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Genetic variants in m^{6}A modification core genes are associated with glioma risk in Chinese children

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Glioma is a highly heritable disease with a strong genetic component. The N6-methyladenosine (m^{6}A) modification core genes play important roles in the context of cancer. However, the effects of polymorphisms in the m^{6}A modification core genes on the risk of pediatric glioma remain undefined. Here, we intended to demonstrate the relationship between 24 functional single-nucleotide polymorphisms (SNPs) in eight m^{6}A modification core genes and glioma risk. Case-control design and multinomial logistic regression were used to develop models to estimate the risk of glioma while accounting for the subtypes of glioma. A total of 171 glioma cases and 228 controls from South China were genotyped using a TaqMan assay. The *WTAP* rs7766006, YTHDF2 rs3738067, and FTO rs9939609 variants conferred a statistically significant increased risk of glioma, respectively. *YTHDC1* rs2293595, YTHDC1 rs3813832, and FTO rs8047395 were associated with a significant inverse association with risk of glioma, respectively. The significant associations were more predominant in stratification analyses of certain subgroups. Functional annotations revealed that *WTAP* rs7766006 and *YTHDF2* rs3738067 could be potential functional variants by increasing expression of *WTAP* and *YTHDF2* mRNA, respectively. Overall, these findings implicate variants in the m^{6}A modification core genes as playing a role in pediatric glioma etiology.

INTRODUCTION

Brain tumors are characterized by high incidence and mortality owing to their notoriously invasive nature.[1] Glioma is the most prevalent primary malignant brain tumor and accounts for almost 30% of all primary brain tumors.[2,3] Gliomas are mainly derived from neuroglial stem or progenitor cells.[4,5] The majority of pediatric gliomas are benign and thus classified as grade I or II by the WHO classification. These pediatric low-grade gliomas (LGGs) rarely undergo malignant transformation and present favorable prognosis under current treatment strategies. However, a significant portion of gliomas are malignant and progress rapidly and are therefore classified as grade III or IV.[6] Despite all therapeutic efforts, patients with high-grade gliomas (HGGs) retain a limited prognosis, with the most aggressive forms being lethal within months.[7]

Many environmental factors have been explored, yet only one definite factor (ionizing radiation) is recognized as a causative agent.[8–10] Hereditary factors, lifestyle, and diet are suggested to confer risk of glioma, but causal relationships should be solidified.[11] Evidence for a genetic component to glioma risk has been growing. Several genome-wide association studies (GWASs) have identified a dozen glioma risk-associated single-nucleotide polymorphisms (SNPs), which are located in genes *CCDC26*, *PHLDB1*, *TP53*, *EGFR*, and *CDKN2A-CDKN2B*.[12–15] Collectively, however, these variants still account for only a small portion of glioma risk, and additional predisposition loci likely remain to be discovered. Exploration of other causative genetic variation is warranted to better understand the etiology of glioma.

N6-methyladenosine (m^{6}A) is the most distributed mRNA post-transcriptional modification in eukaryotic cells.[16] The m^{6}A modification process is accomplished by a series of proteins. According to the different roles of proteins in the RNA methylation process, m^{6}A core proteins are currently mainly divided into writers, erasers, and readers.[17] The m^{6}A methyltransferase complexes methyltransferase-like 3 (METTL3), METTL14, and Wilms tumor 1-associated protein

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Table 1. Association of m^{6}A modification core genes and glioma risk in Southern Chinese children

Gene	Polymorphism	Allele		Case (n = 171)			Control (n = 228)			AOR (95% CI)[a]	p[a]	AOR (95% CI)[b]	p[b]	HWE
		A	B	AA	AB	BB	AA	AB	BB					
ALKBH5	rs1378602	G	A	139	30	2	182	43	2	0.97 (0.58–1.63)	0.920	1.27 (0.17–9.21)	0.815	0.757
ALKBH5	rs8400	G	A	51	85	35	71	118	38	1.14 (0.74–1.77)	0.548	1.37 (0.82–2.31)	0.233	0.350
METTL3	rs1061026	T	G	141	28	2	187	37	4	0.98 (0.58–1.65)	0.935	0.66 (0.12–3.74)	0.641	0.185
METTL3	rs1061027	C	A	112	55	4	139	79	10	0.80 (0.53–1.22)	0.295	0.55 (0.17–1.79)	0.317	0.771
METTL3	rs1139130	A	G	62	85	24	91	102	35	1.13 (0.75–1.71)	0.559	0.88 (0.50–1.56)	0.665	0.470
METTL3	rs1263801	G	C	91	67	13	112	90	26	0.83 (0.55–1.23)	0.349	0.66 (0.33–1.33)	0.241	0.230
METTL14	rs1064034	T	A	77	83	11	112	97	19	1.23 (0.82–1.84)	0.316	0.82 (0.38–1.77)	0.605	0.755
METTL14	rs298982	G	A	135	36	0	179	46	3	1.02 (0.63–1.67)	0.930	–	–	0.982
METTL14	rs62328061	A	G	106	63	2	154	66	8	1.29 (0.85–1.97)	0.232	0.33 (0.07–1.59)	0.168	0.778
METTL14	rs9884978	G	A	110	53	8	155	62	11	1.14 (0.75–1.75)	0.534	0.91 (0.35–2.35)	0.848	0.150
METTL14	rs4834698	T	C	45	81	45	51	118	59	0.79 (0.49–1.25)	0.313	1.03 (0.65–1.63)	0.892	0.583
WTAP	rs9457712	G	A	116	51	4	148	71	9	0.86 (0.56–1.32)	0.487	0.58 (0.17–1.95)	0.381	0.894
WTAP	rs1853259	A	G	57	85	29	62	120	46	0.74 (0.48–1.14)	0.168	0.77 (0.45–1.29)	0.314	0.382
WTAP	rs7766006	G	T	57	88	26	97	106	25	1.58 (1.04–2.40)[c]	0.034[c]	1.46 (0.81–2.65)	0.211	0.620
YTHDC1	rs2293596	T	C	108	55	8	140	76	12	0.91 (0.60–1.38)	0.668	0.84 (0.33–2.12)	0.708	0.689
YTHDC1	rs2293595	T	C	77	74	20	74	112	42	0.59 (0.39–0.90)[c]	0.013[c]	0.61 (0.34–1.08)	0.091	0.974
YTHDC1	rs3813832	T	C	109	53	9	115	96	17	0.61 (0.40–0.91)[c]	0.016[c]	0.72 (0.31–1.67)	0.448	0.619
YTHDF1	rs6011668	C	T	132	32	7	168	56	4	0.81 (0.50–1.29)	0.369	2.66 (0.75–9.43)	0.131	0.787
YTHDF1	rs6090311	A	G	61	75	35	92	103	33	1.20 (0.79–1.81)	0.400	1.58 (0.93–2.68)	0.093	0.633
YTHDF2	rs3738067	A	G	83	78	10	144	74	10	1.86 (1.24–2.80)[c]	0.003[c]	1.31 (0.53–3.25)	0.560	0.900
FTO	rs1477196	G	A	105	58	8	127	87	14	0.76 (0.51–1.15)	0.193	0.80 (0.32–1.96)	0.617	0.860
FTO	rs9939609	T	A	129	33	9	175	51	2	1.07 (0.67–1.72)	0.757	7.39 (1.56–35.11)[c]	0.012[c]	0.411
FTO	rs7206790	C	G	110	53	8	165	59	4	1.45 (0.94–2.24)	0.090	2.73 (0.80–9.38)	0.110	0.626
FTO	rs8047395	A	G	75	78	18	75	116	37	0.63 (0.41–0.95)[c]	0.026[c]	0.63 (0.34–1.16)	0.139	0.481

AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
[a]Adjusted for age and gender for dominant model.
[b]Adjusted for age and gender for recessive model.
[c]Significant results.

(WTAP) act as m^{6}A writers, mainly mediating the m^{6}A methylation of mRNA.[18] m^{6}A demethylase FTO and ALKBH5 act as erasers, mainly mediating m^{6}A demethylation of mRNA. A series of m^{6}A binding proteins, YT521-B homology domain family (YTHDF) 1/2/3, YTHDC1/2, IGF2BP1/2/3, and eIF3, act as readers and are involved in determining the fate of m^{6}A-modified target mRNA transcripts.[19] The effect of abnormal levels of m^{6}A methylated core proteins on cancer progression has also been investigated. Compelling evidence has pointed to the participation of ALKBH5, METTL3, METTL14, WTAP, YTHDC1, YTHDF1, YTHDF2, and FTO in human cancers, including glioma.[20–27] Xi et al.[28] found that WTAP expression predicts poor prognosis in malignant glioma patients. Zhang et al.[27] revealed that m^{6}A demethylase ALKBH5 maintains tumorigenicity of glioblastoma stem-like cells by sustaining FOXM1 expression and cell proliferation program.

Nevertheless, the role of SNPs in m^{6}A modification core genes on glioma risk has been poorly unraveled. Given the evidence that cells modulated by m^{6}A contribute to tumorigenesis, we hypothesize that SNPs of m^{6}A modification core genes may predispose to the risk of glioma. To test this hypothesis, here we conducted a case-control study to investigate the association of SNPs with susceptibility to glioma among children of Chinese ancestry.

RESULTS

Characteristics of the participants

Detailed frequency distributions of demographic and clinical characteristics of glioma cases (n = 171) and cancer-free controls (n = 228) are presented in Table S1. Controls were frequency matched to cases by age (p = 0.623) and gender (p = 0.190). Among these cases, the astrocytic tumors accounted for 125 (73.10%), the ependymoma for 24 (14.62%), the neuronal and mixed neutonal-glial tumors for 14 (8.19%), and embryonal tumors for 7 (4.09%). According to the WHO grades, 103 glioma cases (60.23%) were classified into grade I, 28 (16.37%) into grade II, 15 (8.77%) into grade III, and 25 (14.62%) into grade IV.

Table 2. Stratification analysis between *WTAP* genotypes and glioma risk

Variables	rs7766006 (cases/controls)		AOR (95% CI)[a]	p[a]	Risk genotypes[b] (cases/controls)		AOR (95% CI)[a]	p[a]
	GG	GT/TT			0–2	3		
Age, months								
<60	27/40	58/79	1.09 (0.60–1.98)	0.775	27/40	58/79	1.09 (0.60–1.98)	0.775
≥60	30/57	56/52	2.10 (1.17–3.78)[c]	0.013[c]	30/57	56/52	2.10 (1.17–3.78)[c]	0.013[c]
Gender								
Females	30/40	51/53	1.39 (0.75–2.60)	0.300	30/40	51/53	1.39 (0.75–2.60)	0.300
Males	27/57	63/78	1.76 (1.00–3.12)	0.052	27/57	63/78	1.76 (1.00–3.12)	0.052
Subtypes								
Astrocytic tumors	43/97	82/131	1.54 (0.96–2.45)	0.071	43/97	82/131	1.54 (0.96–2.45)	0.071
Ependymoma	10/97	15/131	0.98 (0.42–2.32)	0.971	10/97	15/131	0.98 (0.42–2.32)	0.971
Neuronal and mixed	3/97	11/131	2.48 (0.66–9.24)	0.177	3/97	11/131	2.48 (0.66–9.24)	0.177
Embryonal tumors	1/97	6/131	17.00 (1.03–281.79)[c]	0.048[c]	1/97[c]	6/131[c]	17.00 (1.03–281.79)[c]	0.048[c]
Tumor grades								
I	31/97	72/131	1.86 (1.12–3.08)	0.017	31/97	72/131	1.86 (1.12–3.08)	0.017
II	12/97	16/131	0.98 (0.44–2.19)	0.964	12/97	16/131	0.98 (0.44–2.19)	0.964
III	5/97	10/131	1.31 (0.43–4.02)	0.632	5/97	10/131	1.31 (0.43–4.02)	0.632
IV	9/97	16/131	1.71 (0.67–4.33)	0.259	9/97	16/131	1.71 (0.67–4.33)	0.259
I+II	43/97	88/131	1.61 (1.02–2.54)[c]	0.041[c]	43/97	88/131	1.61 (1.02–2.54)[c]	0.041[c]
III+IV	14/97	26/131	1.56 (0.76–3.22)	0.226	14/97	26/131	1.56 (0.76–3.22)	0.226

AOR, adjusted odds ratio; CI, confidence interval.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.
[b]Risk genotypes were carriers with rs9457712 GG/GA, rs1853259 AA/AG, and rs7766006 GT/TT genotypes.
[c]Significant results.

Association of m^{6}A modification core genes and glioma risk

Our case-control study successfully genotyped 24 SNPs in the 8 m^{6}A modification core genes. The single-locus analysis was applied to estimate the associations between each selected SNP and glioma risk (Table 1). None of the 24 SNPs violated the Hardy-Weinberg equilibrium (HWE) in control populations (all p values > 0.05). The *WTAP* rs7766006 (adjusted odds ratio [OR] = 1.58, 95% confidence interval [CI] = 1.04–2.40, p = 0.034) and *YTHDF2* rs3738067 (adjusted OR = 1.86, 95% CI = 1.24–2.80, p = 0.003) variants were associated with a statistically significant increased risk of glioma, respectively, in dominant model. *YTHDC1* rs2293595 (adjusted OR = 0.59, 95% CI = 0.39–0.90, p = 0.013), *YTHDC*1 rs3813832 (adjusted OR = 0.61, 95% CI = 0.40–0.91, p = 0.016), and *FTO* rs8047395 (adjusted OR = 0.63, 95% CI = 0.41–0.95, p = 0.026) were associated with a significant inverse association with risk of glioma, respectively, in dominant model. Under recessive model, only *FTO* rs9939609 (adjusted OR = 7.39, 95% CI = 1.56–35.11, p = 0.012) was associated with risk of glioma.

Stratification analysis

Stratification analysis was further performed for those significant SNPs based on age, gender, subtypes, and tumor grades. For *WTAP* genotypes (Table 2), rs7766006 GT/TT increased glioma risk in children aged ≥ 60 months (adjusted OR = 2.10, 95% CI = 1.17–3.78, p = 0.013), subtype of embryonal tumors (adjusted OR = 17.00, 95% CI = 1.03–281.79, p = 0.048), and patients with tumors in the tumor grades I+II (adjusted OR = 1.61, 95% CI = 1.02–2.54, p = 0.041). We then treated rs9457712 GG/GA, rs1853259 AA/AG, and rs7766006 GT/TT as risk genotypes. After combining the risk genotypes, we observed that patients with 3 risk genotypes were more likely to develop glioma in children aged ≥60 months (adjusted OR = 2.10, 95% CI = 1.17–3.78, p = 0.013), subtype of embryonal tumors (adjusted OR = 17.00, 95% CI = 1.03–281.79, p = 0.048), and patients with tumors in the tumor grades I+II (adjusted OR = 1.61, 95% CI = 1.02–2.54, p = 0.041).

For *YTHDC1* genotypes (Table 3), rs2293595 TC/CC was associated with decreased glioma risk in children aged ≥60 months, females, subtype of astrocytic tumors, patients with tumors in tumor grade I, patients with tumors in tumor grade II, and patients with tumors in tumor grade I+II. rs3813832 TC/CC was associated with decreased glioma risk in children aged ≥ 60 months, females, subtype of astrocytic tumors, patients with tumors in tumor grade II, and patients with tumors in tumor grade I+II. rs2293596 TC/CC, rs2293595 TC/CC, and rs3813832 TC/CC were further referred to as protective genotypes. Compared to 0–1 protective genotypes,

Table 3. Stratification analysis between *YTHDC1* genotypes and glioma risk

Variables	rs2293595		AOR (95% CI)[a]	p[a]	rs3813832		AOR (95% CI)[a]	p[a]	Protective genotypes[b]		AOR (95% CI)[a]	p[a]
	(cases/controls)				(cases/controls)				(cases/controls)			
	TT	TC/CC			TT	TC/CC			0–1	2–3		
Age, months												
<60	34/40	51/79	0.76 (0.43–1.35)	0.348	51/58	34/61	0.63 (0.36–1.11)	0.111	45/53	40/66	0.71 (0.4–1.24)	0.229
≥60	43/34	43/75	0.46 (0.25–0.82)[c]	0.009[c]	58/57	28/52	0.55 (0.30–0.99)[c]	0.044[c]	55/48	31/61	0.46 (0.26–0.82)[c]	0.008[c]
Gender												
Females	40/27	41/66	0.42 (0.22–0.80)[c]	0.008[c]	56/46	25/47	0.47 (0.25–0.88)[c]	0.018[c]	52/39	29/54	0.44 (0.24–0.82)[c]	0.010[c]
Males	37/47	53/88	0.77 (0.44–1.34)	0.358	53/69	37/66	0.74 (0.43–1.27)	0.275	48/62	42/73	0.74 (0.43–1.27)	0.271
Subtypes												
Astrocytic tumors	59/74	66/154	0.54 (0.34–0.86)[c]	0.009[c]	83/115	42/113	0.54 (0.34–0.86)[c]	0.009[c]	76/101	49/127	0.53 (0.34–0.84)[c]	0.007[c]
Ependymoma	10/74	15/154	0.76 (0.32–1.78)	0.520	14/115	11/113	0.77 (0.33–1.79)	0.546	12/101	13/127	0.87 (0.38–2.01)	0.744
Neuronal and mixed	6/74	8/154	0.68 (0.22–2.04)	0.489	7/115	7/113	1.01 (0.34–3.00)	0.984	7/101	7/127	0.83 (0.28–2.46)	0.732
Embryonal tumors	2/74	5/154	0.80 (0.13–4.84)	0.806	5/115	2/113	0.48 (0.08–2.85)	0.415	5/101	2/127	0.36 (0.06–2.15)	0.264
Tumor grades												
I	46/74	57/154	0.60 (0.37–0.97)[c]	0.037[c]	63/115	40/113	0.68 (0.42–1.10)	0.112	59/101	44/127	0.61 (0.38–0.98)[c]	0.042[c]
II	15/74	13/154	0.42 (0.19–0.92)[c]	0.031[c]	21/115	7/113	0.34 (0.14–0.83)[c]	0.017[c]	19/101	9/127	0.38 (0.16–0.87)[c]	0.022[c]
III	6/74	9/154	0.74 (0.25–2.17)	0.577	8/115	7/113	0.86 (0.30–2.47)	0.777	7/101	8/127	0.91 (0.32–2.62)	0.859
IV	10/74	15/154	0.72 (0.29–1.78)	0.479	17/115	8/113	0.58 (0.23–1.47)	0.250	15/101	10/127	0.66 (0.27–1.61)	0.355
I+II	61/74	70/154	0.55 (0.35–0.86)[c]	0.009[c]	84/115	47/113	0.59 (0.38–0.92)[c]	0.019[c]	78/101	53/127	0.55 (0.36–0.85)[c]	0.008[c]
III+IV	16/74	24/154	0.73 (0.36–1.47)	0.377	25/115	15/113	0.67 (0.33–1.36)	0.265	22/101	18/127	0.72 (0.36–1.43)	0.342

AOR, adjusted odds ratio; CI, confidence interval.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.
[b]Protective genotypes were carriers with rs2293596 TC/CC, rs2293595 TC/CC, and rs3813832 TC/CC genotypes.
[c]Significant results.

those with 2–3 protective genotypes were less likely to develop glioma in children aged ≥60 months, females, subtype of astrocytic tumors, patients with tumors in tumor grade I, patients with tumors in tumor grade II, and patients with tumors in tumor grade I+II.

For *YTHDF2* rs3738067 A > G polymorphism (Table 4), AG/GG was associated with increased glioma risk in children aged <60 months, children aged ≥60 months, females, subtype of astrocytic tumors, patients with tumors in tumor grade I, patients with tumors in tumor grade IV, patients with tumors in tumor grade I+II, and patients with tumors in tumor grade III+IV.

For *FTO* genotypes (Table 5), rs8047395 AG/GG was associated with decreased glioma risk in subtype of astrocytic tumors, patients with tumors in tumor grade I, and patients with tumors in tumor grade I+II. rs1477196 GA/AA, rs9939609 TT/TA, rs7206790 CC/CG, and rs8047395 GT/TT were further referred to as protective genotypes. Compared to 0–2 protective genotypes, those with 3–4 protective genotypes were less likely to develop glioma in patients with tumors in tumor grade I and patients with tumors in tumor grade I+II.

Expression quantitative trait loci (eQTL) analyses

We further assessed the putative functional relevance of *WTAP* rs7766006 and YTHDF2 rs3738067 using released data from GTEx. Samples with rs7766006 T genotype had significantly higher *WTAP* mRNA levels in the cell-cultured fibroblasts than samples with rs7766006 G genotype (Figure 1A). We also found that rs7766006 T genotype confers to higher mRNA level of neighboring genes, including *PNLDC1* and *ACAT2* (Figure 1B). Samples with rs3738067 A genotype had significantly higher *YTHDF2* mRNA levels in the whole blood than samples with rs3738067 G genotype (Figure 2A). Our *cis*-eQTL analysis also detected an association between rs3738067 A and increased expression of genes *PHACTR4*, *RCC1*, and *PRDX3P2* (Figure 2B).

Functional annotation of WTAP and YTHDF2

Functional annotation of *WTAP* and *YTHDF2* expression was determined in public data deposited in the Chinese Glioma Genome Atlas (CGGA) database (mRNaseq_325). *WTAP* (Figure 3A) and *YTHDF2* (Figure 3B) expression were significantly higher in grade III and IV samples than in grade II samples. We also performed survival analysis to investigate the clinical relevance of *WTAP* and *YTHDF2* expression in patient survival. The results demonstrated that elevated *WTAP*

Table 4. Stratification analysis between *YTHDF2* rs3738067 A > G polymorphism and glioma risk

Variables	rs3738067 (cases/controls)		Crude OR	p	AOR (95% CI)[a]	p[a]
	AA	AG/GG	(95% CI)			
Age, months						
<60	39/73	46/46	1.87 (1.07–3.29)[b]	0.029[b]	1.87 (1.06–3.29)[b]	0.030[b]
≥60	44/71	42/38	1.78 (1.00–3.18)	0.050	1.81 (1.01–3.23)[b]	0.046[b]
Gender						
Females	38/61	43/32	2.16 (1.17–3.97)[b]	0.014[b]	2.27 (1.22–4.25)[b]	0.010[b]
Males	45/83	45/52	1.60 (0.93–2.74)	0.089	1.61 (0.93–2.77)	0.087
Subtypes						
Astrocytic tumors	61/144	64/84	1.80 (1.16–2.80)[b]	0.009[b]	1.90 (1.20–2.99)[b]	0.006[b]
Ependymoma	13/144	12/84	1.58 (0.69–3.63)	0.278	1.58 (0.68–3.64)	0.287
Neuronal and mixed	7/144	7/84	1.71 (0.58–5.06)	0.329	1.63 (0.55–4.84)	0.381
Embryonal tumors	2/144	5/84	4.29 (0.81–22.58)	0.086	6.82 (1.00–46.76)	0.051
Tumor grades						
I	52/144	51/84	1.68 (1.05–2.69)[b]	0.031[b]	1.78 (1.10–2.89)[b]	0.018[b]
II	14/144	14/84	1.71 (0.78–3.77)	0.180	1.71 (0.78–3.77)	0.182
III	8/144	7/84	1.50 (0.53–4.29)	0.449	1.47 (0.51–4.23)	0.475
IV	9/144	16/84	3.05 (1.29–7.20)[b]	0.011[b]	3.08 (1.23–7.71)[b]	0.016[b]
I+II	66/144	65/84	1.69 (1.09–2.61)[b]	0.018[b]	1.77 (1.14–2.75)[b]	0.012[b]
III+IV	17/144	23/84	2.32 (1.17–4.59)[b]	0.016[b]	2.29 (1.14–4.60)[b]	0.019[b]

AOR, adjusted odds ratio; CI, confidence interval.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.
[b]Significant results.

(Figure 3C) and *YTHDF2* (Figure 3D) expression was clinically correlated with unfavorable outcomes of glioma patients.

DISCUSSION

Emerging evidence has been growing regarding the significant contributions of m^{6}A modification core genes to the initiation and development of various cancers. However, no reports have been found in analyzing the impact of these critical gene SNPs on risk of glioma. Discovering genetic variants that distinguish glioma is of critical clinical importance for disease prevention and treatment. In this analysis of Asian children from China, we first identified several glioma risk variants: *WTAP* rs7766006, YTHDF2 rs3738067, FTO rs9939609, YTHDC1 rs2293595, YTHDC1 rs3813832, and FTO rs8047395.

Regarding the epidemiology assessment of m^{6}A modification core gene SNPs on cancer, only two available studies have been conducted. In 2019, Meng et al.[29] performed a first case-control study on m^{6}A modification core gene SNPs and colorectal cancer risk. Their study applied a two-stage design: discovery stage (1,150 cases and 1,342 controls) and replication stage (932 cases and 966 controls). A total of 240 SNPs in 20 m^{6}A modification-related genes were genotyped. Surprisingly, only one variant, *SND1* gene rs118049207, was associated with colorectal cancer risk. *SND1* gene rs118049207 might impact colorectal cancer risk by changing the mRNA expression of the *SND1* gene and then lead to alteration of m^{6}A level. More recently, the group of Yang et al.[30] performed another epidemiology study regarding m6A genes SNPs and cancer risk. They carried out the first study to examine the association between genetic variants in m^{6}A modification genes and esophageal squamous cell carcinoma (ESCC) risk. They observed that one potentially functional SNP located upstream of *YTHDC2*, rs2416282, predisposes to ESCC risk in Chinese population by altering the expression of *YTHDC2*. Given the important role of m^{6}A modification of core gene SNPs in cancer, we aim to investigate the association between m^{6}A modification core gene SNPs and the risk of glioma.

Our study found that *WTAP* rs7766006, YTHDF2 rs3738067, and FTO rs9939609 variants could contribute to glioma risk, respectively. However, we detected a significant inverse association between *YTHDC*1 rs2293595, YTHDC1 rs3813832, FTO rs8047395, and susceptibility to glioma, respectively. We then proceed to determine the possible mechanisms for the conferring risk role of *WTAP* rs7766006 and YTHDF2 rs3738067. The eQTL results indicated that the increased glioma risk was linked to the upregulated expression levels of the *WTAP* and the *YTHDF2* gene. WTAP and its partner Wilms tumor 1 (WT1) protein are present together throughout the nucleoplasm as well as in nuclear speckles and partially colocalize with splicing factors.[31] WTAP is ubiquitously expressed in diverse tissues, rather than the tissue-specific expression pattern of WT1.[31] Substantial evidence supports the implication of WTAP in various cellular processes, including m^{6}A methylation modification,[18] alternative splicing,[32] X chromosome inactivation,[33] and cell cycle regulation.[34] Moreover, WTAP has also been reported to be extensively involved in several cancers. WTAP can be treated as a marker for predicting poor prognosis in malignant glioma patients.[28] Jin et al.[22] suggested that WTAP may play an oncogenic role in glioma. This research provides further support for our result that *WTAP* rs7766006 contributed to increased glioma risk by upregulating expression levels of the *WTAP* gene. The YTHDF proteins are cytoplasmic m^{6}A readers that specifically recognize and bind to m^{6}A within the consensus RR(m^{6}A)CH sequence.[35] Human YTHDF proteins include three members, YTHDF1–3, each of which comprises a highly conserved single-stranded RNA-binding domain located at the carboxy terminus (the YTH domain) and a less

Table 5. Stratification analysis between *FTO* genotypes and glioma risk

Variables	rs8047395 (cases/controls)		AOR (95% CI)[a]	p[a]	Protective genotypes[b] (cases/controls)		AOR (95% CI)[a]	p[a]
	AA	AG/GG			0–2	3–4		
Age, months								
<60	39/41	46/78	0.62 (0.35–1.09)	0.098	38/41	47/78	0.65 (0.37–1.15)	0.138
≥60	36/34	50/75	0.64 (0.35–1.15)	0.133	35/33	51/76	0.64 (0.35–1.16)	0.139
Gender								
Females	36/30	45/63	0.61 (0.32–1.13)	0.116	35/30	46/63	0.64 (0.34–1.19)	0.159
Males	39/45	51/90	0.65 (0.37–1.12)	0.120	38/44	52/91	0.65 (0.37–1.13)	0.128
Subtypes								
Astrocytic tumors	55/75	70/153	0.62 (0.39–0.98)[c]	0.039[c]	53/74	72/154	0.64 (0.41–1.02)	0.060
Ependymoma	12/75	13/153	0.53 (0.23–1.23)	0.141	12/74	13/154	0.53 (0.23–1.22)	0.137
Neuronal and mixed	6/75	8/153	0.66 (0.22–1.99)	0.459	6/74	8/154	0.65 (0.22–1.86)	0.444
Embryonal tumors	2/75	5/153	1.31 (0.22–7.85)	0.767	2/74	5/154	1.23 (0.21–7.26)	0.821
Tumor grades								
I	48/75	55/153	0.58 (0.36–0.93)[c]	0.025[c]	47/74	56/154	0.58 (0.36–0.95)[c]	0.029[c]
II	11/75	17/153	0.76 (0.34–1.70)	0.504	11/74	17/154	0.74 (0.33–1.67)	0.473
III	8/75	7/153	0.44 (0.15–1.26)	0.124	8/74	7/154	0.43 (0.15–1.25)	0.120
IV	8/75	17/153	1.07 (0.42–2.73)	0.892	7/74	18/154	1.27 (0.48–3.34)	0.635
I+II	59/75	72/153	0.60 (0.39–0.94)[c]	0.026[c]	58/74	73/154	0.61 (0.39–0.95)[c]	0.028[c]
III+IV	16/75	24/153	0.72 (0.36–1.45)	0.355	15/74	25/154	0.78 (0.38–1.58)	0.489

AOR, adjusted odds ratio; CI, confidence interval.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.
[b]Protective genotypes were carriers with rs1477196 GA/AA, rs9939609 TT/TA, rs7206790 CC/CG, and rs8047395 GT/TT genotypes.
[c]Significant results.

conserved amino-terminal region.[36] YTHDF2 specifically recognizes m^{6}A by its aromatic cage. Chen et al.[37] showed that METTL3 promotes liver cancer progression through YTHDF2-dependent post-transcriptional silencing of SOCS2. Recently, Chai and his colleagues[38] carried out an observational study to examine if m^{6}A modification genes can be used in a purely prognostic perspective for glioma. They reported a direct correlation between the expression of WTAP, YTHDF2, and the WHO grade of glioma, respectively. In other words, WTAP and YTHDF2 act as "risky" genes in glioma. These data combined with our results shed light on the biological mechanisms of how *WTAP* rs7766006 and YTHDF2 rs3738067 function to enhance glioma risk. We also applied CGGA data to explore whether these genes were associated with glioma progression and prognosis. The result showed that higher expression of *WTAP* and *YTHDF2* were positively correlated to glioma progression and unfavorable overall survival. Taken together, these results suggest the potential value of *WTAP* and *YTHDF2* as markers in the outcome prediction of glioma patients. Of note, specific data regarding the role of *WTAP* and *YTHDF2* in pediatric glioma remains to be explored.

Our study has several limitations. First, while this sample does represent the largest dataset of genotyped Chinese glioma cases, small numbers of subjects in some of the subgroup analysis may have limited the ability to detect associations with certain SNPs. Expansion of sample size is necessary to confirm the associations detected in this analysis, which necessitates additional multicenter collaborations. Second, all the participants were Han Chinese descents. Conclusion obtained from the single population here might not be generalized to overall ethnic groups. Third, environmental factors and environmental-genetic interactions contributed to the glioma, yet these factors were not interrogated in the risk models. Of note, plenty of critical genetic events such as H3K27M mutation, Isocitrate dehydrogenase (IDH) mutation, and v-raf murine sarcoma viral oncogene homolog B1 (BRAF) fusions play an important role in glioma development.[39] However, the impact of these critical genetic events on the risk of glioma remains to be elucidated. Therefore, many other additional variables that likely to impact the glioma phenotype should be considered. Thus, the significant SNPs obtained herein all require additional validation, particularly involving environmental factors and environmental-genetic interaction analysis.

This is the first epidemiology study to assess common variants within m^{6}A modification core genes in relation to different glioma subgroups. In conclusion, we have identified common

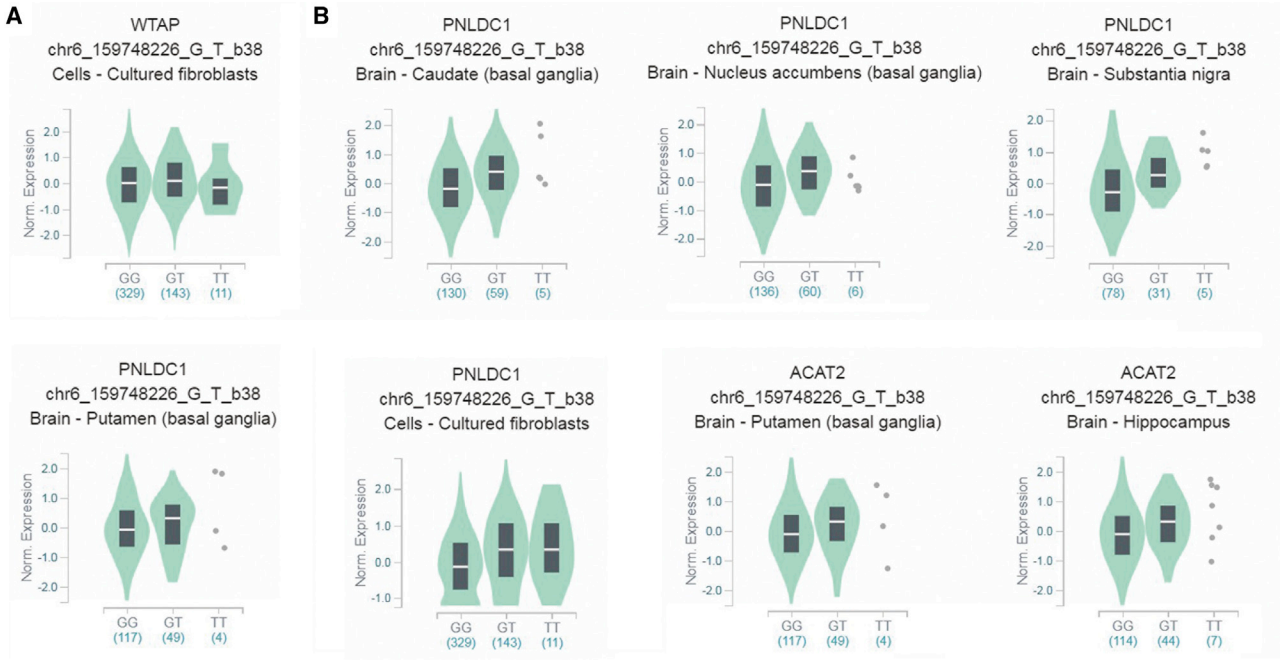

Figure 1. Functional implication of *WTAP* gene rs7766006 polymorphism based on the public database GTEx portal

(A) The genotype of rs7766006 and expression of *WTAP* gene in cell-cultured fibroblasts. (B) The genotype of rs7766006 and expression of its neighboring genes *PNLDC1* and *ACAT2* in different tissues.

variants within m^{6}A modification core genes that are associated with glioma risk. Further functional study of m^{6}A modification core gene SNPs in glioma is warranted and may lead to unearthing the genetic and epigenetic mechanisms underlying this disease.

MATERIALS AND METHODS

Study subjects

In brief, we conducted a hospital-based case-control study in China. 171 children with primary glioma and 228 children who were free of glioma were enrolled. Epidemiological data were collected using structured questionnaires. The inclusion criterion for case subjects was biopsy confirmed or histologically verified glioma. Control subjects, who were recruited concurrently with case subjects, were randomly selected from the volunteers visiting the hospital and matched according to the expected age and gender distribution of cases. All participants gave written informed consent to use their samples for research purposes. The institutional review board of Guangzhou Women and Children's Medical Center approved the current study. A more detailed relevant sample selection could be found in our previous work.[40]

SNP selection and genotyping

Eight m^{6}A modification core genes were contained: Wilms tumor 1-associated protein (WTAP), methyltransferase like 3 (METTL3), methyltransferase like 14 (METTL14), alpha-ketoglutarate dependent dioxygenase (FTO), alkB homolog 5 (ALKBH5), YTH m^{6}A RNA binding protein 1 (YTHDF1), YTH m^{6}A RNA binding protein 2 (YTHDF2), and YTH domain containing 1 (YTHDC1). The potentially functional SNPs were selected by using the NCBI dbSNP database and SNPinfo (https://snpinfo.niehs.nih.gov/). SNPs that fulfilled the following selection criteria were chosen: (1) the minor allele frequency (MAF) reported in HapMap was > 5% for Chinese Han subjects; (2) SNPs located in the 5′ flanking region, exon, 5′ untranslated region (5′ UTR), and 3′ UTR, which might affect transcription activity or binding capacity of the microRNA binding site; and (3) SNPs in low linkage disequilibrium with each other (R^2 < 0.8). A total of 24 SNPs were selected. Specifically, 2 ALKBH5, 4 METTL3, 5 METTL14, 3 WTAP, 3 YTHDC1, 2 YTHDF1, 1 YTHDF2, and 4 FTO SNPs were genotyped. Genomic DNA was prepared from peripheral blood samples using a QIAamp DNA blood mini kit (QIAGEN, Valencia, CA, USA). The TaqMan genotyping for the SNP was performed on an ABI 7900 (Applied Biosystems, Foster City, CA, USA). All case/control status was carried out blind to the laboratory personnel. Genotyping of the proposed SNPs was all performed in the laboratory of Guangzhou. The conditions of reactions were set as follows: pre-read stage at 60°C for 30 s, holding stage at 95°C for 10 min, repeated 45 cycles each of denaturation at 95°C for 15 s, annealing and extension at 60°C for 1 min. 10% duplicate test samples and water samples (negative controls) were included in each 96-well plate. A 100% concordance rate of the duplicated samples was achieved. Detailed information about SNPs selection and genotyping have been described previously.[41–43]

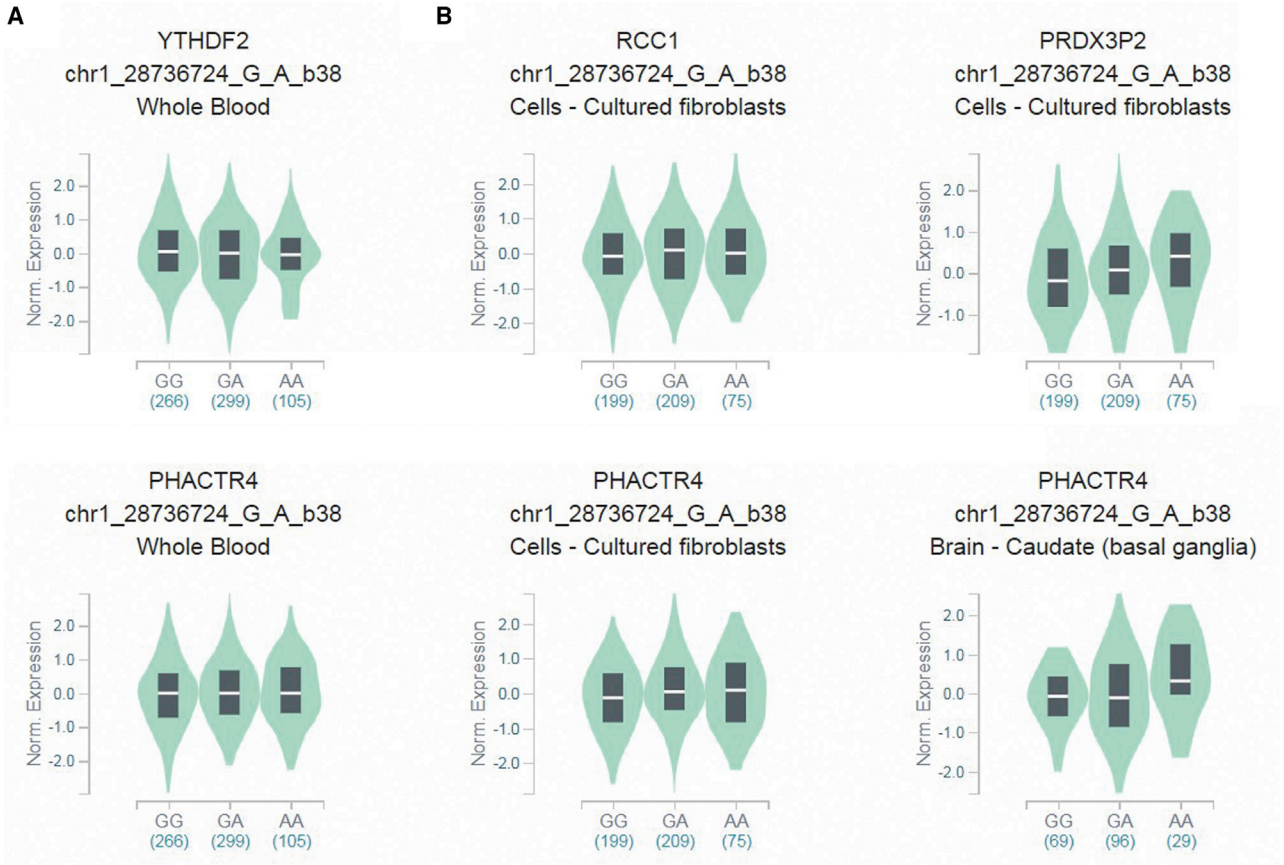

Figure 2. Functional implication of *YTHDF2* gene rs3738067 polymorphism based on the public database GTEx portal

(A) The genotype of rs3738067 and expression of *YTHDF2* gene in whole blood. (B) The genotype of rs3738067 and expression of its neighboring genes *PHACTR4*, *RCC1*, and *PRDX3P2* in different tissues.

Statistical analysis

The compliance of genotypes with HWE among controls was appraised by a χ^2 test. Differences in demographic characteristics between the cases and the controls were evaluated using χ^2 test or t test as appropriate. The age- and gender-adjusted ORs and 95% CIs for the relationships between the SNPs and glioma risk were determined by multivariate logistic regression analysis. We also conducted stratified analyses by reclassifying cases with different subgroups, including age, gender, subtypes, and tumor grade. Further functional annotation of the significant SNPs was performed using the Genotype-Tissue Expression (GTEx) (https://gtexportal.org).[44] Gene expression and glioma patient survival data were obtained from the CGGA database http://www.cgga.org.cn/. All tests for statistical significance used a two-sided alpha of 0.05. All statistical analyses were conducted using the SAS v10.0 (SAS Institute, Cary, NC, USA).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omto.2020.12.013.

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AUTHOR CONTRIBUTIONS

J.H., L.Y., and H.L. designed and performed the study and wrote the manuscript; A. Lin, H.C., A. Luo, and Z.Z. collected the samples and information; J.H. and L.Y. participated in analyzing data; J.H., Z.Z., and X.L. coordinated the study over the entire time. All authors reviewed the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

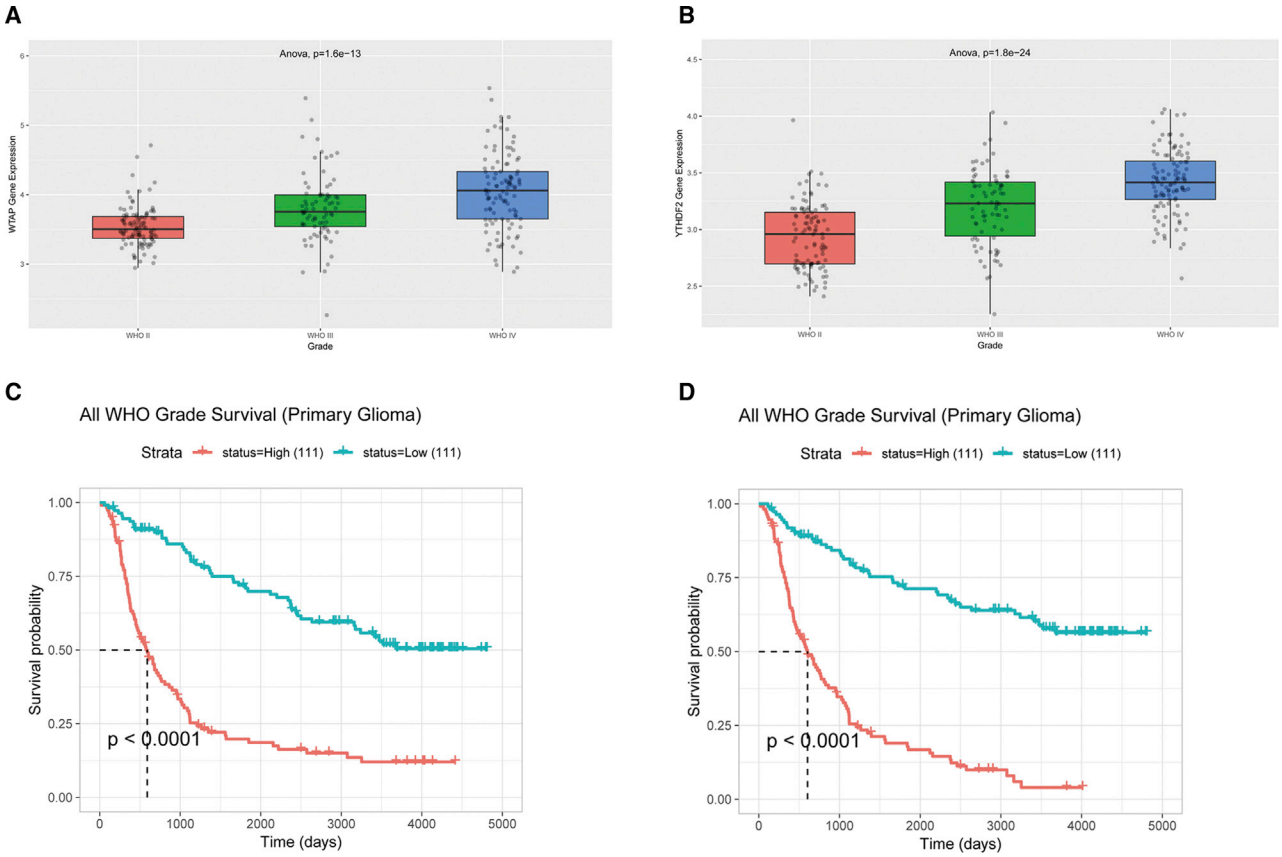

Figure 3. Correlation of *WTAP* and *YTHDF2* with progression and overall survival of glioma based on the CGGA database

(A) *WTAP* expression increased in grades III and IV compared to grade II. (B) *YTHDF2* expression increased in grades III and IV compared to grade II. (C) Correlation between *WTAP* expression and the survival of glioma patients. (D) Correlation between *YTHDF2* expression and the survival of glioma patients.

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