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HOTAIR gene polymorphisms and risk of glioma in Chinese children

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Abstract

Purpose: Glioma is the most common malignant brain tumor in children. Hox transcription antisense intergenic RNA (HOTAIR) has been shown to promote cancers. However, the role of genetic variation of HOTAIR gene on glioma susceptibility has not been fully elucidated. We aimed to evaluate whether HOTAIR gene single nucleotide polymorphisms (SNPs) are associated with susceptibility to glioma.

Methods: The trial included a total of 171 glioma patients and 228 non-glioma controls from Chinese children. Genotyping of HOTAIR gene was evaluated by TaqMan. The strength of the association between HOTAIR gene polymorphism (rs920778 A > G, rs4759314 A > G, rs1899663 C > A) and glioma susceptibility was assessed using odds ratios and 95% confidence intervals. Stratified analysis was further conducted.

Results: Of the three SNPs analyzed, the rs920778 variant and the rs1899663 variant were associated with increased glioma susceptibility. In addition, the combination of two risk genotypes (OR = 1.63, $P = 0.028$) and one to three risk genotypes (OR = 1.58, $P = 0.027$) showed a significantly higher increase in glioma susceptibility than zero risk genotypes. These two SNPs (rs920778 A > G, rs1899663 C > A) were significantly associated with increased glioma susceptibility in certain subgroups in stratified analysis. Similar results were found in stratified analyses for one to three risk genotypes compared with zero risk genotypes. Splice quantitative trait loci (sQTLs) indicated rs920778 A > G and rs1899663 C > A are associated with splicing events in certain genes (HOTAIR, HOXC5, HOXC10, HOXC6, and HOXC4).

Conclusion: Overall, our results suggest that some HOTAIR SNPs are associated with increased glioma susceptibility.

Keywords: Glioma, Risk, HOTAIR, Polymorphism, Case-control study

1 Introduction

Brain tumors are known to be common in children, and they account for a high proportion of childhood cancer-related deaths (Albright, 1993; Wang et al., 2019). It is reported that gliomas account for about 28% of all brain

tumors, and they are the most common tumors among all malignant primary brain tumors (Ostrom et al., 2014b; Ostrom et al., 2014a). It is widely believed that gliomas originate from neural stem cells (Xu et al., 2020), (Le Rhun et al., 2019). Gliomas, the most common central nervous system tumors in children, present a high degree of heterogeneity (Sturm et al., 2017). They exhibit different histological, biological, and clinical features (Malta et al., 2018). According to the WHO classification of malignancy of central nervous system (CNS) tumors, pediatric gliomas are mostly classified as grade I or II, low-grade gliomas (LGG), because of their benign characteristics (Sturm et al., 2017). For example, histologically classified ependymomas, and low-grade astrocytoma can

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be classified as LGG. Unlike LGG in children, isocitrate dehydrogenase (IDH) mutant LGG, defined by WHO in 2016 based on molecular characteristics, often develops malignant transformation in adults (Sturm et al., 2017), (Louis et al., 2016). However, there are still a considerable number of gliomas in the development of malignant. They can be classified as high-grade gliomas (HGG) according to the WHO classification, including grade III or IV (Sturm et al., 2017). For example, the glioblastoma that belongs to astrocytoma can be divided into IV-grade high-grade glioma. Current treatments for HGG remain limited, with the most aggressive type of glioma dying within months (Sturm et al., 2017). Various studies are underway for new precision treatments for gliomas, such as combinations of molecularly targeted therapies that promise improved outcomes (Lapointe et al., 2018), (Lin et al., 2021).

The etiology and pathogenesis of glioma remain unclear. The risk of glioma is related to environmental factors, such as ionizing radiation, which can increase the risk of glioma (Ostrom et al., 2014b; Ostrom et al., 2014a). In recent years, the genetics of glioma has made important progress with molecular research. It was reported that IDH1/2 (IDH) mutation, 1P / 19Q co-deletion, ATRX expression deletion, TERT promoter mutation, and BRAF mutation are important biomarkers of glioma (Reifenberger et al., 2017). Importantly, some genome-wide association studies (GWAS) have provided us with results that single nucleotide polymorphisms (SNPs) are associated with glioma susceptibility (Kinnerley et al., 2015; Wrensch et al., 2009; Shete et al., 2009; Melin et al., 2017). These SNPs are located in the gene regions of CDKN2B, RTEL1, TP53, PHLDB1, CCDC26, and EGFR (Wrensch et al., 2009), (Shete et al., 2009; Melin et al., 2017). A recent study has shown that SNPs located in the WTAP, YTHDC1, YTHDF2, and FTO genes are associated with glioma susceptibility (He et al., 2021). These studies indicate the importance of SNPs in gliomas, but they are far from sufficient to elucidate the genetic mechanisms of gliomas. More molecular genetic studies are needed to explore the unknown genetic variation associated with gliomas.

In human chromosomes, homologous genes (HOX) have four loci located on different chromosomes. The HOXC gene on chromosome 12 can transcribe a non-coding RNA (ncRNA) called Hox transcribed antisense RNA (HOTAIR) (Rinn et al., 2007). HOTAIR is about 2.2 kilobases long and can influence tumor and embryonic development through gene regulation (Rinn et al., 2007; Shah & Sukumar, 2010). HOTAIR has trans-action and silences expression of HOXD loci through interaction with Polycomb Repressive Complex 2 (PRC2) (Rinn et al., 2007). High expression of

HOTAIR is associated with poor prognosis in bladder cancer, breast cancer, lung cancer, gastric cancer, and other tumors (Qu et al., 2019). HOTAIR may promote the growth, invasion, and apoptosis inhibition of cancers including glioma by regulating other genes (Huang et al., 2017; Angelopoulou et al., 2020; Chi-yomaru et al., 2014). Aberrant expression of HOTAIR is associated with poor progression of glioma (Tan et al., 2018; Zhang et al., 2013; Pastori et al., 2015). High HOTAIR level was significantly associated with glioblastoma multiforme and its poor prognosis, and HOTAIR level was proved to be a new prognostic and diagnostic biomarker for glioblastoma multiforme (Tan et al., 2018). Zhang et al. found that the expression of HOTAIR can predict the grade and prognosis of glioma (Zhang et al., 2013). Interestingly, BET protein has also been shown to directly bind to the HOTAIR promoter to regulate the expression of the long-chain non-coding RNA (lncRNA) HOTAIR, which reveals the mechanism of the new HOTAIR expression affecting the growth of glioblastoma (Pastori et al., 2015). HOTAIR has become a hot therapeutic target for glioma (Li et al., 2019; Zhao et al., 2021). A study found that a small molecule AC1Q3QWB (AQB) can effectively inhibit the HOTAIR-EZH2 interaction to prevent the recruitment of the multi comb inhibition complex 2 (PRC2), which shows effective anti-tumor activity in vitro (Li et al., 2019). In conclusion, HOTAIR is a biomarker with great potential for diagnosis, prognosis, and treatment of glioma (Tan et al., 2018; Zhang et al., 2013; Pastori et al., 2015; Li et al., 2019; Zhao et al., 2021). It is necessary to further explore the important role of the HOTAIR gene in gliomas. SNP is currently an important genetic factor for predicting disease risk and poor prognosis (Kreile et al., 2016; Grotenhuis et al., 2016). Importantly, multiple studies have shown that multiple SNPs in the HOTAIR gene are associated with cancer risk (Zhang et al., 2014; Bayram et al., 2016; Taheri et al., 2017). One of our previous studies showed that HOTAIR gene polymorphism was associated with increased susceptibility to neuroblastoma (Yang et al., 2018). To the best of our knowledge, there is only one study on HOTAIR gene polymorphisms and glioma. This study showed that HOTAIR SNPs rs920778 and rs12826786 were not associated with glioma susceptibility (Xavier-Magalhaes et al., 2017). The role of HOTAIR gene variations in glioma is an interesting and novel study.

Therefore, the effect of the HOTAIR gene SNP on glioma risk remains to be studied. Given the cancer-promoting effect of the HOTAIR gene, we hypothesized that the SNP of the HOTAIR gene might be susceptible to the risk of glioma. To test this hypothesis, we

conducted a case-control study to explore whether the SNPs of HOTAIR increase glioma susceptibility in Chinese children.

2 Materials and methods

2.1 Study subjects

This study was a hospital-based case-control study in China. A total of 171 children with primary glioma and 228 children without glioma were enrolled. Epidemiological data were collected through a structured questionnaire. The case group that met the criteria for inclusion was children with glioma confirmed by biopsy or histologically. Subjects in the control group were recruited at the same time as the case group, randomly selected from hospital volunteers, and matched according to the expected demographic characteristics (sex and age) distribution of the case group. All participants have signed informed consent for us to use their samples for the study. The current study was approved by the Institutional Review Committee of Guangzhou Women and Children's Medical Center.

2.2 Polymorphism selection and genotyping

We selected three SNPs (rs920778 A>G, rs4759314 A>G, rs1899663 C>A) in the HOTAIR gene from previous studies (Zhang et al., 2014; Yang et al., 2018; Guo et al., 2015; Yan et al., 2015). QIAamp DNA blood mini kit (QIAGEN, Valencia, CA, USA) was used to extract genomic DNA from peripheral blood samples. These 3 SNPs were genotyped using TaqMan real-time polymerase chain reaction (Takara, Dalian, China). Laboratory personnel were not informed of the status of any cases or controls. All SNP genotyping was performed in the same laboratory. We randomly selected 10% of the samples for repeated genotyping, and their agreement rate was 100%.

2.3 Statistical analysis

The 2-sided chi-square test was used to assess differences in genotype frequency distribution and demographic characteristics between cases and controls. Univariate and multivariate logistic regression analysis were used to calculate the OR value and 95% CI value before and after adjustment, respectively, to assess the relationship between HOTAIR single nucleotide polymorphism and glioma risk. We divided them into different subgroups based on different ages, sex, subtype, and tumor grade for further stratified analysis. Genotypic tissue expression (GTEx) (<https://gtexportal.org>) was used to preliminarily explore how important HOTAIR SNPs affect glioma susceptibility. All statistical significance tests were 2-sided, and the significant level was 0.05. SAS V10.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

3 Results

3.1 Characteristics of the participants

In our previous study, we described the clinical and demographic characteristics of glioma cases and cancer-free controls. The sample size of glioma cases was 171 and that of non-glioma controls was 228. The specific frequency distribution is shown in Table S1. There were no statistically significant differences in the frequency distribution of gender ($P=0.190$) and age ($P=0.623$) between the case group and the control group. According to clinical and biological classification, 125 (73.10%) glioma cases could be classified as astrocytic tumors, 24 (14.62%) as ependymomas, 14 (8.19%) as neuronal and neuronal and mixed neutral gliomas, and 7 (4.09%) as embryonal tumors. According to WHO classification, there were 103 (60.23%) cases in grade I, 28 (16.37%) cases in grade II, 15 (8.77%) cases in grade III, and 25 (14.62%) cases in grade IV.

3.2 Associations of HOTAIR polymorphisms with glioma susceptibility

We successfully genotyped the HOTAIR gene SNPs (rs920778 A>G, rs4759314 A>G, rs1899663 C>A) in 171 cases and 228 controls. Table 1 shows the genotype frequency of HOTAIR gene polymorphism and its association with the risk of glioma. The AG/GG genotype of rs920778 ($P=0.019$), CA/AA genotype of rs1899663 ($P=0.043$) showed statistically significant differences in frequency distribution between glioma patients and controls. The variation of HOTAIR rs920778 was significantly associated with increased glioma susceptibility (AG vs. AA: adjusted OR=1.77, 95%CI=1.15-2.73, $P=0.010$; AG/GG vs. AA: adjusted OR=1.58, 95%CI=1.05-2.38, $P=0.027$). Similarly, we found that the rs1899663 variant allele was significantly associated with an increased risk of glioma (CC/CA/AA vs. CC: adjusted OR=1.46, 95%CI=1.04-2.04, $P=0.030$; CA/AA vs. CC: adjusted OR=1.53, 95%CI=1.02-2.31, $P=0.041$). HOTAIR rs920778 AG/GG, rs4759314 GG, and rs1899663 CA/AA were defined as risk genotypes based on their ORs, although the association between rs4759314 variant alleles and increased risk of glioma was not statistically significant. This analysis is not shown in this paper due to the small sample size of cases and controls with all three risk genotypes. The difference in the frequency distribution of risk genotypes between the case group and the control group was statistically significant ($P=0.019$). Compared with participants with zero risk genotypes, participants with two protective genotypes had a 0.63-fold increased risk of developing glioma (95% CI=1.05 to 2.52, $P=0.028$), participants with 1-3 protective genotypes had a

Table 1 Associations of *HOTAIR* polymorphisms with glioma susceptibility in Chinese children

Genotype	Cases (N = 171)	Controls (N = 227)	P ^a	Crude OR (95% CI)	P	Adjusted OR (95% CI) ^b	P ^b
rs920778							
AA	65 (38.01)	113 (49.78)		1.00		1.00	
AG	87 (50.88)	84 (37.00)		1.80 (1.17-2.76)	0.007	1.77 (1.15-2.73)	0.010
GG	19 (11.11)	30 (13.22)		1.10 (0.57-2.11)	0.772	1.07 (0.55-2.06)	0.844
Additive			0.162	1.23 (0.92-1.65)	0.163	1.21 (0.90-1.63)	0.203
Dominant	106 (61.99)	114 (50.22)	0.019	1.62 (1.08-2.42)	0.020	1.58 (1.05-2.38)	0.027
AA/AG	152 (88.89)	197 (86.78)		1.00		1.00	
GG	19 (11.11)	30 (13.22)	0.527	0.82 (0.45-1.51)	0.527	0.80 (0.43-1.49)	0.489
rs4759314							
AA	138 (80.70)	180 (79.30)		1.00		1.00	
AG	29 (16.96)	43 (18.94)		0.88 (0.52-1.48)	0.629	0.84 (0.50-1.43)	0.521
GG	4 (2.34)	4 (1.76)		1.30 (0.32-5.31)	0.711	1.34 (0.33-5.49)	0.688
Additive			0.859	0.96 (0.62-1.48)	0.859	0.94 (0.61-1.45)	0.775
Dominant	33 (19.30)	47 (20.70)	0.729	0.92 (0.56-1.51)	0.729	0.88 (0.53-1.46)	0.627
AA/AG	167 (97.66)	223 (98.24)		1.00		1.00	
GG	4 (2.34)	4 (1.76)	0.685	1.34 (0.33-5.42)	0.686	1.38 (0.34-5.65)	0.655
rs1899663							
CC	92 (53.80)	145 (63.88)		1.00		1.00	
CA	67 (39.18)	73 (32.16)		1.45 (0.95-2.21)	0.087	1.46 (0.95-2.24)	0.083
AA	12 (7.02)	9 (3.96)		2.10 (0.85-5.18)	0.107	2.11 (0.85-5.26)	0.110
Additive			0.030	1.45 (1.04-2.02)	0.030	1.46 (1.04-2.04)	0.030
Dominant	79 (46.20)	82 (36.12)	0.043	1.52 (1.01-2.28)	0.043	1.53 (1.02-2.31)	0.041
CC/CA	159 (92.98)	218 (96.04)		1.00		1.00	
AA	12 (7.02)	9 (3.96)	0.178	1.83 (0.75-4.44)	0.183	1.83 (0.74-4.49)	0.188
Combined effect of risk genotypes ^c							
0	65 (38.01)	113 (49.78)		1.00		1.00	
1	24 (14.04)	28 (12.33)		1.49 (0.80-2.78)	0.211	1.39 (0.74-2.62)	0.307
2	81 (47.37)	86 (37.89)		1.64 (1.07-2.52)	0.025	1.63 (1.05-2.52)	0.028
3	1 (0.58)	0 (0.00)		/	/	/	/
1-3	106 (61.99)	114 (50.22)	0.019	1.62 (1.08-2.42)	0.020	1.58 (1.05-2.38)	0.027

OR odds ratio, CI confidence interval

^a χ^2 test for genotype distributions between glioma cases and cancer-free controls^b Adjusted for age and gender^c Risk genotypes were carriers with rs920778 AG/GG, rs4759314 GG, and rs1899663 CA/AA genotypes

0.58-fold increased risk of developing glioma (95%CI =1.05-2.38, $P=0.027$).

3.3 Stratification analysis of significant SNPs

We further stratified the association between *HOTAIR* gene polymorphism and glioma susceptibility in different ages, sex, and clinical stage (Table 2). Our results showed that the rs920778 AG/GG genotype was significantly associated with an increased risk of glioma in groups with age ≥ 60 months, male, clinical stage IV glioma, and clinical stage III + IV glioma. Similarly, the rs1899663 CA/AA genotype was significantly associated with increased susceptibility to gliomas in the groups of

age ≥ 60 months, male, and clinical stage IV. More importantly, we found a statistical increase in glioma risk with 1-3 risk genotypes in the subgroups of ≥ 60 months, male, stage IV, and stage III + IV.

3.4 Effect of SNPs on gene splicing (sQTLs)

We further explored the cis-expression quantitative trait loci (eQTLs) and splice quantitative trait loci (sQTLs) of rs920778 and rs1899663 on GTEx. *HOTAIR* rs920778 and rs1899663 were found to be associated with important gene splicing events (Figs. 1 and 2). Importantly, we found that rs920778 (Fig. 1A) and rs1899663 (Fig. 1B) were significantly associated with the splicing event of the

Table 2 Stratification analysis for the association between risk genotypes for *HOTAIR* polymorphisms and glioma susceptibility

Variables	rs920778 (cases/controls)		AOR (95% CI) ^a		P ^a	rs1899663 (cases/controls)		AOR (95% CI) ^a		P ^a	Risk genotypes ^b (cases/controls)		AOR (95% CI) ^a		P ^a
	AA	AG/GG	AA	AG/GG		CC	CA/AA	CC	CA/AA		0	1-3	0	1-3	
Age, month															
<60	35/57	50/62	1.30 (0.74-2.29)		0.358	47/71	38/48			0.529	35/57	50/62	1.30 (0.74-2.29)		0.358
≥60	30/56	56/52	1.99 (1.11-3.57)		0.022	45/74	41/34			0.026	30/56	56/52	1.99 (1.11-3.57)		0.022
Sex															
Females	31/41	50/51	1.26 (0.68-2.34)		0.458	46/55	35/37			0.683	31/41	50/51	1.26 (0.68-2.34)		0.458
Males	34/72	56/63	1.88 (1.09-3.25)		0.023	46/90	44/45			0.018	34/72	56/63	1.88 (1.09-3.25)		0.023
Subtypes															
Astrocytic tumors	50/113	75/114	1.45 (0.92-2.28)		0.108	72/145	53/82			0.270	50/113	75/114	1.45 (0.92-2.28)		0.108
Ependymoma	10/113	15/114	1.44 (0.62-3.36)		0.402	11/145	14/82			0.095	10/113	15/114	1.44 (0.62-3.36)		0.402
Neuronal and mixed	4/113	10/114	2.66 (0.80-8.83)		0.110	6/145	8/82			0.114	4/113	10/114	2.66 (0.80-8.83)		0.110
Embryonal tumors	1/113	6/114	7.95 (0.75-84.02)		0.085	3/145	4/82			0.070	1/113	6/114	7.95 (0.75-84.02)		0.085
Clinical stage															
I	41/113	62/114	1.46 (0.91-2.36)		0.119	59/145	44/82			0.249	41/113	62/114	1.46 (0.91-2.36)		0.119
II	13/113	15/114	1.15 (0.52-2.53)		0.732	15/145	13/82			0.285	13/113	15/114	1.15 (0.52-2.53)		0.732
III	5/113	10/114	1.98 (0.65-6.02)		0.229	7/145	8/82			0.249	5/113	10/114	1.98 (0.65-6.02)		0.229
IV	6/113	19/114	3.52 (1.26-9.82)		0.016	11/145	14/82			0.023	6/113	19/114	3.52 (1.26-9.82)		0.016
I+II	54/113	77/114	1.39 (0.90-2.15)		0.141	74/145	57/82			0.167	54/113	77/114	1.39 (0.90-2.15)		0.141
III+IV	11/113	29/114	2.63 (1.24-5.62)		0.012	18/145	22/82			0.016	11/113	29/114	2.63 (1.24-5.62)		0.012

OR odds ratio, CI confidence interval

^a Adjusted for age and sex

^b Risk genotypes were carriers with rs920778 AG/GG, rs4759314 GG, and rs1899663 CA/AA genotypes

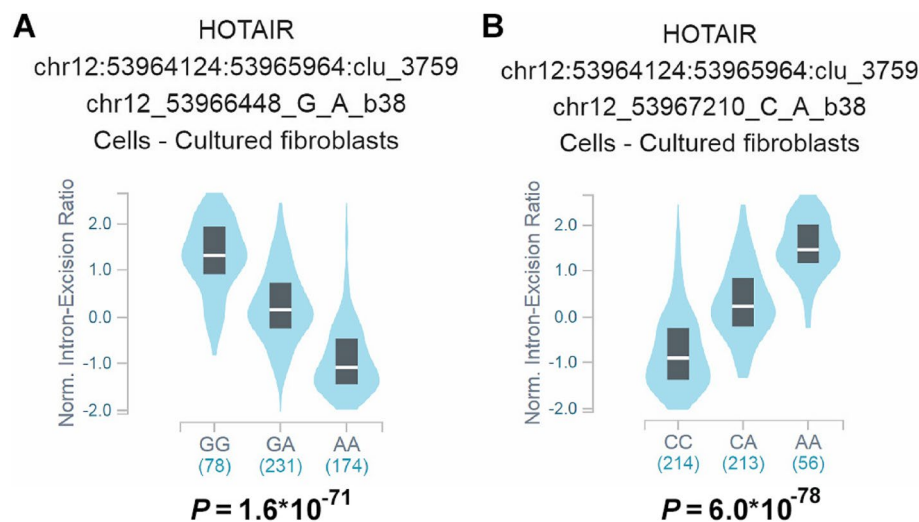


Fig. 1 Functional correlation between two SNPs (rs920778 and rs1899663) and HOTAIR gene splicing events from the GTEx database. **A** rs920778 can affect the splicing event of the HOTAIR gene in cell culture fibroblasts ($P = 1.6 \times 10^{-71}$). **B** rs1899663 is associated with splicing events of the HOTAIR gene in cell culture fibroblasts ($P = 6.0 \times 10^{-78}$)

HOTAIR gene in cell-cultured fibroblasts. Interestingly, SNP RS1899663 may also affect the splicing events of HOXC5 (Fig. 2A), HOXC10 (Fig. 2B), HOXC6 (Fig. 2C), and HOXC4 (Fig. 2D) genes in cultured fibroblasts.

4 Discussion

There are more and more studies and relevant evidence on the influence of the HOTAIR gene on the occurrence and development of various cancers, including glioma. However, HOTAIR polymorphism and glioma susceptibility have not been studied. The discovery of important genetic variants related to gliomas, such as HOTAIR SNP, is of vital clinical significance for the prevention, prognosis, and treatment of gliomas. We first identified three SNPs of the HOTAIR gene (rs920778, rs4759314, and rs1899663) to study glioma susceptibility in Asian children in China.

HOTAIR, as a long-chain non-coding RNA (lncRNA), has been increasingly demonstrated to play a carcinogenic role in the occurrence and development of cancer (Qu et al., 2019; Loewen et al., 2014; Yang et al., 2019; Liu et al., 2014). Liu et al. found that the successful secretion of exosomes requires the regulation of HOTAIR (Yang et al., 2019). For example, HOTAIR can regulate RAB35 and SNAP23 to achieve the docking and fusion of multivesicular bodies containing exosomes, which plays an important role in the progression of hepatocellular carcinoma. HOTAIR acts as a cancer-promoting agent, regulating genes involved in the proliferation and invasion of lung cancer (Loewen et al., 2014). HOTAIR inhibited HOXA5 and p21^{WAF1/CIP1} genes, thus promoting the

growth of lung cancer (Loewen et al., 2014; Liu et al., 2013). Wang et al. found that overexpression of HOTAIR was associated with adverse progression of gastric cancer (Liu et al., 2014). Importantly, they also found that HOTAIR could act as a receptor of Mir-331-3p to regulate HER2, which has important implications for understanding the carcinogenic mechanism of HOTAIR. In preclinical studies, Kang et al. found that HOTAIR is an activator of the NF- κ B inflammatory signaling pathway and is involved in immune escape in gliomas (Wang et al., 2021).

The genetic mechanism of HOTAIR overexpression in tumors remains unclear. However, more and more pieces of evidence indicate the association between polymorphism of the HOTAIR gene and tumor susceptibility. Sharma et al. found that HOTAIR gene polymorphism was associated with low expression of HOTAIR and mir-22 overexpression in cervical cancer (Sharma Saha et al., 2016). In a case-control study of 393 neuroblastoma cases and 812 healthy controls, our team identified the HOTAIR gene SNPs (rs12826786 C>T, rs874945 C>T and rs1899663 C>A) increased risk of neuroblastoma (Yang et al., 2018). Previous studies have shown that glioma in the European population is associated with a risk of 25 heritable loci (Melin et al., 2017). A recent study showed that the susceptibility of glioma in China was related to the SNP of the CYP4F12 gene (rs688755) (Li et al., 2021). To our knowledge, there was only one previous study on the association between HOTAIR gene polymorphism and susceptibility to glioma. Ana Xavier Magalhães et al. genotyped two SNPs (rs920778 C>T

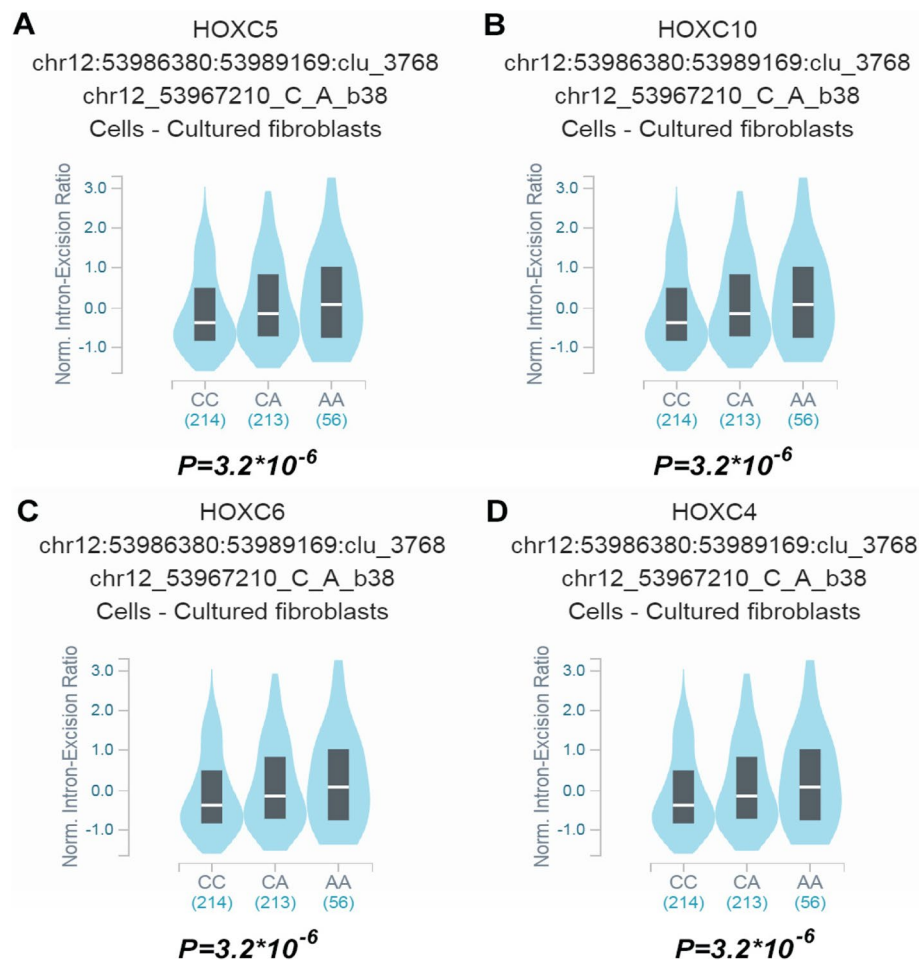


Fig. 2 Functional correlation between rs1899663 and splicing events of some important genes from GTEx database. HOTAIR gene rs1899663 was associated with splicing events of **A** HOXC5 ($P=3.2*10^{-6}$), **B** HOXC10 ($P=3.2*10^{-6}$), **C** HOXC6 ($P=3.2*10^{-6}$), and **D** HOXC4 ($P=3.2*10^{-6}$) genes in cultured fibroblasts

and rs12826786 C>T) in 177 Portuguese glioma patients and 199 cancer-free controls by PCR and restriction fragment length polymorphism (RFLP) (Xavier-Magalhaes et al., 2017). However, the results showed that these SNPs were not associated with glioma risk. HOTAIR rs920778 and rs12826786 polymorphisms showed a significant correlation with the good prognosis of WHO III anaplastic oligodendroglioma patients (Xavier-Magalhaes et al., 2017). The relationship between these HOTAIR SNPs (rs920778 A>G, rs4759314 A>G, rs1899663 C>A) and glioma susceptibility has not been studied. Therefore, we conducted a case-control study to explore the relationship between three HOTAIR SNPs (rs920778 A>G, rs4759314 A>G, rs1899663 C>A) and the susceptibility of glioma. First, we found statistically significant differences in the frequency distribution of some SNP genotypes in HOTAIR (rs920778 AG/GG, rs1899663 CA/AA) between the case group and the control

group. Subsequent risk analysis and stratified analysis showed that HOTAIR polymorphism (rs920778 A>G and rs1899663 C>A) could affect glioma susceptibility. Interestingly, participants with two and one to three risk genotypes had an increased risk of glioma compared to participants with none of the risk genotypes. These results further suggest that the SNPs of the HOTAIR gene increased susceptibility to glioma. Participants with two and one to three risk genotypes were significantly associated with an increased risk of glioma compared to participants with none of the risk genotypes. Interestingly, although having one genotype was not statistically associated with an increased risk of glioma, the trend showed that having more risk genotypes seemed to increase the risk of glioma. In theory, the combined effect of HOTAIR SNP may be stronger, but this experimental species did not show a statistically significant correlation. It may be due to the problem of sample size, so more experiments

are needed to explore. Here, we use the GTEx database to evaluate the influence of HOTAIR SNP rs920778 and rs1899663 on variable splicing events. We found that rs920778 and rs1899663 were significantly correlated with gene HOTAIR, HOXC5, HOXC10, HOXC6, and HOXC4 splicing. The expression of HOTAIR, HOXC5, HOXC10, HOXC6, and HOXC4 plays an important role in tumor progression (Loewen et al., 2014; Bijl et al., 1998; Yan et al., 2018; Fang et al., 2021). HOTAIR SNPs rs920778 and rs1899663 may affect glioma by affecting the splicing events of HOTAIR, HOXC5, HOXC10, HOXC6, and HOXC4. Our study provides new clues to the genetic mechanism of HOTAIR gene polymorphism in glioma. Importantly, our results identify the HOTAIR SNPs (rs920778 A > G and rs1899663 C > A) as risk markers for glioma. These findings not only shed light on the relationship between some HOTAIR SNPs and glioma susceptibility but also contribute to the assessment of risk stratification in patients with glioma tumors. In conclusion, the specific mechanism by which HOTAIR SNP affects the risk of glioma by regulating gene expression and splicing pattern remains to be clarified.

Although to our knowledge there are no studies linking these HOTAIR SNPs to glioma risk, some limitations should be acknowledged. Firstly, only 3 SNPs were genotyped in this study, which is far from enough to explain the genetic role of the HOTAIR gene in glioma. More potential functional SNPs in the HOTAIR gene should be used to explore their roles in glioma. Secondly, although the sample size is still relatively large, the sample size of this study is not enough to represent glioma patients. Future studies with larger sample sizes are needed. Finally, the results are limited to Chinese patients, and the conclusions should be extended with caution to other populations. Therefore, a larger multicenter study is needed to further confirm the role of HOTAIR in glioma susceptibility. In conclusion, the important role of the HOTAIR gene and these SNPs in gliomas requires further investigation.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44194-022-00019-6>.

Additional file 1: Table S1. Frequency distribution of selected variables in glioma patients and cancer-free controls.

Authors' contributions

Lei Lin, Yongping Chen, and Yuxiang Liao conducted the study; Li Yuan, Xiaokai Huang, Qin Tian, and Meng Li participated in analyzing data; Lei Lin drafted the manuscript; Hui-Ran Lin, Ji-Chen Ruan, Lei Miao coordinated the study over the entire time. All authors reviewed the final manuscript. The authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors made no disclosures.

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