Introduction

According to the classification by the World Health Organization (WHO), glioma encompasses all tumors that are thought to be of glial cell origin, including Astrocytic tumors [Astrocytoma grades I, II (Astrocytoma), III (Anaplastic astrocytoma), and IV (Glioblastoma or GBM)], Oligodendrogiomas, Ependymomas, and Mixed gliomas (Central Brain Tumor Registry of the United States). Gliomas are common tumors and account for almost 80% of primary malignant brain tumors, usually resulting in poor survival compared to other types of brain tumors.

Current evidence suggests that inherited risks play a role in glioma susceptibility, as with other cancers. A majority of the inherited risk is due to the co-inheritance of multiple low-risk variants, some of which are commonly seen gene variants and hence can be identified through association studies [1]. The epidemiology of glioma has focused on identifying factors that can be modified to prevent this disease [2–4]. Recent research has focused on identifying germline polymorphisms associated with the risk of glioma and defining molecular markers to classify glial tumors in more homogenous groups [2–4].

The epidermal growth factor receptor (EGFR) regulates important cellular processes and is implicated in human tumors. Several previous studies have assessed single nucleotide polymorphisms (SNPs) in the EGFR gene for the association with the risk of cancers, such as lung cancer [5,6], breast cancer [7], prostate cancer [8], and esophageal cancer [9]. Somatic alterations of the EGFR gene are common in glioma and influence several mechanisms of malignant transformation [10]. Previous studies have shown that regulation of the EGFR pathway plays an important role in glioma progression [11], and certain EGFR genotypes may be related to glioblastoma risk, indicating that germline EGFR polymorphisms may have important implications in carcinogenesis of glioma [12].

In addition, it is possible that haplotypes and locus–locus interactions within the EGFR gene may be correlated with the development of glioma. To investigate potential relationships between EGFR SNP polymorphisms, haplotypes, locus–locus interactions, and their role in the etiology of gliomas, we performed a comprehensive association analysis in a case–control study in the Han Chinese population. Our study indicated important evidence for the association between EGFR gene polymorphisms and the risk of glioma.

Results

A total of 301 cases (157 male, 144 female; median age at diagnosis 41.5 yrs) and 302 controls (155 male, 147 female; median age 42.3 yrs) were included in the current study. Basic characteristics of the cases and controls were listed in Table 1.
including gender, age, and pathology. As listed in Table 2, a multiplexed SNP MassEXTEND assay was designed with the Sequenom MassARRAY Assay Design 3.0 Software. Nine SNPs in the EGFR gene in glioma patients and the control group were genotyped (raw genotype data are listed in Table S1 and Table S2). The average tSNPs call rate was 90.5% in cases and controls. All of the tested SNPs are in Hardy–Weinberg equilibrium (HWE) in the control population of this study (Table 3). We compared the differences in frequency distributions of alleles between cases and controls by $\chi^2$ test and found two significant tSNPs in the EGFR gene at a 5% level ($rs1468727, p = 0.003$, odds ratio [OR]: 1.31, 95% confidence interval [CI]: 1.04–1.65 and $rs730437, p = 0.016$; OR: 1.32, 95% CI: 1.05–1.66). After a strict Bonferroni correction analysis was applied, we found no association between tSNPs and risk of glioma (Table 3). We further analyzed the allele frequency differentiation of rs730437 and rs1468727 between diverse groups of cases with varying aggressive grades and found no association between tumor aggressiveness and presence of the risk allele (Table S3).

Association results between tSNPs and risk of glioma were listed in Table 4. We identified two significant SNP genotypes associated with the risk of glioma, one was genotype “CC” of rs1468727 (OR, 1.68; 95% CI, 1.04–2.69; $p = 0.032$) and additive model analyses (OR, 1.33; 95% CI, 1.05–1.72; $p = 0.019$). We also observed another susceptibility SNP, rs1468727, by reccessive model analyses (OR, 1.88; 95% CI, 1.22–2.89; $p = 0.004$) and additive model analyses (OR, 1.37; 95% CI, 1.07–1.76; $p = 0.012$).

Three blocks were detected in studied EGFR SNPs by haplotype analyses (Figure 1). The global result for Block 1 ($rs1947492$ and $rs12718943$) was: total case = 594, total control = 596, global $\chi^2 = 0.106$ while df = 1, Fisher’s $p$ value = 0.744, and Pearson’s $p$ value = 0.744. The global result for Block 2 ($rs170437$, $rs11506105$, $rs3752651$, and $rs1466727$) was: total case = 502, total control = 559, global $\chi^2 = 6.384$ while df = 2, Fisher’s $p$ value = 0.037, and Pearson’s $p$ value = 0.037. The global result for Block 3 ($rs845552$ and $rs9642393$) was: total case = 576, total control = 572, global $\chi^2 = 2.79$ while df = 1, Fisher’s $p$ value = 0.095, and Pearson’s $p$ value = 0.095. The global result was: total case = 545, total control = 517, global $\chi^2 = 18.81$ while df = 6, Fisher’s $p$ value = 0.005, and Pearson’s $p$ value = 0.005 (frequency <0.03 in both the control and case was dropped).

The results of the association between the EGFR haplotype and the risk of glioma were listed in Table 6. Haplotype “CGTC” in Block 2 was found to be associated with the risk of glioma (OR, 1.321; 95% CI, 1.033–1.688; Fisher’s $p$ value = 0.026; Pearson’s $p$ value = 0.026). In Block 2, we also found a protective haplotype “AATT” associated with the risk of glioma (OR, 0.732; 95% CI, 0.576–0.929; Fisher’s $p$ value = 0.01; Pearson’s $p$ value = 0.01). Haplotype association analyses showed that haplotype “TGTAATTGC” was associated with an increased risk of glioma at a 1% level (OR, 0.286; 95% CI, 0.135–0.609; Fisher’s $p$ value = 0.001; Pearson’s $p$ value = 0.001).

### Table 1. Basic characteristics of case and control patients.

|            | Cases (n = 301) | Controls (n = 302) | $p$ value from $\chi^2$ |
|------------|-----------------|-------------------|-------------------------|
| **Sex**    |                 |                   |                         |
| Male       | 157             | 155               | 0.837                   |
| Female     | 144             | 147               |                          |
| **Age**    |                 |                   | 0.063                   |
| > = 50     | 117             | 140               | 46.4                    |
| < 50       | 184             | 162               | 53.6                    |
| Median age | 41.5            | 42.3              |                          |
| **Histologic type** |         |                   |                         |
| Astrocytoma| 173             | 57.5              |                          |
| Ependymoma | 20              | 6.6               |                          |
| Glioblastoma| 42             | 14.0              |                          |
| Oligodendroglia| 9            | 3.0               |                          |
| others     | 57              | 18.9              |                          |

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### Discussion

In this case–control study in a Han Chinese population, we identified for the first time rs1468727 and rs730437 in the EGFR gene associated with an increased risk of glioma. A protective effect was also observed for the haplotype “AATT” of the EGFR gene that was associated with a 29% reduction in the risk of developing glioma. Additionally, we also observed a strong effect of the “CGTC” haplotype, which increased the risk of developing glioma by 36%.

Our study adopted a genotype and haplotype based approach. To the best of our knowledge, this study was the first haplotype-based study that described the association between tSNPs in the EGFR gene and glioma risk in a Chinese population. Previous studies focused only on one or two variants in the EGFR gene, which might not sufficiently capture the effect of susceptibility loci in Chinese glioma patients. A haplotype-based association approach is an increasingly accepted approach for genetic association studies [13]. Using this approach, we provided strong support that EGFR gene variations contributed to the susceptibility to glioma.

It is important to note two SNPs (rs1468727 and rs730437) and their relationship with glioma risk in this study. We found that genotype “CC” of rs1468727 in intron 13 of the EGFR gene was associated with the risk of glioma in Chinese patients. Interestingly, genotype “TT” of rs1468727 was found to be associated with a decreased risk of glioma in a previous study in a European population (OR, 0.61; 95% CI, 0.40–0.93; $p = 0.017$) [12]. These results supported our findings that rs1468727 was a susceptibility loci and the genotype “CC” of this locus was a risk genotype for glioma. Another SNP, rs730437, located in intron 4 of the EGFR gene was identified in both studies. In our study, the genotype “CC” of rs730437 was identified as the risk genotype with frequencies of 0.43 in glioma patients and 0.36 in controls. However, in the European population, the risk genotype was “AA” (OR, 1.32; 95% CI, 1.03–1.68; $p = 0.032$), with frequencies of 0.27 in glioma patients and 0.23 in controls [12]. Together, these findings indicate that ethnic differences among the EGFR gene variants may affect the development of glioma in diverse populations. Furthermore, tSNPs rs1468727 and rs730437 may have a tight linkage with other functional SNPs. Therefore, the
Table 2. PCR primers.

| SNP_ID   | 1st-PCR primer sequences | 2nd-PCR primer sequences | UEP sequences |
|----------|--------------------------|--------------------------|---------------|
| rs7340373 | ACGTTGATGAGGAGCAATCGAGCTCA | ACGTTGATGAGGAGCAATCGAGCTCA | CATATGGTCGGTGAAG |
| rs855552  | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACGTTGATGACCTTTCACTTCTGAGCTCA | TGGATCTCAGACCATCT |
| rs1468727 | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACTCTGCTCTCTCCCT |
| rs3752651 | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACGTTGATGACCTTTCACTTCTGAGCTCA | ATCTGGTTCAGCTTATG |
| rs497492  | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACGTTGATGACCTTTCACTTCTGAGCTCA | AGTGGAGATGTCACATATG |
| rs9642393 | ACGTTGATGACCTTTCACTTCTGAGCTCA | ACGTTGATGACCTTTCACTTCTGAGCTCA | TCCCAGTGTGCTGCTGCG |
| rs11506105| ACGTTGATGAGGAGCAATCGAGCTCA | ACGTTGATGAGGAGCAATCGAGCTCA | GGAAGAGAGATTATTTAATAAGG |
| rs12718945| ACGTTGATGAGGAGCAATCGAGCTCA | ACGTTGATGAGGAGCAATCGAGCTCA | CAATTACTATCATAATCCATAG |

UEP: Unextended mini-sequencing primer.

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Table 3. Examined tSNPs examined in the EGFR gene.

| SNP_ID   | Location | Position (Genome build 36.3) | HWE p value | p value from $\chi^2$ | p value adj.* | OR (95%CI) |
|----------|----------|------------------------------|-------------|-----------------------|---------------|------------|
| rs11506105 | 7p11.2   | 55187671(boundary)         | 0.909       | 0.053                 | 0.477         | 1.23(0.97–1.56) |
| rs12718945 | 7p11.2   | 55160457(Intron 1)         | 0.904       | 0.777                 | 1             | 1.04(0.82–1.32) |
| rs1468727  | 7p11.2   | 55197599(Intron 13)        | 0.757       | 0.008                 | 0.072         | 1.31(1.04–1.65) |
| rs17172432 | 7p11.2   | 55108811(Intron 1)         | 0.926       | 0.563                 | 1             | 0.88(0.61–1.28) |
| rs3752651  | 7p11.2   | 55197037(Intron 13)        | 0.295       | 0.232                 | 1             | 1.11(0.73–1.69) |
| rs497492   | 7p11.2   | 55155486(Intron 1)         | 0.882       | 0.723                 | 1             | 1.04(0.82–1.32) |
| rs730437   | 7p11.2   | 55182512(Intron 4)         | 0.960       | 0.016                 | 0.144         | 1.32(1.05–1.66) |
| rs845552   | 7p11.2   | 55213001(Intron 19)        | 0.643       | 0.105                 | 0.945         | 1.24(0.98–1.56) |
| rs9642393  | 7p11.2   | 55213141(Intron 19)        | 0.979       | 0.115                 | 1             | 1.20(0.95–1.51) |

Note: *p value was adjusted by Bonferroni corrections.
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phosphorylation by the intracellular kinase domain, resulting in receptor activation. The EGFR gene was identified to be instrumental in glioma formation by EGFR transgenic rats (or mice) that developed cerebellar glioma [16–17]. In a previous study, a polymorphism in the 5'-untranslated region of the epidermal growth factor (EGF) gene, a natural ligand of the EGFR, was identified to play an important role in the pathogenesis of malignant gliomas [18]. They found that patients with the “GA” or “GG” genotype had higher EGF levels, irrespective of the EGFR status, were more likely to recur after surgery, and had a statistically significant shorter overall progression-free survival than patients with the “AA” genotype. Their findings, combined with our results, indicate that EGFR pathways may play a key role in the development of glioma.

**Table 4.** Association between EGFR tSNP genotypes and the risk of glioma.

| SNP_ID | Genotype | No. (frequency) | OR (95% CI) | p value | Case | Control |
|--------|----------|----------------|-------------|---------|------|---------|
| rs11506105 | GG | 50 (16.9) | 37 (12.5) | 1.56 (0.94–2.56) | 0.081 |
|          | AG | 140 (47.3) | 137 (46.3) | 1.18 (0.83–1.67) | 0.365 |
|          | AA | 106 (35.8) | 122 (41.2) | 1 (referent) | - |
| rs12718945 | TT | 36 (12.1) | 35 (11.7) | 1.07 (0.63–1.81) | 0.801 |
|          | CT | 138 (46.3) | 135 (45.2) | 1.06 (0.76–1.5) | 0.725 |
|          | GG | 124 (41.6) | 129 (43.1) | 1 (referent) | - |
| rs1468727 | CC | 77 (25.8) | 50 (17.2) | 1.78 (1.11–2.84) | 0.016 |
|          | TT | 78 (26.2) | 90 (31) | 1 (referent) | - |
| rs1712432 | CC | 20 (0.7) | 4 (1.3) | 0.48 (0.09–2.67) | 0.659 |
|          | TT | 245 (81.4) | 237 (79.8) | 1 (referent) | - |
| rs3752651 | CC | 20 (0.7) | 10 (0.3) | 2.02 (0.18–22.37) | 0.997 |
|          | CT | 46 (15.3) | 43 (14.4) | 1.08 (0.69–1.69) | 0.743 |
|          | TT | 252 (84) | 254 (85.2) | 1 (referent) | - |
| rs4947492 | CC | 37 (12.3) | 36 (12) | 1 (referent) | - |
|          | GT | 138 (46.3) | 135 (45.2) | 1.06 (0.76–1.5) | 0.725 |
|          | GG | 124 (41.6) | 129 (43.1) | 1 (referent) | - |
| rs730437  | CC | 56 (18.6) | 40 (13.3) | 1.74 (1.07–2.83) | 0.024 |
|          | CA | 147 (48.8) | 139 (46.2) | 1.32 (0.93–1.87) | 0.126 |
|          | AA | 98 (32.6) | 122 (40.5) | 1 (referent) | - |
| rs845552  | AA | 57 (19.1) | 43 (14.8) | 1 (referent) | - |
|          | GA | 132 (44.3) | 125 (43) | 1.19 (0.84–1.7) | 0.333 |
|          | GG | 109 (36.6) | 123 (42.5) | 1 (referent) | - |
| rs9642393 | TT | 57 (19.5) | 43 (14.5) | 1.48 (0.92–2.39) | 0.106 |
|          | CT | 135 (46.1) | 140 (47.3) | 1.08 (0.75–1.54) | 0.677 |
|          | CC | 101 (34.5) | 113 (38.2) | 1 (referent) | - |

OR: odd ratio; CI: confidence interval.

**Table 5.** Association between EGFR tSNPs and the risk of glioma based on logistic tests and their heterozygote and homozygote odds ratios, per allele odds ratios and confidence intervals.

| SNP No. | Minor Allele | MAF Case | MAF Control | Dominant Model OR 95% CI | p | Recessive Model OR 95% CI | p | Additive Model OR 95% CI | p |
|---------|--------------|----------|-------------|--------------------------|----|--------------------------|----|--------------------------|----|
| rs11506105 | G | 0.41 | 0.36 | 1.25 | 0.88 | 1.78 | 0.218 | 1.58 | 0.97 | 2.58 | 0.069 | 1.26 | 0.98 | 1.62 | 0.071 |
| rs12718945 | T | 0.35 | 0.34 | 1.03 | 0.73 | 1.45 | 0.881 | 1.08 | 0.64 | 1.83 | 0.781 | 1.03 | 0.80 | 1.33 | 0.807 |
| rs1468727  | C | 0.50 | 0.43 | 1.29 | 0.88 | 1.89 | 0.190 | 1.88 | 1.22 | 2.89 | 0.004 | 1.37 | 1.07 | 1.76 | 0.012 |
| rs1712432  | C | 0.10 | 0.11 | 0.92 | 0.60 | 1.42 | 0.702 | 0.61 | 0.11 | 3.38 | 0.571 | 0.91 | 0.61 | 1.35 | 0.624 |
| rs3752651  | C | 0.08 | 0.08 | 1.09 | 0.67 | 1.75 | 0.739 | 4.52 | 0.38 | 33.81 | 0.233 | 1.14 | 0.72 | 1.79 | 0.579 |
| rs4947492  | G | 0.36 | 0.35 | 1.02 | 0.72 | 1.44 | 0.917 | 1.11 | 0.66 | 1.87 | 0.701 | 1.04 | 0.80 | 1.33 | 0.793 |
| rs730437   | C | 0.43 | 0.36 | 1.38 | 0.97 | 1.97 | 0.077 | 1.68 | 1.04 | 2.69 | 0.032 | 1.35 | 1.05 | 1.72 | 0.019 |
| rs845552   | A | 0.41 | 0.36 | 1.34 | 0.94 | 1.91 | 0.105 | 1.34 | 0.84 | 2.12 | 0.221 | 1.24 | 0.97 | 1.58 | 0.081 |
| rs9642393  | T | 0.42 | 0.38 | 1.25 | 0.87 | 1.79 | 0.225 | 1.38 | 0.87 | 2.20 | 0.169 | 1.22 | 0.95 | 1.56 | 0.119 |

MAF: minor allele frequency; OR: odd ratio; CI: confidence interval.

Polymorphisms of EGFR and Glioma Risk

**Figure 1.** Haplotype block map for all the tSNPs of the EGFR gene. Block 1 includes rs4947492 and rs12718945; Block 2 includes rs730437, rs11506105, rs3752651 and rs1468727; and Block 3 includes rs845552 and rs9642393. The LD between two SNPs is standardized D9 scheme.

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phosphorylation by the intracellular kinase domain, resulting in receptor activation. The EGFR gene was identified to be instrumental in glioma formation by EGFR transgenic rats (or mice) that developed cerebellar glioma [16–17]. In a previous study, a polymorphism in the 5'-untranslated region of the epidermal growth factor (EGF) gene, a natural ligand of the EGFR, was identified to play an important role in the pathogenesis of malignant gliomas [18]. They found that patients with the “GA” or “GG” genotype had higher EGF levels, irrespective of the EGFR status, were more likely to recur after surgery, and had a statistically significant shorter overall progression-free survival than patients with the “AA” genotype. Their findings, combined with our results, indicate that EGFR pathways may play a key role in the development of glioma.
Table 6. EGFR haplotype frequencies and the association with the risk of glioma in case and control patients.

| Block | Haplotype | freq(case) | freq(control) |  χ² | Fisher’s p | Pearson’s p | OR | [95%CI] |
|-------|-----------|------------|---------------|-----|------------|-------------|----|--------|
| 1     | A G       | 0.645      | 0.653         | 0.106 | 0.744      | 0.744       | 0.961 | [0.757,1.221] |
|       | G T       | 0.352      | 0.342         | 0.106 | 0.744      | 0.744       | 1.041 | [0.819,1.322] |
| 2     | A A C C   | 0.084      | 0.073         | 0.514 | 0.474      | 0.474       | 1.171 | [0.76,1.084] |
|       | A A T T   | 0.471      | 0.546         | 6.571 | 0.01       | 0.01        | 0.732 | [0.576,0.929] |
|       | C G T C   | 0.394      | 0.333         | 4.945 | 0.026      | 0.026       | 1.321 | [1.033,1.688] |
| 3     | A T       | 0.412      | 0.362         | 2.79  | 0.095      | 0.095       | 1.226 | [0.965,1.556] |
|       | G C       | 0.578      | 0.622         | 2.79  | 0.095      | 0.095       | 0.816 | [0.643,1.036] |
| Total | C A G A T T G C | 0.04 | 0.048         | 0.515 | 0.473      | 0.473       | 0.808 | [0.452,1.447] |
|       | T A G A C C G C | 0.046 | 0.028        | 2.359 | 0.125      | 0.125       | 1.661 | [0.864,3.193] |
|       | T A G A T T G C | 0.395 | 0.421        | 1.669 | 0.196      | 0.196       | 0.843 | [0.65,1.093] |
|       | T A G C G T C A T | 0.086 | 0.063 | 1.831 | 0.176 | 0.176 | 1.374 | [0.866,2.18] |
|       | T G T A A T T G C | 0.016 | 0.053 | 11.841 | 0.001 | 0.001 | 0.286 | [0.135,0.609] |
|       | T G T C G T C A T | 0.229 | 0.188 | 2.239 | 0.135 | 0.135 | 1.258 | [0.931,1.7] |
|       | T G T C G T C G C | 0.036 | 0.027 | 0.530 | 0.467 | 0.467 | 1.29 | [0.649,2.564] |

OR: odd ratio; CI: confidence interval.
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The EGFR gene has been reported as one of the major genes responsible for malignant progression and phenotype reversion of gliomas, and has been used as one of the most important therapeutic targets. However, the mechanism how germline EGFR variants contribute to gliomagenesis remains unclear. Since EGFR gene amplifications were observed commonly in glioblastoma multiform, we hypothesized that certain mutations or haplotypes rendered the receptor susceptible to EGFR amplification. In future studies, to uncover the role of the EGFR gene in gliomagenesis, serum EGFR expression levels between different mutations or haplotype groups will be compared. We will also investigate the association between germline EGFR variants and somatic EGFR mutations, and the relationship between serum EGFR expression and somatic EGFR expression in the same glioma subjects.

In conclusion, our comprehensive analysis of SNPs in the EGFR gene suggests that EGFR genotypes and haplotypes are associated with glioma risk. These findings indicate that germ-line genetic variants of the EGFR gene play a complex role in the development of glioma, and that interactions of loci in the EGFR gene may be more important than a single locus. Our study offers important insights into the etiology of glioma.

Materials and Methods

Ethics Statement

The use of human tissue and the protocol in this study were strictly conformed to the principles expressed in the Declaration of Helsinki and were approved by the Ethical Committee of Xijing Hospital for approval of research involving human subjects. Signed informed consent was obtained from each participant.

Study population

In our study population, all analyses were restricted to Han Chinese. A total of 301 patients with glioma between November 2008 and December 2010 were recruited into an ongoing molecular epidemiological study at the Department of Neurosurgery of the Xijing Hospital affiliated with The Fourth Military Medical University (FMMU) in Xi’an city, China. All glioma cases had no previous history of other cancers, or prior chemotherapy or radiotherapy. There were no age, sex, or disease stage restrictions for case recruitment. All patients were recently diagnosed and histologically confirmed to have glioma.

A random sample of 500 healthy unrelated individuals were recruited between June 2010 and August 2010 from the medical examination center at Xijing Hospital, for genetic association research of human complex diseases, such as lung cancer, stomach cancer, and glioma. All of the chosen subjects were Han Chinese living in Xi’an city and its surrounding areas. A detailed recruitment and exclusion criteria were used. Generally, subjects with chronic diseases and conditions involving vital organs (heart, lung, liver, kidney, and brain) and severe endocrinological, metabolic, and nutritional diseases were excluded from this study. The purpose of the above exclusion procedures was to minimize the known environmental and therapeutic factors that influence the variation of human complex diseases. A total of 302 unrelated healthy subjects were recruited as controls in this study.

Demographic and clinical data

Demographic and personal data were collected through an in-person interview using a standardized epidemiological questionnaire, including age, sex, ethnicity, residential region, smoking status, alcohol use, education status, and family history of cancer. For patients, detailed clinical information was collected through a medical chart review or consultation with treating physicians. Plasma carcinoembryonic antigen and alpha-fetoprotein were tested in control subjects to make sure they did not have any cancers.

SNP selection and genotyping

Candidate tSNPs in the EGFR gene were selected from previously published polymorphisms associated with glioma [12]. Validated tSNPs were selected with a MAF >5% in the HapMap Asian population. A total of 9 tSNPs in the EGFR gene were selected for further genotyping. Genomic DNA was extracted from whole blood using the phenol-chloroform extraction method [19]. DNA concentration was measured by spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). A multiplexed SNP MassEXTEND assay was designed...
with the Sequenom MassARRAY Assay Design 3.0 Software [20]. SNP genotyping was performed using the Sequenom MassARRAY RS1000 with a standard protocol recommended by the manufacturer [20]. Data management and analyses were performed using the Sequenom Typer 4.0 software as previously described [20–21].

Statistical analysis

Statistical analyses were performed using Microsoft Excel and SPSS 16.0 statistical packages (SPSS, Chicago, IL). All p values in this study were two-sided. A p<0.05 was considered the threshold for statistical significance. Genotypic frequencies in control subjects for each SNP were tested for departure from HWE using the exact test [19,22]. ORs and 95% CIs were calculated by unconditional logistic regression analyses adjusted for age and sex [23]. We did not divide subjects into subgroups because of the limited sample size. The possibility of sex differences as a source of population sub-structure was evaluated by a genotype test for each SNP in male and female controls, and the number of significant results at the 5% level was compared with the number expected by the χ² test. We did not detect population stratification because all participants’ ethnicity was Han Chinese.

The three genetic models (dominant, recessive and additive) were applied by PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) to assess the association of single tSNPs with the risk of glioma. ORs and 95% CIs were calculated by unconditional logistic regression analyses adjusted for age and sex [23,24].

We used the Haploview software package (version 4.2) and SHEsis software platform (http://www.nhgg.org/analysis/) for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci [25,26].

Supporting Information

Table S1 Raw genotype data of 301 glioma cases.

Table S2 Raw genotype data of 302 controls.

Table S3 Allele frequency differentiation of rs730437 and rs1468727 between diverse groups of cases with varying aggressive grades.

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Author Contributions

Conceived and designed the experiments: WH YZ LX. Performed the experiments: WH WA XB. Analyzed the data: WH WA XB. Contributed reagents/materials/analysis tools: HD ZL. Wrote the paper: WH WA.