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METTL1 gene polymorphisms and Wilms tumor susceptibility in Chinese children: A five-center case-control study

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To the Editor: Wilms tumor, or nephroblastoma, is the most frequently diagnosed pediatric kidney cancer, accounting for >90% of all renal tumors in children 1–7 years old, and the disease may be related to genetic factors.[1] As an emerging research spot, the epitranscriptome has been implicated in different RNA modifications in human diseases, including cancers.[2] Methyltransferase-like 1 (*METTL1*), a critical RNA N7-methylguanosine (m7G) methyltransferase, can catalyze the formation of a m7G modification (mostly at nucleotide position 46 in the variable region of transfer RNAs [tRNAs]) by complexing with WD repeat domain 4 (*WDR4*). Depending on the different positions, the tRNA m7G modifications have various functions. In particular, the tRNA modifications located in the anticodon region play a crucial role in translation and growth, while those outside the anticodon region are responsible for the regulation of tRNA folding, stability, and protein synthesis. The m7G modification is evolutionarily conserved and has more complicated and important physiological functions in mammals.[3] Increasing evidence strongly supports that the dysregulation or mutation of *METTL1* m7G methyltransferase is closely associated with tumorigenesis and poor prognosis.[2] Mechanistically, an underlying molecular mechanism has been identified for how *METTL1*-mediated m7G modification leads to malignant transformation and tumorigenesis. Orellana *et al*[2] discovered that an m7G modification of tRNAs is upregulated in certain cancers, and this process is catalyzed by the *METTL1/WDR4* complex. The m7G-modified tRNAs can decode the corresponding oncogenic and cell cycle-regulating messenger RNAs (mRNAs) that are enriched in m7G-tRNAs-dependent codons without ribosomal collisions, thereby facilitating the translation of these mRNAs and potential malignant transformations of cells.

Considering that the transformation of normal cells into cancer cells is an important factor in the initiation of cancer, we hypothesized that the *METTL1*-mediated m7G modification that leads to malignant transformations of cells and tumorigenesis may also be a potential mechanism for *METTL1* involvement in the development of Wilms tumor [Supplementary Figure 1, http://links.lww.com/CM9/B595]. The single-nucleotide polymorphisms (SNPs) of *METTL1* genes may be correlated with the alteration of Wilms tumor risk. To date, the relationship between *METTL1* gene polymorphisms and Wilms tumor has never been described. Therefore, we carried out a large case-control study to explore their relationship and the etiology of Wilms tumor.

A total of 414 patients with Wilms tumor and 1199 age- and sex-matched healthy controls were included in this case-control study [Supplementary Table 1, http://links.lww.com/CM9/B595]. All the subjects were genetically unrelated and Han Chinese. Cases were diagnosed with a Wilms tumor and were confirmed by histopathology. Controls were healthy volunteers that were recruited from the same hospital as the cases were during the same period, and the control individuals did not have a family history of Wilms tumor. All the procedures in the

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study were based on the principles of the *Declaration of Helsinki*, and the study was approved by the Ethics Committee of Guangzhou Women and Children Medical Center (No. 202016601). Written consent was obtained from the parents or other legal guardians of all the subjects.

Three potentially functional SNPs in the *METTL1* gene (rs2291617 G > T, rs10877013 T > C, rs10877012 T > G) were selected from the dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP) and SNPinfo (https://snpinfo.niehs.nih.gov/snpinfo/snpfunc.html). Genomic DNA from all the participants was extracted from their peripheral blood using the Genomic DNA kit (Tian Gen Biotech Co. Ltd., Beijing, China). Then, we genotyped the *METTL1* gene polymorphisms by TaqMan real-time polymerase chain reaction (PCR) (Applied Biosystems, Foster City, CA, USA). Ten percents of all samples were randomly selected to undergo further genotyping to improve the accuracy, and the results of the regenotyped samples were 100% concordant with the original genotypes.

A two-sided χ^2 test was performed to determine the difference in demographic characteristics and genotype frequency distribution between the cases and controls. We used a goodness-of-fit chi-squared test to assess Hardy–Weinberg equilibrium (HWE) for SNP genotype frequency in the control group. The association between *METTL1* gene polymorphisms and the risk of Wilms tumor was assessed by an odds ratio (OR) with a 95% confidence interval (CI) using a multivariate logistic regression analysis method. Statistical significance for all the tests was set at a two-sided P <0.05 with SAS 9.1 software (SAS Institute, Cary, NC, USA).

We successfully genotyped 3 *METTL1* SNPs in 400 cases and 1198 controls. All the SNP genotype frequencies were consistent with the HWE in controls ($P = 0.832$ for rs2291617 G > T, $P = 0.945$ for rs10877013 T > C, $P = 0.934$ for rs10877012 T > G). The relationship between the above SNPs and the susceptibility of Wilms tumor is shown in Table 1. None of these three SNPs were significantly associated with the risk of Wilms tumor. We then considered rs2291617 GT/TT, rs10877013 TC/CC, and rs10877012 TG/GG as protective genotypes according to the ORs. The results indicated that carriers with 1, 2, and 3 protective genotypes did not show a reduced risk of developing Wilms tumor. However, the combination of 3 protective genotypes exhibited more protective effects against Wilms tumor than that of the 0–2 protective genotypes, with an OR of 0.8.

To further explore the association between the *METTL1* gene polymorphisms and the susceptibility of Wilms tumor to age, sex, and clinical stage, we performed a stratification analysis, and the results are displayed in Supplementary Table 2, http://links.lww.com/CM9/B595. When compared to healthy controls, rs10877012 TG/GG genotypes significantly reduced the risk of developing Wilms tumor in clinical stage III (adjusted OR = 0.64, 95% CI = 0.42–0.99, $P = 0.042$) and clinical stage III + IV (adjusted OR = 0.64, 95% CI = 0.45–0.91, $P = 0.014$) subgroups. However, either the rs2291617 genotype or the rs10877013 genotype failed to decrease the risk of Wilms tumor susceptibility in the subgroups classified by age, sex, and clinical stage. Moreover, subjects carrying three protective genotypes did not show a significantly decreased risk of developing Wilms tumor compared to that of those carrying 0–2 combined protective genotypes.

Previous studies revealed that METTL1 deficiency or mutations caused the misregulation of m7G modifications, leading to genetic defects and certain cancers.[2] *METTL1*, located at human chromosome 12, forms a complex with *WDR4*, which functions as a m7G methyltransferase to catalyze the m7G modification, especially at nucleotide position 46 in the tRNA variant region in humans. To our knowledge, *METTL1* can catalyze the installation of m7G at specific sites of tRNA, ribosomal RNA (rRNA), and mRNA, which is crucial for mRNA translation and tRNA regulation folding, stability, and protein synthesis.[3] Ying *et al*[4] discovered that the expression levels of *METTL1* were obviously elevated in bladder cancer (BC) and associated with poor survival and advanced tumor stage. They indicated that the oncogenic functions of *METTL1* and *METTL1* promote the proliferation, invasion, and migration of BC cells via the *METTL1*-m7G/*EGFR*/*EFEMP1* axis, thereby contributing to BC progression. In fact, the *METTL1* gene not only served as a potential oncogene but also acted as a tumor suppressor. Pandolfini *et al*[5] demonstrated that *METTL1* methylates and positively regulates a specific subset of tumor-suppressive microRNAs (miRNAs), including the *let-7* miRNA family, to promote miRNA maturation and inhibit cancer cell migration. They found that *METTL1* knockdown in A549 cells significantly increased their cell migration capacity without affecting mRNA translation and cell proliferation. Importantly, the expression of wild-type *METTL1* rather than catalytic death mutants could rescue the increased migration, suggesting that *METTL1* has a suppressive role in tumorigenesis via the regulation of special subsets of miRNAs, particularly the *let-7* miRNA family. Hence, it is reasonable to speculate that METTL1 may be

Table 1: Associations between *METTL1* gene polymorphisms and Wilms tumor susceptibility.

	Allele		Case ($N = 400$)			Control ($N = 1198$)							
Polymorphism	A	B	AA	AB	BB	AA	AB	BB	AOR (95% CI)*	*P* value*	AOR (95% CI)†	*P* value*	HWE
rs2291617	G	T	171	175	54	470	558	170	0.86 (0.69–1.09)	0.213	0.94 (0.68–1.31)	0.724	0.832
rs10877013	T	C	185	167	48	495	551	152	0.82 (0.65–1.03)	0.088	0.94 (0.66–1.33)	0.715	0.945
rs10877012	T	G	174	177	49	462	565	171	0.82 (0.65–1.03)	0.082	0.84 (0.60–1.18)	0.307	0.934

AOR: Adjusted odds ratio; CI: Confidence interval; HWE: Hardy–Weinberg equilibrium. *Adjusted for age and gender for dominant model. †Adjusted for age and gender for recessive model.

involved in the occurrence of Wilms tumor, but we have not confirmed that *METTL1* gene SNPs play a cancer-suppressing or cancer-promoting role in Wilms tumor.

In recent years, the utility of SNPs for genome-wide association studies (GWASs) based on the principle of human genome linkage disequilibrium (LD) has validated hundreds of predisposition loci for complex diseases, including cancers. The potential mechanism for the effect of SNP variants on target gene expression is as follows. When SNP variants appear in the protein-coding region, they change the protein structure or lead to modified proteins with functional defects. In contrast, non-coding SNP variants are located in transcriptional regulatory regions, modulating the expression level of nearby or distant target genes. In fact, most variants of SNPs that are associated with cancer susceptibility are always located in non-coding regulatory regions, suggesting that transcriptional regulation plays a key role in cancer susceptibility.[6] In addition to explaining the genetic basis of cancer susceptibility, applications of SNPs can also provide many clinical benefits, including individualized drug therapy guidance, reliable biomarkers, and effective screening and disease prevention strategies.[7]

To date, few publications have investigated the effect of *METTL1* gene SNPs on genetic susceptibility to tumors. Herein, we performed the first case-control, five-center study among Han Chinese children to determine the effect of SNPs in *METTL1* genes on Wilms tumor risk. None of the three SNPs showed a meaningful association with Wilms tumor risk. Stratification analysis revealed that individuals with the rs10877012 variant alleles exhibited significantly reduced Wilms tumor risk in certain subgroups, suggesting that *METTL1* SNPs might exert a slight effect on Wilms tumor susceptibility. This result may be explained by the heterogeneity of the study population and the low frequency of high-risk genotypes, leading to reduced statistical power.

There are still several limitations in the current study. First, the sample size was not large enough, and all subjects in this study were Han Chinese, limiting the power of the stratified analysis. Second, the Wilms tumor is influenced by a combination of genetic and environmental factors, and only the SNPs in *METTL1* genes cannot fully explain the risk of the Wilms tumor. The effect of environment and lifestyle on the risk of Wilms tumor should be considered. Moreover, only three SNPs in *METTL1* genes were selected for study, and additional polymorphisms of the *METTL1* gene contributing to Wilms tumor risk need to be further investigated. In addition, we lack further experiments to validate how Wilms tumor is affected. Ultimately, all subjects in this study were Han Chinese. The findings could not directly be applied to non-Han Chinese individuals.

In summary, the current study was the first large-scale and well-designed evaluation attaching *METTL1* gene SNPs to Wilms tumor susceptibility, which only revealed a relatively weak association between *METTL1* gene polymorphisms and Wilms tumor susceptibility. The rs10877012 TG/GG genotypes significantly reduced Wilms tumor risk and protected against the progression of Wilms tumor in some special subgroups, implying that METTL1 may be a potential gene target for special genotypes in Wilms tumor patients; thus, these results provide insight for new therapeutic options. Our conclusion should be further verified in larger, well-designed case-control studies that control for other confounding factors.

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Conflicts of interest

None.

References

1. Nakata K, Colombet M, Stiller CA, Pritchard-Jones K, Steliarova-Foucher E. Incidence of childhood renal tumours: An international population-based study. Int J Cancer 2020; 147:3313–3327. doi: 10.1002/ijc.33147.
2. Orellana EA, Liu Q, Yankova E, Pirouz M, De Braekeleer E, Zhang W, *et al*. METTL1-mediated m(7)G modification of Arg-TCT tRNA drives oncogenic transformation. Mol Cell 2021; 81:3323.e–3338.e. doi: 10.1016/j.molcel.2021.06.031.
3. Tomikawa C. 7-Methylguanosine modifications in transfer RNA (tRNA). Int J Mol Sci 2018;19:4080. doi: 10.3390/ijms19124080.
4. Ying X, Liu B, Yuan Z, Huang Y, Chen C, Jiang X, *et al*. METTL1-m(7) G-EGFR/EFEMP1 axis promotes the bladder cancer development. Clin Transl Med 2021;11:e675. doi: 10.1002/ctm2.675.
5. Pandolfini L, Barbieri I, Bannister AJ, Hendrick A, Andrews B, Webster N, *et al*. METTL1 promotes let-7 microRNA processing via m7G methylation. Mol Cell 2019;74:1278.e–1290.e. doi: 10.1016/j.molcel.2019.03.040.
6. Monteiro AN, Freedman ML. Lessons from postgenome-wide association studies: Functional analysis of cancer predisposition loci. J Intern Med 2013;274:414–424. doi: 10.1111/joim.12085.
7. Freedman ML, Monteiro AN, Gayther SA, Coetzee GA, Risch A, Plass C, *et al*. Principles for the post-GWAS functional characterization of cancer risk loci. Nat Genet 2011;43:513–518. doi: 10.1038/ng.840.

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