

HMGA2 Gene rs8756 A>C Polymorphism Reduces Neuroblastoma Risk in Chinese Children: A Four-Center Case-Control Study

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Background: Neuroblastoma, mainly affecting children, is a lethal malignancy arising from the developing sympathetic nervous system. The genetic etiology of neuroblastoma remains mostly obscure. High mobility group AT-hook 2 (HMGA2), an oncogenic gene, is up-regulated in many tumors. Single nucleotide polymorphisms (SNPs) often modify cancer susceptibility. However, no studies are investigating the association between *HMGA2* SNPs and neuroblastoma susceptibility.

Methods: We conducted a four-center case-control study to evaluate the association between three *HMGA2* polymorphisms (rs6581658 A>G, rs8756 A>C and rs968697 T>C) and neuroblastoma susceptibility in a Chinese population with 505 cases and 1070 controls. Logistic regression was performed to evaluate the strength of the association.

Results: We found that the rs8756 AC/CC genotypes were associated with a reduced neuroblastoma risk when compared to rs8756 AA genotype [Adjusted odds ratio (OR) =0.74, 95% confidence interval (CI)=0.56–0.99, *P*=0.039]. Carriers with 3 protective genotypes have lower neuroblastoma susceptibility than those without or with 0–2 protective genotypes. The stratified analysis revealed that the protective effects of rs8756 AC/CC genotypes were more predominant among children of age > 18 months, males, and subgroups with the tumor in the mediastinum. Furthermore, haplotype analysis uncovered that haplotype ACC significantly reduced neuroblastoma risk.

Conclusion: Our study indicated *HMGA2* rs8756 A>C polymorphism is significantly associated with decreased neuroblastoma risk.

Keywords: neuroblastoma, susceptibility, *HMGA2*, polymorphism

Introduction

Neuroblastoma is one of the most common pediatric extracranial solid tumors, which is derived from primordial sympathetic neural precursors.¹ The incidence of neuroblastoma is approximately 1/7000 in the USA² and 1/13,000 in China.^{3,4} It is the third leading cause of tumor-related death in children, account for 15% of all cases.^{5,6} Neuroblastoma is a highly heterogeneous disorder characterized by diverse clinical symptoms. For instance, most of the low-risk patients have spontaneous regression without chemotherapy.⁷ However, high-risk patients, constituting near 50% of neuroblastoma, have widely disseminated disease at diagnosis and have survival rates of less than 40% despite intensive therapies.⁸ Moreover, the lifelong serious co-existing health issues often affect survivors' social life, including marriage and employment.⁹ Therefore, neuroblastoma remains a great burden for affected families and public health.¹⁰

The pathogenesis of neuroblastoma is not fully understood. Approximately 1–2% of neuroblastoma cases are familial,¹¹ which was reported to associate with the mutation of *PHOX2B*¹² and *ALK*¹³ genes. Sporadic neuroblastoma is the primary form of neuroblastoma. Environmental factors such as radiation sources, wood dust, and hydrocarbons^{14,15} have been thought to predispose individuals to neuroblastoma. However, not all offsprings of exposed parents develop neuroblastoma.¹⁶ It suggests that genetic factors may play a role in the occurrence of neuroblastoma. Increasing evidence indicates that the genetic polymorphisms may somehow contribute to the neuroblastoma susceptibility.^{17–19}

Genome-wide association study (GWAS) has shed more light on the genetic etiology of human diseases including cancers.²⁰ It now is a powerful tool to study the genetic mechanisms of neuroblastoma. To date, six neuroblastoma GWASs have been performed and several inherited common variants in susceptibility genes were identified. *CASC15* was the first variant discovered to predispose to neuroblastoma by Maris et al in 2008.²¹ Later on, the same group found that several common variants in *BARD1* gene²² were related to high-risk neuroblastoma; moreover, the polymorphisms within *DUSP12*, *DDX4*, *IL31RA*, and *HSD17B12* contributed to the low-risk neuroblastoma.²³ In 2011, Wang et al demonstrated that single nucleotide polymorphisms (SNPs) in the *LMO1* gene could modify the neuroblastoma susceptibility.²⁴ Diskin et al indicated that the polymorphisms in *LIN28B* and *HACE1* genes also altered susceptibility to neuroblastoma.²⁵ More recent GWAS performed by McDaniel et al revealed that common variants within the *CPZ* gene at 4p16 and upstream of the *MLF1* gene at 3q25 could modify neuroblastoma susceptibility.²⁶ More importantly, the GWAS results are very useful in discovering novel biological processes underlying the malignant transformation of neuroblastoma. For example, Cimmino et al performed a fine-mapping analysis of *BARD1* locus (2q35) using GWAS data from 556 high-risk neuroblastoma patients and 2575 controls of European-American ancestry recently. They identified a potentially causative SNP rs17489363 C>T in the canonical promoter region that associated with high-risk neuroblastoma. They demonstrated that the risk allele T of rs17489363 altered binding sites of the transcription factor *HSF1* and lead to low expression of full-length *BARD1* mRNA and protein, and the decreased expression of full-length *BARD1* might contribute to neuroblastoma progression through promoting cell proliferation and invasion, the full-length *BARD1* may function as a tumor suppressor.²⁷

Furthermore, candidate gene approaches also discovered *NEFL*¹⁸ and *CDKN1B*²⁸ gene polymorphisms could influence neuroblastoma susceptibility.

Epithelial-to-mesenchymal transition (EMT) is a critical step in the progression of cancer.²⁹ EMT confers cancer cells specific mesenchymal characteristics, such as increased cell motility, resistance to apoptosis, and resistance to therapy.³⁰ The high mobility group AT-hook 2 (*HMGA2*), located in chromosome 12q13-15, has been involved in the EMT.^{31,32} The *HMGA2* is a member of the high motility group (HMG) protein family and abundantly expressed in the undifferentiated mesenchymal tissues.³³ One AT-hook basic domain in *HMGA2* binds to DNA minor groove at sequences abundant with A and T nucleotides, which helps to install transcriptional or enhancer complexes on chromatin.³⁴ Furthermore, *HMGA2* functions as a transcription co-regulator by recruiting other transcription-associated proteins.³⁵ Apart from EMT, *HMGA2* also regulates cell proliferation and differentiation, overexpression of which is observed in numerous human tumor tissues. Sarhadi et al reported that intense *HMGA2* expression contributed to the metastasis and poor prognosis in lung cancer.³⁶ Elevated *HMGA2* expression promoted metastasis and drug resistance in gastrointestinal tumors.^{37,38} Up-regulation of *HMGA2* often results from genetic alterations such as gene amplification and translocation. Besides, previous researches showed that some SNPs in genes are able to influence the gene expression and protein structure. There are some studies to evaluate the association between SNPs in the *HMGA2* gene and complex human diseases, such as childhood and adult height,³⁹ bone mineral density,⁴⁰ and nephropathy.⁴¹ However, there are no publications regarding the association between *HMGA2* gene polymorphisms and cancer susceptibility, including neuroblastoma. Therefore, we performed this four-center case-control study to evaluate the association between SNPs in the *HMGA2* gene and neuroblastoma susceptibility in Chinese children.

Materials and Methods

Study Subjects

In total, the current study included 505 clinically and histopathologically diagnosed neuroblastoma cases and 1070 cancer-free controls.⁴² As described previously, participants were recruited from four centers of China: Guangzhou Women and Children's Medical Center, The First Affiliated Hospital of Zhengzhou University, The Second Affiliated Hospital, and Yuying Children's Hospital of Wenzhou Medical University, and the Second Affiliated

Hospital of Xi'an Jiaotong University. The eligibility criteria for the included subjects were described previously.⁴³ Written informed consent was acquired before the study from all participants or their parents. And the study protocols were ratified by the Institutional Review Board of each participating institution. This study was conducted in accordance with the Declaration of Helsinki.

Polymorphism Selection and Genotyping

We searched for potentially functional *HMGA2* polymorphisms in the dbSNP database (<https://www.ncbi.nlm.nih.gov/snp/>) and SNPinfo (<https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html>) using the selection criteria described in the previous publication.⁴⁴ Three polymorphisms in the *HMGA2* gene were ultimately selected. The rs8756 A>C, located in 3' untranslated region (UTR) of the *HMGA2* gene, may affect the microRNA binding affinity, and thereby influence the expression and stabilization of the *HMGA2* gene. The rs6581658 A>G and rs968697 T>C, located in the 5' near gene region, may affect the binding of transcription factors and the transcription of the *HMGA2* gene. As showed in [Supplemental Figure 1](#), there was no significant linkage disequilibrium ($R^2 < 0.8$) among these three included SNPs ($R^2 = 0.001$ between rs6581658 and rs968697; $R^2 = 0.008$ between rs6581658 and rs8756; $R^2 = 0.001$ between rs968697 and rs8756).

For genotyping, the genomic DNA was purified from venous blood of participants by a TIANamp Blood DNA Kit (TianGen Biotech Co. Ltd., Beijing, China) and genotyped following the standard TaqMan real-time PCR methods.⁴⁴⁻⁴⁶ To assure the authenticity of the result, 10% of the samples were selected randomly to perform a second-time analysis. All repeated samples obtained a 100% concordance.

Statistical Analysis

Whether the selected polymorphisms were in Hardy-Weinberg equilibrium (HWE) in all control was assessed by the goodness-of-fit χ^2 test. And the distributions of demographics and allele frequencies between all cases and controls were compared through a two-sided chi-square test. A logistic regression analysis was conducted. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the association between the *HMGA2* polymorphisms and neuroblastoma risk. Moreover, stratified analysis was also carried out regarding age, gender, tumor origin site, and clinical stage. All statistical analyses were conducted using SAS software (version 9.4 SAS Institute, NC, USA). And a result was thought to be statistically significant when the *P* value < 0.05.

Results

Associations Between *HMGA2* Polymorphisms and Neuroblastoma Risk

In the current case-control study, 505 cases and 1070 controls were successfully genotyped ([Supplemental Table 1](#)). The genotype frequencies distribution of three selected SNPs were in accordance with HWE among the controls ($P = 0.365$ for rs6581658 A>G, $P = 0.811$ for rs8756 A>C and $P = 0.780$ for rs968697 T>C). The genotype frequencies of the SNPs in neuroblastoma cases and cancer-free controls were shown in [Table 1](#). In single locus analysis, the rs8756 A>C was associated with decreased neuroblastoma susceptibility; carriers with rs8756 AC/CC genotypes had significantly reduced neuroblastoma risk when compared with subjects with AA genotype [Adjusted OR (AOR)=0.74, 95% CI=0.56–0.99, $P = 0.039$]. We further evaluated the combined effect of protective genotypes of *HMGA2* on neuroblastoma risk. The results showed that individuals carrying 3 protective genotypes were at significantly lower risk of developing neuroblastoma than those without protective genotypes (AOR=0.33, 95% CI=0.13–0.84, $P = 0.020$) and those with 0–2 protective genotypes (AOR=0.35, 95% CI=0.18–0.70, $P = 0.003$).

Stratification Analysis

We investigated the effects of rs8756 A>C polymorphism and combined protective genotypes on the neuroblastoma risk among different subgroups defined by age, gender, site of tumor origin, and clinical stage. As shown in [Table 2](#), the rs8756 AC/CC genotypes were significantly associated with decreased neuroblastoma risk in children older than 18 months (AOR=0.65, 95% CI=0.45–0.93, $P = 0.020$), male (AOR=0.63, 95% CI=0.43–0.91, $P = 0.014$) and those with tumor of mediastinum origin (AOR=0.58, 95% CI=0.34–0.99, $P = 0.044$). When the protective genotypes were combined, we observed that subjects harboring 3 protective genotypes had a significant lower neuroblastoma risk than those with 0–2 protective genotypes among the following subgroup: age >18 months (AOR=0.33, 95% CI=0.14–0.78, $P = 0.012$), male (AOR=0.18, 95% CI=0.06–0.60, $P = 0.005$), tumor of adrenal gland-origin (AOR=0.31, 95% CI=0.09–0.99, $P = 0.048$) and early-stage tumor (AOR=0.28, 95% CI=0.10–0.79, $P = 0.016$).

HMGA2 Haplotypes and Neuroblastoma Risk

As shown in [Table 3](#), eight haplotypes were observed in the studied subjects. In comparison with the reference haplotype GAT, a significant association was observed

Table 1 Association Between *HMGA2* Gene Polymorphisms and Neuroblastoma Risk

| Genotype | Cases (N=505) | Controls (N=1070) | P ^a | Crude OR (95% CI) | P | Adjusted OR (95% CI) ^b | P ^b |
|--|------------------|----------------------|----------------|-------------------------|--------------|--------------------------------------|----------------|
| rs6581658 A>G (HWE=0.365) | | | | | | | |
| AA | 319 (63.17) | 666 (62.24) | | 1.00 | | 1.00 | |
| AG | 158 (31.29) | 350 (32.71) | | 0.98 (0.80–1.21) | 0.860 | 0.98 (0.79–1.21) | 0.839 |
| GG | 28 (5.54) | 54 (5.05) | | 1.13 (0.71–1.80) | 0.615 | 1.12 (0.71–1.79) | 0.622 |
| Additive | | | 0.893 | 0.99 (0.83–1.18) | 0.894 | 0.99 (0.83–1.18) | 0.899 |
| Dominant | 186 (36.83) | 404 (37.76) | 0.723 | 0.96 (0.77–1.20) | 0.724 | 0.96 (0.77–1.20) | 0.729 |
| Recessive | 477 (94.46) | 1016 (94.95) | 0.678 | 1.10 (0.69–1.77) | 0.678 | 1.11 (0.69–1.77) | 0.676 |
| rs8756 A>C (HWE=0.811) | | | | | | | |
| AA | 425 (84.16) | 854 (79.81) | | 1.00 | | 1.00 | |
| AC | 76 (15.05) | 203 (18.97) | | 0.79 (0.60–1.05) | 0.100 | 0.79 (0.60–1.04) | 0.093 |
| CC | 4 (0.79) | 13 (1.21) | | 0.65 (0.21–2.00) | 0.454 | 0.64 (0.21–1.98) | 0.439 |
| Additive | | | 0.038 | 0.76 (0.58–0.99) | 0.038 | 0.76 (0.58–0.99) | 0.038 |
| Dominant | 80 (15.84) | 216 (20.19) | 0.039 | 0.74 (0.56–0.99) | 0.040 | 0.74 (0.56–0.99) | 0.039 |
| Recessive | 501 (99.21) | 1057 (98.79) | 0.448 | 0.65 (0.21–2.00) | 0.452 | 0.65 (0.21–1.99) | 0.447 |
| rs968697 T>C (HWE=0.780) | | | | | | | |
| TT | 390 (77.23) | 799 (74.67) | | 1.00 | | 1.00 | |
| TC | 107 (21.19) | 250 (23.36) | | 0.92 (0.72–1.17) | 0.488 | 0.92 (0.72–1.17) | 0.474 |
| CC | 8 (1.58) | 21 (1.96) | | 0.82 (0.36–1.85) | 0.628 | 0.84 (0.37–1.90) | 0.666 |
| Additive | | | 0.258 | 0.88 (0.70–1.10) | 0.259 | 0.88 (0.70–1.10) | 0.266 |
| Dominant | 115 (22.77) | 271 (25.33) | 0.271 | 0.87 (0.68–1.12) | 0.272 | 0.87 (0.68–1.12) | 0.276 |
| Recessive | 497 (98.42) | 1049 (98.04) | 0.602 | 0.80 (0.35–1.83) | 0.603 | 0.81 (0.36–1.85) | 0.622 |
| Combined effect of protective genotypes ^c | | | | | | | |
| 0 | 14 (2.77) | 27 (2.52) | | 1.00 | | 1.00 | |
| 1 | 320 (63.37) | 641 (59.91) | | 0.96 (0.50–1.86) | 0.910 | 0.96 (0.50–1.86) | 0.901 |
| 2 | 161 (31.88) | 344 (32.15) | | 0.90 (0.46–1.77) | 0.765 | 0.90 (0.46–1.77) | 0.763 |
| 3 | 10 (1.98) | 58 (5.42) | | 0.33 (0.13–0.84) | 0.021 | 0.33 (0.13–0.84) | 0.020 |
| 0–2 | 495 (98.02) | 1012 (94.58) | | 1.00 | | 1.00 | |
| 3 | 10 (1.98) | 58 (5.42) | 0.002 | 0.35 (0.18–0.70) | 0.003 | 0.35 (0.18–0.70) | 0.003 |

Notes: The results were in bold, if the 95% CI excluded 1 or $P < 0.05$. ^a χ^2 test for genotype distributions between neuroblastoma patients and cancer-free controls.

^bAdjusted for age and gender. ^cRisk genotypes were rs6581658 AA/AG, rs8756 AC/CC and rs968697 TC/CC

Abbreviations: OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

for the haplotype ACC (AOR=0.36, 95% CI=0.18–0.72, $P=0.004$).

Discussion

We conducted this four-center case-control study to investigate the association between *HMGA2* gene polymorphisms and neuroblastoma susceptibility. Here, we found that rs8756 AC/CC genotypes could reduce the risk of neuroblastoma, especially among subgroups with age > 18 months, male, and subjects with the mediastinum-origin tumor. To the best of our knowledge, the current study is the first investigation to explore the association between *HMGA2* polymorphisms and neuroblastoma risk in the Chinese population.

HMGA2, as one of the major nonhistone chromosomal proteins, has been implicated in many fundamental cellular processes, including gene regulation, cell cycle, differentiation, and viral integration.⁴⁷ This chromatin-associated protein binds to AT-rich DNA sequences and potentiates the effects of transcription factors by altering local chromatin structure. Monzen et al demonstrate that *HMGA2* cooperated with the Smad transcription factor to induce the expression of *Nkx2.5*, which encodes an important early transcription factor for cardiac development. This is accomplished through *HMGA2*'s binding to the conserved AT-rich region in the *Nkx2.5* promoter. The knockdown of *HMGA2* blocks cardiomyocyte differentiation in an embryonal carcinoma cell line and completely abrogates

Table 2 Stratification Analysis for Association Between HMGA2 Gene Genotypes and Neuroblastoma Susceptibility

| Variables | rs8756 (Case/Control) | | OR (95% CI) | P | AOR (95% CI) ^a | P ^a | Protective Genotypes (Case/Control) | | OR (95% CI) | P | AOR (95% CI) ^a | P ^a |
|-----------------|-----------------------|---------|-------------------------|--------------|---------------------------|----------------|-------------------------------------|------|-------------------------|--------------|---------------------------|----------------|
| | AA | AC/CC | | | | | 0-2 | 3 | | | | |
| Age, month | ≤18 | 34/81 | 0.93 (0.60-1.45) | 0.754 | 0.94 (0.60-1.46) | 0.772 | 185/403 | 4/22 | 0.40 (0.14-1.17) | 0.093 | 0.40 (0.14-1.18) | 0.097 |
| | >18 | 270/510 | 0.64 (0.45-0.93) | 0.018 | 0.65 (0.45-0.93) | 0.020 | 310/609 | 6/36 | 0.33 (0.14-0.79) | 0.012 | 0.33 (0.14-0.78) | 0.012 |
| Gender | Female | 176/366 | 0.94 (0.61-1.44) | 0.771 | 0.94 (0.61-1.44) | 0.767 | 206/423 | 7/25 | 0.58 (0.25-1.35) | 0.205 | 0.57 (0.24-1.35) | 0.202 |
| | Male | 249/488 | 0.63 (0.43-0.92) | 0.016 | 0.63 (0.43-0.91) | 0.014 | 289/589 | 3/33 | 0.19 (0.06-0.61) | 0.006 | 0.18 (0.06-0.60) | 0.005 |
| Sites of origin | Adrenal gland | 145/854 | 0.76 (0.50-1.18) | 0.220 | 0.76 (0.49-1.17) | 0.206 | 170/1012 | 3/58 | 0.31 (0.10-0.99) | 0.049 | 0.31 (0.09-0.99) | 0.048 |
| | Retropitoneal | 124/854 | 0.73 (0.46-1.17) | 0.196 | 0.73 (0.46-1.17) | 0.191 | 145/1012 | 2/58 | 0.24 (0.06-0.996) | 0.049 | 0.24 (0.06-1.01) | 0.052 |
| | Mediastinum | 118/854 | 0.57 (0.34-0.97) | 0.037 | 0.58 (0.34-0.99) | 0.044 | 133/1012 | 2/58 | 0.26 (0.06-1.09) | 0.065 | 0.26 (0.06-1.08) | 0.063 |
| | Others | 32/854 | 1.24 (0.60-2.55) | 0.568 | 1.25 (0.60-2.58) | 0.554 | 41/1012 | 1/58 | 0.43 (0.06-3.15) | 0.403 | 0.43 (0.06-3.18) | 0.408 |
| Clinical stage | I+II+4s | 208/854 | 0.80 (0.56-1.15) | 0.225 | 0.81 (0.56-1.16) | 0.248 | 246/1012 | 4/58 | 0.28 (0.10-0.79) | 0.016 | 0.28 (0.10-0.79) | 0.016 |
| | III+IV | 195/854 | 0.75 (0.51-1.10) | 0.140 | 0.74 (0.51-1.09) | 0.128 | 226/1012 | 6/58 | 0.46 (0.20-1.09) | 0.077 | 0.46 (0.20-1.09) | 0.077 |

Notes: The results were in bold, if the 95% CI excluded 1 or P<0.05. ^aAdjusted for age and gender; omitting the corresponding stratify factor.
Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.

Table 3 The Frequency of Inferred Haplotypes of *HMGA2* Gene Based on Observed Genotypes and Their Association with the Neuroblastoma Susceptibility

| Haplotypes ^a | Cases (n=1010) | Controls (n=2140) | Crude OR (95% CI) | P | Adjusted OR ^b (95% CI) | P ^b |
|-------------------------|----------------|-------------------|-------------------------|--------------|-----------------------------------|----------------|
| GAT | 199 (19.70) | 416 (19.44) | 1.00 | | 1.00 | |
| GAC | 6 (0.59) | 12 (0.56) | 1.05 (0.39–2.84) | 0.923 | 1.06 (0.39–2.86) | 0.911 |
| GCT | 8 (0.79) | 28 (1.31) | 0.60 (0.27–1.34) | 0.213 | 0.60 (0.27–1.34) | 0.210 |
| GCC | 1 (0.10) | 2 (0.09) | 1.05 (0.10–11.65) | 0.968 | 1.07 (0.10–11.92) | 0.954 |
| AAT | 615 (60.89) | 1263 (59.02) | 1.02 (0.84–1.24) | 0.820 | 1.02 (0.84–1.24) | 0.824 |
| AAC | 106 (10.50) | 220 (10.28) | 1.01 (0.76–1.35) | 0.935 | 1.01 (0.76–1.35) | 0.927 |
| ACT | 65 (6.44) | 141 (6.59) | 0.97 (0.69–1.36) | 0.852 | 0.97 (0.69–1.36) | 0.849 |
| ACC | 10 (0.99) | 58 (2.71) | 0.36 (0.18–0.72) | 0.004 | 0.36 (0.18–0.72) | 0.004 |

Notes: The results were in bold, if the 95% CI excluded 1 or P<0.05. ^aThe haplotypes order were rs6581658, rs8756 and rs968697. ^bObtained in logistic regression models with adjustment for age and gender.

Abbreviations: OR, odds ratio; CI, confidence interval.

in vivo cardiogenesis in embryos of the frog *Xenopus laevis*.⁴⁸ Dong et al proved that the interaction between *HMGA2* and pRb facilitated the transcriptional activation of *FOXL2* by E2F1, which exert critical effects on the metastases and EMT of chemo-resistant gastric cancer.⁴⁹ Further studies confirmed that *HMGA2* could also modify the expression of *Bcl-2*, EMT-associated proteins, and caspase activity, indicating that *HMGA2* plays a direct role in regulating cell apoptosis and EMT.⁵⁰

Here, our research data showed that rs8756 A>C, one SNP located at 3' untranslated region (UTR) of the *HMGA2* gene, was related to the reduced susceptibility of neuroblastoma. It should be noted that *HMGA2* is a functional target of several microRNAs, which target the 3'UTR of genes for degradation. Yu et al found that miRNA *let-7* could reduce breast carcinoma cells proliferation and self-renewal partly by posttranscriptional regulation of *HMGA2*.⁵¹ And one research performed by Kang et al indicated *miR-490-3p* could act on the 3' UTR of *HMGA2* and inhibit its expression, then inhibit the proliferation, invasion, migration, and EMT of esophageal squamous cell carcinoma cells.⁵² A recent study confirmed that *miR-495* could be directly associated with the 3' UTR of *HMGA2*. Upregulated expression of *miR-495* significantly downregulated the mRNA and protein expression levels of *HMGA2* in A549 cells, and then suppressed the proliferation of lung cancer cells.⁵³ These above studies all indicated that miRNA is an important regulatory mechanism for the expression of *HMGA2*. It is reasonable to speculate that the rs8756 A>C in the 3' UTR of the *HMGA2* gene may affect some miRNA's binding to *HMGA2*, thereby alternating gene expression level.

This was the first research to investigate the association between SNPs in the *HMGA2* gene and neuroblastoma

susceptibility. However, the relationship between *HMGA2* polymorphisms and other complex human diseases has been explored, such as nanism. Bouatia-Naji et al showed that rs1042725 in the 3' UTR of the *HMGA2* gene contributed to height variability in European populations.⁵⁴ Kuipers et al further demonstrated that *HMGA2* polymorphism rs1042725 may be involved in bone metabolism; A novel association between rs1042725 and trabecular bone mineral density in ethnically diverse older men was suggested.⁴⁰ Further study by Hendriks et al indicated that rs1042725 is not only associated with height variation in the general population but also plays an important role in one of the extremes of the height distribution.⁵⁵ Alkayyali et al found *HMGA2* rs1531343 polymorphism was associated with increased risk of developing nephropathy in patients with type 2 diabetes.⁴¹ Moreover, another 3' UTR polymorphism in *HMGA2*, rs8756 was shown to be associated with human stature in an Icelandic population.⁵⁶ Our results showed that rs8756 A>C polymorphism was associated with neuroblastoma susceptibility. The rs8756 C allele exerted protective effects against neuroblastoma. However, the other two SNPs rs6581658 A>G and rs968697 T>C were not associated with neuroblastoma risk. These results should be further validated by the well-designed studies with larger sample size.

Limitations of the current study should be notified. First, selection bias is inevitable as it is a hospital-based case-control study. Second, even we enrolled participants from four independent hospitals, the sample size is still relatively small, especially for the stratified analysis. The statistical power might be compromised. Third, only three SNPs in the *HMGA2* gene were investigated; more potentially functional polymorphisms in the *HMGA2* gene should be assessed in the future study. Fourth, impacts of

some environmental factors such as living environment, dietary intake, and childhood or parental exposure should be taken into account, as neuroblastoma is a heterogeneous disease with complex etiology. Such information was not available due to the nature of the retrospective investigation. Fifth, the conclusions obtained from this study may not be directly applied to other ethnicities, as only Chinese Han ethnicity was included in this study. In the last, functional experiments should be performed to further elucidate the role of *HMGA2* gene polymorphisms and the underlying mechanisms in neuroblastoma carcinogenesis.

Conclusions

In summary, we firstly provide evidence that polymorphism in the *HMGA2* gene could affect neuroblastoma risk. The *HMGA2* rs8756 AC/CC genotypes are associated with decreased neuroblastoma susceptibility. It suggests that *HMGA2* gene polymorphisms might be potential biomarkers for neuroblastoma susceptibility.

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Disclosure

The authors report no conflicts of interest in this work.

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