RESEARCH ARTICLE

ALKBH5 gene polymorphisms and Wilms tumor risk in Chinese children: A five-center case-control study

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Abstract

Background: Wilms tumor is a frequently diagnosed renal cancer among children with unclear genetic causes. N6-methyladenosine (m6A) modification genes play critical roles in tumorigenesis. However, whether genetic variations of m6A modification genes predispose to Wilms tumor remain unclear. ALKBH5 (AlkB homolog 5), a crucial member of m6A modification genes, encodes a demethylase that functions to reverse m6A RNA methylation.

Methods: Herein, we evaluated the association of single nucleotide polymorphisms (SNPs) in the m6A modification gene ALKBH5 and Wilms tumor susceptibility in a large multi-center case-control study. A total of 414 Wilms tumor cases and 1199 healthy controls were genotyped for ALKBH5 rs1378602 and rs8400 polymorphisms by TaqMan.

Results: No significant association was detected between these two polymorphisms and Wilms tumor risk. Moreover, 1, 2, and 1-2 protective genotypes (rs1378602 AG/AA or rs8400 GG) did not significantly reduce Wilms tumor risk, compared with risk genotypes only. Stratification analysis revealed a significant relationship between rs1378602 AG/AA genotypes and decreased Wilms tumor risk in children in clinical stage I diseases [adjusted odds ratio (OR) = 0.56, 95% confidence interval (CI) = 0.32-0.98, P = .042]. The presence of 1-2 protective genotypes was correlated with
1 | INTRODUCTION

Wilms tumor is a common embryonal kidney that mostly affects children. It is typically characterized by the disorganized and dysregulated development of a kidney. The prevalence of Wilms tumor is about 7-10 per million in Western countries, while it is only 3.3 per million in China. Nearly 95% of cases are diagnosed under ten years old, with mean diagnosis age at 43-48 months. Survival rates of Wilms tumor in Western countries have reached over 90%, while the survival rate for relapsed cases is much lower. What is more frustrated, survivors may be subject to chronic severe health conditions. Strong evidence has been increasingly added in supporting the contribution of genetic variants to Wilms tumor. The Wilms tumor 1 (WT1) gene, mapped to chromosome 11p13, was first identified in 1990 as a tumor suppressor gene in Wilms tumor. Subsequently, mutations in the WTX, CTNNB1, and TP53, as well as abnormalities of 11p15 methylation have been discovered in Wilms tumor. Apart from these, many other novel gene mutations also have been revealed to be involved in Wilms tumorigenesis. However, all of the above gene mutations affect fewer than 50% of Wilms tumor cases. Therefore, identifying more variants is indispensable in better understanding the Wilms tumor susceptibility.

N6-methyladenosine (m^6^A) is the most prevalent and enriched mRNA post-transcriptional modification. First discovered in 1974, m^6^A modification is now found to be extensively spread in both prokaryotes and eukaryotes. The m^6^A modification-related enzymes are mainly composed of m^6^A methyltransferase ("writers": METTL3, METTL14, and WTAP), m^6^A demethylases ("erasers": FTO and ALKBH5), and m^6^A-binding proteins ("readers": IGF2BP1 and YTHDF1). The m^6^A modification plays a critical role in mRNA stability, mRNA translation, and many other important processes. Dysregulated m^6^A is closely related to various diseases, especially cancers. Genetic variants in the m^6^A genes may change the RNA sequences of the target sites or key flanking nucleotide and thereby influence m^6^A modification. The aberrant m^6^A modification level may have impacts on individuals’ cancer susceptibility. The genetic variants in the m^6^A genes are referred to as the m^6^A-associated SNPs (m^6^A-SNPs). The m^6^A modification has become a research hotspot yet studies on the association between m^6^A-SNPs and cancer risk are very scarce. Therefore, it is urgent to explore the effect of m^6^A-SNPs on cancer risk, which can provide a new perspective of not only the etiology of cancer but also of the role of m^6^A.

The roles of m^6^A modification gene ALKBH5 SNPs were recently investigated in the risk of major depressive disorder, rheumatoid arthritis, and colorectal cancer. Till now, no studies have explored the potential relationship of m^6^A modification gene ALKBH5 SNPs with Wilms tumor risk. In this study, we conducted the first case-control study of 414 Wilms tumor cases and 1199 controls to yield new insights into the role of m^6^A modification gene ALKBH5 SNPs in Wilms tumorigenesis.

2 | METHODS

2.1 | Study subjects

Wilms tumor cases were enrolled from five hospitals located in Guangzhou, Zhengzhou, Wenzhou, Xi’an, and Taiyuan, respectively. The current study included a total of 414 cases and 1199 controls of Chinese ancestry, matched on age and gender (Table S1). All Wilms tumor cases were newly diagnosed and pathologically confirmed. No preoperative treatment such as chemotherapy or radiation was performed on the cases before the collection of the blood sample. Healthy controls were recruited in the same period and geographical region. We obtained written informed consent from all participants’ parents or guardians prior to enrolment. Recruitment details and patient characteristics were available in the previously published study. The study was approved by the Ethics Committee of Guangzhou Women and Children’s Medical Center.

2.2 | Genotyping

Potentially functional SNPs in ALKBH5 were chosen from the dbSNP database following the criteria described in our previous studies. Briefly, criteria were as follows: (a) located at the two ends of the ALKBH5 gene (ie, the 5’ near gene, 5’ UTR, 3’ UTR and 3’ near gene); (b) the minor allele frequency (MAF) reported in 1000 Genomes (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/)
was ≥ 5% for Chinese Han subjects; and (c) affecting transcription factor binding sites (TFBS) activity or the miRNA binding sites activity. As a result, two SNPs (rs1378602 and rs8400) met these criteria. Genomic DNA was extracted from participants’ blood. Samples were genotyped for the rs1378602 and rs8400 SNPs by a custom ria. Genomic DNA was extracted from participants’ blood. Samples were re-genotyped for the SNPs to assess the genotyping error rate. All re-genotyped SNPs achieved 100% concordance.

2.3 | Statistical analysis

Characteristics of cases and controls were compared using the chi-square test or t test as appropriate. Compliance of individual SNPs with the Hardy-Weinberg equilibrium was measured in controls using a chi-square test. To estimate the association of SNP with Wilms tumor risk, we conducted the unconditional logistic regression analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the association. We also investigated the effect of ALKBH5 gene SNPs on Wilms tumor susceptibility across strata of age, sex, and clinical stage. False-positive report probability (FPRP) analysis was further explored to test whether the significant findings were just chance or noteworthy observations. All tests for statistical significance used a two-sided P < .05. Statistical analyses were completed in SAS 9.1 (SAS Institute Inc).

3 | RESULTS

3.1 | Association between the ALKBH5 SNPs and Wilms tumor risk

Patient characteristics were summarized in our previous publication.33 The results of the correlation between ALKBH5 gene polymorphisms and Wilms tumor susceptibility were presented in Table 1. Finally, 413 cases and 1198 controls were successfully genotyped for rs1378602 and rs8400. The genotype frequencies of both SNPs were in accordance with the Hardy-Weinberg equilibrium in control subjects (P = .488 for rs1378602 and P = .963 for rs8400). Neither of these two polymorphisms displayed a significant association with Wilms tumor risk. We then regarded rs1378602 AG/AA or rs8400 GG genotypes as protective genotypes to further explore the combined effects of the two SNPs. However, carriers with 1, 2, and 1-2 protective genotypes did not have a lower risk in Wilms tumor than those without protective genotype.

3.2 | Stratification analysis

We further performed a stratified analysis by age, gender and clinical stages (Table 2). The protective effect of rs1378602 AG/AA genotypes was pronounced in the subgroup of children with clinical stage I diseases (adjusted OR = 0.56, 95% CI = 0.32-0.98, P = .042). However, no significant association with Wilms tumor risk was found for the rs8400 in the stratification analysis. In subgroups of age > 18 months, the existence of 1-2 protective genotypes was associated with 0.74-fold decreased risk of Wilms tumor, when compared to 0 protective genotypes (adjusted OR = 0.74, 95% CI = 0.56-0.98, P = .035).

3.3 | False-positive report probability results

We preset 0.2 as the FPRP threshold. As shown in Table S2, at the prior probability of 0.1, all of the significant findings disappeared. At a prior probability level of 0.25, the decreased Wilms tumor risk remains noteworthy in carriers with protective genotypes 1-2 for the children > 18-month subgroup.

4 | DISCUSSION

This work was motivated by the discovery of m6A modification genes as critical cancer regulators and the emerging role of m6A gene SNPs in cancer susceptibility. Thus, we proposed a potential contributing role of m6A SNPs in Wilms tumor risk. Herein, we attempted to investigate whether ALKBH5 gene SNPs could link to the risk of Wilms tumor. Our data suggested a weak association between ALKBH5 gene SNPs and Wilms tumor risk in Chinese children. To date, this is the first report focusing on the association between the ALKBH5 gene SNPs and Wilms tumor risk.

The m6A demethylases include FTO and ALKBH5, both of which belong to the AlkB family.36 ALKBH5 was firstly found to have demethylation activity in 2013.24 FTO-mediated m6A demethylation generates two intermediates, N6-hydroxymethyladenosine (hm6A) and N6-formyladenosine (fA), which were finally hydrolyzed into adenine.37,38 Unlike FTO, ALKBH5 catalyzes the direct removal of m6A without generating an intermediate.36 Silencing of ALKBH5 led to the increase in the total m6A levels on RNA as well as the boost of RNAs exportation from the nucleus to the cytoplasm.24 Moreover, ALKBH5 also significantly affects RNA metabolism and the assembly of mRNA processing factors.24 ALKBH5 is critically implicated in the development and progression of several malignancies. Zhang et al found that expression of ALKBH5 was upregulated in glioblastoma stem-like cells (GSCs). ALKBH5 regulates FOXM1 gene expression, consequently affecting GSC tumorigenesis. Enhanced ALKBH5 induced by hypoxia decreases the level of methylated NANOG mRNA. The increased NANOG protein levels promote the enrichment of breast cancer stem cell (BCSC) population. Conversely, knockdown of ALKBH5 impairs tumor formation in vivo by decreasing hypoxia-induced NANOG expression and BCSC enrichment.41 It was also reported that overexpression of ALKBH5 promotes invasion and metastasis of gastric cancer by demethylating the IncRNA NEAT1.42 Panneerdoss et al revealed that ALKBH5 exerts its pro-tumorigenic
role by regulating m^6^A levels of angiogenesis-associated and epithelial-mesenchymal transition transcripts. They provided evidence that collaboration among writers, erasers, and readers regulates cancer growth and progression. Although the significance of the m^6^A gene in cancer is highly appreciated, the study of m^6^A-SNPs is a nascent field as yet.

FTO, as well as its SNPs, were revealed to be strongly associated with various human diseases, mainly obesity, and cancer. Unlike FTO, information of ALKBH5 SNPs was still limited. Only until recently has it begun to be realized that m^6^A-SNPs in the ALKBH5 account for genetic predisposition to complex traits, such as cancer. Du et al.\(^{30}\) reported that SNP rs12936694 in the FTO gene plays a significant role in conferring to the risk of major depressive disorder in the Chinese Han population. A recent study has shown that 21 SNPs in the ALKBH5 gene were significantly associated with the risk of rheumatoid arthritis in Asian and European populations.\(^{31}\) Most recently, Meng et al. performed the first case-control study regarding m^6^A SNPs and cancer risk. Their study comprised of two stages, discovery stage with 1150 colorectal cancer cases and 1342 controls, and validation stage with 932 colorectal cancer cases and 966 controls. They comprehensively analyzed 240 SNPs in 20 m^6^A modification-related genes. Among them, only the SND1 gene rs118049207 contributes to the development of colorectal cancer in the Chinese population. They circumstantiatiated that rs118049207 change the mRNA of SND1 gene, and then lead to m^6^A level alteration. SNPs rs2124370, rs8400, rs9899249, rs9913266, and rs2925137 in the ALKBH5 gene were not associated with colorectal cancer risk.\(^{32}\) Given that FTO-SNPs are involved in cancer risk, we have reason to believe that ALKBH5 gene SNPs exert a similar role. Due to extremely low prevalence, studies specifically in this area of Wilms tumor have not been conducted. Thus, it is of a great necessity to investigate the association between ALKBH5 gene SNPs and the risk of Wilms tumor. The current clinical analysis provided only a weak impact of ALKBH5 gene SNPs on susceptibility to Wilms tumor. We speculate the insufficient statistical power caused by the moderate sample size, relative weak effects of single polymorphism, and the influence of other potential pertinent factors may work together to generate such results. To be noted, positive associations were only detected for rs1378602 AG/AA or rs8400 GG genotypes.

### TABLE 1 Association between ALKBH5 gene polymorphisms and Wilms tumor susceptibility

| Genotype         | Cases (N = 413) | Controls (N = 1198) | P\(^a\) | Crude OR (95% CI) | P | Adjusted OR (95% CI)\(^b\) | P\(^b\) |
|------------------|-----------------|---------------------|--------|------------------|---|------------------------------|------|
| rs1378602 (HWE = 0.488) |                 |                     |        |                  |   |                              |      |
| GG               | 352 (85.23)     | 991 (82.72)         | 1.00   | 0.85 (0.62-1.17) | .319 | 0.84 (0.61-1.15)             | .281 |
| AG               | 59 (14.29)      | 195 (16.28)         | 0.47 (0.11-2.11) | 0.323 | 0.46 (0.10-2.05) | .307 |
| AA               | 2 (0.48)        | 12 (1.00)           |        |                  |   |                              |      |
| Dominant         | 61 (14.77)      | 207 (17.28)         | 0.83 (0.61-1.13) | 0.238 | 0.82 (0.60-1.12) | .205 |
| Recessive        | 411 (99.52)     | 1186 (99.00)        | 0.48 (0.11-2.16) | 0.339 | 0.47 (0.10-2.11) | .324 |
| rs8400 (HWE = 0.963) |                 |                     |        |                  |   |                              |      |
| GG               | 136 (32.93)     | 403 (33.64)         | 1.00   | 1.04 (0.81-1.34) | .749 | 1.04 (0.81-1.33)             | .783 |
| AG               | 205 (49.64)     | 583 (48.66)         | 1.01 (0.72-1.40) | 0.970 | 1.00 (0.72-1.40) | .986 |
| AA               | 72 (17.43)      | 212 (17.70)         | 1.01 (0.86-1.19) | 0.911 | 1.01 (0.86-1.18) | .933 |
| Dominant         | 277 (67.07)     | 795 (66.36)         | 1.03 (0.81-1.31) | 0.793 | 1.03 (0.81-1.30) | .825 |
| Recessive        | 341 (82.57)     | 986 (82.30)         | 0.98 (0.73-1.32) | 0.904 | 0.98 (0.73-1.32) | .905 |

**Protective genotypes**

|     |   |     |     |
|-----|---|-----|-----|
| 0   |   |     |     |
| 1   |   |     |     |
| 2   |   |     |     |
| 1-2 |   |     |     |

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

\(^a\)chi-square test for genotype distributions between Wilms tumor cases and cancer-free controls.

\(^b\)Adjusted for age and gender.

Protective genotypes were carriers with rs1378602 AG/AA or rs8400 GG genotypes.
**TABLE 2** Stratification analysis of ALKBH5 gene polymorphisms with Wilms tumor susceptibility

| Variables | rs1378602 (cases/controls) | rs8400 (cases/controls) | Protective genotypes (cases/controls) |
|-----------|-----------------------------|-------------------------|--------------------------------------|
|           | GG | AG/AA | AOR (95% CI) | P | GG | AG/AA | AOR (95% CI) | P | 0 | 1-2 | AOR (95% CI) | P |
| Age, month |    |       |             |   |    |       |             |   |    |     |             |   |
| ≤18       | 118/377 | 25/89 | 0.90 (0.55-1.47) | .673 | 53/147 | 90/319 | 0.79 (0.54-1.17) | .245 | 66/230 | 77/236 | 1.13 (0.77-1.64) | .535 |
| >18       | 234/614 | 36/118 | 0.78 (0.52-1.16) | .220 | 83/256 | 187/476 | 1.22 (0.90-1.65) | .199 | 151/358 | 119/374 | 0.74 (0.56-0.98) | .035 |
| Gender    |    |       |             |   |    |       |             |   |    |     |             |   |
| Females   | 166/417 | 28/102 | 0.69 (0.44-1.09) | .112 | 61/172 | 133/347 | 1.09 (0.76-1.55) | .654 | 105/245 | 89/274 | 0.76 (0.54-1.05) | .099 |
| Males     | 186/574 | 33/105 | 0.96 (0.63-1.47) | .856 | 75/231 | 144/448 | 0.99 (0.72-1.37) | .966 | 112/343 | 107/336 | 0.97 (0.71-1.31) | .837 |
| Clinical stages | |       |             |   |    |       |             |   |    |     |             |   |
| I         | 122/991 | 15/207 | 0.56 (0.32-0.98) | .042 | 52/403 | 85/795 | 0.81 (0.56-1.17) | .256 | 70/588 | 67/610 | 0.92 (0.64-1.31) | .638 |
| II        | 93/991 | 23/207 | 1.15 (0.71-1.86) | .576 | 32/403 | 84/795 | 1.31 (0.84-2.00) | .214 | 61/588 | 55/610 | 0.87 (0.59-1.27) | .459 |
| III       | 81/991 | 13/207 | 0.79 (0.43-1.44) | .438 | 29/403 | 65/795 | 1.17 (0.74-1.84) | .507 | 52/588 | 42/610 | 0.77 (0.51-1.18) | .229 |
| IV        | 40/991 | 9/207 | 1.09 (0.52-2.29) | .821 | 13/403 | 36/795 | 1.42 (0.75-2.72) | .284 | 28/588 | 21/610 | 0.72 (0.40-1.28) | .262 |
| I + II    | 215/991 | 38/207 | 0.81 (0.56-1.18) | .279 | 84/403 | 169/795 | 1.00 (0.75-1.33) | .995 | 131/588 | 122/610 | 0.89 (0.68-1.17) | .418 |
| III + IV | 121/991 | 22/207 | 0.89 (0.55-1.43) | .620 | 42/403 | 101/795 | 1.24 (0.85-1.82) | .267 | 80/588 | 63/610 | 0.76 (0.53-1.07) | .115 |

Abbreviations: AOR, adjusted odds ratio; CI, confidence interval.
The values that are 95% CIs excluded 1 or P<.05 are indicated in bold.
*aAdjusted for age and gender, omitting the corresponding stratify factor.
The strengths of our study include its good design, multicentric analysis, and relatively large sample size. However, we cannot neglect its accompanied shortcomings. First, although our study was large, the stratified analyses were still limited in power due to the relatively small sample size. The significant findings might be chance observations (FPRP values larger than 0.2 at the prior probability level of 0.1). Therefore, the conclusion obtained here must be viewed as preliminary and needs to be confirmed. Second, all the included participants were Chinese based population. The single population here limits the applicability of the findings to other ethnicities. Last, the current study focuses on only the relationship of m^6A-SNPs with cancer risk. The specific mechanisms underlying the effect of the abovementioned m^6A-SNPs genotypes on cancer risk should be investigated.

To the best of our knowledge, this is the first large-scale and multi-center evaluation of SNPs of key candidate genes involved in the m^6A pathway and Wilms tumor susceptibility. The observed association should be further validated in another well-designed analysis with other larger ethnicities.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

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