RESEARCH ARTICLE

# CCDC26 Gene Polymorphism and Glioblastoma Risk in the Han Chinese Population

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## Abstract

**Background: Glioblastoma (GBM) is an immunosuppressive tumor whose median survival time is only 12-15 months, and patients with GBM have a uniformly poor prognosis. It is known that heredity contributes to formation of glioma, but there are few genetic studies concerning GBM. Materials and Methods: We genotyped six tagging SNPs (tSNP) in Han Chinese GBM and control patients. We used Microsoft Excel and SPSS 16.0 statistical package for statistical analysis and SNP Stats to test for associations between certain tSNPs and risk of GBM in five different models. ORs and 95%CIs were calculated for unconditional logistic-regression analysis with adjustment for age and gender. The SHEsis software platform was applied for analysis of linkage disequilibrium, haplotype construction, and genetic associations at polymorphism loci. Results: We found rs891835 in CCDC26 to be associated with GBM susceptibility at a level of $p$=0.009. The following genotypes of rs891835 were found to be associated with GBM risk in four different models of gene action: *i*) genotype GT (OR=2.26; 95%CI, 1.29-3.97; $p$=0.019) or GG (OR=1.33; 95%CI, 0.23-7.81; $p$=0.019) in the codominant model; *ii*) genotypes GT and GG (OR=2.18; 95%CI, 1.26-3.78; $p$=0.0061) in the dominant model; *iii*) GT (OR=2.24; 95%CI, 1.28-3.92; $p$=0.0053) in the overdominant model; *iv*) the allele G of rs891835 (OR=1.85; 95%CI, 1.14-3.00; $p$=0.015) in the additive model. In addition, "CG" and "CGGAG" were found by haplotype analysis to be associated with increased GBM risk. In contrast, genotype GG of CCDC26 rs6470745 was associated with decreased GBM risk (OR=0.34; 95%CI, 0.12-1.01; $p$=0.029) in the recessive model. Conclusions: Our results, combined with those from previous studies, suggest a potential genetic contribution of CCDC26 to GBM progression among Han Chinese.**

**Keywords:** Single-nucleotide polymorphism (SNP) - glioblastoma (GBM) - CCDC26 - case-control studies

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## Introduction

Glioma is one of the most aggressive human tumors, and it consists of several subtypes, e.g., glioblastoma (GBM), astrocytoma, medulloblastoma, ependymoma, and pinealoma (Li et al., 2012). The most common primary brain tumor in adults is GBM, which is highly lethal (Colman et al., 2010; Verhaak et al., 2010). Previous studies have shown that exposure to ionizing radiation and history of familial cancer, such as Li-Fraumeni or Turcot syndrome, are risk factors, but this explains only a very small proportion of glioma cases (Relling et al., 1999; Liu et al., 2010). A strong genetic component, which is manifested by co-inheritance of multiple low-risk genetic variants, contributes to glioma susceptibility (Liu et al., 2010; Li et al., 2012; Li et al., 2012). Mutations in IDH1/IDH2 have becom molecular markers of significant diagnostic and prognostic relevance in the assessment of human gliomas (Das et al., 2013). With respect to GBM survival, the latest findings implicate such genes as G-protein coupled receptor 98, epidermal growth factor, and ABC transporters (Sadeque et al., 2012).

The CCDC26 gene, which modulates cell differentiation and death, has been associated with glioma (Li et al., 2012). Here, we investigate in a case-control study whether specific known tagging SNPs (tSNP) of CCDC26 are also associated with GBM, and according to which genetic model. Our data are the first to show a significant association between CCDC26 and GBM susceptibility in the Chinese Han population.

## Materials and Methods

*Study participants*

We recruited a total of 100 GBM patients from October 2011 to September 2012 for an ongoing molecular

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epidemiological study at the Department of Neurosurgery of the Tangdu Hospital, affiliated with The Fourth Military Medical University, in Xi'an, China. All of the study participants are Han Chinese living in the area of Xi'an, and GBM patients had been recently diagnosed and histologically confirmed. After exclusion of 28 cases because of unclear pathologic diagnoses or poor DNA quality, 72 GBM cases were successfully genotyped.

We also recruited a random group of 320 unrelated healthy individuals from June 2011 to July 2012 as controls, according to standard recruitment and exclusion criteria. Generally, all subjects were healthy without diseases related to vital organs. Levels of alpha-fetoprotein and plasma carcinoembryonic antigen were tested to ensure quality of controls. After exclusion of 18 patients because of incomplete case information, we successfully genotyped a total of 302 healthy control subject participants in the study.

We used a standardized epidemiological questionnaire, including such information as age, sex, ethnicity, residential region, and family history of cancer, to collect demographic and personal data. All participants were informed of the purpose and experimental procedures of the study. The Human Research Committee of the Tangdu Hospital for Approval of Research Involving Human Subjects gave permission for use of human tissue in this study. We obtained signed informed consent from each study participant.

*SNP selection and genotyping*

Six tSNPs in CCDC26 with minor allele frequencies (MAF) of >5% in the Asian-population HapMap, and which had previously been reported to be associated with glioma, were selected for genotyping (Liu et al., 2010; Lachance et al., 2011; Di Stefano et al., 2012). Genomic DNA from whole blood was extracted using the GoldMag® nanoparticles method, and concentration was measured by spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). Sequenom MassARRAY Assay Design 3.0 Software was used to design primers for amplification and extension reactions (Gabriel et al., 2009). We used Sequenom MassARRAY RS1000 (Sequenom Co. Ltd., San Diego, California, USA) to genotype the SNPs, following the standard protocol recommended by the manufacturer. Finally, Sequenom Typer 4.0 Software was used for data management and analysis (Thomas et al., 2007; Gabriel et al., 2009).

*Statistical analysis*

We used Microsoft Excel and SPSS 16.0 statistical package (SPSS; Chicago, IL, USA) for statistical analysis. All *p* values presented in our results are two-sided, and $p \leq 0.05$ was used as threshold of statistical significance. Departure from Hardy-Weinberg equilibrium (HWE) of each tSNP frequency was assessed using an exact test in control subjects. We calculated genotype frequencies among the cases and controls using a $\chi^2$ test (Adamec et al., 1964). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic-regression analysis, with adjustments for age and gender (Bland et al., 2000). The possibility of gender-related differences as a source of population substructure was also evaluated, by genotype testing of each tSNP in males versus females, followed by comparing the number of significant results at the 5% level with the number expected from $\chi^2$ test (Adamec et al., 1964).

We used SNP Stats (http://bioinfo.iconcologia.net/SNPstats_web), which is a web-based software tool (Bland et al., 2000), to test the associations between certain tSNPs and risk of GBM in five different models (codominant, dominant, recessive, overdominant, and additive) (Sole et al., 2006). We calculated ORs and 95%CIs using unconditional logistic-regression analysis with adjustments for age and gender (Bland et al., 2000).

Finally, we used the SHEsis software platform (http://www.nhgg.org/analysis/) for analysis of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Linkage disequilibrium was followed by the D' statistic, and a D' value of ≥0.8 indicated the related tSNPs formed one block (Shi et al., 2005).

## Results and Discussion

A total of 72 GBM patients were included in the study. Of these, there were 34 males and 38 females, with a mean age at diagnosis of 41±18 (SD) y. There were also 302 control patients with a mean age of 55±12 y, consisting of 150 males and 152 females, in the study. Six tSNPs were successfully genotyped in the GBM cases and control participants. The tSNP primer sequences are listed in Table 1 (Gabriel et al., 2009). Table 2 summarizes the MAF distribution of cases and controls. We found there was a correlation between rs891835 and increased GBM susceptibility (OR, 1.82; 95%CI, 1.16-2.86; $p$=0.009). The rs9656979 data were excluded at the 1% HWE *p* level.

We further analyzed data with SNP stats according to five gene models (dominant, recessive, additive, codominant, and overdominant models) to determine association of tSNPs and GBM risk by unconditional logistic regression analysis, with adjustments for age and gender (Sole et al., 2006). The miner allele (MA) of each tSNP was assumed to be a risk factor. We found one

**Table 1. Sequence of Oligonucleotide Primers Used in this Study**

| SNP_ID | 2nd-PCRP | 1st-PCRP | UEP_SEQ |
|---|---|---|---|
| rs891835 | ACGTTGGATGGGAAACACTGGTCATTATTGC* | ACGTTGGATGAATCCTTGCTACATTCCCCC | gaccTTATTGCAGTACAGCAATACACAG |
| rs4295627 | ACGTTGGATGTCTTGTTCAGTGACCAAGGG | ACGTTGGATGGGACAATAGTGTATGATAGC | TTACACTGCAAAAGCCA |
| rs6470745 | ACGTTGGATGATCCTTCCAACAACACCCTG | ACGTTGGATGTAAGCCACTCCTCTGTGCCT | cctcCCAACAACACCCTGAGCAGTA |
| rs9656979 | ACGTTGGATGGCTATTGCCCAGAAGCACAG | ACGTTGGATGTAGAGACAATCACCCTGGGC | CAAACCCTGCTCTATACTCC |
| rs10464870 | ACGTTGGATGCAAAAAACCCTGGGTTTTTC | ACGTTGGATGAGATTGCTGGGTGTCCCAC | CCTGGGTTTTTCTAAATCATTA |
| rs16904140 | ACGTTGGATGTGGAAACATTTTGCTCTTGC | ACGTTGGATGGAAATAAAGAGGGACTTTGG | aacgTGCTACTAAAATCAAGTGC |

*Sequences are written in the 5'→3' (left to right) orientation. UEP, unextended minisequencing primer

protective allele, rs6470745, and one risk allele, rs891835.

Data showing the relationship between greater GBM risk and tSNP rs891835 is presented in Table 3. We found the allele G of is associated with increased GBM risk. In the codominant model, genotypes GT (OR=2.26; 95%CI, 1.29-3.97; *p*=0.019) and GG (OR=1.33; 95%CI, 0.23-7.81; *p*=0.019) associate with increased risk by 2.26- and 1.33-fold, respectively. In the dominant model, genotypes GT and GG associate with a >2-fold increase in risk (OR=2.18; 95%CI, 1.26-3.78; *p*=0.0061). In the overdominant model, genotype GT increases risk by 2.24-fold (OR=2.24; 95%CI, 1.28-3.92; *p*=0.0053). Finally, in the additive model, allele G increases risk by 1.85-fold (OR=1.85; 95%CI, 1.14-3.00; *p*=0.015).

In contrast, we found the allele G of CCDC26 tSNP rs6470745 in our study population decreased GBM risk. Only one gene model showed a statistically significant association, and this was the recessive model. From those data, the genotype GG is found to associate with a 0.34-fold decrease in GBM risk (OR=0.34; 95%CI, 0.12-1.01; *p*=0.029).

Two blocks were detected in CCDC26 tSNPs by

**Table 2. Basic Information about Candidate SNPs Used in this Study**

| SNP ID | Gene Name | Chromosome position | Base change | MAF (Case) | MAF (Control) | HWE *p* | ORs | 95%CI | | *p* |
|---|---|---|---|---|---|---|---|---|---|---|
| rs10464870 | CCDC26 | 130477823 | T/C | 0.243 | 0.202 | 0.233 | 1.27 | 0.83 | 1.95 | 0.273 |
| rs891835 | CCDC26 | 130491752 | T/G | 0.229 | 0.14 | 0.998 | 1.82 | 1.16 | 2.86 | 0.009* |
| rs6470745 | CCDC26 | 130641921 | A/G | 0.306 | 0.343 | 0.575 | 0.84 | 0.57 | 1.25 | 0.396 |
| rs9656979 | CCDC26 | 130664407 | T/C | 0.493 | 0.465 | 0.000# | - | - | - | - |
| rs16904140 | CCDC26 | 130665643 | G/A | 0.285 | 0.29 | 0.843 | 0.98 | 0.65 | 1.46 | 0.905 |
| rs4295627 | CCDC26 | 130685457 | T/G | 0.278 | 0.287 | 0.796 | 0.95 | 0.64 | 1.43 | 0.819 |

*$p$ value≤0.05 indicates statistical significance. #Site with HWE p≤0.01 is excluded; OR, odd ratio; CI, confidence interval; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium

**Table 3. Relationship between SNPs rs891835 and rs6470745 and GBM Risk (Adjusted for Gender and Age)**

| SNP ID | Model | Genotype | Control n (%) | Case n (%) | OR (95%CI) | *p* | AIC | BIC |
|---|---|---|---|---|---|---|---|---|
| rs891835 | Codominant | T/T | 221 (73.9%) | 41 (56.9%) | 1 | 0.019* | 350.2 | 369.8 |
| | | G/T | 72 (24.1%) | 29 (40.3%) | 2.26 (1.29-3.97) | | | |
| | | G/G | 6 (2%) | 2 (2.8%) | 1.33 (0.23-7.81) | | | |
| | Dominant | T/T | 221 (73.9%) | 41 (56.9%) | 1 | 0.0061* | 348.6 | 364.2 |
| | | G/T-G/G | 78 (26.1%) | 31 (43.1%) | 2.18 (1.26-3.78) | | | |
| | Recessive | T/T-G/T | 293 (98%) | 70 (97.2%) | 1 | 0.98 | 356.1 | 371.8 |
| | | G/G | 6 (2%) | 2 (2.8%) | 1.03 (0.18-5.94) | | | |
| | Overdominant | T/T-G/G | 227 (75.9%) | 43 (59.7%) | 1 | 0.0053* | 348.3 | 364 |
| | | G/T | 72 (24.1%) | 29 (40.3%) | 2.24 (1.28-3.92) | | | |
| | Log-additive | - | - | - | 1.85 (1.14-3.00) | 0.015* | 350.1 | 365.8 |
| rs6470745 | Codominant | A/A | 134 (44.4%) | 32 (44.4%) | 1 | 0.09 | 354.1 | 373.8 |
| | | G/A | 129 (42.7%) | 36 (50%) | 1.07 (0.61-1.85) | | | |
| | | G/G | 39 (12.9%) | 4 (5.6%) | 0.35 (0.12-1.08) | | | |
| | Dominant | A/A | 134 (44.4%) | 32 (44.4%) | 1 | 0.66 | 356.8 | 372.5 |
| | | G/A-G/G | 168 (55.6%) | 40 (55.6%) | 0.89 (0.52-1.51) | | | |
| | Recessive | A/A-G/A | 263 (87.1%) | 68 (94.4%) | 1 | 0.029* | 352.2 | 367.9 |
| | | G/G | 39 (12.9%) | 4 (5.6%) | 0.34 (0.12-1.01) | | | |
| | Overdominant | A/A-G/G | 173 (57.3%) | 36 (50%) | 1 | 0.37 | 356.2 | 371.9 |
| | | G/A | 129 (42.7%) | 36 (50%) | 1.28 (0.75-2.17) | | | |
| | Log-additive | - | - | - | 0.76 (0.51-1.15) | 0.19 | 355.2 | 370.9 |

*$p$ value ≤ 0.05 indicates statistical significance; OR, odd ratio; CI, confidence interval; AIC, Akaike's information criterion; BIC, Bayesian information criterion

**Table 4. CDC26 Haplotype Frequencies and Association with Risk of GBM in Case and Control Patients**

| Block | Haplotype | Freq (case) | Freq (control) | c2 | Fisher's *p* | Pearson's *p* | OR | 95%CI |
|---|---|---|---|---|---|---|---|---|
| 1 | C G | 0.192 | 0.12 | 5.237 | 0.022* | 0.022* | 1.75 | 1.08-2.83 |
| | C T | 0.051 | 0.08 | 1.48 | 0.224 | 0.224 | 0.61 | 0.27-1.36 |
| | T G | 0.037 | 0.018 | 1.901 | 0.168 | 0.168 | 2.07 | 0.72-5.95 |
| | T T | 0.72 | 0.782 | 2.465 | 0.116 | 0.116 | 0.72 | 0.48-1.09 |
| 2 | A G T | 0.687 | 0.653 | 0.735 | 0.391 | 0.391 | 1.19 | 0.80-1.77 |
| | G A G | 0.271 | 0.282 | 0.064 | 0.8 | 0.8 | 0.95 | 0.63-1.43 |
| | G G T | 0.028 | 0.055 | 0.76 | 0.185 | 0.185 | 0.5 | 0.17-1.42 |
| Total | C G A G T | 0.121 | 0.092 | 1.279 | 0.258 | 0.258 | 1.39 | 0.78-2.48 |
| | C G G A G | 0.062 | 0.025 | 5.247 | 0.022* | 0.022* | 2.62 | 1.12-6.13 |
| | C T A G T | 0.024 | 0.052 | 1.971 | 0.16 | 0.16 | 0.45 | 0.15-1.41 |
| | T T A G T | 0.542 | 0.49 | 1.85 | 0.174 | 0.174 | 1.3 | 0.89-1.91 |
| | T T G A G | 0.16 | 0.229 | 2.981 | 0.084 | 0.084 | 0.65 | 0.41-1.06 |
| | T T G G T | 0.018 | 0.056 | 3.485 | 0.062 | 0.062 | 0.32 | 0.09-1.13 |

*$p$ value≤0.05 Indicates statistical significance; Freq, frequency; OR, odd ratio; CI, confidence interval.

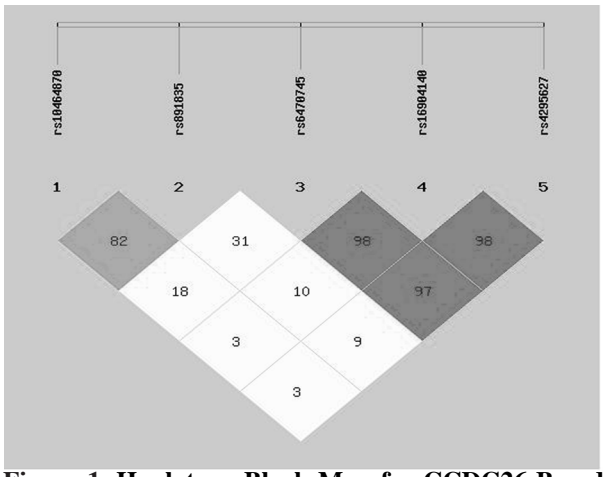


**Figure 1. Haplotype-Block Map for CCDC26 Based on SNPs rs891835, rs429562, rs6470745, rs10464870, and rs16904140**

haplotype analysis (Figure 1). Block 1 was found to contain rs10464870 and rs891835, and Block 2 contains rs429562, rs6470745, and rs16904140. The global result for Block 1 is: total cases=594, total controls=144, global $\chi^2$=8.332 while degrees of freedom (df)=3, Fisher's *p* value=0.040, and Pearson's *p* value=0.040. The global result for Block 2 is: total cases=602, total controls=144, global $\chi^2$=1.962 while df=2, Fisher's *p* value=0.375, and Pearson's *p* value=0.375. The overall global result is: total cases=592, total controls=144, global $\chi^2$=14.56 while df=5, Fisher's *p* value=0.013, and Pearson's *p* value=0.012. Data with frequency of <0.03, in both control and GBM cases, were discarded.

Results showing association between CCDC26 haplotype and risk of GBM are summarized in Table 4. Haplotype CG in Block 1 increases risk by 1.75-fold (OR, 1.75; 95%CI, 1.08-2.83; Fisher's *p*=0.020; Pearson's *p*=0.026). In Block 2, no haplotype is associated with GBM risk. By global haplotype association, haplotype CGGAG was seen to increase risk by 2.62-fold (OR, 2.62; 95%CI, 1.12-6.13; Fisher's *p*=0.022; Pearson's *p*=0.022).

CCDC26 is a retinoic acid modulator of cell differentiation and death. Retinoic acid induces caspase-8 transcription through phosphorylation of cAMP response element-binding protein, and it increases apoptosis following death stimuli in neuroblastoma and glioblastoma cells, accompanied by downregulation of telomerase activity (Jiang et al., 2008). Genetic variants of CCDC26 are associated with a number of common tumors, such as colorectal, breast, bladder, and prostate cancers (Easton et al., 2007; Tomlinson et al., 2007; Yeager et al., 2007; Kiemeney et al., 2008). A metastudy in the USA found polymorphisms in a region of CCDC26 containing rs4295627 to be strongly associated with oligodendroglial tumor risk (OR=2.05, *p*=8.3×10-11), but not with risk for glioblastoma (Jenkins et al., 2011). A study in Dutch participants reported an association between bizygomatic distance and rs987525 at 8q24.21, near the CCDC26 gene (*p*=0.017) (Boehringer et al., 2011). Our findings provide the first evidence in a limited Chinese population for association between CCDC26 SNPs and GBM risk. Because CCDC26 is a retinoic acid modulator related to telomerase activity, we predict that changes in CCDC26 may lead to altered telomerase activity, which then causes a change in the GBM cell state. Further mechanistic studies are required to test this hypothesis.

In conclusion, we found one risk allele and one protective allele in CCDC26 related to GBM susceptibility. Our study provides new evidence for a relationship between CCDC26 and GBM onset, which may shed light on the etiology of GBM. Furthermore, the studies validate use of several tSNPs in CCDC26 as relevant genetic markers for additional studies of GBM progression among Chinese populations.

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