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. 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 PHOX2B13 and ALK14,15 genes frequently predispose children to familial neuroblastoma. The introduction of single-nucleotide
polymorphism (SNP) array technology in the 1990s enabled genome-wide association studies (GWASs) in the late 2000s. In 2008, the first GWAS regarding neuroblastoma was performed, with 1,752 European American neuroblastoma cases and 4,171 cancer-free controls. 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. 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 (m6A) 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). Key regulators of m6A modification mainly fall into three categories: RNA methyltransferases (METTL3, METTL14, and WTAP, known as “writers”), demethylases (FTO and ALKBH5, known as “erasers”), and m6A-binding proteins (YTHDF1/2/3 and IGFB2BP1, known as “readers”). Strong evidence suggests that aberrant expression of m6A key regulators could cause dysregulated RNA m6A modification, resulting in a variety of diseases, including cancers. Some SNPs located in specific regions of m6A key regulator genes may alter m6A methylation and subsequently affect related biological processes. A large number of m6A gene SNPs have been identified to associate with major depressive disorder, osteoporosis, and obesity. However, evidence regarding m6A modification key regulators genes SNPs and risk of cancer is very scarce.

To identify novel susceptibility m6A 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 contributed 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 (<18 months: adjusted OR = 1.49, 95% CI = 1.02–2.22, 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, AGGCT, 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...
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.

| Genotype | Cases (N = 896) | Controls (N = 1,733) | p | Crude OR (95% CI) | p | Adjusted OR (95% CI) | p |
|----------|-----------------|----------------------|---|-------------------|---|----------------------|---|
| 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) | 1.07 (0.82–1.40) | 0.063 | 1.07 (0.82–1.40) | 0.061 | |
| 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) | 1.00 (0.75–1.32) | 0.989 | 1.00 (0.75–1.32) | 0.992 | |
| Additive | | | 0.271 | 0.91 (0.77–1.09) | 0.271 | 0.91 (0.77–1.09) | 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) | 1.00 (0.75–1.32) | 0.989 | 1.00 (0.75–1.32) | 0.992 | |
| rs62328061 A>G (HWE = 0.600) | | | | | | | |
| AA | 645 (71.99) | 1,176 (67.86) | 1.00 | 1.00 | | | |
| AG | 217 (24.22) | 403 (23.25) | 0.78 (0.65–0.94) | 0.009 | 0.77 (0.64–0.93) | 0.007 | |
| GG | 34 (3.79) | 59 (3.29) | 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) | 1.00 (0.75–1.32) | 0.989 | 1.00 (0.75–1.32) | 0.992 | |
| 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 | | | 0.085 | 1.02 (0.89–1.16) | 0.084 | 1.02 (0.89–1.16) | 0.078 |
| 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) | 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 (48.92) | 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 (1.02–1.60) | 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 (74.12) | 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

- 0: 100 (11.16) 269 (15.52) 1.00
- 2: 796 (88.84) 1,464 (84.48) 0.002

Values were in bold if the 95% CIs excluded 1 or P values less than 0.05.

*χ² test for genotype distributions between neuroblastoma patients and controls.

bAdjusted for age and gender.

cRisk genotypes were rs1064034 AA, rs298982 GG/GA, rs62328061 GG, rs9884978 AA, and rs4834698 TC/CC.
Effect of rs298982 G>A on the Expression of Surrouding 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 METTL14 in colorectal cancer tissues and cell lines. The low expression had worse recurrence-free survival and 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 associated with poor overall survival.

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 m6A-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 gene SNP rs298982 G>A enhances hepatoblastoma risk. This is the most extensive study to date.
There is only one publication regarding the association of METTL14 gene SNPs with cancer risk so far. In brief, Meng et al. explored the association between m6A gene SNPs and colorectal cancer risk in a two-stage case-control study with 1,150 cases and 1,342 controls in the validation stage. Out of 240 SNPs in 20 m6A-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. Moreover, the discovery stage, with 932 cases and 966 controls in the validation stage, suggested that the G allele in rs298982 is significantly associated with neuroblastoma risk. Expectedly, METTL14 gene polymorphisms are associated with neuroblastoma risk. Therefore, we explored whether various haplotypes consisting of the five METTL14 gene polymorphisms 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 (IncRNA SNHG8) level in the cultured fibroblasts. IncRNA SNHG8 was documented to play oncogenic roles in several kinds of cancers. Therefore, we propose the higher expression of IncRNA 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-
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

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

| Genotype                  | Crude OR (95% CI) | p    | Statistical Power | Prior Probability |
|---------------------------|-------------------|------|-------------------|-------------------|
| rs298982 G>A              |                   |      |                   |                   |
| AA versus GG              | 0.40 (0.19–0.83)  | 0.013| 0.113             | 0.263             |
| AA versus GG/GA           | 0.40 (0.19–0.82)  | 0.013| 0.109             | 0.259             |
| >18                       | 0.28 (0.11–0.73)  | 0.009| 0.063             | 0.299             |
| Stages I+II+4S            | 0.17 (0.04–0.70)  | 0.014| 0.056             | 0.430             |
| rs62328061 A>G            |                   |      |                   |                   |
| AG versus AA              | 0.78 (0.65–0.94)  | 0.009| 0.944             | 0.027             |
| AG/GG versus AA           | 0.82 (0.69–0.98)  | 0.030| 0.986             | 0.083             |
| >18                       | 0.78 (0.62–0.99)  | 0.037| 0.994             | 0.109             |
| Mediastinum               | 0.70 (0.51–0.97)  | 0.032| 0.605             | 0.137             |
| rs4834698 T>C             |                   |      |                   |                   |
| CC versus TT              | 1.28 (1.02–1.60)  | 0.035| 0.987             | 0.095             |
| CC/CT versus TT           |                   |      |                   |                   |
| >18                       | 1.33 (1.05–1.69)  | 0.020| 0.833             | 0.068             |
| Risk Genotypes            |                   |      |                   |                   |
| 2–5 versus 0–1            | 1.46 (1.14–1.87)  | 0.002| 0.582             | 0.012             |
| ≤18                       | 1.50 (1.01–2.23)  | 0.047| 0.507             | 0.216             |
| >18                       | 1.44 (1.06–1.97)  | 0.021| 0.593             | 0.096             |
| Female                    | 1.55 (1.06–2.26)  | 0.022| 0.439             | 0.132             |
| Male                      | 1.40 (1.01–1.93)  | 0.044| 0.667             | 0.164             |
| Adrenal gland             | 1.57 (1.02–2.40)  | 0.039| 0.427             | 0.213             |
| Mediastinum               | 1.68 (1.05–2.69)  | 0.030| 0.330             | 0.215             |
| Stages I+II+4S            | 1.54 (1.12–2.12)  | 0.008| 0.446             | 0.053             |
| Risk Haplotypes           |                   |      |                   |                   |
| AAAGC versus AAAGT        | 1.95 (1.17–3.25)  | 0.011| 0.884             | 0.035             |
| AAAAC versus AAAGT        | 7.39 (4.27–42.91) | 0.026| 0.041             | 0.651             |
| AAGGT versus AAAGT        | 2.04 (1.13–3.70)  | 0.019| 0.485             | 0.104             |
| AGAGT versus AAAGT        | 2.50 (1.39–4.48)  | 0.002| 0.227             | 0.027             |
| AGACC versus AAAGT        | 70.22 (8.92–552.56)| <0.0001| 0.000             | 0.443             |
| AGAAT versus AAAGT        | 35.26 (4.01–276.19)| 0.001| 0.003             | 0.574             |
| AGAAC versus AAAGT        | 36.96 (4.50–303.80)| 0.0008| 0.002            | 0.557             |
| AAGGTT versus AAAGT       | 2.31 (1.32–4.05)  | 0.003| 0.383             | 0.025             |
| TGAAT versus AAAGT        | 1.75 (1.09–2.80)  | 0.021| 1.000             | 0.058             |
| TGAGT versus AAAGT        | 2.10 (1.30–3.37)  | 0.002| 0.999             | 0.007             |
| TGAAAT versus AAAGT       | 2.04 (1.21–3.43)  | 0.007| 0.784             | 0.027             |
| TGACG versus AAAGT        | 1.98 (1.21–3.25)  | 0.007| 0.955             | 0.022             |
| TGGAC versus AAAGT        | 11.09 (1.10–111.65)| 0.041| 0.048             | 0.719             |

aX² test was used to calculate the genotype frequency distributions.
bStatistical power was calculated using the number of observations in the subgroup and the OR and p values in this table.
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. Is worth pointing out that information from diverse ethnic backgrounds here may strengthen the reliability of the conclusion in the study. It was reduced. On the other hand, a single population background 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.

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 = 0.236). According to the International Neuroblastoma Staging System (INSS), 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.

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 × 10⁻⁴), but a significantly lower RP11–38K6.6 level in the (B) whole blood (p = 3.9 × 10⁻⁵) and (C) cell-cultured fibroblasts (p = 9.4 × 10⁻⁵).

Polymorphism Selection and Genotyping

METTL14 gene SNPs with potential functions were retrieved from the dbSNP database and SNPinfo software, with details reported elsewhere. 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 (R² < 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 (R² < 0.8) among these five METTL14 SNPs (R² = 0.033 between rs298982 G>A and rs9884978 G>A; R² = 0.023 between rs298982 G>A and rs62328061 A>G; R² = 0.135 between rs298982 G>A and rs4834698 T>C; R² = 0.322 between rs298982 G>A and rs1064034 T>A; R² = 0.521 between rs9884978 G>A and rs4834698 T>C; R² = 1.03 between rs9884978 G>A and rs1064034 T>A; R² = 0.218 between rs62328061 A>G and rs4834698 T>C; R² = 0.521 between rs62328061 A>G and rs1064034 T>A; R² = 0.419 between rs4834698 T>C and rs1064034 T>A).}

Statistical Analysis

We checked HWE for each SNP in controls using a goodness-of-fit χ² test. A two-sided χ² 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...
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. We also applied the FPRP analysis to evaluate noteworthy associations by the means as described elsewhere. In brief, three parameters were employed to determine FPRP values, statistical power, p value, and prior probability 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. 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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