**METTL14** gene polymorphisms decrease Wilms tumor susceptibility in Chinese children

Zhenjian Zhuo¹, Rui-Xi Hua¹†, Huizhu Zhang²†, Huiran Lin³, Wen Fu¹, Jinhong Zhu⁴, Jiwen Cheng⁵, Jiao Zhang⁶, Suhong Li⁷, Haixia Zhou⁸, Huimin Xia¹, Guochang Liu¹, Wei Jia¹* and Jing He¹*

**Abstract**

**Background:** Wilms tumor is a highly heritable malignancy. Aberrant METTL14, a critical component of N6-methyladenosine (m⁶A) methyltransferase, is involved in carcinogenesis. The association between genetic variants in the **METTL14** gene and Wilms tumor susceptibility remains to be fully elucidated. We aimed to assess whether variants within this gene are implicated in Wilms tumor susceptibility.

**Methods:** A total of 403 patients and 1198 controls were analyzed. **METTL14** genotypes were assessed by TaqMan genotyping assay.

**Result:** Among the five SNPs analyzed, rs1064034 T > A and rs298982 G > A exhibited a significant association with decreased susceptibility to Wilms tumor. Moreover, the joint analysis revealed that the combination of five protective genotypes exerted significantly more protective effects against Wilms tumor than 0–4 protective genotypes with an OR of 0.69. The stratified analysis further identified the protective effect of rs1064034 T > A, rs298982 G > A, and combined five protective genotypes in specific subgroups. The above significant associations were further validated by haplotype analysis and false-positive report probability analysis. Preliminary mechanism exploration indicated that rs1064034 T > A and rs298982 G > A are correlated with the expression and splicing event of their surrounding genes.

**Conclusions:** Collectively, our results suggest that **METTL14** gene SNPs may be genetic modifiers for the development of Wilms tumor.

**Keywords:** Wilms tumor, Risk, **METTL14**, Polymorphism, Case-control study

**Introduction**

Wilms tumor, also known as nephroblastoma, is the most common pediatric kidney cancer [1]. It accounts for over 90% of all the diagnosed kidney tumors in children [2]. The incidence rate of Wilms' tumor varies geographically [3, 4]. The prevalence of Wilms tumor is about 7 cases per million children in the United States. Wilms tumor is also one of the most common renal tumors in children in China, with an incidence rate of ~ 3.3 per million. Wilms tumors are frequently diagnosed in young children with an average age of 2–3 years [5]. At present, long-term overall survival for the localized Wilms tumors exceeds 90% due to the improved risk stratification-adapted treatment [6]. However, nearly 20% of Wilms tumors are classified into high-risk subtype with frequent metastasis. Patients with high-risk tumors still subject to suboptimal outcomes [7–9]. Chronic health conditions secondary to intensified therapeutic regimens impact nearly 25% of Wilms tumor survivors [10].
The genetics of Wilms tumor tumorigenesis is complex, with multiple oncogenic drivers identified over the years. The currently known repertoire of oncogenic Wilms tumor driver alterations includes mutations in the \( WT1, CTNNB1, TP53, AMER1 \), as well as an abnormality of 11p15 methylation [11–15]. Apart from these, genetic association analyses in case-control studies also unveiled some Wilms tumor susceptibility loci [16–19]. Nevertheless, the well-established risk factors for Wilms tumor probably are only the tip of the iceberg. So far, all the known gene mutations can only explain less than 50% of Wilms tumor. Therefore, it is imperative to identify more causative variants to improve the understanding of the genetic susceptibility to Wilms tumor. In addition, detailed genetic information leads to new druggable targets, facilitating the development of more effective treatments for Wilms tumor.

N6-methyladenosine (m6A) is the most common internal chemical modification on eukaryotic mRNA [20]. m6A is mainly involved in the regulation of splicing, subcellular localization, translation, stability, and degradation of mRNA. m6A modulators are mainly classified into methyltransferase (writer), demethylase (eraser), and binding protein (reader). Methyltransferases include METTL3, METTL14, and WTAP, which mainly mediate m6A methylation of mRNA adenylicate. Demethylases, consisting of FTO and ALKBH5, mainly remove m6A modification installed on RNA. Binding proteins include YTHDF1/2/3, YTHDC1/2, IGF2BP1/2/3, and elf3, which are responsible for recognizing bases modified by m6A and regulating downstream pathways [21, 22]. The m6A modulator proteins play an important role in the occurrence and development of a variety of tumors [23–25]. However, research on the expression and function of m6A modulator genes in Wilms tumor has not yet been reported. The scarcity of investigation prompted us to contribute to our current report on associations between genetic variability of METTL14 and the risk of Wilms tumor. To this end, a total of five common SNPs in the METTL14 gene were genotyped and tested for their association with Wilms tumor susceptibility.

Methods

Sample selection

The study was carried out based on the principles of the Declaration of Helsinki. Approval of the study protocol was obtained from the institutional review board of Guangzhou Women and Children’s Medical Center (Ethics Approval No: 202016600). Eligible cases were all children newly diagnosed with a histologically confirmed Wilms tumor. Controls, recruited from the same hospital, were healthy volunteers of Chinese origin, without family history of Wilms tumor. Written informed consent was signed by all subjects’ guardians. All the subjects were enrolled from March 2001 to March 2018 and were genetically unrelated ethnic Han Chinese from China. A total of 414 cases diagnosed with Wilms tumor and 1199 hospital-based controls were included. They were enrolled from five hospitals (Guangzhou Women and Children’s Medical Center, The Second Affiliated Hospital and Yuying Children’s Hospital of Wenzhou Medical University, The First Affiliated Hospital of Zhengzhou University, Second Affiliated Hospital of Xi’an Jiao Tong University, and Shanxi Provincial Children’s Hospital) in five different cities of China. Detailed information was previously reported [26, 27].

Polymorphism selection and genotyping

The selection of the five potentially functional METTL14 gene SNPs (rs1064034 T > A, rs298982 G > A, rs62328061 A > G, rs9884978 G > A, and rs4834698 T > C) was described in detail in our previous studies [28–30]. Genomic DNA from each sample was extracted from peripheral blood. Genotypes were determined using the TaqMan method. Replicate samples (10% of the samples) were picked out of all genotyping batches, and the concordance levels for blind duplicate samples were 100% for all SNPs assayed.

Statistical analysis

SNP genotypes were tested for consistency with Hardy-Weinberg equilibrium (HWE) within the control sample using a Goodness-of-fit \( \chi^2 \) test. Differences between cases and controls in the distribution of demographic and clinical variables were checked using a two-sided \( \chi^2 \) test. Adjusted odds ratios (ORs) with 95% confidence intervals (CIs) and two-sided \( P \)-values were calculated using unconditional logistic regression to estimate the relative risk associated with each genotype. Associations were further estimated in the groups stratified by age, gender, and clinical stages. Haplotype frequency distributions were deduced from observed genotypes using logistic regression analyses [31, 32]. False-positive report probability (FPRP) analysis was applied to assess noteworthy associations with detailed methods presented elsewhere [33, 34]. We performed expression quantitative trait loci (eQTL) and splicing quantitative trait loci (sQTLs) analyses through the Genotype-Tissue Expression (GTEx) project (http://www.gtexportal.org/) to evaluate the correlations between genotypes of candidate SNPs and genes expression as well as alternative splicing (AS) events of genes [35]. A probability value (\( P \) value) less than 0.05 was considered significant. All statistical analyses were performed using SAS version 9.1 software (SAS Institute, Inc., Cary, North Carolina).
Results

Effect of METTL14 gene SNPs on Wilms tumor risk

Clinical characteristics of the participants were depicted in our previous study (Table S1) [27]. Here, 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 403 cases and 1198 controls, out of 414 cases and 1199 controls samples. The correlation between these SNPs and Wilms tumor risk is shown in Table 1. All these SNPs followed Hardy-Weinberg equilibrium (HWE) in controls (HWE P > 0.05). The rs1064034 variant alleles were remarkably

| Genotype          | Cases (N = 403) | Controls (N = 1198) | Crude OR (95% CI) | Adjusted OR (95% CI) | P     |
|-------------------|-----------------|---------------------|-------------------|----------------------|-------|
| rs1064034 T > A (HWE = 0.715) |                |                     |                   |                      |       |
| TT                | 216 (53.60)     | 564 (47.08)         | 1.00              | 1.00                 |       |
| TA                | 152 (37.72)     | 512 (42.74)         | 0.78 (0.61–0.99)  | 0.037                | 0.78 (0.61–0.99) | 0.041 |
| AA                | 35 (8.68)       | 122 (10.18)         | 0.75 (0.50–1.13)  | 0.164                | 0.76 (0.51–1.15) | 0.198 |
| Additive          |                 |                     | 0.035             |                      | 0.83 (0.70–0.99) | 0.044 |
| Dominant          | 187 (46.40)     | 634 (52.92)         | 0.77 (0.61–0.97)  | 0.024                | 0.78 (0.62–0.97) | 0.029 |
| Recessive         | 368 (91.32)     | 1076 (89.82)        | 0.84 (0.57–1.24)  | 0.382                | 0.86 (0.58–1.27) | 0.438 |
| rs298982 G > A (HWE = 0.155) |                |                     |                   |                      |       |
| GG                | 321 (79.65)     | 873 (72.87)         | 1.00              | 1.00                 |       |
| GA                | 66 (16.38)      | 292 (24.37)         | 0.62 (0.46–0.83)  | 0.001                | 0.62 (0.46–0.84) | 0.002 |
| AA                | 16 (3.97)       | 33 (2.75)           | 1.32 (0.72–2.43)  | 0.375                | 1.32 (0.72–2.43) | 0.373 |
| Additive          |                 |                     | 0.061             |                      | 0.80 (0.64–1.01) | 0.071 |
| Dominant          | 82 (20.35)      | 325 (27.13)         | 0.69 (0.52–0.90)  | 0.007                | 0.69 (0.53–0.91) | 0.009 |
| Recessive         | 387 (96.03)     | 1165 (97.25)        | 1.46 (0.80–2.68)  | 0.220                | 1.46 (0.79–2.68) | 0.225 |
| rs62328061 A > G (HWE = 0.819) |                |                     |                   |                      |       |
| AA                | 281 (69.73)     | 830 (69.28)         | 1.00              | 1.00                 |       |
| AG                | 109 (27.05)     | 333 (27.80)         | 0.97 (0.75–1.25)  | 0.796                | 0.97 (0.75–1.25) | 0.812 |
| GG                | 13 (3.23)       | 35 (2.92)           | 1.10 (0.57–2.10)  | 0.780                | 1.12 (0.58–2.15) | 0.736 |
| Additive          |                 |                     | 0.963             |                      | 1.00 (0.81–1.24) | 0.998 |
| Dominant          | 122 (30.27)     | 368 (30.72)         | 0.867             | 0.867                | 0.98 (0.77–1.26) | 0.894 |
| Recessive         | 390 (96.03)     | 1163 (97.08)        | 1.11 (0.58–2.12)  | 0.757                | 1.13 (0.59–2.16) | 0.714 |
| rs9884978 G > A (HWE = 0.412) |                |                     |                   |                      |       |
| GG                | 252 (62.53)     | 758 (63.27)         | 1.00              | 1.00                 |       |
| GA                | 131 (32.51)     | 384 (32.05)         | 1.03 (0.80–1.31)  | 0.836                | 1.03 (0.81–1.31) | 0.826 |
| AA                | 20 (4.96)       | 56 (4.67)           | 1.07 (0.63–1.83)  | 0.791                | 1.06 (0.62–1.80) | 0.826 |
| Additive          |                 |                     | 0.759             |                      | 1.03 (0.85–1.25) | 0.773 |
| Dominant          | 151 (37.47)     | 440 (36.73)         | 1.03 (0.82–1.30)  | 0.789                | 1.03 (0.82–1.30) | 0.791 |
| Recessive         | 383 (95.04)     | 1142 (95.33)        | 1.01 (0.63–1.80)  | 0.814                | 1.05 (0.62–1.78) | 0.851 |
| rs4834698 T > C (HWE = 0.827) |                |                     |                   |                      |       |
| TT                | 107 (26.55)     | 329 (27.46)         | 1.00              | 1.00                 |       |
| TC                | 193 (47.89)     | 594 (49.58)         | 1.00 (0.76–1.31)  | 0.995                | 0.99 (0.75–1.30) | 0.921 |
| CC                | 103 (25.56)     | 275 (22.95)         | 1.15 (0.84–1.58)  | 0.379                | 1.14 (0.83–1.56) | 0.425 |
| Additive          |                 |                     | 0.392             |                      | 1.07 (0.92–1.26) | 0.438 |
| Dominant          | 296 (73.45)     | 869 (72.54)         | 1.05 (0.81–1.35)  | 0.724                | 1.03 (0.80–1.34) | 0.798 |
| Recessive         | 300 (74.44)     | 923 (77.05)         | 1.15 (0.89–1.50)  | 0.287                | 1.15 (0.88–1.49) | 0.304 |
| Combined effect of protective genotypes  |                |                     |                   |                      |       |
| 0–4               | 322 (79.90)     | 875 (73.04)         | 1.00              | 1.00                 |       |
| S                 | 81 (20.10)      | 323 (26.96)         | 0.006             | 0.68 (0.52–0.90)     | 0.006 | 0.69 (0.52–0.91) | 0.008 |

OR Odds ratio. CI Confidence interval. HWE Hardy-Weinberg equilibrium

* p < 0.05 for genotype distributions between Wilms tumor patients and controls

** Adjusted for age and gender

Protective genotypes were carriers with rs1064034 TA/AA, rs298982 GA/AA, rs62328061 AG/AA, rs9884978 GA/GG and rs4834698 TT/TC
Table 2  Stratification analysis of protective genotypes with Wilms tumor susceptibility

| Variables | rs1064034 | AOR (95% CI) a | P a | rs298982 | AOR (95% CI) a | P a | Combined (95% CI) a | P a |
|-----------|------------|----------------|------|----------|----------------|------|---------------------|------|
|           | TT         | TA/AA          |      | GG       | GA/AA         |      |                     |      |
| Age, month|            |                |      |          |                |      |                     |      |
| ≤ 18      | 72/243     | 66/222         | 1.00 (0.68–1.47) | 0.995 | 105/356      | 33/109 | 1.01 (0.65–1.58) | 0.971 |
| > 18      | 144/321    | 121/412        | 0.67 (0.50–0.88) | 0.005 | 216/517      | 49/216 | 0.56 (0.39–0.79) | 0.001 |
| Gender    |            |                |      |          |                |      |                     |      |
| Females   | 109/251    | 80/270         | 0.68 (0.49–0.95) | 0.025 | 159/394      | 30/127 | 0.59 (0.38–0.91) | 0.017 |
| Males     | 107/313    | 107/364        | 0.87 (0.64–1.18) | 0.371 | 162/479      | 52/198 | 0.78 (0.55–1.11) | 0.172 |
| Clinical stages | | | | | | | | |
| I         | 73/564     | 64/634         | 0.81 (0.57–1.15) | 0.239 | 111/873      | 26/325 | 0.64 (0.41–1.01) | 0.053 |
| II        | 61/564     | 52/634         | 0.77 (0.52–1.14) | 0.193 | 88/873       | 25/325 | 0.78 (0.49–1.23) | 0.285 |
| III       | 44/564     | 48/634         | 0.94 (0.61–1.44) | 0.781 | 74/873       | 18/325 | 0.64 (0.38–1.10) | 0.105 |
| IV        | 28/564     | 17/634         | 0.53 (0.29–0.98) | 0.043 | 37/873       | 8/325  | 0.58 (0.27–1.26) | 0.171 |
| I+II      | 134/564    | 116/634        | 0.68 (0.60–1.04) | 0.093 | 199/873      | 51/325 | 0.70 (0.50–0.98) | 0.037 |
| III+IV    | 72/564     | 65/634         | 0.79 (0.55–1.12) | 0.183 | 111/873      | 26/325 | 0.62 (0.40–0.98) | 0.039 |

AOR Adjusted odds ratio, CI Confidence interval
a Adjusted for age and gender, omitting the corresponding factor

Table 3  The frequency of inferred haplotypes of METTL14 gene based on observed genotypes and their association with the risk of Wilms tumor

| Haplotypes a | Cases (n = 806) | Controls (n = 2396) | Crude OR (95% CI) | P | Adjusted OR b (95% CI) | P b |
|--------------|-----------------|---------------------|------------------|---|------------------------|-----|
| TGAAC        | 78 (9.68)       | 233 (9.72)          | 1.00             | 1.00 |
| TGAAT        | 41 (5.09)       | 111 (4.63)          | 0.88 (0.57–1.34) | 0.542 | 0.87 (0.57–1.33) | 0.516 |
| TGGC         | 209 (25.93)     | 550 (22.95)         | 0.90 (0.68–1.20) | 0.468 | 0.90 (0.68–1.19) | 0.464 |
| TGGT         | 242 (30.02)     | 744 (31.05)         | 0.77 (0.59–1.02) | 0.064 | 0.77 (0.59–1.02) | 0.066 |
| TGGAT        | 4 (0.50)        | 0 (0.00)            | /                | /    | /                      |    |
| TGGGC        | 5 (0.62)        | 1 (0.04)            | 11.85 (1.37–102.72) | 0.025 | 11.15 (1.28–96.76) | 0.029 |
| TGGGT        | 3 (0.37)        | 1 (0.04)            | 7.11 (0.73–69.18) | 0.091 | 7.50 (0.77–73.05) | 0.083 |
| TAAAT        | 1 (0.12)        | 0 (0.00)            | /                | /    | /                      |    |
| TAAGC        | 1 (0.12)        | 0 (0.00)            | /                | /    | /                      |    |
| AGGTC        | 23 (2.85)       | 79 (3.30)           | 0.69 (0.41–1.16) | 0.162 | 0.70 (0.41–1.16) | 0.172 |
| AGGCC        | 65 (8.06)       | 193 (8.06)          | 0.80 (0.55–1.15) | 0.227 | 0.80 (0.55–1.15) | 0.221 |
| AGGCT        | 23 (2.85)       | 69 (2.88)           | 0.79 (0.47–1.34) | 0.380 | 0.80 (0.47–1.36) | 0.417 |
| AGAAC        | 3 (0.37)        | 0 (0.00)            | /                | /    | /                      |    |
| AGAAT        | 2 (0.25)        | 1 (0.04)            | 4.74 (0.43–52.87) | 0.206 | 5.23 (0.47–58.94) | 0.180 |
| AGAGC        | 1 (0.12)        | 1 (0.04)            | 2.37 (0.15–38.27) | 0.543 | 2.46 (0.15–39.70) | 0.527 |
| AGAGT        | 9 (1.12)        | 55 (2.30)           | 0.39 (0.19–0.82) | 0.012 | 0.40 (0.19–0.84) | 0.016 |
| AAGAC        | 1 (0.12)        | 0 (0.00)            | /                | /    | /                      |    |
| AAGGC        | 2 (0.25)        | 2 (0.08)            | 2.37 (0.33–17.06) | 0.392 | 2.32 (0.32–16.75) | 0.403 |
| AAGGT        | 9 (1.12)        | 58 (2.42)           | 0.37 (0.18–0.77) | 0.008 | 0.38 (0.18–0.80) | 0.010 |
| AAAC         | 0 (0.00)        | 2 (0.08)            | /                | /    | /                      |    |
| AAAAT        | 18 (2.23)       | 70 (2.92)           | 0.61 (0.35–1.08) | 0.088 | 0.62 (0.35–1.09) | 0.096 |
| AAAGC        | 34 (4.22)       | 162 (6.76)          | 0.50 (0.32–0.77) | 0.002 | 0.50 (0.32–0.77) | 0.002 |
| AAAGT        | 32 (3.97)       | 64 (2.67)           | 1.19 (0.73–1.92) | 0.492 | 1.19 (0.73–1.93) | 0.488 |

a The haplotype order were rs1064034, rs298982, rs62328061, rs9884978, and rs4834698
b Obtained in logistic regression models with adjustment for age and gender
associated with reduced risk of Wilms tumor (TA vs. TT: adjusted OR = 0.78, 95% CI = 0.61–0.99, P = 0.041; TA/AA vs. TT: adjusted OR = 0.83, 95% CI = 0.70–0.95, P = 0.044). Similar association was found for the rs298982 (GA/AA vs. GG: adjusted OR = 0.69, 95% CI = 0.53–0.91, P = 0.009). We then defined rs1064034 TA/AA, rs298982 GA/AA, rs62328061 AG/AA, rs9884978 GA/GG, and rs4834698 TT/TC as protective genotypes based on their ORs. Participants with 5 protective genotypes showed a 0.69-fold decrease in the risk of developing Wilms tumor when compared with those with 0–4 protective genotypes (95% CI = 0.52–0.91, P = 0.008).

Stratification analysis of significant SNPs
We analyzed the association between the METTL14 gene polymorphisms and susceptibility to Wilms tumor in subgroups separated by age, gender, and clinical stages (Table 2). Further stratification study revealed that the rs1064034 was associated with reduced Wilms tumor risk in groups with age > 18 months, female, and clinical stage IV diseases. Moreover, stronger protective effects was found for the GA/AA genotypes of rs298982 and combined five protective genotypes among children age > 18 months, females, clinical stage I+II tumors, and clinical stage III+IV tumors.

**METTL14 haplotype analysis**
We next evaluated whether the haplotypes of the five METTL14 gene SNPs are linked with Wilms tumor risk (Table 3). When compared to reference haplotype TGAAC, haplotypes AGAGT (P = 0.016), AAGGT (P = 0.010), and AAAGC (P = 0.002) were linked with significantly decreased Wilms tumor risk.

### Table 4 False-positive report probability analysis for significant findings

| Genotype | OR (95% CI) | P | Statistical power | Prior probability |
|----------|-------------|---|------------------|-----------------|
| rs1064034 T > A | | | | |
| TA vs. TT | 0.78 (0.61–0.99) | 0.0372 | 0.899 | 0.110 | 0.071 | 0.804 | 0.976 | 0.998 |
| TA/AA vs. TT | 0.77 (0.61–0.97) | 0.0237 | 0.886 | 0.074 | 0.194 | 0.726 | 0.964 | 0.996 |
| > 18 | 0.66 (0.49–0.87) | 0.0033 | 0.441 | 0.022 | 0.063 | 0.426 | 0.882 | 0.987 |
| Females | 0.68 (0.49–0.96) | 0.0257 | 0.544 | 0.124 | 0.298 | 0.824 | 0.979 | 0.998 |
| Stage IV | 0.54 (0.29–0.997) | 0.049 | 0.255 | 0.366 | 0.634 | 0.950 | 0.995 | 0.999 |
| rs298982 G > A | | | | |
| GA vs. GG | 0.62 (0.46–0.83) | 0.0013 | 0.307 | 0.013 | 0.037 | 0.295 | 0.809 | 0.977 |
| GA/AA vs. GG | 0.69 (0.52–0.90) | 0.0071 | 0.571 | 0.036 | 0.101 | 0.552 | 0.926 | 0.992 |
| > 18 | 0.54 (0.38–0.77) | 0.0006 | 0.134 | 0.013 | 0.039 | 0.308 | 0.818 | 0.978 |
| Female | 0.59 (0.38–0.91) | 0.0167 | 0.287 | 0.149 | 0.344 | 0.852 | 0.983 | 0.998 |
| Stage I | 0.63 (0.40–0.98) | 0.0416 | 0.399 | 0.238 | 0.484 | 0.912 | 0.990 | 0.999 |
| Stage I+II | 0.69 (0.49–0.96) | 0.028 | 0.566 | 0.129 | 0.308 | 0.830 | 0.980 | 0.998 |
| Stage III+IV | 0.63 (0.40–0.98) | 0.0416 | 0.400 | 0.238 | 0.484 | 0.911 | 0.990 | 0.999 |
| Protective genotypes | | | | |
| 5 vs. 0–4 | 0.68 (0.52–0.90) | 0.0063 | 0.552 | 0.033 | 0.093 | 0.531 | 0.919 | 0.991 |
| > 18 | 0.54 (0.38–0.77) | 0.0006 | 0.134 | 0.013 | 0.039 | 0.308 | 0.818 | 0.978 |
| Female | 0.60 (0.39–0.93) | 0.0216 | 0.318 | 0.169 | 0.379 | 0.871 | 0.985 | 0.999 |
| Stage I | 0.64 (0.41–0.99) | 0.0455 | 0.413 | 0.248 | 0.498 | 0.916 | 0.991 | 0.999 |
| Stage I+II | 0.69 (0.50–0.97) | 0.0318 | 0.585 | 0.140 | 0.329 | 0.843 | 0.982 | 0.998 |
| Stage III+IV | 0.61 (0.39–0.95) | 0.0291 | 0.338 | 0.205 | 0.437 | 0.895 | 0.989 | 0.999 |
| Haplotypes | | | | |
| TGGGC vs. TGAAC | 11.85 (1.37–102.72) | 0.025 | 0.035 | 0.683 | 0.866 | 0.986 | 0.999 | 1.000 |
| AGAGT vs. TGAAC | 0.39 (0.19–0.82) | 0.012 | 0.089 | 0.295 | 0.557 | 0.932 | 0.993 | 0.999 |
| TGGGC vs. TGAAC | 0.37 (0.18–0.77) | 0.008 | 0.070 | 0.256 | 0.508 | 0.919 | 0.991 | 0.999 |
| TGGGC vs. TGAAC | 0.50 (0.32–0.77) | 0.002 | 0.148 | 0.035 | 0.099 | 0.547 | 0.924 | 0.992 |

OR Odds ratio, CI Confidence interval

* Chi-square test was used to calculate the genotype frequency distributions

Statistical power was calculated using the number of observations in each subgroup and the corresponding ORs and P values in this table
False-positive report probability (FPRP) analysis
The obtained significant findings above were further assessed using false-positive report probability (FPRP) analysis (Table 4). At the prior probability of 0.1 and FPRP threshold value of 0.2, the associations between rs1064034 and Wilms tumor risk remained noteworthy in models TA/AA vs. TT and subgroup of children >18 months in TA/AA vs. TT. Noteworthy results were also found for the GA vs. GG, GA/AA vs. GG, and subgroup of children >18 months in GA/AA vs. GG. In addition, a significant decrease of Wilms tumor risk was detected in the carrier of 5 vs. 0–4 protective genotypes and subgroup of children >18 months in 5 vs. 0–4 protective genotypes. Significant findings remained noteworthy in the haplotype TGGGC when compared to reference haplotype TGAAC.

Effect of SNPs on gene expression (eQTLs) and splicing (sQTLs)
We further used GTEx to analyze the expression quantitative trait loci (eQTLs) and splicing quantitative trait loci (sQTLs) of rs1064034 and rs298982. Interestingly, rs1064034 was significantly associated with mRNA expression of RP11-384K6.6 in the whole blood (Fig. 1A) and cells-cultured fibroblasts (Fig. 1B), as well as SNHG8 in cells-cultured fibroblasts (Fig. 1C). We found that the rs1064034 could affect the splicing events of RP11-384K6.6 (Fig. 1D) and SNHG8 (Fig. 1E) genes.
in cells-cultured fibroblasts. Similarly, rs298982 was significantly associated with mRNA expression of \textit{RP11-384K6.6} in the whole blood (\(P = 3.9 \times 10^{-9}\)) and cells-cultured fibroblasts (\(P = 9.4 \times 10^{-9}\)), as well as \textit{SNHG8} mRNA level in cells-cultured fibroblasts (\(P = 1.8 \times 10^{-6}\)). rs1064034 can affect the splicing events of \textit{RP11-384K6.6} (\(P = 8.7 \times 10^{-7}\)) and \textit{SNHG8} (\(P = 4.3 \times 10^{-6}\)) genes in cells-cultured fibroblasts.

\textbf{Discussion}  
This is the first genetic epidemiological study on the association of genetic variants in the \textit{METTL14} gene and Wilms tumor risk. We found that common variants in the \textit{METTL14} gene were significantly associated with susceptibility to this malignancy. This study may contribute to uncovering the underlying biology and genetics of Wilms tumor.

\textit{METTL14} is a key component of the m\(^6\)A methyltransferase complex. \textit{METTL14} has different roles in different tumors and can be either a cancer promoter or suppressor. Chen et al. [36] identified \textit{METTL14} as a tumor suppressor in colorectal cancer. The low \textit{METTL14} was significantly associated with poor overall survival. Further functional experiments demonstrated that \textit{METTL14} inhibited the progression of colorectal cancer by regulating the production process of m\(^6\)A-dependent precursor miR-375. Ma et al. [37] found that \textit{METTL14} was remarkably downregulated in hepatocellular carcinoma. The reduced \textit{METTL14}
expression was significantly associated with unfavorable recurrence-free survival and overall survival. The inhibitory role of METTL14 on hepatocellular carcinoma may be partly attributed to its facilitation of the primary miR-126 maturation in a m6A-dependent manner. METTL14 exerted an oncogenic role in acute myeloid leukemia via mRNA m6A modification [38]. Lang et al. [39] observed that METTL14 was an important driver in EBV-induced oncogenesis. They found that knockdown of METTL14 caused a decreased tumorigenic activity of EBV-transformed cells in the xenograft animal model systems. METTL14 could promote the growth and metastasis of pancreatic cancer by up regulating the m6A level of PERP mRNA [40].

Since the function and mechanism of m6A modification in mammals have not been studied for a long time, the effect of SNPs of m6A modification genes on genetic susceptibility to tumors has been hardly understood. Through adopting a two-stage case-control study, Meng et al. [41] conducted the first study to explore whether m6A gene SNPs could predispose to colorectal cancer in the Chinese population. All the five METTL14 gene SNPs (rs115267066, rs167246, rs2029399, rs298981, and rs441216) failed to show impacts on colorectal cancer risk. By enrolling 898 patients with neuroblastoma and 1734 controls, our group found that the METTL14 gene rs298982 G > A and rs62328061 A > G could significantly reduce the risk of neuroblastoma in children, while rs9884978 G > A and rs4834698 T > C could significantly increase the risk of neuroblastoma [28]. Regarding Wilms tumor, no studies investigating the role of METTL14 gene SNPs were available by far.

In the current study, rs1064034 and rs298982 variant alleles were found to protect from developing Wilms tumor. The combination of five protective genotypes led to a 0.69-fold decrease in the risk of developing Wilms tumor in comparison to 0–4 protective genotypes, indicating the stronger effect of the combined SNPs. It is believed that association studies based on haplotypes of multiple SNPs instead of individual SNP remarkably strengthen the power for mapping and characterizing disease-causing genes [42, 43]. Thus, we examined whether haplotypes of METTL14 gene are associated with Wilms tumor risk. Expectedly, METTL14 gene haplotypes showed a significantly increased protection against Wilms tumor, indicating the synergistic effects of these SNPs. Genetic variation can modulate gene expression, thereby affecting phenotypes and susceptibility to complex diseases such as Wilms tumor. Here we harnessed the GTEx database to evaluate the effect of SNPs rs1064034 and rs298982 on expression and alternative splicing events of genes. We found that rs1064034 and rs298982 were significantly correlated with the expression and splicing of its nearby genes SNHG8 and RP11-384K6.6. LncRNA SNHG8 acts as a vital role in tumorigenesis [44–48]. Thus, it is biologically possible that changes of the expression and
splicing of SNHG8 and RP11-384 K6.6 caused by SNP rs1064034 and rs298982 may influence Wilms tumor risk (Fig. 3). Our results bring new insights into genetic mechanisms of how METTL14 affects Wilms tumor risk. Our findings identify METTL14 gene SNPs as risk markers in pediatric Wilms tumor. These findings not only show the relationship between some METTL14 gene SNPs and Wilms tumor risk but also can help to improve risk stratification strategies for Wilms tumor patients. In all, in-depth mechanism of how METTL14 SNPs affects Wilms tumor risk by regulating the gene expression and splicing pattern awaits to be elucidated. Potential limitations of our study include relatively small sample size, a lack of independent validation, and failure to incorporate other confounders. We also acknowledged that the conclusion obtained here was limited to Chinese. Cautions should be taken when interpreting this conclusion in other populations.

Conclusion
In summary, we demonstrated the significant effects of METTL14 gene SNPs on the risk of Wilms tumor. However, further validation studies with larger sample size and involving different populations are required to strengthen this association.

Abbreviations
m6A: N6-methyladenosine; HWE: Hardy-Weinberg equilibrium; ORs: Odds ratios; CIs: Confidence intervals; FPRP: False-positive report probability analysis; eQTL: Expression quantitative trait loci; sQTL: Splicing quantitative trait loci; GTEx: Genotype-Tissue Expression; AS: Alternative splicing.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12885-021-09019-5.

Received: 2 June 2021 Accepted: 18 November 2021
Published online: 04 December 2021

Acknowledgements
Not applicable.

Authors’ contributions
Conceptualization: HX, GL and JH; Data curation: JH; Formal analysis: ZZ and JH; Funding acquisition: RH, WF, HX, and JH; Investigation: ZZ, RH, HZ, WJ, HL, WF and JH; Methodology: JHZ and JH; Project administration: JHZ; Resources: ZZ and JH; Supervision: ZZ and JH; Validation: ZZ and RH; Visualization: ZZ; Roles/Writing - original draft: ZZ, JHZ and JH; Writing - review & editing: All authors. All authors had given final approval of the version to be published.

Funding
This work was supported by grants from the National Natural Science Foundation of China (No. 82003523, 81803520), National Science Foundation of Guangdong Province (No. 2021A1515010860), and Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease (No. 2019B030301004).

Availability of data and materials
All data and material are available from the corresponding author on reasonable request. The datasets generated or analyzed during the current study are not publicly available but are available with the corresponding author and can be provided on reasonable request.

Declarations
Ethics approval and consent to participate
Written informed consent was signed by all subjects' guardians. The study was carried out based on the principles of the Declaration of Helsinki. Approval of the study protocol was obtained from the institutional review board of Guangzhou Women and Children's Medical Center (Ethics Approval No: 202016600).

Consent for publication
Not applicable.

Competing interests
The author(s) declare that they have no conflict of interest.

Author details
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, 9 Jinsi Road, Guangzhou 510623, Guangdong, China. 2 Department of Gynaecology and Obstetrics, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China. 3 Faculty of Medicine, Macau University of Science and Technology, Macau 999078, China. 4 Department of Clinical Laboratory, Biobank, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China. 5 Department of Pediatric Surgery, the Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an 710068, Shaanxi, China. 6 Department of Pediatric Surgery, the First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan, China. 7 Department of Pathology, Children Hospital and Women Health Center of Shanxi, Shannxi, Taiyuan 030013, China. 8 Department of Hematology, The Second Affiliated Hospital and Vuying Children's Hospital of Wenzhou Medical University, Wenzhou 325027, Zhejiang, China.

References
1. Aldrink JH, Heaton TE, Dasgupta R, Lautz TB, Malek MM, Abdessalam SF, et al. Update on Wilms tumor. J Pediatr Surg. 2019;54:390–7.
2. Phelps HM, Kavanya S, Borinstein SC, Lovorn HN 3rd. Biological Drivers of Wilms Tumor Prognosis and Treatment. Children (Basel). 2019;5:145.
3. Breslow N, Olshan A, Beckwith JB, Green DM. Epidemiology of Wilms tumor. Med Pediatr Oncol. 1993;21:112–81.
4. Bao PP, Li K, Wu CX, Huang ZZ, Wang CF, Xiang YM, et al. Recent incidences and trends of childhood malignant solid tumors in Shanghai, 2002–2010. Zhonghua Er Ke Za Zhi. 2013;51:288–94.
5. Hohenstein P, Pritchard-Jones K, Charlton J. The yin and yang of kidney development and Wilms’ tumors. Genes Dev. 2015;29:467–82.
6. Dome JS, Graff N, Geller J, Fernandez CV, Mullen EA, Spreafico F, et al. Advances in Wilms tumor treatment and biology: Progress through international collaboration. J Clin Oncol. 2015;33:2999–3007.
7. Spiegel HR, Murphy AJ, Yanishevski D, Brennan RC, Li C, Lu Z, et al. Complications following nephron-sparing surgery for Wilms tumor. J Pediatr Surg. 2020;55:126–9.
8. Saltzman AF, Carrasco A Jr, Amini A, Cost NG. Patterns of care and survival comparison of adult and pediatric Wilms tumor in the United States: a study of the National Cancer Database. Urology. 2020;135:50–6.
9. Sonn G, Shortliffe LM. Management of Wilms tumor: current standard of care. Nat Clin Pract Urol. 2008;5:551–60.
10. Wong KF, Reulen RC, Winter DL, Guha J, Fidler MM, Kelly J, et al. Risk of adverse health and social outcomes up to 50 years after Wilms tumor: the British childhood Cancer survivor study. J Clin Oncol. 2016;34:1772–9.
11. Haber DA, Buckler AJ, Glaser T, Call KM, Pelletier J, Sohn RL, et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms’ tumor. Cell. 1990;61:1257–69.

12. Pelletier J, Bruening W, Li FP, Haber DA, Glaser T, Housman DE. WT1 mutations contribute to abnormal genital system development and hereditary Wilms’ tumour. Nature. 1991;353:431–4.

13. Treger TD, Chowdhury T, Pritchard-Jones K, Behati S. The genetic changes of Wilms tumour. Nat Rev Nephrol. 2019;15:240–51.

14. Ruesho¨ user EC, Robinson SM, Huff V. Wilms tumor genetics: mutations in WT1, WTX, and CNOT1 account for only about one-third of tumors. Genes Chromosomes Cancer. 2008;47:461–70.

15. Turnbull C, Perdeaux ER, Pernet D, Naranjo A, Renwick A, Seal S, et al. A genome-wide association study identifies susceptibility loci for Wilms tumor. Nat Genet. 2012;44:681–4.

16. Fu W, Zhuo Z, Hua RX, Fu K, Jia W, Zhu J, et al. Association of KRAS and NRAS gene polymorphisms with Wilms tumor risk: a four-center case-control study. Aging (Albany NY). 2019;11:1551–63.

17. Liu GC, Zhuo ZJ, Zhu SB, Zhu J, Jia W, Zhao Z, et al. Associations between LMO1 gene polymorphisms and Wilms’ tumor susceptibility. Oncotarget. 2017;8:50665–72.

18. Liu F, Zhuo Z, Li W, Cheng J, Zhou H, He J, et al. TP53 rs1042522 C>G polymorphism and Wilms tumor susceptibility in Chinese children: a four-center case-control study. J Pediatr Hematol Oncol. 2019;31:256–8.

19. Ferrara M, Capozzi L, Russo R. Impact of the MTHFR C677T polymorphism on risk of Wilms tumor: case-control study. J Pediatr Hematol Oncol. 2017;109:31–9.

20. Cao G, Li HB, Yin Z, Flavell RA. Recent advances in dynamic m6A RNA modification. Open Biol. 2016;6:160003.

21. Lang F, Singh RK, Pei Y, Zhang S, Sun K, Robertson ES. EBV epitranscriptional regulation of METTL14. PLoS Pathog. 2019;15:e1007796.

22. Wang M, Liu J, Zhao Y, He R, Xu X, Guo X, et al. Upregulation of METTL14 mediates the elevation of PERP mRNA N (6) adenosine methylation promoting the growth and metastasis of pancreatic cancer. Mol Cancer. 2020;19:130.

23. Tao L, Mu X, Chen H, Jin D, Zhang R, Zhao Y, et al. FTO modifies the m6A modification of the TRIM7 positively regulates tumorigenesis and chemoresistance in osteosarcoma through ubiquitination of BRMS1. Mol Ther. 2019;28:609–22.

24. Cheng C, Su Z, Xiao F, Zhang X, Wang H, et al. EBV epitranscriptional regulation of METTL14 is critical for viral-associated tumor development and metabolism. PLoS Pathog. 2019;15:e1007796.

25. Song H, Song J, Liu L, Li S. SNHG8 is upregulated in esophageal squamous cell carcinoma and directly sponges microRNA-411 to inhibit the growth of esophageal squamous cell carcinoma. Oncotarget. 2017;65:529–43.

26. Zhuo Z, Lu H, Zhu J, Hua RX, Ge L, Zhu J, Yuan L, Chen C, et al. LIN28A gene polymorphisms confer neuroblastoma susceptibility in Chinese children. Cancer Med. 2019;8:50665–72.

27. Zhuo Z, Zhou C, Fang Y, Zhu J, Lu H, Zhou H, et al. Correlation between the ERCC1/XPF gene polymorphisms and Wilms’ tumor susceptibility. Onco Targets Ther. 2019;12:144839.

28. Zhuo Z, Lu H, Zhu J, Hua RX, Li Y, Yang Z, et al. METTL14 gene polymorphisms and Wilms’ tumor susceptibility. Aging (Albany NY). 2019;11:1551–63.

29. Zhuo ZJ, Liu W, Zhang J, Zhu J, Zhang R, Tang J, et al. Functional polymorphisms at ERCC1/XPF genes confer neuroblastoma risk in Chinese children. Mol Carcinog. 2013;52(Suppl 1):E70–9.

30. Zhuo Z, Zhou C, Fang Y, Zhu J, Lu H, Zhou H, et al. Correlation between the ERCC1/XPF gene polymorphisms and Wilms’ tumor susceptibility. Aging (Albany NY). 2019;11:1551–63.

31. Zhuo ZJ, Liu W, Zhang J, Zhu J, Zhang R, Tang J, et al. Functional polymorphisms at ERCC1/XPF genes confer neuroblastoma risk in Chinese children. Mol Carcinog. 2013;52(Suppl 1):E70–9.