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# *METTL14* Gene Polymorphisms Confer Neuroblastoma Susceptibility: An Eight-Center Case-Control Study

Zhenjian Zhuo,[1,11] Hongting Lu,[2,11] Jinhong Zhu,[1,3,11] Rui-Xi Hua,[1] Yong Li,[4] Zhonghua Yang,[5] Jiao Zhang,[6] Jiwen Cheng,[7] Haixia Zhou,[8] Suhong Li,[9] Li Li,[10] Huimin Xia,[1] and Jing He[1]

[1]Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China; [2]Department of Pediatric Surgery, The Affiliated Hospital of Qingdao University, Qingdao 266000, Shandong, China; [3]Department of Clinical Laboratory, Biobank, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China; [4]Department of Pediatric Surgery, Hunan Children's Hospital, Changsha 410004, Hunan, China; [5]Department of Pediatric Surgery, Shengjing Hospital of China Medical University, Shenyang 110004, Liaoning, China; [6]Department of Pediatric Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China; [7]Department of Pediatric Surgery, The Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710004, Shaanxi, China; [8]Department of Hematology, The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang, China; [9]Department of Pathology, Children Hospital and Women Health Center of Shanxi, Taiyuan 030013, Shannxi, China; [10]Kunming Key Laboratory of Children Infection and Immunity, Yunnan Key Laboratory of Children's Major Disease Research, Yunnan Institute of Pediatrics Research, Yunnan Medical Center for Pediatric Diseases, Kunming Children's Hospital, Kunming 650228, Yunnan, China

**Neuroblastoma is the primary cause of cancer death in childhood. METTL14 is tightly linked to cancer. However, whether single-nucleotide polymorphisms (SNPs) in the *METTL14* gene could predispose to neuroblastoma susceptibility lacks evidence. With an epidemiology case-control study, associations between *METTL14* gene SNPs and overall risk for neuroblastoma were estimated in 898 cases and 1,734 controls. Following that, stratified analysis was performed. Among the five analyzed SNPs, rs298982 G>A and rs62328061 A>G exhibited a significant association with decreased susceptibility to neuroblastoma, whereas the associations with increased neuroblastoma susceptibility were observed for rs9884978 G>A and rs4834698 T>C. Moreover, subjects carrying two to five risk genotypes were more inclined to develop neuroblastoma than those with zero to one risk genotypes. The stratified analysis further demonstrated the protective effect of rs298982 G>A and rs62328061 A>G, as well as the predisposing effect of rs4834698 T>C and two to five risk genotypes, in certain subgroups. Haplotype analysis was performed. Moreover, false-positive report probability analysis validated the reliability of the significant results. The expression quantitative trait locus analysis revealed that rs298982 is correlated with the expression levels of its surrounding genes. Our results suggest that some SNPs in the *METTL14* gene are associated with predisposition to neuroblastoma.**

## INTRODUCTION

Neuroblastoma is predominantly a cancer of infants and young children, because most of the affected children were diagnosed at under five years of age.[1] The incidence of neuroblastoma is about 11–13 in 1 million among children less than 15 years old in developed countries.[2] In China, neuroblastoma represents approximately 10% of childhood tumors, with an incidence of approximately 7.7 cases per 1 million children.[3] Neuroblastoma is the primary cause of cancer death in childhood, accounting for approximately 15% of all pediatric cancer mortality.[4,5] Normally, neural crest cell precursors migrate from the dorsal neural tube and then start to differentiate upon reaching their appropriate locations in the sympathetic nervous system. However, neuroblastoma may occur sometimes because of the defects in migration, maturation, or differentiation of neural crest cells.[6,7] So far, the long-term survival of high-risk neuroblastoma is less than 40%. What is worse, this type of neuroblastoma represents about 50% of all of the newly diagnosed patients.[8,9]

Remarkable advancement has been achieved in the ever-greater understanding of the genetic etiology of neuroblastoma.[10–12] Mutations in the *PHOX2B*[13] and *ALK*[14,15] genes frequently predispose children to familial neuroblastoma. The introduction of single-nucleotide

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[11]These authors contributed equally to this work.

**Correspondence:** Jing He, Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, Guangdong, China.
**E-mail:** hejing198374@gmail.com
**Correspondence:** Huimin Xia, Department of Pediatric Surgery, Guangzhou Institute of Pediatrics, Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, 9 Jinsui Road, Guangzhou 510623, Guangdong, China.
**E-mail:** xia-huimin@foxmail.com

polymorphism (SNP) array technology in the 1990s[16] enabled genome-wide association studies (GWASs) in the late 2000s.[17] In 2008, the first GWAS regarding neuroblastoma was performed, with 1,752 European American neuroblastoma cases and 4,171 cancer-free controls.[18] This study identified that a chromosome 6p22 locus was strongly associated with neuroblastoma predisposition, specifically aggressive subtype. To date, plenty of neuroblastoma susceptibility SNPs have been identified by GWASs.[19–23] However, so far all of the genetic variations have not been sufficient to fully understand the genetic landscape of neuroblastoma. Therefore, a better understanding of the genetic alterations that shape neuroblastoma risk becomes necessary to clarify its etiology. Ongoing research identifying novel genetic variations may lead to new approaches to diagnosis, prevention, and treatment of neuroblastoma.

N6-methyladenosine ($m^6A$) refers to methylation at the sixth position of N on adenosine, which is the most prevalent RNA posttranscriptional modification in eukaryotic organisms, especially in messenger RNA (mRNA).[24] Key regulators of $m^6A$ modification mainly fall into three categories: RNA methyltransferases (METTL3, METTL14, and WTAP, known as "writers"), demethylases (FTO and ALKBH5, known as "erasers"), and $m^6A$-binding proteins (YTHDF1/2/3 and IGF2BP1, known as "readers").[25,26] Strong evidence suggests that aberrant expression of $m^6A$ key regulators could cause dysregulated RNA $m^6A$ modification, resulting in a variety of diseases, including cancers.[27–29] Some SNPs located in specific regions of $m^6A$ key regulator genes may alter $m^6A$ methylation and subsequently affect related biological processes.[30] A large number of $m^6A$ gene SNPs have been identified to associate with major depressive disorder,[31] osteoporosis,[32] and obesity.[33] However, evidence regarding $m^6A$ modification key regulator genes SNPs and the risk of cancer is very scarce.

To identify novel susceptibility $m^6A$ modification key regulator gene *METTL14* SNPs to neuroblastoma, we performed this multi-center epidemiology study. We addressed the associations between the *METTL14* gene SNPs and neuroblastoma risk in a Chinese population of children.

## RESULTS

### Effect of *METTL14* Gene SNPs on Neuroblastoma Risk

We successfully genotyped the five *METTL14* gene SNPs (rs1064034 T>A, rs298982 G>A, rs62328061 A>G, rs9884978 G>A, and *rs4834698* T>C) in 896 cases and 1,733 controls. The associations of these SNPs with neuroblastoma risk are shown in Table 1. The actual SNP genotype frequencies were in accordance with Hardy-Weinberg equilibrium (HWE) in controls (HWE $p > 0.05$), except for *rs9884978* G>A. In the single-locus analysis, carriers of the rs298982 (AA versus GG: adjusted odds ratio [OR] = 0.40, 95% confidence interval [CI] = 0.20–0.83, p = 0.014; AA versus GG/GA: adjusted OR = 0.40, 95% CI = 0.20–0.83, p = 0.014) or rs62328061 (AG versus AA: adjusted OR = 0.77, 95% CI = 0.64–0.93, p = 0.007; AG/GG versus AA: adjusted OR = 0.82, 95% CI = 0.68–0.97, p = 0.024) variant alleles showed decreased susceptibility to neuroblastoma. On the contrary, the rs9884978 (AA versus GG/GA: adjusted OR = 1.39, 95% CI = 1.00–1.94, p = 0.048) and rs4834698 (CC versus TT: adjusted OR = 1.28, 95% CI = 1.02–1.61, p = 0.034) variant alleles contribute to increased susceptibility to neuroblastoma. We then defined rs1064034 AA, rs298982 GG/GA, rs62328061 GG, rs9884978 AA, and rs4834698 TC/CC as risk genotypes. We observed that participants with two to five risk genotypes experienced a 1.47-fold increase in the risk of developing neuroblastoma in comparison with zero to one risk genotypes (adjusted OR = 1.47, 95% CI = 1.15–1.88, p = 0.002).

### Stratification Analysis of Identified SNPs

We further explored the association between *METTL14* gene polymorphisms and susceptibility to neuroblastoma in certain subgroups classified by age, gender, sites of origins, and clinical stages (Table 2). The protective effect of rs298982 remains prominent in children aged 18 months and over (adjusted OR = 0.29, 95% CI = 0.11–0.75, p = 0.011), male (adjusted OR = 0.38, 95% CI = 0.15–0.99, p = 0.048), and patients in clinical stages I+II+4S (adjusted OR = 0.17, 95% CI = 0.04–0.70, p = 0.014), while the association with rs62328061 was observed for subgroups over 18 months of age (adjusted OR = 0.78, 95% CI = 0.62–0.98, p = 0.033), with tumors originating from the mediastinum (adjusted OR = 0.70, 95% CI = 0.50–0.97, p = 0.032) and with clinical stages III+IV neuroblastoma (adjusted OR = 0.77, 95% CI = 0.60–0.98, p = 0.037). As for the rs4834698 polymorphism, a stronger risk effect of TC/CC genotypes was found among children older than 18 months (adjusted OR = 1.31, 95% CI = 1.03–1.67, p = 0.026) and patients in clinical stages III+IV (adjusted OR = 1.31, 95% CI = 1.01–1.70, p = 0.043). The combined analysis stated that the presence of two to five risk genotypes was associated with an enhanced neuroblastoma risk in all age ($\leq$18 months: adjusted OR = 1.49, 95% CI = 1.002–2.22, p = 0.049; >18 months: adjusted OR = 1.43, 95% CI = 1.04–1.95, p = 0.026) and gender groups (female: adjusted OR = 1.54, 95% CI = 1.06–2.25, p = 0.024; male: adjusted OR = 1.41, 95% CI = 1.02–1.95, p = 0.039), children with tumors originating from the adrenal gland (adjusted OR = 1.59, 95% CI = 1.04–2.44, p = 0.034), tumors originating from the mediastinum (adjusted OR = 1.66, 95% CI = 1.04–2.66, p = 0.034), and patients in clinical stages I+II+4S (adjusted OR = 1.53, 95% CI = 1.11–2.11, p = 0.009).

### *METTL14* Haplotype Analysis

We next determined whether the haplotypes of the five *METTL14* gene SNPs are linked to neuroblastoma risk. As shown in Table 3, the wild-type allele AAAGT was defined as the reference group. When compared with the reference haplotype AAAGT, the following haplotypes were significantly associated with enhanced neuroblastoma risk: AAAGC, AAAAC, AAGGT, AGAGT, AGAGC, AGAAT, AGAAC, AGGGT, TGAGT, TGAGC, TGAAT, TGAAC, and TGGAC.

### False-Positive Report Probability (FPRP) Analysis

FPRP analysis was carried out to interrogate whether the significant findings are deserving attention (Table 4). At the prior probability level of 0.1, the significant association for rs62328061 A>G (AG versus AA) remained noteworthy. It was also the case for the association with rs4834698 T>C (CC/CT versus TT) in children over 18 months of

**Table 1. Association of *METTL14* Gene Polymorphisms with Neuroblastoma Susceptibility**

| Genotype | Cases (N = 896) | Controls (N = 1,733) | p[a] | Crude OR (95% CI) | p | Adjusted OR (95% CI) [b] | p[b] |
|---|---|---|---|---|---|---|---|
| rs1064034 T>A (HWE = 0.619) | | | | | | | |
| TT | 446 (49.78) | 812 (46.86) | | 1.00 | | 1.00 | |
| TA | 359 (40.07) | 755 (43.57) | | 0.87 (0.73–1.03) | 0.098 | 0.86 (0.73–1.02) | 0.084 |
| AA | 91 (10.16) | 166 (9.58) | | 1.00 (0.75–1.32) | 0.989 | 1.00 (0.75–1.32) | 0.992 |
| Additive | | | 0.386 | 0.95 (0.84–1.07) | 0.386 | 0.95 (0.84–1.07) | 0.367 |
| Dominant | 450 (50.22) | 921 (53.14) | 0.155 | 0.89 (0.76–1.05) | 0.155 | 0.89 (0.75–1.04) | 0.138 |
| Recessive | 805 (89.84) | 1,567 (90.42) | 0.637 | 1.07 (0.82–1.40) | 0.634 | 1.07 (0.82–1.40) | 0.619 |
| rs298982 G>A (HWE = 0.092) | | | | | | | |
| GG | 672 (75.00) | 1,287 (74.26) | | 1.00 | | 1.00 | |
| GA | 215 (24.00) | 403 (23.25) | | 1.02 (0.85–1.24) | 0.824 | 1.02 (0.84–1.24) | 0.833 |
| AA | 9 (1.00) | 43 (2.48) | | **0.40 (0.19–0.83)** | **0.013** | **0.40 (0.20–0.83)** | **0.014** |
| Additive | | | 0.271 | 0.91 (0.77–1.08) | 0.271 | 0.91 (0.77–1.08) | 0.273 |
| Dominant | 224 (25.00) | 446 (25.74) | 0.682 | 0.96 (0.80–1.16) | 0.683 | 0.96 (0.80–1.16) | 0.678 |
| Recessive | 887 (99.00) | 1,690 (97.52) | 0.010 | **0.40 (0.19–0.82)** | **0.013** | **0.40 (0.20–0.83)** | **0.014** |
| rs62328061 A>G (HWE = 0.600) | | | | | | | |
| AA | 645 (71.99) | 1,176 (67.86) | | 1.00 | | 1.00 | |
| AG | 217 (24.22) | 507 (29.26) | | **0.78 (0.65–0.94)** | **0.009** | **0.77 (0.64–0.93)** | **0.007** |
| GG | 34 (3.79) | 50 (2.89) | | 1.24 (0.79–1.94) | 0.345 | 1.24 (0.79–1.94) | 0.346 |
| Additive | | | 0.145 | 0.89 (0.77–1.04) | 0.145 | 0.89 (0.76–1.04) | 0.128 |
| Dominant | 251 (28.01) | 557 (32.14) | 0.030 | **0.82 (0.69–0.98)** | **0.030** | **0.82 (0.68–0.97)** | **0.024** |
| Recessive | 862 (96.21) | 1,683 (97.11) | 0.209 | 1.33 (0.85–2.07) | 0.210 | 1.33 (0.85–2.07) | 0.207 |
| rs9884978 G>A (HWE = 0.028) | | | | | | | |
| GG | 576 (64.29) | 1,092 (63.01) | | 1.00 | | 1.00 | |
| GA | 255 (28.46) | 548 (31.62) | | 0.88 (0.74–1.06) | 0.171 | 0.88 (0.74–1.05) | 0.166 |
| AA | 65 (7.25) | 93 (5.37) | | 1.33 (0.95–1.85) | 0.097 | 1.34 (0.96–1.87) | 0.087 |
| Additive | | | 0.805 | 1.02 (0.89–1.16) | 0.804 | 1.02 (0.89–1.16) | 0.787 |
| Dominant | 320 (35.71) | 641 (36.99) | 0.520 | 0.95 (0.80–1.12) | 0.521 | 0.95 (0.80–1.12) | 0.521 |
| Recessive | 831 (92.75) | 1,640 (94.63) | 0.054 | 1.38 (0.99–1.91) | 0.054 | **1.39 (1.00–1.94)** | **0.048** |
| rs4834698 T>C (HWE = 0.587) | | | | | | | |
| TT | 222 (24.78) | 484 (27.93) | | 1.00 | | 1.00 | |
| TC | 442 (49.33) | 853 (49.22) | | 1.13 (0.93–1.37) | 0.223 | 1.13 (0.93–1.37) | 0.235 |
| CC | 232 (25.89) | 396 (22.85) | | **1.28 (1.02–1.60)** | **0.035** | **1.28 (1.02–1.61)** | **0.034** |
| Additive | | | 0.035 | **1.13 (1.01–1.27)** | **0.035** | **1.13 (1.01–1.27)** | **0.034** |
| Dominant | 674 (75.22) | 1,249 (72.07) | 0.084 | 1.18 (0.98–1.42) | 0.084 | 1.18 (0.98–1.41) | 0.088 |
| Recessive | 664 (74.11) | 1,337 (77.15) | 0.083 | 1.18 (0.98–1.42) | 0.083 | 1.18 (0.98–1.43) | 0.077 |
| Combined Effect of Risk Genotypes[c] | | | | | | | |
| 0–1 | 100 (11.16) | 269 (15.52) | | 1.00 | | 1.00 | |
| 2–5 | 796 (88.84) | 1,464 (84.48) | 0.002 | **1.46 (1.14–1.87)** | **0.002** | **1.47 (1.15–1.88)** | **0.002** |

Values were in bold if the 95% CIs excluded 1 or P values less than 0.05.
[a]$\chi^2$ test for genotype distributions between neuroblastoma patients and controls.
[b]Adjusted for age and gender.
[c]Risk genotypes were rs1064034 AA, rs298982 GG/GA, rs62328061 GG, rs9884978 AA, and rs4834698 TC/CC.

age. Regarding the combined analysis, findings for the presence of two to five genotypes in the overall analysis and patients in stages I+II+4S in the stratified analysis could be called noteworthy. Significant findings remained noteworthy for the haplotypes AAAGC, AGAGT, AGGGT, TGAGT, TGAGC, TGAAT, and TGAAC when compared with reference haplotype AAAGT.

**Table 2. Stratification Analysis for Association between *METTL14* Gene Genotypes and Neuroblastoma Susceptibility**

| | rs298982 (Case/Control) | | | | rs62328061 (Case/Control) | | | | rs4834698 (Case/Control) | | | | Risk Genotypes (Case/Control) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variables | GG/GA | AA | AOR (95% CI)[a] | p[a] | AA | AG/GG | AOR (95% CI)[a] | p[a] | TT | TC/CC | AOR (95% CI)[a] | p[a] | 0–1 | 2–5 | AOR (95% CI)[a] | p[a] |
| Age, Months | | | | | | | | | | | | | | | | |
| ≤18 | 340/702 | 4/11 | 0.77 (0.24–2.44) | 0.656 | 244/487 | 100/226 | 0.88 (0.67–1.17) | 0.380 | 93/190 | 251/523 | 0.98 (0.73–1.31) | 0.880 | 37/109 | 307/604 | **1.49 (1.002–2.22)** | **0.049** |
| >18 | 547/988 | 5/32 | **0.29 (0.11–0.75)** | **0.011** | 401/689 | 151/331 | **0.78 (0.62–0.98)** | **0.033** | 129/294 | 423/726 | **1.31 (1.03–1.67)** | **0.026** | 63/160 | 489/860 | **1.43 (1.04–1.95)** | **0.026** |
| Gender | | | | | | | | | | | | | | | | |
| Female | 402/727 | 4/17 | 0.44 (0.15–1.33) | 0.146 | 293/501 | 113/243 | 0.79 (0.60–1.03) | 0.077 | 101/197 | 305/547 | 1.07 (0.81–1.42) | 0.620 | 42/113 | 364/631 | **1.54 (1.06–2.25)** | **0.024** |
| Male | 485/963 | 5/26 | **0.38 (0.15–0.99)** | **0.048** | 352/675 | 138/314 | 0.84 (0.66–1.07) | 0.150 | 121/287 | 369/702 | 1.25 (0.98–1.60) | 0.074 | 58/156 | 432/833 | **1.41 (1.02–1.95)** | **0.039** |
| Sites of Origin | | | | | | | | | | | | | | | | |
| Adrenal gland | 246/1,690 | 2/43 | 0.32 (0.08–1.34) | 0.120 | 181/1,176 | 67/557 | 0.77 (0.57–1.04) | 0.085 | 55/484 | 193/1249 | 1.37 (1.00–1.88) | 0.054 | 26/269 | 222/1,464 | **1.59 (1.04–2.44)** | **0.034** |
| Retroperitoneal | 312/1,690 | 6/43 | 0.76 (0.32–1.81) | 0.540 | 222/1,176 | 96/557 | 0.90 (0.69–1.17) | 0.416 | 90/484 | 228/1,249 | 0.99 (0.76–1.29) | 0.942 | 40/269 | 278/1,464 | 1.29 (0.90–1.84) | 0.163 |
| Mediastinum | 212/1,690 | 1/43 | 0.19 (0.03–1.37) | 0.099 | 160/1,176 | 53/557 | **0.70 (0.50–0.97)** | **0.032** | 48/484 | 165/1,249 | 1.31 (0.94–1.84) | 0.115 | 21/269 | 192/1,464 | **1.66 (1.04–2.66)** | **0.034** |
| Others | 105/1,690 | 0/43 | / | / | 71/1,176 | 34/557 | 1.02 (0.67–1.55) | 0.943 | 27/484 | 78/1,249 | 1.11 (0.71–1.74) | 0.649 | 13/269 | 92/1,464 | 1.29 (0.71–2.34) | 0.400 |
| Clinical Stages | | | | | | | | | | | | | | | | |
| I+II+4S | 467/1,690 | 2/43 | **0.17 (0.04–0.70)** | **0.014** | 333/1,176 | 136/557 | 0.86 (0.69–1.08) | 0.194 | 124/484 | 346/1,249 | 1.09 (0.86–1.37) | 0.479 | 50/269 | 419/1,464 | **1.53 (1.11–2.11)** | **0.009** |
| III+IV | 387/1,690 | 7/43 | 0.74 (0.33–1.66) | 0.464 | 286/1,176 | 108/557 | **0.77 (0.60–0.98)** | **0.037** | 91/484 | 303/1,249 | **1.31 (1.01–1.70)** | **0.043** | 48/269 | 346/1,464 | 1.36 (0.97–1.89) | 0.073 |

AOR, adjusted odds ratio.
[a]Adjusted for age and gender, omitting the corresponding stratify factor.

### Effect of rs298982 G>A on the Expression of Surrounding Genes

To further assess whether the functional relevance of rs298982 G>A affects mRNA expression, we explored Cis-expression quantitative trait loci (eQTLs) target genes of the rs298982 G>A using released data from GTEx. It showed that the rs298982 G allele was significantly associated with increased *SNHG8* mRNA levels in the cultured fibroblasts (Figure 1A). However, the rs298982 G allele was significantly associated with lower expression levels of *RP11-384K6.6* in the whole blood (Figure 1B) and cultured fibroblasts (Figure 1C). The overall workflow is shown in Figure 2.

## DISCUSSION

More and more novel neuroblastoma susceptibility genetic variants have been identified. Yet it remains a challenge to unearth the full range of neuroblastoma susceptibility variations. In this study, we provided evidence that common variations in the *METTL14* gene were significantly associated with the risk of neuroblastoma. Our data also shed light on the biological mechanisms by which *METTL14* gene SNP rs298982 G>A enhances hepatoblastoma risk. This is the most extensive study to date studying the association of *METTL14* gene SNPs and neuroblastoma risk.

Recent research has uncovered the importance of METTL14 in cancer development. Chen et al.[34] detected a lower expression level of METTL14 in colorectal cancer tissues and cell lines. The low METTL14 was significantly associated with poor overall survival. Furthermore, functional experiments demonstrated that METTL14 suppressed colorectal cancer via the miR-375/SP1 and miR-375/YAP1 pathways. Ma et al.[35] reported that METTL14 was significantly downregulated in hepatocellular carcinoma. Reduced METTL14 expression had worse recurrence-free survival and overall survival. Functionally, METTL14 facilitates the maturation of primary miR-126 in an m$^6$A-dependent manner by binding to microprocessor protein DGCR8. In contrast, Weng et al.[29] found that METTL14 was highly expressed in acute myeloid leukemia cells and played an oncogenic role. Lang et al.[36] indicated that METTL14 was an important driver in Epstein-Barr virus (EBV)-induced oncogenesis. They showed that knockdown of METTL14 reduced tumorigenic activity of EBV-transformed cells in the xenograft animal model systems.

**Table 3. The Frequency of Inferred Haplotypes of *METTL14* Gene Based on Observed Genotypes and Their Association with the Risk of Neuroblastoma**

| Haplotypes[a] | Cases (N = 1,792) | Controls (N = 3,466) | Crude OR (95% CI) | p | Adjusted OR[b] (95% CI) | p[b] |
|---|---|---|---|---|---|---|
| AAAGT | 23 (1.28) | 85 (2.45) | 1.00 | | 1.00 | |
| AAAGC | 117 (6.53) | 222 (6.41) | **1.95 (1.17–3.25)** | **0.011** | **1.95 (1.17–3.25)** | **0.011** |
| AAAAT | 35 (1.95) | 100 (2.89) | 1.29 (0.71–2.35) | 0.401 | 1.29 (0.71–2.36) | 0.403 |
| AAAAC | 4 (0.22) | 2 (0.06) | **7.39 (1.27–42.91)** | **0.026** | **7.30 (1.25–42.51)** | **0.027** |
| AAGGT | 42 (2.34) | 76 (2.19) | **2.04 (1.13–3.70)** | **0.019** | **2.01 (1.11–3.64)** | **0.022** |
| AAGGC | 3 (0.17) | 3 (0.09) | 3.70 (0.70–19.54) | 0.124 | 3.70 (0.70–19.57) | 0.124 |
| AAGAT | 0 (0.00) | 1 (0.03) | / | / | / | / |
| AGAGT | 50 (2.79) | 74 (2.14) | **2.50 (1.39–4.48)** | **0.002** | **2.47 (1.38–4.44)** | **0.002** |
| AGAGC | 19 (1.06) | 1 (0.03) | **70.22 (8.92–552.56)** | **<0.0001** | **70.21 (8.91–553.13)** | **<0.0001** |
| AGAAT | 9 (0.50) | 1 (0.03) | **33.26 (4.01–276.19)** | **0.001** | **32.69 (3.93–271.78)** | **0.001** |
| AGAAC | 10 (0.56) | 1 (0.03) | **36.96 (4.50–303.80)** | **0.0008** | **35.34 (4.29–290.73)** | **0.0009** |
| AGGGT | 62 (3.46) | 99 (2.86) | **2.31 (1.32–4.05)** | **0.003** | **2.34 (1.34–4.10)** | **0.003** |
| AGGGC | 123 (6.86) | 300 (8.66) | 1.52 (0.91–2.51) | 0.108 | 1.50 (0.90–2.49) | 0.118 |
| AGGAT | 43 (2.40) | 122 (3.52) | 1.30 (0.73–2.32) | 0.369 | 1.29 (0.73–2.32) | 0.390 |
| AGGAC | 1 (0.06) | 0 (0.00) | / | / | / | / |
| TAAGT | 3 (0.17) | 0 (0.00) | / | / | / | / |
| TAAGC | 2 (0.11) | 0 (0.00) | / | / | / | / |
| TAAAT | 4 (0.22) | 0 (0.00) | / | / | / | / |
| TGAGT | 509 (28.40) | 1,077 (31.07) | **1.75 (1.09–2.80)** | **0.021** | **1.74 (1.08–2.79)** | **0.022** |
| TGAGC | 448 (25.00) | 790 (22.79) | **2.10 (1.30–3.37)** | **0.002** | **2.09 (1.30–3.37)** | **0.002** |
| TGAAT | 102 (5.69) | 185 (5.34) | **2.04 (1.21–3.43)** | **0.007** | **2.05 (1.22–3.45)** | **0.007** |
| TGAAC | 172 (9.60) | 321 (9.26) | **1.98 (1.21–3.25)** | **0.007** | **1.97 (1.20–3.24)** | **0.007** |
| TGGGT | 2 (0.11) | 1 (0.03) | 7.39 (0.64–85.16) | 0.109 | 5.89 (0.51–68.68) | 0.157 |
| TGGGC | 4 (0.22) | 4 (0.12) | 3.70 (0.86–14.92) | 0.079 | 3.65 (0.85–15.74) | 0.082 |
| TGGAT | 2 (0.11) | 0 (0.00) | / | / | / | / |
| TGGAC | 3 (0.17) | 1 (0.03) | **11.09 (1.10–111.65)** | **0.041** | **10.84 (1.07–109.36)** | **0.043** |

[a]The haplotypes order was rs1064034, rs298982, rs62328061, rs9884978, and rs4834698.
[b]Obtained in logistic regression models with adjustment for age and gender.

There is only one publication regarding the association of *METTL14* gene SNPs with cancer risk so far. In brief, Meng et al.[37] explored the association between $m^6A$ gene SNPs and colorectal cancer risk in a two-stage case-control study with 1,150 cases and 1,342 controls in the discovery stage, and with 932 cases and 966 controls in the validation stage. Out of 240 SNPs in 20 $m^6A$ modification-related genes, only 1 SNP, rs118049207, located in the *SND1* gene, was identified to predispose to colorectal cancer in the Chinese population. None of the five studied *METTL14* gene SNPs (rs115267066, rs298981, rs2029399, rs167246, and rs441216) was associated with colorectal cancer risk. Clearly, these results can provide genetic insights into the origins of colorectal cancer risk. However, to date, the roles of the *METTL14* gene SNPs in neuroblastoma risk are unknown. To fill this gap, we designed the current case-control investigation to determine the correlation of *METTL14* gene polymorphisms and neuroblastoma risk in the Chinese population. In the present study, significant relationships were detected among neuroblastoma risk and the four *METTL14* gene polymorphisms (rs298982 G>A, rs62328061 A>G, rs9884978 G>A, and rs4834698 T>C). Compared with the genotypes of single SNPs, association studies based on haplotypes of multiple markers significantly improve the power of mapping and characterizing disease-causing genes.[38,39] Therefore, we explored whether various haplotypes consisting of the five *METTL14* gene polymorphisms are associated with neuroblastoma risk. Expectedly, *METTL14* gene haplotypes significantly confer a higher risk of neuroblastoma. These results suggest that these variants may interact with each other to modify the risk of neuroblastoma. We further attempted to interpret the possible mechanism of *METTL14* gene SNP-mediated neuroblastoma risk. eQTLs evidence suggested that the G allele in rs298982 is significantly associated with increased long noncoding RNA *SNHG8* (lncRNA *SNHG8*) level in the cultured fibroblasts. lncRNA *SNHG8* was documented to play oncogenic roles in several kinds of cancers.[40–44] We propose the higher expression of lncRNA *SNHG8* caused by G allele in rs298982 may facilitate the development of neuroblastoma. On the contrary, with the increase of G genotype, the average expression of *RP11-*

**Table 4. False-Positive Report Probability Values for the Associations between *METTL14* Gene Polymorphisms and Neuroblastoma Susceptibility**

| | | | | Prior Probability | | | | |
|---|---|---|---|---|---|---|---|---|
| Genotype | Crude OR (95% CI) | p[a] | Statistical Power[b] | 0.25 | 0.1 | 0.01 | 0.001 | 0.0001 |
| rs298982 G>A | | | | | | | | |
| AA versus GG | 0.40 (0.19–0.83) | 0.013 | 0.113 | 0.263 | 0.517 | 0.922 | 0.992 | 0.999 |
| AA versus GG/GA | 0.40 (0.19–0.82) | 0.013 | 0.109 | 0.259 | 0.512 | 0.920 | 0.991 | 0.999 |
| >18 | 0.28 (0.11–0.73) | 0.009 | 0.063 | 0.299 | 0.561 | 0.934 | 0.993 | 0.999 |
| Stages I+II+4S | 0.17 (0.04–0.70) | 0.014 | 0.056 | 0.430 | 0.694 | 0.961 | 0.996 | 1.000 |
| rs62328061 A>G | | | | | | | | |
| AG versus AA | 0.78 (0.65–0.94) | 0.009 | 0.944 | **0.027** | **0.078** | 0.483 | 0.904 | 0.989 |
| AG/GG versus AA | 0.82 (0.69–0.98) | 0.030 | 0.986 | **0.083** | 0.214 | 0.750 | 0.968 | 0.997 |
| >18 | 0.78 (0.62–0.99) | 0.037 | 0.904 | **0.109** | 0.268 | 0.801 | 0.976 | 0.998 |
| Mediastinum | 0.70 (0.51–0.97) | 0.032 | 0.605 | **0.137** | 0.323 | 0.840 | 0.981 | 0.998 |
| rs4834698 T>C | | | | | | | | |
| CC versus TT | 1.28 (1.02–1.60) | 0.035 | 0.987 | **0.095** | 0.239 | 0.776 | 0.972 | 0.997 |
| CC/CT versus TT | | | | | | | | |
| >18 | 1.33 (1.05–1.69) | 0.020 | 0.833 | **0.068** | **0.178** | 0.705 | 0.960 | 0.996 |
| Risk Genotypes | | | | | | | | |
| 2–5 versus 0–1 | 1.46 (1.14–1.87) | 0.002 | 0.582 | **0.012** | **0.036** | 0.290 | 0.805 | 0.976 |
| ≤18 | 1.50 (1.01–2.23) | 0.047 | 0.507 | 0.216 | 0.452 | 0.901 | 0.989 | 0.999 |
| >18 | 1.44 (1.06–1.97) | 0.021 | 0.593 | **0.096** | 0.242 | 0.778 | 0.973 | 0.997 |
| Female | 1.55 (1.06–2.26) | 0.022 | 0.439 | **0.132** | 0.314 | 0.834 | 0.981 | 0.998 |
| Male | 1.40 (1.01–1.93) | 0.044 | 0.667 | **0.164** | 0.370 | 0.866 | 0.985 | 0.998 |
| Adrenal gland | 1.57 (1.02–2.40) | 0.039 | 0.427 | 0.213 | 0.448 | 0.899 | 0.989 | 0.999 |
| Mediastinum | 1.68 (1.05–2.69) | 0.030 | 0.330 | 0.215 | 0.451 | 0.900 | 0.989 | 0.999 |
| Stages I+II+4S | 1.54 (1.12–2.12) | 0.008 | 0.446 | **0.053** | **0.145** | 0.651 | 0.949 | 0.995 |
| Risk Haplotypes | | | | | | | | |
| AAAGC versus AAAGT | 1.95 (1.17–3.25) | 0.011 | 0.884 | **0.035** | **0.098** | 0.545 | 0.924 | 0.992 |
| AAAAC versus AAAGT | 7.39 (1.27–42.91) | 0.026 | 0.041 | 0.651 | 0.848 | 0.984 | 0.998 | 1.000 |
| AAGGT versus AAAGT | 2.04 (1.13–3.70) | 0.019 | 0.485 | **0.104** | 0.258 | 0.793 | 0.975 | 0.997 |
| AGAGT versus AAAGT | 2.50 (1.39–4.48) | 0.002 | 0.227 | **0.027** | **0.077** | 0.478 | 0.902 | 0.989 |
| AGAGC versus AAAGT | 70.22 (8.92–552.56) | <0.0001 | 0.000 | 0.443 | 0.705 | 0.963 | 0.996 | 1.000 |
| AGAAT versus AAAGT | 33.26 (4.01–276.19) | 0.001 | 0.003 | 0.574 | 0.802 | 0.978 | 0.998 | 1.000 |
| AGAAC versus AAAGT | 36.96 (4.50–303.80) | 0.0008 | 0.002 | 0.557 | 0.790 | 0.976 | 0.998 | 1.000 |
| AGGGT versus AAAGT | 2.31 (1.32–4.05) | 0.003 | 0.383 | **0.025** | **0.072** | 0.460 | 0.896 | 0.989 |
| TGAGT versus AAAGT | 1.75 (1.09–2.80) | 0.021 | 1.000 | **0.058** | **0.157** | 0.672 | 0.954 | 0.995 |
| TGAGC versus AAAGT | 2.10 (1.30–3.37) | 0.002 | 0.999 | **0.007** | **0.020** | **0.186** | 0.697 | 0.958 |
| TGAAT versus AAAGT | 2.04 (1.21–3.43) | 0.007 | 0.784 | **0.027** | **0.077** | 0.480 | 0.903 | 0.989 |
| TGAAC versus AAAGT | 1.98 (1.21–3.25) | 0.007 | 0.955 | **0.022** | **0.062** | 0.420 | 0.880 | 0.987 |
| TGGAC versus AAAGT | 11.09 (1.10–111.65) | 0.041 | 0.048 | 0.719 | 0.885 | 0.988 | 0.999 | 1.000 |

[a]$\chi^2$ test was used to calculate the genotype frequency distributions.
[b]Statistical power was calculated using the number of observations in the subgroup and the OR and p values in this table.

*384K6.6* is gradually decreased. This conclusion requires further interpretation as the role of *RP11-384K6.6* remains to be revealed. In all, more functional experiments are needed to support this possible mechanism.

The major strength of this study includes novelty, the relatively large sample size in neuroblastoma cases, and multiple-center participants in a single Chinese population. However, our results should be interpreted in light of three limitations. First, the study population

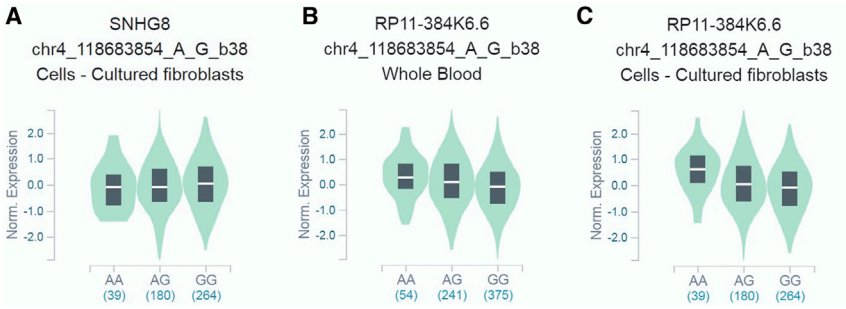


**Figure 1. Functional Relevance of rs298982 G>A on Genes Expression in GTEx Database**

(A–C) The rs298982 G genotype had a significantly (A) higher *SNHG8* mRNA level in the cell-cultured fibroblasts ($p = 1.8 \times 10^{-6}$), but a significantly lower *RP11-384K6.6* level in the (B) whole blood ($p = 3.9 \times 10^{-9}$) and (C) cell-cultured fibroblasts ($p = 9.4 \times 10^{-9}$).

involved only Chinese subjects and was limited to volunteers. Therefore, the generalizability of these findings to the general population was reduced. On the other hand, a single population background here may strengthen the reliability of the conclusion in the study. It is worth pointing out that information from diverse ethnic backgrounds is very useful for elucidating pathogenetic mechanisms in greater detail. Further comparative studies are needed to clarify whether the SNPs are also associated with neuroblastoma in other ethnicities with different genetic backgrounds. Second, only genetic factors, but not environmental factors, were taken into account. Third, the number of SNPs included was relatively small.

In this study, we, for the first time, identified a significant association of *METTL14* gene SNPs with neuroblastoma risk. These SNPs in the *METTL14* gene are intriguing loci for further studies, and the underlying biological mechanisms should be revealed.

## MATERIALS AND METHODS

### Sample Selection

The current study was approved by the institutional review board of Guangzhou Women and Children's Medical Center. We recruited 898 neuroblastoma patients registered in eight hospitals located in eight cities (Guangzhou, Zhengzhou, Wenzhou, Xi'an, Taiyuan, Kunming, Changsha, and Shenyang). We also included 1,734 age- and gender-matched healthy controls having visited the participating hospitals. Written consent was obtained from all participants at enrolment into the study. More details could refer to our previous work.[45,46]

### Population Characteristics

The clinical characteristics of the participants are depicted in Table S1. The mean age for cases was 33.11 ± 28.07 months, and the mean age for controls was 30.41 ± 24.90 months. Overall, 898 cases and 1,734 controls were well matched in terms of age ($p = 0.155$) and gender ($p = 0.236$). According to the International Neuroblastoma Staging System (INSS),[47] 310 neuroblastoma cases (34.52%) were diagnosed with clinical stage I, 160 (17.82%) with clinical stage II, 163 (18.15%) with clinical stage III, 231 (25.72%) with clinical stage IV, 18 (2.00%) with clinical stage 4S disease, and 16 (1.78%) without adequate information. Overall, 248 (27.62%) neuroblastomas occurred in the adrenal gland, 319 (35.52%) in the retroperitoneal region, 214 (23.83%) in the mediastinum, 105 (11.69%) in other regions, and 12 (1.34%) to be determined.

### Polymorphism Selection and Genotyping

*METTL14* gene SNPs with potential functions were retrieved from the dbSNP database and SNPinfo software, with details reported elsewhere.[48] In brief, selection criteria were as follows: (1) located at the two ends of the *METTL14* gene (i.e., the 5′ near gene, 5′ untranslated region [UTR], 3′ UTR, and 3′ near gene); (2) the minor allele frequency ≥ 5% for Chinese Han subjects reported in 1000 Genomes (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) and (3) affecting transcription factor binding site (TFBS) activity or the miRNA binding sites activity; and (4) SNPs in low linkage disequilibrium (LD) with each other ($R^2 < 0.8$). Five SNPs (rs1064034 T>A, rs298982 G>A, rs62328061 A>G, rs9884978 G>A, and rs4834698 T>C) fell into the scope of criteria. Among them, rs298982 G>A and rs9884978 G>A are located in the 5′ near gene, rs1064034 T>A and rs4834698 T>C are located in the 3′ UTR, and the rs62328061 A>G is located in the coding region. Moreover, rs1064034 T>A and rs4834698 T>C affect miRNA binding sites activity, whereas rs298982 G>A and rs9884978 G>A affect TFBS activity. In the last, rs62328061 A>G modulates splicing activity. There was no significant LD ($R^2 < 0.8$) among these five *METTL14* SNPs ($R^2 = 0.033$ between rs298982 G>A and rs9884978 G>A; $R^2 = 0.023$ between rs298982 G>A and rs62328061 A>G; $R^2 = 0.135$ between rs298982 G>A and rs4834698 T>C; $R^2 = 0.322$ between rs298982 G>A and rs1064034 T>A; $R^2 = 0.054$ between rs9884978 G>A and rs62328061 A>G; $R^2 = 0.252$ between rs9884978 G>A and rs4834698 T>C; $R^2 = 0.103$ between rs9884978 G>A and rs1064034 T>A; $R^2 = 0.218$ between rs62328061 A>G and rs4834698 T>C; $R^2 = 0.521$ between rs62328061 A>G and rs1064034 T>A; $R^2 = 0.419$ between rs4834698 T>C and rs1064034 T>A) (Figure S1). Genomic DNA was extracted from peripheral blood using the standard procedure. The genotyping of the SNPs was conducted using the TaqMan SNP genotyping assay.[49–51] Laboratory technicians were blind to the sample information, including the identities of the replicate aliquots. A repeated genotyping was analyzed by an arbitrarily chosen 10% of the samples from both cases and controls. A concordance rate of 100% was obtained.

### Statistical Analysis

We checked HWE for each SNP in controls using a goodness-of-fit $\chi^2$ test. A two-sided $\chi^2$ test was used to analyze the difference in demographic and clinical variables between the cases and controls. The homozygotes of the common allele served as the reference group. The

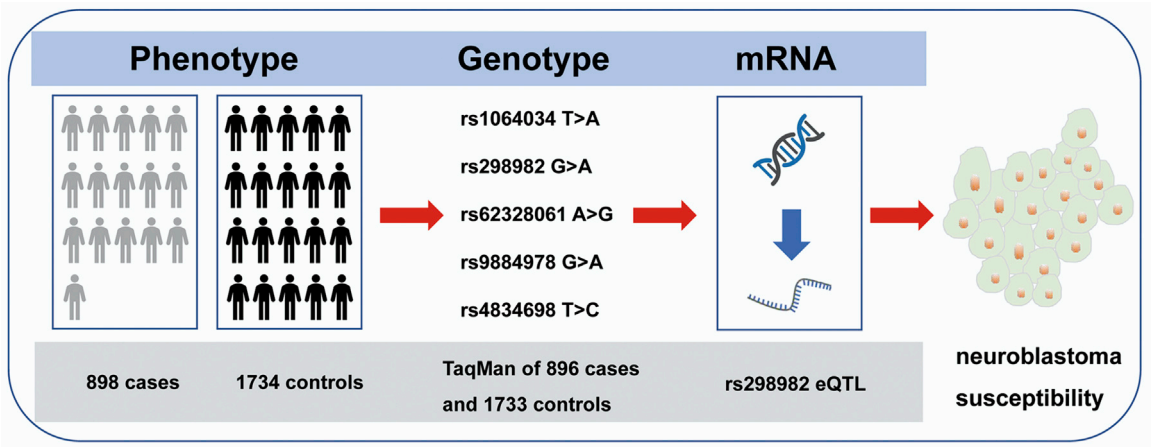


**Figure 2. The Workflow of the Current Study**

remaining genotypes were classified as variants. ORs and 95% CIs were calculated for the variant compared with the reference category using logistic regression analyses. Data were further stratified by age, gender, sites of origins, and clinical stages. The estimation of haplotype frequency and the analysis of their effect on neuroblastoma risk were performed using logistic regression analysis.[46,52] We also applied the FPRP analysis to evaluate noteworthy associations by the means as described elsewhere.[53,54] In brief, three parameters were employed to determine FPRP values, statistical power, p value, and prior probability $\pi$ representing a real association between the SNP and a disease. We set 0.2 as an FPRP threshold and assigned a prior probability of 0.1 to detect an OR of 1.50 (for risk effects) or 0.67 (for protective effects) for an association with genotypes under investigation. eQTLs analysis in the GTEx portal (https://www.gtexportal.org/home/) was adopted to determine the correlation between the SNPs and levels of nearby genes expression.[55] p values below 0.05 were considered significant. All statistical calculations were carried out using SAS 9.1 (SAS Institute, Cary, NC, USA).

### Novelty

We genotyped five *METTL14* polymorphisms in 898 neuroblastoma cases and 1,734 controls enrolled from eight hospitals. We found that rs298982, rs62328061, rs9884978, and rs4834698 were associated with neuroblastoma susceptibility. The significant associations were further validated by stratified analyses, haplotype analyses, and FPRP analyses. eQTL analysis suggested a potential functional role of rs298982 in neuroblastoma. Our results first highlight the critical roles of *METTL14* polymorphisms in the etiology of neuroblastoma.

## SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.omtn.2020.08.009.

## AUTHOR CONTRIBUTIONS

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

## CONFLICTS OF INTEREST

The authors declare no competing interests.

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