS by a multivariate Cox proportional hazard model, adjusted for patient surgical method and chemotherapy (Table 5). The rs7115578 heterozygous was significantly associated with the poorer PFS of glioma (HR = 1.25, 95% CI: 1.02–1.53, P = 0.031) and high-grade glioma (HR = 1.45, 95% CI: 1.03–2.03, P = 0.032). Additionally, astrocytoma patients carrying the AG genotype had significantly decreased OS (HR = 1.40, 95% CI: 1.08–1.81, P = 0.010) and PFS (HR = 1.38, 95% CI: 1.07–1.78, P = 0.014) compared with those with the AA genotype.

Discussion
In the present study, we first investigated the association between MAML2 genetic variants and glioma risk or prognosis among the Chinese Han population. We found that rs7938889, rs485842, rs7115578 and rs4598633 polymorphisms were significantly related to the risk of glioma. Moreover, we demonstrated that rs7115578 and rs11021499 variants...
represented a poorer prognosis of glioma. To the best of our knowledge, no previous studies have investigated the role of MAML2 variants for glioma. Our study addressed a gap in knowledge of the MAML2 gene polymorphisms and the susceptibility and prognosis of glioma, indicating that MAML2 genetic variations might play an important role in the development of glioma.

MAML2, located at 11q21, normally acts as a co-activator of Notch receptor and transactivates Notch target gene, participating in the formation of Notch-associated RBP-J/CBF complex [19,20]. The oncogenic role of MAML2 was first described in mucoepidermoid carcinoma, in which a fusion oncogene MECT1-MAML2 that was involved in disrupting the normal cell cycle, differentiation and tumor development [21]. In addition, MAML2 was previously found to participate in a fusion with CRTCI, which was important for cell growth, proliferation, survival, migration and metabolism [22]. These studies provided some biologic evidence for the role played by MAML2 in possible molecular mechanisms of carcinogenesis. A recent study showed that the CRTCI-MAML2 gene fusion was also identified in the brain tumors [23]. Additionally, MAML2 as a novel gene was abnormal expressed in glioma [14]. By bioinformatics analysis, we found that MAML2 gene expression is up-regulated in glioma compared with normal tissue. Moreover, low expression of MAML2 was associated with a poor OS for glioma, especially lower grade glioma. These results hinted that MAML2 plays an important role in the progression and prognosis of glioma, but more studies are needed to validate.

Previous studies have confirmed that the genetic variability of MAML2, including structural variation, copy number variation and SNPs, were associated with the occurrence and progression of disease [16,24,25]. In the present study, four SNPs in MAML2 (rs7938889 and rs485842, rs7115578 and rs4598633) were found to be significantly associated with glioma risk. Specifically, carriers of the rs7938889 TT and rs485842 TT genotypes reduced the risk of the overall glioma and astrocytoma. Furthermore, the association between glioma risk and rs7938889 genotypes was more profound in the individuals at age \( \leq 40 \) years, while rs485842 variant in the subjects at age \( > 40 \) years. We also found that rs7938889 and rs7115578 polymorphisms had a lower susceptibility to glioma in males, while rs4598633 variant was associated with a higher risk for glioma in females. There are differences of glioma incidence in gender and age [26]. This result suggested the risk association of MAML2 polymorphisms with glioma might be dependent on age or gender. More importantly, we revealed that rs7115578 AG genotype represented a poorer prognosis of glioma, particularly among astrocytoma and high-grade glioma. Rs11021499 polymorphism had lower OS and PFS in patients with low-grade glioma. Although MAML2 polymorphisms were found to be significantly associated with the risk and prognosis of glioma, the mechanism of MAML2 underlying the effect on the glioma risk and patients survival was not identified in the present study, nor has not been reported in the literature. Several studies supported that intronic SNPs confer susceptibilities by affecting gene expression [27–29]. The online prediction tool Haploreg showed that rs7938889 and rs485842, rs7115578, rs4598633 and rs11021499 polymorphisms, located in the intron region, were associated with the regulation of promoter histone marks, enhancer histone marks, DNase and/or motifs changed, suggesting their possible functions in glioma patients. However, further study is necessary to confirm the function of these variant in glioma.

| SNP ID | Genotype | OS HR (95% CI) | OS P | PFS HR (95% CI) | PFS P |
|--------|----------|----------------|------|----------------|------|
| rs7115578 | AA | 1 | 1 | 1.25 (1.02–1.53) | 0.031 |
| | AG | 1.21 (1.00–1.49) | 0.056 | 1.07 (0.84–1.37) | 0.572 |
| | GG | 1.06 (0.83–1.36) | 0.627 |
| rs7115578 | AA | 1 | 1 | 1.38 (1.07–1.78) | 0.014 |
| | AG | 1.40 (1.08–1.81) | 0.010 | 1.19 (0.87–1.61) | 0.275 |
| | GG | 1.17 (0.86–1.60) | 0.306 |
| rs7115578 | AA | 1 | 1 | 1.45 (1.03–2.03) | 0.032 |
| | AG | 1.35 (0.96–1.89) | 0.080 | 1.28 (0.82–1.99) | 0.284 |

Log-rank P-values were calculated using the Chi-Square test. 

P-values were calculated by Cox multivariate analysis with adjustments for surgical method and use of chemotherapy. 

P < 0.05 indicates statistical significance.
Strengths of the current study include the relatively large sample size considering the rarity of glioma, and the first demonstration on the associations of MAML2 polymorphisms with glioma risk and prognosis among Chinese Han population. However, several limitations should be addressed in the present study. First, the potential selection bias might have occurred because the study subjects in our study were hospital-based, thus the conclusion of the present study warrants further confirmation in a larger scale population. Second, the detailed molecular mechanism under which MAML2 polymorphisms affect glioma risk and prognosis needs further studies to elucidate.

Conclusion
In conclusion, these results suggested that MAML2 polymorphisms might contribute to glioma susceptibility and prognosis. We found that MAML2 rs7938889 and rs485842 polymorphisms were significantly associated with the reduced risk of glioma. Moreover, rs7115578 seem to confer a worse prognosis for glioma and astrocytoma. Although these data need confirmation by independent studies, our results hint MAML2 genetic variants might play an important role in the development of glioma among Chinese Han population, and add to the body of knowledge surrounding genetic polymorphisms as putative player affecting the risk or prognosis of glioma.

Ethical Approval
The protocol of the present study was approved by the institutional Ethnics Committee of both the People’s Hospital of Xinjiang Uygur Autonomous Region and Northwest University, and carried out in accordance with the World Medical Association Declaration of Helsinki.

Informed Consent
Written informed consent was obtained from all of the subjects before participating.

Author Contribution
The work presented here was carried out in collaboration among all authors. M.Z. carried out the molecular genetic studies and drafted the manuscript. Y.Z. performed the statistical analysis and interpreted the results. J.Z. and T.H. designed primers and performed the SNP genotyping experiments. X.G. and X.M. collected clinical information about patients and performed the SNP genotyping experiments. Y.W. conceived the study, worked on associated data collection and their interpretation, participated in the design and coordination of the study, and funded the study. All authors read and approved the final manuscript.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Funding
This work was supported by the Fundamental Research Funds for the Central Universities [grant number xj2015018]; the Special Research Fund for Personnel Training of the Second Affiliated Hospital of Xi’an Jiaotong University [grant number RC (XM) 201603]; and the Special Research Fund for the Youth Sciences Foundation of the Second Affiliated Hospital of Xi’an Jiaotong University [grant number YJ (QN) 201402].

Abbreviations
CI, confidence interval; CNS, central nervous system; HR, hazard ratio; HWE, Hardy–Weinberg equilibrium; MAML2, mastermind-like transcriptional co-activator 2; OR, odds ratio; OS, overall survival; PFS, progression-free survival; SNP, single-nucleotide polymorphism.

References
1 Weller, M., Wick, W., Aldape, K., Brada, M., Berger, M., Pfister, S.M. et al. (2015) Glioma. *Nat. Rev. Dis. Primers* **1**, 15017, https://doi.org/10.1038/nrdp.2015.17
2 Chen, W., Zheng, R., Baade, P.D., Zhang, S., Zeng, H., Bray, F. et al. (2016) Cancer statistics in China, 2015. *CA Cancer J. Clin.* **66**, 115–132, https://doi.org/10.3322/caac.21338
3 Reni, M., Mazza, E., Zanon, S., Gatta, G. and Vecht, C.J. (2017) Central nervous system gliomas. *Crit. Rev. Oncol. Hematol.* **113**, 213–234, https://doi.org/10.1016/j.critrevonc.2017.03.021
4 Ostrom, Q.T., Gittleman, H., Stetson, L., Virk, S.M. and Barnholtz-Sloan, J.S. (2015) Epidemiology of gliomas. *Cancer Treat. Res.* **163**, 1–14, https://doi.org/10.1007/978-3-319-12048-5_1
5 Li, S., Jin, T., Zhang, J., Lou, H., Yang, B., Li, Y. et al. (2012) Polymorphisms of TREH, IL4R and CCDC26 genes associated with risk of glioma. *Cancer Epidemiol. Biomarkers Prev.* **21**, 283–287, https://doi.org/10.1095/1016671122112011
6 Li, G., Jin, T., Liang, H., Zhang, Z., He, S., Tu, Y. et al. (2013) RTEL1 tagging SNPs and haplotypes were associated with glioma development. *Diagn. Pathol.* **8**, 83, https://doi.org/10.1186/1746-1596-8-83

7 Jin, T., Wang, Y., Li, G., Du, S., Yang, H., Geng, T. et al. (2015) Analysis of difference of association between polymorphisms in the XRCC5, RPA3 and RTEL1 genes and glioma, astrocytoma and glioblastoma. *Am. J. Cancer Res.* **5**, 2294–2300

8 Lin, S.E., Oyama, T., Nagase, T., Harigaya, K. and Kitagawa, M. (2002) Identification of new human mastermind proteins defines a family that consists of positive regulators for notch signaling. *J. Biol. Chem.* **277**, 50612–50620, https://doi.org/10.1074/jbc.M209529200

9 Li, L., Tang, P., Li, S., Qin, X., Yang, H., Wu, C. et al. (2017) Notch signaling pathway networks in cancer metastasis: a new target for cancer therapy. *Med. Oncol.* **34**, 180

10 Yan, D., Hao, C., Xiao-Feng, L., Yu-Chen, L., Yu-Bin, F. and Lei, Z. (2018) Molecular mechanism of Notch signaling with special emphasis on microRNAs: Implications for glioma. *J. Cell. Physiol.* **234**, 158–170, https://doi.org/10.1002/jcp.26775

11 Bishop, J.A., Cowan, M.L., Shum, C.H. and Westra, W.H. (2018) MAML2 rearrangements in variant forms of mucoepidermoid carcinoma: ancillary diagnostic testing for the ciliated and warthin-like variants. *Am. J. Surg. Pathol.* **42**, 130–136

12 Kuma, Y., Yamada, Y., Yamamoto, H., Kohashi, K., Ito, T., Furue, M. et al. (2017) A novel fusion gene CRTC3-MAML2 in hidradenoma: histopathological significance. *Hum. Pathol.* **70**, 55–61, https://doi.org/10.1016/j.humpath.2017.10.004

13 Lubecka, K., Kurzawa, L., Flower, K., Buvala, H., Zhang, H., Teegarden, D. et al. (2016) Stibensoids remodel the DNA methylation patterns in breast cancer cells and inhibit oncogenic NOTCH signaling through epigenetic regulation of MAML2 transcriptional activity. *Carcinogenesis* **37**, 656–668, https://doi.org/10.1039/c3canw04684

14 Jiang, C.M., Wang, X.H., Shu, J., Yang, W.X., Fu, P., Zhuang, L.L. et al. (2015) Analysis of differentially expressed genes based on microarray data of glioma. *Int. J. Clin. Exp. Med.* **8**, 17321–17332

15 Zhang, W., Liu, H., Liu, Z., Zhu, D., Amos, C.I., Fang, S. et al. (2015) Functional variants in Notch Pathway Genes NCO2, NCSTN, and MAML2 predict survival of patients with cutaneous melanoma. *Cancer Epidemiol. Biomarkers Prev.* **24**, 1101–1110

16 Xu, Y., Cheng, L., Dai, H., Zhang, R., Wang, M., Shi, T. et al. (2018) Variants in Notching signalling pathway genes, PSEN1 and MAML2, predict overall survival in Chinese patients with epithelial ovarian cancer. *J. Cell. Mol. Med.* **22**, 4975–4984

17 Gabriel, S., Ziaugra, L. and Tabbaa, D. (2009) SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Curr. Protoc. Hum. Genet. Chapter 2*, Unit 2.12

18 Dai, Z.J., Liu, X.H., Ma, Y.F., Kang, H.F., Jin, T.B., Dai, Z.M. et al. (2016) Association between single nucleotide polymorphisms in DNA polymerase kappa gene and breast cancer risk in Chinese Han population: a STROBE-Compliant Observational Study. *Medicine (Baltimore)* **95**, e2466, https://doi.org/10.1097/MD.000000000002466

19 Wu, L., Sun, T., Kobayashi, K., Gao, P. and Griffin, J.D. (2002) Identification of a family of mastermind-like transcriptional coactivators for mammalian notch receptors. *Mol. Cell. Biol.* **22**, 7688–7770, https://doi.org/10.1128/MCB.22.21.7688-7700.2002

20 Enlund, F., Behboudi, A., Andren, Y., Oberg, C., Lendahl, U., Mark, J. et al. (2004) Altered Notch signaling resulting from expression of a novel fusion product that disrupts a Notch signaling pathway. *Genes Chromosomes Cancer* **37**, 730–749

21 Tonon, G., Modi, S., Wu, L., Kubo, A., Coxon, A.B., Komiya, T. et al. (2003) t(11;19)(q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. *Nat. Genet.* **33**, 208–213, https://doi.org/10.1038/ng1083

22 Chen, J., Li, J.J., Chen, Z., Griffin, J.D. and Wu, L. (2015) Gene expression profiling analysis of CRTC1-MAML2 fusion oncogene-induced transcriptional program in human mucoepidermoid carcinoma cells. *BMC Cancer* **15**, 803, https://doi.org/10.1186/s12885-015-1827-3

23 Busse, T.M., Roth, J.J., Wilmoth, D., Wainwright, L., Took, L. and Biegel, J.A. (2017) Copy number alterations determined by single nucleotide polymorphism array testing in the clinical laboratory are indicative of gene fusions in pediatric cancer patients. *Genes Chromosomes Cancer* **56**, 730–749

24 Nalesnik, M.A., Tseng, G., Ding, Y., Xiang, G.S., Zheng, Z.L., Yu, Y. et al. (2012) Gene deletions and amplifications in human hepatocellular carcinomas: correlation with hepatocyte growth regulation. *Am. J. Pathol.* **180**, 1495–1508, https://doi.org/10.1016/j.ajpath.2011.12.021

25 Davis, M.E. (2018) Epidemiology and overview of gliomas. *Semin. Oncol. Nurs.* **34**, 420–429, https://doi.org/10.1016/j.socn.2018.10.001

26 Zhao, H., Yang, W., Qiu, R., Li, J., Xin, Q., Wang, X. et al. (2012) An intronic variant associated with systemic lupus erythematosus changes the binding affinity of Yin1/y to downregulate WDFY4. *Genes Immun.* **13**, 536

27 Seo, S., Takayama, K., Uno, K., Ohi, K., Hashimoto, R., Nishizawa, D. et al. (2013) Functional analysis of deep intronic SNP rs13438494 in intron 24 of PCLO gene. *PLoS ONE* **8**, e76960, https://doi.org/10.1371/journal.pone.0076960

28 Wang, D. and Sadee, W. (2016) CYP3A4 intrinsic SNP rs35599367 (CYP3A4*22) alters RNA splicing. *Pharmacogenet. Genom. 26*, 40–43