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Original Article

Association of NFIL3, XRCC5 polymorphisms with glioblastoma in Han Chinese population

Bin Li[1,2,3*], Yang Li[4*], Mengdan Yan[2,3], Jing Li[2,3], Jingjie Li[2,3], Shuli Du[2,3], Tianbo Jin[2,3], Yongkuan Gong[1]

[1]Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an, China; [2]Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, School of Life Sciences, Northwest University, Xi'an, China; [3]School of Life Sciences, Northwest University, Xi'an, China; [4]Department of Anesthesiology, Shaanxi Provincial People's Hospital, Xi'an, China. [*]Equal contributors.

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Abstract: Background: Glioblastoma (GBM) is the most common primary glial brain tumor and the most common primary malignant brain tumor. We wanted to evaluate the potential association between gene polymorphisms and glioblastoma in Chinese population. Materials and methods: We evaluated and validated 84 tag single nucleotide polymorphisms (tSNPs) associated with glioma susceptibility in Han Chinese population, including 122 glioblastoma cases and 298 controls. The effects of the polymorphisms on the risk of GBM cancer were expressed as odds ratios (ORs) with 95% confidence intervals (95% CIs), evaluated by genetic models using unconditional logistic regression analysis adjusted for age and gender. Results: We analyzed all of the tSNPs using the SNPStats software, we found that rs9288516 in *XRCC5* decreased the risk of glioblastoma (OR=0.72, *P*=0.038), and rs7021746 in *NFIL3* increased the risk of glioblastoma in log-additive model (OR=1.38, *P*=0.049). Besides, we found that *CCDC26* rs891835 GT-GG genotype (OR=1.80, *P*=0.014) and *TP53* rs1042522 CC genotype (OR=1.91, *P*=0.023) were increased the risk of glioblastoma and *IL4R* rs1801275 AG genotype (OR=0.48, *P*=0.0049) decreased the risk of glioblastoma.Conclusions: Our results indicate for the first time that *NFIL3* associated with a risk of glioblastoma. And also verified the *XRCC5*, *CCDC26*, *TP53* and *IL4R* genes associated with the risk of glioblastoma.

Keywords: Glioblastoma, single nucleotide polymorphism (SNP), *XRCC5*, *NFIL3,* case-control study

Introduction

In the brain cancer, glioblastoma is the most common, which is a heterogeneous tumor characterized by uncontrolled cellular proliferation, diffuse infiltration, prominent microvascular proliferation, propensity for necrosis and intense resistance to apoptosis [1]. Although surgical resection and radiation and chemotherapy can treat glioma patients, however, the prognosis of glioblastoma patients is still poor, and the median survival in patients with only 12 months. The mainly reason is due to the rapid growth of tumor cell and infiltration of tumor cells [2].

The occurrence of glioblastoma is extremely complex, including environmental factors and genetic factors. As is known to all, exposed to ionizing radiation conditions, will increase the risk of glioblastoma development [3]. However, many patients have not been exposed to known environmental factors, and finally developed into glioma. So, Genetic factors may also affect the occurrence of glioma. Genome-wide association studies determined that inherited variants in some chromosomal regions, such as *RTEL*, *TERT*, *CDKN2BAS*, *EGFR*, *CCDC26* and *PHLDB1* have a significant association with the risk of adult glioma [4-6].

Although GWAS found that some sites have relationships with glioma, and there are significant differences between the Europeans and the Chinese peoples in genetic background. Therefore, in this study, we aimed to determine the relationship between gene polymorphisms and glioblastoma risk in Han Chinese population from the northwestern China.

Table 1. Clinical and demographic characteristics of Han Chinese patients with glioblastoma (n=122) and healthy control subjects (n=298)

Characteristic	Patients with GBM (n=122)	Healthy control subjects (n=298)
Sex		
Female	52 (42.6)	179 (60.1)
Male	70 (57.4)	119 (39.9)
Age, years	46.89±15.072	49.91±7.582

Data presented as mean ± SD for age; other data presented as number (%) of patients or controls.

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 Tangdu 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 122 patients with glioblastoma between December 2010 and April 2014 were recruited into an ongoing molecular epidemiological study at the Department of Neurosurgery of the Tangdu Hospital affiliated with The Fourth Military Medical University in Xi'an city, China. All glioblastoma 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 glioblastoma.

Random samples of 298 healthy unrelated individuals were recruited from the medical examination center at Tangdu Hospital between December 2010 and April 2014. All of the chosen subjects were Han Chinese living in Xi'an city and its surrounding areas. 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.

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. We tested the alpha-fetoprotein and plasma carcinoembryonic antigen to reduce the risk of including patients with tumors in the control group.

SNP selection and genotyping

We selected 84 tSNPs, with minor allele frequencies > 5% in the Han Chinese in Beijing, China population, previously reported to be associated with glioma [4, 5, 7-20]. We extracted genomic DNA from whole blood using the GoldMag® nanoparticle method and measured the nanoparticle concentration by spectrometry (DU530 UV/VIS spectrophotometer, Beckman Instruments, Fullerton, CA, USA). We designed primers for amplification and extension reactions using Sequenom Mass ARRAY Assay Design 3.0 Software [21]. We performed Sequenom Mass ARRAY RS1000 to genotype the SNPs using the standard protocol recommended by the manufacturer. We used Sequenom Typer 4.0 Software to perform the data management and analysis [21, 22].

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 an exact test. Allele frequencies and genotype frequencies for each SNP of glioma patients and control subjects were compared using the x^2 test [23]. ORs and 95% CIs were calculated by unconditional logistic regression analyses adjusted for age and sex [24]. We did

Table 2. Basic information about candidate SNPs used in this study

SNP	Position	Band	Alleles A/B	Gene (s)	Role	MAF (case)	MAF (control)	HWE *p*-value	OR (95% CI)	Allele model *p*-value
rs12022378	114448389	1p13.2	C/T	DCLRE1B	Coding exon	0.402	0.398	0.47	1.02 (0.75-1.38)	0.91
rs1980444	186850960	1q31.1	C/T	PLA2G4A	Intron	0.430	0.443	1.00	0.95 (0.7-1.28)	0.74
rs1800871	206946634	1q32.1	C/T	IL10	Promoter	0.316	0.334	0.04#	0.92 (0.67-1.27)	0.61
rs1136410	226555302	1q42.12	C/T	PARP1	Coding exon	0.434	0.435	0.23	1.00 (0.74-1.35)	0.98
rs2110922	40363644	2p22.1	G/T	SLC8A1	Intron	0.434	0.431	0.91	1.01 (0.75-1.37)	0.93
rs2072532	40366301	2p22.1	C/T	SLC8A1	Intron	0.164	0.204	0.86	0.77 (0.52-1.14)	0.18
rs3755377	70732852	2p13.3	T/C	TGFA	Intron	0.418	0.462	0.10	0.84 (0.62-1.13)	0.25
rs2066804	191841759	2q32.2	T/C	STAT1	Intron (boundary)	0.463	0.447	0.72	1.07 (0.79-1.44)	0.68
rs3770502	217045059	2q35	A/G	XRCC5	Intron	0.172	0.166	0.68	1.04 (0.70-1.55)	0.83
rs9288516	217053264	2q35	A/T	XRCC5	Intron	0.389	0.456	0.64	0.76 (0.56-1.03)	0.08
rs568408	159713467	3q25.33	A/G	IL12A	3'UTR	0.123	0.160	0.83	0.74 (0.47-1.14)	0.17
rs3828550	55976451	4q12	T/C	KDR	Intron	0.299	0.317	0.35	0.92 (0.67-1.27)	0.61
rs10516950	95254730	4q22.3	A/G	HPGDS	Intron	0.336	0.330	0.29	1.03 (0.75-1.41)	0.86
rs4444903	110834110	4q25	G/A	EGF	5'UTR	0.484	0.473	0.00#	1.04 (0.77-1.41)	0.78
rs12645561	178260872	4q34.3	T/C	NEIL3	Intron (boundary)	0.287	0.275	0.19	1.06 (0.76-1.47)	0.73
rs2853676	1288547	5p15.33	A/G	TERT	Intron	0.209	0.171	0.10	1.28 (0.88-1.86)	0.20
rs6869535	40597618	5p13.1	A/G			0.037	0.047	0.12	0.78 (0.36-1.68)	0.52
rs1056503	82648977	5q14.2	T/G	XRCC4	Coding exon	0.279	0.284	0.78	0.98 (0.70-1.36)	0.89
rs2266690	86695274	5q14.3	C/T	CCNH	Coding exon	0.078	0.091	0.28	0.84 (0.49-1.46)	0.54
rs2069812	131879916	5q31.1	C/T	IL5	Promoter	0.320	0.349	0.80	0.88 (0.64-1.20)	0.41
rs20541	131995964	5q31.1	T/C	IL13	Coding exon	0.303	0.339	0.90	0.85 (0.62-1.17)	0.32
rs2243248	132008644	5q31.1	G/T	IL4	Promoter	0.057	0.065	0.62	0.87 (0.46-1.63)	0.66
rs2070874	132009710	5q31.1	C/T	IL4	5'UTR	0.209	0.215	1.00	0.97 (0.67-1.39)	0.85
rs1801270	36651971	6p21.2	A/C	CDKN1A	Coding exon	0.471	0.424	0.41	1.21 (0.90-1.63)	0.21
rs6931798	40665415	6p21.1	T/C			0.230	0.267	0.56	0.82 (0.58-1.16)	0.26
rs9369226	40670716	6p21.1	A/G			0.316	0.359	0.45	0.82 (0.60-1.13)	0.23
rs4140805	7727101	7p21.3	G/T	RPA3	Intron	0.258	0.206	0.15	1.34 (0.94-1.90)	0.10
rs6947203	7737048	7p21.3	T/C	RPA3	Intron	0.164	0.133	1.00	1.28 (0.85-1.93)	0.24
rs2160138	7755797	7p21.3	C/T	RPA3	Intron	0.262	0.213	0.12	1.31 (0.93-1.86)	0.12
rs17172432	55141317	7p11.2	C/T	EGFR	Intron	0.102	0.108	0.76	0.94 (0.58-1.53)	0.81
rs4947492	55187992	7p11.2	G/A	EGFR	Intron	0.344	0.347	0.90	0.99 (0.72-1.35)	0.94
rs12718945	55192963	7p11.2	T/G	EGFR	Intron	0.340	0.343	0.90	0.99 (0.72-1.35)	0.94
rs730437	55215018	7p11.2	C/A	EGFR	Intron	0.422	0.364	1.00	1.28 (0.94-1.73)	0.12
rs11506105	55220177	7p11.2	G/A	EGFR	Intron (boundary)	0.406	0.356	0.90	1.23 (0.91-1.68)	0.18
rs3752651	55229543	7p11.2	C/T	EGFR	Intron	0.078	0.074	1.00	1.06 (0.60-1.85)	0.85
rs1468727	55230105	7p11.2	C/T	EGFR	Intron	0.475	0.430	0.28	1.20 (0.89-1.62)	0.24
rs845552	55245507	7p11.2	A/G	EGFR	Intron	0.410	0.361	0.37	1.23 (0.91-1.67)	0.18
rs7003908	48770702	8q11.21	C/A	PRKDC	Intron	0.230	0.225	0.32	1.03 (0.72-1.46)	0.88
rs10464870	130477823	8q24.21	C/T	CCDC26		0.225	0.199	0.14	1.17 (0.81-1.68)	0.40
rs891835	130491752	8q24.21	G/T	CCDC26		0.201	0.139	0.81	1.55 (1.05-2.29)	0.03*
rs6470745	130641921	8q24.21	G/A	CCDC26		0.336	0.342	0.30	0.97 (0.71-1.33)	0.86
rs9656979	130664407	8q24.21	C/T	CCDC26		0.492	0.465	0.00#	1.12 (0.83-1.50)	0.47
rs16904140	130665643	8q24.21	A/G	CCDC26		0.295	0.289	0.40	1.03 (0.74-1.43)	0.85
rs4295627	130685457	8q24.21	G/T	CCDC26		0.291	0.287	0.32	1.02 (0.73-1.42)	0.91
rs1063192	22003367	9p21.3	C/T	CDKN2B	3'UTR	0.213	0.190	0.70	1.15 (0.80-1.66)	0.45
rs2157719	22033366	9p21.3	G/A	CDKN2BAS	Intron	0.152	0.117	1.00	1.34 (0.87-2.06)	0.18
rs1412829	22043926	9p21.3	C/T	CDKN2BAS	Intron	0.152	0.116	1.00	1.36 (0.88-2.09)	0.16
rs4977756	22068652	9p21.3	G/A	CDKN2BAS	Intron	0.221	0.218	0.50	1.02 (0.71-1.46)	0.91
rs7021746	94176261	9q22.31	A/G	NFIL3	Intron	0.430	0.361	0.61	1.34 (0.99-1.82)	0.06
rs2291427	45936224	10q11.21	A/G	ALOX5	Intron	0.340	0.339	0.15	1.01 (0.73-1.38)	0.97
rs1509937	67055905	10q21.3	G/A			0.148	0.101	1.00	1.54 (0.99-2.40)	0.06
rs701848	89726745	10q23.31	C/T	PTEN	3'UTR	0.418	0.408	0.90	1.04 (0.77-1.41)	0.78
rs12917	131506283	10q26.3	T/C	MGMT	Coding exon	0.102	0.107	0.76	0.95 (0.58-1.55)	0.83
rs1695	67352689	11q13.2	G/A	GSTP1	Coding exon	0.189	0.232	0.33	0.77 (0.53-1.11)	0.16

rs603965	69462910	11q13.3	G/A	CCND1	Coding exon	0.434	0.441	0.91	0.97 (0.72-1.31)	0.86
rs7124728	70876435	11q13.4	C/T	SHANK2	Intron	0.504	0.470	0.64	1.15 (0.85-1.55)	0.37
rs498872	118477367	11q23.3	T/C	ARCN1	Downstream	0.295	0.279	0.57	1.08 (0.78-1.51)	0.63
rs17748	118528424	11q23.3	T/C	TREH	Downstream	0.266	0.273	0.02#	0.97 (0.69-1.35)	0.85
rs2701652	66580877	12q14.3	C/G	IRAK3	Promoter	0.135	0.135	0.80	1.00 (0.65-1.55)	0.98
rs7989882	24214603	13q12.12	A/G	TNFRSF19	Intron	0.225	0.233	0.63	0.96 (0.67-1.36)	0.81
rs3829382	28577688	13q12.2	G/T	FLT3	3'UTR	0.520	0.453	0.41	1.31 (0.97-1.77)	0.08
rs3212092	104168644	14q32.33	T/C	XRCC3	Intron	0.057	0.040	1.00	1.45 (0.74-2.85)	0.28
rs861530	104174123	14q32.33	G/A	XRCC3	Intron	0.393	0.423	0.91	0.89 (0.65-1.2)	0.43
rs12439272	40584804	15q15.1	A/G	PLCB2	Intron	0.115	0.087	0.47	1.36 (0.84-2.21)	0.22
rs1805015	27374180	16p12.1	C/T	IL4R	Coding exon	0.098	0.099	0.51	0.99 (0.6-1.63)	0.97
rs1801275	27374400	16p12.1	G/A	IL4R	Coding exon	0.152	0.197	0.06	0.73 (0.49-1.09)	0.12
rs171125	34760967	16p11.1	A/G			0.082	0.070	1.00	1.18 (0.68-2.05)	0.56
rs1042522	7579472	17p13.1	C/G	TP53	Coding exon	0.467	0.408	0.23	1.27 (0.94-1.72)	0.11
rs8079544	7580052	17p13.1	T/C	TP53	Intron	0.086	0.084	0.71	1.03 (0.60-1.75)	0.92
rs854692	34323944	17q12	T/C	CCL15	Downstream	0.442	0.433	0.19	1.04 (0.77-1.40)	0.81
rs1476278	37836243	17q12	A/G	PGAP3	Intron	0.496	0.495	0.29	1.00 (0.74-1.35)	0.98
rs2952155	37861718	17q12	C/T	ERBB2	Intron	0.492	0.486	0.49	1.02 (0.76-1.38)	0.89
rs7216389	38069949	17q12	C/T	GSDMB	Intron	0.291	0.277	0.77	1.07 (0.77-1.49)	0.68
rs7257116	2028985	19p13.3	T/C	MKNK2	Downstream	0.336	0.337	0.04#	0.99 (0.73-1.36)	0.97
rs105038	4414710	19p13.3	C/T	CHAF1A	Intron	0.443	0.436	0.48	1.03 (0.76-1.39)	0.86
rs2992	4443046	19p13.3	A/G	UBXN6	Downstream	0.430	0.430	0.72	1.00 (0.74-1.36)	0.98
rs3212986	45912736	19q13.32	T/G	CD3EAP	Coding exon	0.336	0.305	0.04#	1.15 (0.84-1.58)	0.38
rs2206920	6668179	20p12.3	A/G			0.090	0.101	0.20	0.88 (0.53-1.47)	0.63
rs6010620	62309839	20q13.33	G/A	RTEL1	Intron	0.320	0.264	0.00#	1.31 (0.94-1.81)	0.11
rs2297440	62312299	20q13.33	C/T	RTEL1	Intron	0.316	0.260	0.00#	1.31 (0.95-1.82)	0.10
rs4809324	62318220	20q13.33	C/T	RTEL1	Intron	0.090	0.112	0.56	0.78 (0.47-1.30)	0.34
rs202445	33025667	21q22.11	G/A	SOD1	Promoter	0.016	0.012	1.00	1.39 (0.40-4.80)	0.85
rs2267130	29099754	22q12.1	C/T	CHEK2	Intron	0.303	0.268	1.00	1.19 (0.85-1.65)	0.31
rs6519265	42025350	22q13.2	A/G	XRCC6	Intron	0.111	0.086	0.25	1.33 (0.81-2.18)	0.26

A/B stands for minor/major alleles on the control sample frequencies; OR, odd ratio; CI, confidence interval; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium. #P<0.05 indicates deviate from Hardy-Weinberg equilibrium. *P<0.05 indicates statistical significance.

not divide subjects into subgroups because of the limited sample size. The possibility of sex differences as a source of population substructure 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 x^2 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 [24, 25].

Results

In our research, we total recruited 122 glioblastoma patients, which including 70 male and 52 female, and the mean age is 46.89 yrs; At the same time, 298 health population as control group, consists of 119 male and 179 female, the man age of control is 49.91 yrs. Basic characteristics of participants were shown in **Table 1**.

In our research, we analyzed the association between 84 gene polymorphism loci and glioblastoma.The information of eighty-fourt SNPs was listed in **Table 2**, including the Chromosomal position, minor allele frequency, and HWE test results. Eight of the tSNPs displayed significant deviation from HWE among controls (P< 0.05). These eight loci were *IL10* rs1800871, *EGF* rs4444903, *CCDC26* rs9656979, *TREH* rs17748, *MKNK2* rs7257116, *CD3EAP* rs3212986, *RTEL1* rs6010620, and *RTEL1* rs2297440. We also used χ^2 test to compare the difference of allele frequency distributions in cases and controls groups. Through the analysis, we found that "G" allele *CCDC26* rs891835

Table 3. Relationship between gene polymorphisms and the risk of GBM (adjusted by sex + age)

SNP	Model	Genotype	Control	Case	OR (95% CI)	*P*-value
XRCC5 rs9288516	Codominant	T/T	90 (30.3%)	45 (36.9%)	1	0.12
		A/T	143 (48.1%)	59 (48.4%)	0.73 (0.45-1.18)	
		A/A	64 (21.6%)	18 (14.8%)	0.51 (0.27-0.99)	
	Dominant	T/T	90 (30.3%)	45 (36.9%)	1	0.081
		A/T-A/A	207 (69.7%)	77 (63.1%)	0.66 (0.42-1.05)	
	Recessive	T/T-A/T	233 (78.5%)	104 (85.2%)	1	0.1
		A/A	64 (21.6%)	18 (14.8%)	0.62 (0.35-1.11)	
	Log-additive	---	---	---	0.72 (0.53-0.98)	0.038*
NFIL3 rs7021746	Codominant	G/G	118 (40.1%)	39 (32%)	1	0.14
		A/G	140 (47.6%)	61 (50%)	1.38 (0.85-2.25)	
		A/A	36 (12.2%)	22 (18%)	1.90 (0.98-3.69)	
	Dominant	G/G	118 (40.1%)	39 (32%)	1	0.088
		A/G-A/A	176 (59.9%)	83 (68%)	1.49 (0.94-2.36)	
	Recessive	G/G-A/G	258 (87.8%)	100 (82%)	1	0.14
		A/A	36 (12.2%)	22 (18%)	1.57 (0.86-2.85)	
	Log-additive	---	---	---	1.38 (1.00-1.90)	0.049*
CCDC26 rs891835	Codominant	T/T	221 (74.2%)	76 (62.3%)	1	0.043
		G/T	71 (23.8%)	43 (35.2%)	1.84 (1.15-2.95)	
		G/G	6 (2%)	3 (2.5%)	1.31 (0.29-5.90)	
	Dominant	T/T	221 (74.2%)	76 (62.3%)	1	0.014*
		G/T-G/G	77 (25.8%)	46 (37.7%)	1.80 (1.13-2.85)	
	Recessive	T/T-G/T	292 (98%)	119 (97.5%)	1	0.9
		G/G	6 (2%)	3 (2.5%)	1.10 (0.25-4.89)	
	Log-additive	---	---	---	1.61 (1.06-2.43)	0.025
IL4R rs1801275	Codominant	A/A	184 (62.6%)	91 (74.6%)	1	0.0049*
		A/G	104 (35.4%)	25 (20.5%)	0.48 (0.29-0.80)	
		G/G	6 (2%)	6 (4.9%)	2.05 (0.60-7.00)	
	Dominant	A/A	184 (62.6%)	91 (74.6%)	1	0.016
		A/G-G/G	110 (37.4%)	31 (25.4%)	0.56 (0.34-0.90)	
	Recessive	A/A-A/G	288 (98%)	116 (95.1%)	1	0.14
		G/G	6 (2%)	6 (4.9%)	2.53 (0.75-8.53)	
	Log-additive	---	---	---	0.70 (0.46-1.08)	0.099
TP53 rs1042522	Codominant	G/G	99 (33.2%)	36 (29.5%)	1	0.075
		C/G	155 (52%)	58 (47.5%)	0.99 (0.60-1.63)	
		C/C	44 (14.8%)	28 (22.9%)	1.89 (1.01-3.54)	
	Dominant	G/G	99 (33.2%)	36 (29.5%)	1	0.49
		C/G-C/C	199 (66.8%)	86 (70.5%)	1.18 (0.74-1.88)	
	Recessive	G/G-C/G	254 (85.2%)	94 (77%)	1	0.023*
		C/C	44 (14.8%)	28 (22.9%)	1.91 (1.10-3.29)	
	Log-additive	---	---	---	1.33 (0.97-1.82)	0.08

OR odds ratio, 95% CI 95% confidence interval. *P value<0.05 indicates statistical significance.

increased the risk of glioblastoma (odds ratio [OR]: 1.55, 95% confidence interval [CI]: 1.05-2.29, P=0.03), when compared the "T" allele *CCDC26* rs891835.

Next, we performed unconditional logistic regression adjusted for age and gender to analysis the association between 84 gene polymorphism loci and glioblastoma in different genetic model. We found that, compared with the subjects with the allele "T", the subjects of carrier allele "A" decreased glioblastoma risk, which is a protective gene (OR=0.72, 95% CI: 0.53-0.98, P=0.038). We also found that *NFIL3* rs7021746

allele "A" increased glioblastoma risk in log-additive model (OR=1.38, 95% CI: 1.00-1.90, *P*=0.049), which is arisk gene for glioblastoma. Besides, we found that *CCDC26* rs891835 GG-GT genotype and *TP53* rs1042522 CC genotype increased the risk of glioblastoma. For the *IL4R* rs1801275, compared with the subjects with the homozygous AA genotype the odds of having glioblastoma would be 0.48-fold (95% CI: 0.29-0.80, *P*=0.0049) with heterozygote AG genotype (**Table 3**).

Discussion

We adopted case-control method to analysis of the effects of 84 loci on glioblastoma, we found that the "A" allele of *XRCC5* rs9288516 decreased the risk of glioblastoma and the A allele of *NFIL3* rs7021746 increased the risk of glioblastoma. And we also found that *CCDC26* rs891835, *IL4R* rs1801275, and *TP53* rs1042522 were significant influence the risk of glioblastoma. The results of *CCDC26* rs891835, *IL4R* rs1801275, and *TP53* rs1042522 are consistent with previous studies [26, 27].

XRCC5 gene is located on chromosome 2q33~35, encoding Ku80 protein. XRCC6 encoding Ku70 protein, Ku80 protein and Ku70 protein composed of two heterologous dimers (Ku protein). Ku protein as a conserved DNA protein, which involved in gene recombination and double strand break repair, to promote double chain reconnection and recombination, play an important role in maintaining genomic integrity and repairing the damage caused by the carcinogenic factors. When the rays caused DSB, Ku80 and Ku70 subunit quickly form a heterodimer, combined with damaged DNA ends to activate DNA-PKes. Guide *XRCC4* and DNA-LigaseIV complex composition, final positioning by DNA-LigaseIV, connection DNA double-strand break ends to complete the repair [28]. Any alteration in binding could affect the assembly of the DNA-PK complex, leading to inefficient processing by the ligation complex, resulting in accumulation of unprocessed DSBs and recruitment of the apoptotic machinery [29].

Liu Y et al. [19] analyzed the effect of *XRCC5*, *XRCC6* and *XRCC7* gene on glioma in 771 case and 752 controls, found that *XRCC5* tSNPs (rs828704, rs3770502 and rs9288516) were associated with the risk of glioma. In our study, we only found *XRCC5* rs9288516 decreased the risk of glioblastoma. However, we did not find any association between *XRCC5* rs3770-502 and glioblastoma. We suspect this may be caused by sample size. Liu Y et al. surmised that rs9288516 had a strongest association with risk of glioma, it may have a tight linkage with other functional SNPs [19]. FASTSNP software predicted that the allele mutation of rs9288516 loci can result in a change in the potential binding site of the transcription factor [30], to cause the abnormal expression of Ku protein and the disorder of activity, which resulting in the occurrence of tumor. Therefore, the exact location and biological functions of the real causal SNPs in *XRCC5* is of great interest and warrant further investigation.

NFIL3 (nuclear factor IL-3 regulated), which is also called E4BP4, is a basic leucine zipper transcription repressor, and glucocorticoid can induce E4BP4 expression up-regulation by regulate intracellular calcium ion concentration. According to a study has demonstrated E4BP4 involved in regulating the development and function of some immune cell lineage, and can also regulate the expression of IgE like transformation of B cells, T cell polarization response and cytokine expression [31, 32]. Besides, *NFIL3* may also influence the nerve cell growth and survival [33, 34]. In our study, we found that *NFIL3* rs7021746 increased the risk of GBM, so, we guess *NFIL3* mutant may influence the Glioblastoma occurs. In the future, we will further explore the mechanism of action.

In summary, our study suggested that *NFIL3* and *XRCC5* are associated with glioblastoma. And also verified *CCDC26*, *IL4R*, and *TP53* gene were influence the risk of glioblastoma. In the future, we will continue to research the association through enough samples and research the mechanism of the polymorphisms in the related gene regulate glioblastoma occur.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tianbo Jin, Key Laboratory of Resource Biology and Biotechnology in Western China (Northwest University), Ministry of Education, School of Life Sciences, Northwest University, #229 North Taibai Road, Xi'an 710069, China. Tel: +86-29-33755247; Fax: +86-29-33755-247; E-mail: jintianbo@gmail.com; Yongkuan Gong, Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, Northwest University, #229 North Taibai Road, Xi'an 710069, China. Tel: +86 29 88302109; Fax: +86 29 88302604; E-mail: dr_gongyongkuan@163.com

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