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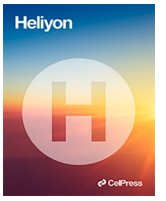

Genetic variants of m7G modification genes influence neuroblastoma susceptibility

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ABSTRACT

Objective: Neuroblastoma is a life-threatening pediatric solid tumor whose etiology remains unclear. N7-methylguanosine (m7G) is one of the most important epigenetic modifications of RNA, which plays a crucial role in tumorigenesis. The m7G-mediated genes *METTL1* and *WDR4* also have been reported to be dysregulated in various cancers. However, the implications of *METTL1* and *WDR4* in neuroblastoma have not been clarified.

Methods: Given the oncogenic potential of m7G modification, we performed a case-control study to assess the association of *METTL1* and *WDR4* genes polymorphisms with neuroblastoma risk in a Chinese population consisting of 402 cases and 473 controls. Odds ratios (ORs) and 95 % confidence intervals (CIs) were applied to evaluate the associations between studied polymorphisms and neuroblastoma risk. The adjusted odds ratio (AOR) was adjusted for age and gender.

Results: Overall, four polymorphisms were significantly associated with neuroblastoma risk, including *METTL1* rs2291617 (recessive model: adjusted OR = 1.59, 95 % CI = 1.08–2.34, P = 0.019), *WDR4* rs2156316 (dominant model: adjusted OR = 0.74, 95 % CI = 0.57–0.97, P = 0.028), *WDR4* rs6586250 (dominant model: adjusted OR = 0.59, 95 % CI = 0.42–0.84, P = 0.004) and *WDR4* rs15736 (dominant model: adjusted OR = 0.60, 95 % CI = 0.42–0.85, P = 0.004). Stratified analysis showed stronger correlations between significant polymorphisms and neuroblastoma risk among subgroups divided by age, gender, tumor origin, and clinical stage. Furthermore, expression quantitative trait loci (eQTL) analysis revealed that significant polymorphisms were associated with the expression of the adjacent genes.

Conclusions: Our study indicated that four polymorphisms in m7G-mediated genes contribute to neuroblastoma susceptibility in the eastern Chinese population. However, our findings should be verified further by large-scale and well-designed studies.

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1. Introduction

Neuroblastoma is the most common extracranial pediatric malignancy originating from embryonic sympathetic crest progenitor and composes about 10 % of all pediatric malignancies [1]. It ranks third leading cause of neoplastic death in children, accounting for almost 15 % of all cases [2]. The incidence of neuroblastoma is approximately $7.7/10^6$ in China, being considered the fourth most prevalent pediatric tumor [3]. Neuroblastoma is a highly heterogeneous disorder with diverse clinical manifestations, which can be divided into low-, intermediate- and high-risk subgroups according to the clinical symptoms, pathological characteristics, and prognosis [4]. Some low-risk patients can spontaneously recover from the disease without radiotherapy and chemotherapy. However, the prognosis of high-risk patients remains unsatisfactory, even after having undergone multiple combined therapies. In recent years, great progress has been made in treating neuroblastoma, following intensive investigations, with a five-year survival of nearly 70 %. However, its survival rate also shows a sharp downward trend with the malignant progress of neuroblastoma. The five-year survival rates of patients in stages III and IV drop to 60 % and 20 %, respectively. In addition, about half of the patients were diagnosed as high-risk patients, and their five-year survival rate was less than 40 % [2]. The widespread metastasis at the diagnosis may explain this poor prognosis [5]. Therefore, it is urgent to explore the genetic variants associated with neuroblastoma susceptibility for its early diagnosis and risk assessment to improve the therapeutic efficacy and survival rate.

To date, the etiology of neuroblastoma is not fully understood. Studies showed that the genetic variations in the *PHOX2B* [6] and *ALK* [7] genes are associated with familial neuroblastoma. However, familial neuroblastoma is rare, making up roughly 1–2% of all cases [8]. The genetic mechanisms regarding sporadic neuroblastoma remain poorly understood. Although some environmental factors such as wood dust, diesel oil, paint thinner, turpentine, and welding flux have been suggested as potential risk factors predisposing individuals to neuroblastoma [9,10], no direct evidence indicates exposure to these factors leads to neuroblastoma ultimately. And only a few offspring developed neuroblastoma after their parents were exposed to the environmental risk factors [11]. These hint to us that genetic factors, not only environmental factors, may play a decisive role in the occurrence and progression of neuroblastoma. Growing evidence showed that genetic polymorphisms might predispose individuals to neuroblastoma. In other words, the acquisition of genetic susceptibility largely determines whether the offspring suffers from neuroblastoma [8,12].

Single nucleotide polymorphism (SNP) is the most common genetic variation which may affect the genes expression or protein structure and activity, thus leading to individual differences in disease susceptibility and prognosis. With the rapid development of high-throughput sequencing technology and bioinformatic analysis, the genome-wide association study (GWAS) has become a powerful tool for studying human diseases' genetic mechanisms, including neuroblastoma. Increasing studies reported the close relationship between SNP and neuroblastoma susceptibility. Over the recent ten years, six GWASs have identified a series of neuroblastoma susceptibility SNPs in the following genes, *CASC15* [13], *BARD1* [14], *DDX4*, *DUSP12*, *HSD17B12*, *IL31RA* [15], *LMO1* [16], *HACE1*, *LIN28B* [17], *MLF1*, *CPZ* [18]. Furthermore, using candidate gene approaches, many other potential susceptibility SNPs have also been discovered in some vital functional genes, such as *FAS*, *FASL* [19], *NEFL* [20], *XPG* [21], *ALKBH5* [22], and *CDKN1B* [23]. In brief, SNP plays a key role in neuroblastoma susceptibility, and it should be one of the entry points to explore the genetic etiology of neuroblastoma.

N7-methylguanosine (m7G) is the most conservative and common modification of tRNA in prokaryotes and eukaryotes [24]. In addition, studies have found that m7G also widely exists in mRNA, miRNA, and 18 S rRNA. The m7G is referred to as methylated modification at the N7 position of guanine within various RNAs, which is catalyzed by the METTL1/WDR4 methyltransferase complex. In this process, *S*-adenosylmethionine acts as the methyl donor. Methyltransferase *METTL1* and the cofactor *WDR4* transfer the methyl to the 7th N of guanine. Lin et al. found that m7G modification is widespread in mammals. Knockout of *METTL1* or *WDR4* in mouse embryonic stem cells caused the absence of tRNA m7G modifications, which decreased mRNA translation [25]. Zhang et al. revealed that m7G modifications lead to local structure recombination of mRNA, which changed the interaction between regulatory protein and mRNA, thereby regulating the processing, splicing, translation, transport, and stability of mRNA [26]. In addition, Pandolfini et al. discovered that METTL1-mediated pri-miRNA m7G modification promotes miRNA maturation by destroying an inhibitory secondary structure [27]. Increasingly studies have proved that m7G modification is critical in oncogenesis. Pandolfini et al. reported that METTL1-mediated m7G modification is involved in the processing and maturation of mi-RNA *let-7*, inhibiting lung cancer cell migration [27]. Dai et al. demonstrated that METTL1/WDR4-mediated m7G modification of tRNA could increase the translation of downstream oncogenes *CCNA2* and *EGFR*, thus promoting the progression of intrahepatic cholangiocarcinoma [28]. Similarly, Ma et al. also demonstrated that METTL1/WDR4-mediated tRNA m7G modification improves the translation efficiency of downstream oncogenes *CCND3* and *CCNE1*, thus promoting lung cancer cell proliferation, migration, and invasion [29]. However, no study has reported the role of m7G modification in neuroblastoma. Considering the importance of RNA m7G modification and SNPs in tumorigenesis, it is reasonable to speculate that functional SNPs in *METTL1/WDR4* genes may change the expression or structure of their protein, then deregulate downstream target genes, leading to cell dysfunction and eventually carcinogenesis. We conducted this case-control study to assess the association between SNPs in the *METTL1/WDR4* genes and neuroblastoma risk in Chinese children.

2. Materials and methods

2.1. Study subjects

This case-control study involved a total of 402 histopathologically and clinically diagnosed neuroblastoma cases and 473 cancer-free controls [30,31]. All the subjects were recruited from the Jiangsu province of Eastern China. The criteria of acceptability for the

Table 1
Association of m7G modification genes and neuroblastoma risk in children from Jiangsu province.

Gene	Polymorphism	Allele		Case (N = 401)			Control (N = 473)			AOR (95 % CI)[a]	P^a	AOR (95 % CI)[b]	P^b	HWE
		A	B	AA	AB	BB	AA	AB	BB					
METTL1	rs2291617	G	T	176	157	68	206	213	54	0.99 (0.76–1.29)	0.923	**1.59 (1.08–2.34)**	**0.019**	0.925
METTL1	rs10877013	T	C	179	169	53	212	210	51	1.01 (0.77–1.32)	0.954	1.26 (0.84–1.90)	0.267	0.925
METTL1	rs10877012	T	G	178	159	64	203	211	59	0.94 (0.72–1.23)	0.664	1.34 (0.91–1.96)	0.137	0.717
WDR4	rs2156315	C	T	246	125	30	276	173	24	0.88 (0.67–1.16)	0.370	1.52 (0.87–2.65)	0.141	0.641
WDR4	rs2156316	C	G	202	154	45	203	219	51	**0.74 (0.57–0.97)**	**0.028**	1.05 (0.68–1.60)	0.833	0.478
WDR4	rs6586250	C	T	343	48	10	368	97	8	**0.59 (0.42–0.84)**	**0.004**	1.49 (0.58–3.81)	0.407	0.584
WDR4	rs15736	G	A	342	51	8	367	98	8	**0.60 (0.42–0.85)**	**0.004**	1.18 (0.44–3.19)	0.737	0.624
WDR4	rs2248490	C	G	198	162	41	207	216	50	0.80 (0.61–1.04)	0.098	0.96 (0.62–1.49)	0.869	0.566

AOR, adjusted odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.
Values were in bold if the *P*-values less than 0.05 or the 95 % CIs excluding 1.00.
[a] Adjusted for age and gender for dominant model.
[b] Adjusted for age and gender for recessive model.

study subjects were described previously [21]. The demographic characteristics of participants are shown in Table S1. Before the study, participants or their guardians signed the written informed consent. Moreover, the study protocol was authorized by the institutional review boards of the participating institution (Approval No: 202112141–1).

2.2. Polymorphism selection and genotyping

The potential functional SNPs in *METTL1/WDR4* genes were identified by applying the dbSNP database (http://www.ncbi.nlm.nih.gov/) and SNPinfo (http://snpinfo.niehs.nih.gov/). The selection criteria referred to previous publications [32]. In brief, all the functional SNPs were selected from the two terminals, 5' untranslated regions (5' UTR), 3' UTR, or exon of the *METTL1/WDR4* genes. Ultimately, eight potential functional SNPs in *METTL1/WDR4* genes were identified for the final study. In genotyping, we used the TIANamp Blood DNA Kit (TianGen Biotech, Beijing, China) for extracting the genomic DNA from the peripheral blood of the study population. The purified DNA samples were diluted to 5 ng/μL in 96-well plates, and all DNA samples were genotyped for the 8 SNPs in the 384-well format by the TaqMan real-time PCR method [33]. To ensure the authenticity and reliability of the results, we conducted a second-time genotyping in 10 % of the DNA samples chosen randomly. Two genotyping results were 100 % consistent.

2.3. Statistical analysis

We assessed the deviation from the Hardy-Weinberg equilibrium (HWE) of the selected SNPs among control subjects by a goodness-of-fit χ^2 test. The two-sided chi-square test was applied to evaluate the differences in demographics and genotype frequency distributions between cases and control subjects. The associations between the studied SNPs and neuroblastoma risk were assessed by the odds ratios (ORs) and 95 % confidence intervals (CIs) calculated in the unconditional logistic regression model. Moreover, the unconditional multivariate logistic regression analysis was used for calculating the adjusted ORs and corresponding 95 % CIs, which were adjusted for age and sex. Furthermore, the stratification analysis was carried out according to age, gender, sites of origin, and clinical stage. Moreover, we conducted the eQTL (Expression Quantitative Trait Loci) analysis from the GTEx (Genotype-Tissue Expression) platform to assess the potential effects of the significant SNPs on the expressions of neighboring genes in normal human cells or tissue. The detail of the analysis referred to previous studies [34,35]. All statistic analyses were conducted by applying version 9.4 SAS software (SAS Institute, NC, USA). The results with a *P* value less than 0.05 was considered statistically significant.

3. Results

3.1. Associations between SNPs in METTL1/WDR4 genes and neuroblastoma risk

In this case-control study, 402 neuroblastoma cases and 473 healthy controls were included, and 401 cases and 473 controls were successfully genotyped for analyzing the associations between 8 candidate SNPs and neuroblastoma risk. As shown in Table 1, the genotype frequencies of all the SNPs among the controls were coincident with Hardy-Weinberg equilibrium (HWE) ($P > 0.05$). Four SNPs (1 in the *METTL1* gene and 3 in the *WDR4* gene) were related to neuroblastoma risk significantly. For *METTL1* rs2291617 G > T, we found that subjects with rs2291617 TT genotype had significantly increased neuroblastoma risk compared to subjects with GG/GT genotype in the recessive mode (AOR = 1.59, 95 % CI = 1.08–2.34, $P = 0.019$). Moreover, all other three SNPs (rs2156316 C > G,

Table 2
Stratification analysis for the association between *METTL1* genotypes and neuroblastoma risk.

Variables	rs2291617 (cases/controls)		AOR (95 % CI)[a]	*P*[a]	Risk genotypes[b] (cases/controls)		AOR (95 % CI)[a]	*P*[a]
	GG/GT	TT			0	1–3		
Age, month								
≤18	111/127	28/12	**2.70 (1.31–5.58)**	**0.007**	108/125	31/14	**2.60 (1.31–5.16)**	**0.006**
>18	222/292	40/42	1.25 (0.79–2.00)	0.345	215/283	47/51	1.21 (0.79–1.87)	0.384
Gender								
Females	153/195	37/30	1.58 (0.93–2.68)	0.091	147/189	43/36	1.54 (0.94–2.52)	0.087
Males	180/224	31/24	1.61 (0.91–2.84)	0.102	176/219	35/29	1.50 (0.88–2.55)	0.134
Subtypes								
Adrenal gland	81/419	12/54	1.17 (0.60–2.29)	0.647	79/408	14/65	1.13 (0.60–2.12)	0.703
Retroperitoneal	134/419	32/54	**1.85 (1.15–2.99)**	**0.012**	132/408	34/65	**1.62 (1.02–2.56)**	**0.040**
Mediastinum	99/419	21/54	1.66 (0.96–2.88)	0.072	93/408	27/65	**1.84 (1.11–3.05)**	**0.018**
Others	16/419	2/54	0.96 (0.21–4.30)	0.957	16/408	2/65	0.78 (0.18–3.48)	0.744
Clinical stages								
I + II+4s	147/419	26/54	1.36 (0.82–2.26)	0.230	145/408	28/65	1.20 (0.74–1.94)	0.467
III + IV	135/419	27/54	1.60 (0.97–2.65)	0.068	133/408	29/65	1.40 (0.87–2.27)	0.169

AOR, adjusted odds ratio; CI, confidence interval.
Values were in bold if the *P*-values less than 0.05 or the 95 % CIs excluding 1.00.
[a] Adjusted for age and gender, omitting the corresponding stratify factor.
[b] Risk genotypes were carriers with rs2291617 TT, rs10877013 CC and rs10877012 GG genotypes.

Table 3
Stratification analysis for the association between *WDR4* gene polymorphisms and neuroblastoma susceptibility.

Variables	rs2156316 (cases/controls)		AOR (95 % CI)[a]	*P*[a]	rs6586250 (cases/controls)		AOR (95 % CI)[a]	*P*[a]	rs15736 (cases/controls)		AOR (95 % CI)[a]	*P*[a]
	CC	CG/GG			CC	CT/TT			GG	GA/AA		
Age, month												
≤18	70/62	69/77	0.80 (0.50–1.28)	0.341	122/112	17/27	0.57 (0.30–1.11)	0.098	122/110	17/29	0.52 (0.27–1.01)	0.053
>18	132/141	130/193	**0.72 (0.52–0.996)**	**0.047**	221/256	41/78	**0.61 (0.40–0.93)**	**0.020**	220/257	42/77	**0.64 (0.42–0.97)**	**0.034**
Gender												
Females	97/92	93/133	**0.66 (0.45–0.98)**	**0.038**	167/181	23/44	**0.57 (0.33–0.98)**	**0.042**	167/179	23/46	**0.54 (0.31–0.92)**	**0.024**
Males	105/111	106/137	0.82 (0.57–1.18)	0.285	176/187	35/61	**0.61 (0.38–0.97)**	**0.037**	175/188	36/60	0.65 (0.41–1.02)	0.063
Sites of origin												
Adrenal gland	54/203	39/270	**0.55 (0.35–0.86)**	**0.009**	86/368	7/105	**0.28 (0.13–0.63)**	**0.002**	84/367	9/106	**0.37 (0.18–0.76)**	**0.007**
Retroperitoneal	80/203	86/270	0.81 (0.57–1.15)	0.236	139/368	27/105	0.68 (0.43–1.09)	0.107	139/367	27/106	0.67 (0.42–1.07)	0.096
Mediastinum	57/203	63/270	0.83 (0.56–1.24)	0.369	99/368	21/105	0.74 (0.44–1.25)	0.257	101/367	19/106	0.65 (0.38–1.11)	0.115
Others	8/203	10/270	0.93 (0.36–2.39)	0.874	15/368	3/105	0.70 (0.20–2.48)	0.583	14/367	4/106	0.99 (0.32–3.06)	0.981
Clinical stages												
I + II+4s	83/203	90/270	0.83 (0.58–1.17)	0.284	146/368	27/105	0.66 (0.41–1.05)	0.080	145/367	28/106	0.68 (0.43–1.08)	0.101
III + IV	89/203	73/270	**0.62 (0.44–0.89)**	**0.010**	142/368	20/105	**0.49 (0.29–0.82)**	**0.007**	141/367	21/106	**0.51 (0.31–0.85)**	**0.010**

AOR, adjusted odds ratio; CI, confidence interval.
Values were in bold if the *P*-values less than 0.05 or the 95 % CIs excluding 1.00.
[a] Adjusted for age and gender, omitting the correspondence factor.

rs6586250 C > T, rs15736 G > A) in the *WDR4* gene were demonstrated to be associated with reduced neuroblastoma risk in the dominant model. Detailedly, carriers with rs2156316 CG/GG genotype have a lower neuroblastoma risk than those with rs2156316 CC genotype (AOR = 0.74, 95 % CI = 0.57–0.97, $P = 0.028$); rs6586250 CT/TT genotype decreased neuroblastoma risk significantly when compared with CC genotype (AOR = 0.59, 95 % CI = 0.42–0.84, $P = 0.004$); rs15736 GA/AA genotype also reduced neuroblastoma risk significantly compared to GG genotype (AOR = 0.60, 95 % CI = 0.42–0.85, $P = 0.004$). No significant association with neuroblastoma risk was found for the rest SNPs ($P > 0.05$).

3.2. SNPs in METTL1/WDR4 genes are associated with neuroblastoma risk among different subgroups

To evaluate the risk or protective effects of associated SNPs on neuroblastoma susceptibility among different subgroups, we performed the stratification analysis according to age, gender, sites of origin, and clinical stages. We found that the effect of rs2291617 TT genotype on neuroblastoma risk was more evident in the following subgroup: age ≤18 months (AOR = 2.70, 95 % CI = 1.31–5.58, $P = 0.007$) and those with a tumor of retroperitoneal origin (AOR = 1.85, 95 % CI = 1.15–2.99, $P = 0.012$). Moreover, subjects with 1–3 risk genotypes have a higher neuroblastoma risk than those without risk genotype among the following subgroups: age ≤18 months (AOR = 2.60, 95 % CI = 1.31–5.16, $P = 0.006$), those with a tumor of retroperitoneal (AOR = 1.62, 95 % CI = 1.02–2.56, $P = 0.040$) and mediastinum (AOR = 1.84, 95 % CI = 1.11–3.05, $P = 0.018$) origin (Table 2).

As shown in Table 3, *WDR4* rs2156316 C > G decreased the neuroblastoma risk among the following subgroups: age >18 months (AOR = 0.72, 95 % CI = 0.52–0.996, $P = 0.047$), females (AOR = 0.66, 95 % CI = 0.45–0.98, $P = 0.038$), neuroblastoma of adrenal origin (AOR = 0.55, 95 % CI = 0.35–0.86, $P = 0.009$), patients of III + IV stage (AOR = 0.62, 95 % CI = 0.44–0.89, $P = 0.010$). Similar results were observed for rs6586250 C > T and rs15736 G > A in the same subgroups. In addition, rs6586250 C > T was also associated with decreased neuroblastoma risk in the male subgroup (AOR = 0.61, 95 % CI = 0.38–0.97, $P = 0.037$).

3.3. Associated SNPs influence the expressions of the host and adjacent genes

To further assess the potential effects of the associated SNPs on the adjacent gene expressions and explore the possible mechanism by which these significant SNPs modify neuroblastoma susceptibility, we carried out the eQTL analysis applying the GTEx platform. We found that the *METTL1* rs2291617 G allele was associated with lower mRNA levels of *METTL1* in the thyroid (Fig. 1A) and *TSFM* in the adrenal gland (Fig. 1B). However, it was related to higher mRNA levels of *METTL21B* in the adrenal gland (Fig. 1C). We also observed that the *WDR4* mRNA levels in whole blood with the *WDR4* rs6586250 T allele were significantly higher than those with the *WDR4* rs6586250 C allele (Fig. S1A). The *WDR4* rs6586250 T allele also increased the *CBS* mRNA compared to the *WDR4* rs6586250 C allele in cultured fibroblasts (Fig. S1B). In addition, the *WDR4* rs15736 A allele was related to higher *WDR4* mRNA in whole blood (Fig. 2A) and increased *NDUFV3* mRNA in cultured fibroblasts (Fig. 2B) and adrenal gland (Fig. 2C) than the rs15736 G allele.

4. Discussion

The genetic predisposition of neuroblastoma still needs further elucidation. However, it is promising that identify genetic susceptibility locus and combine multiple loci for clinical diagnosis, grading, and prognosis of neuroblastoma. In this study, we comprehensively evaluated the associations between 8 functional SNPs in *METTL1/WDR4* genes and neuroblastoma susceptibility for the first time. And we identified one SNP (rs2291617 G > T) in the *METTL1* gene, and three SNPs (rs2156316 C > G, rs6586250 C > T,

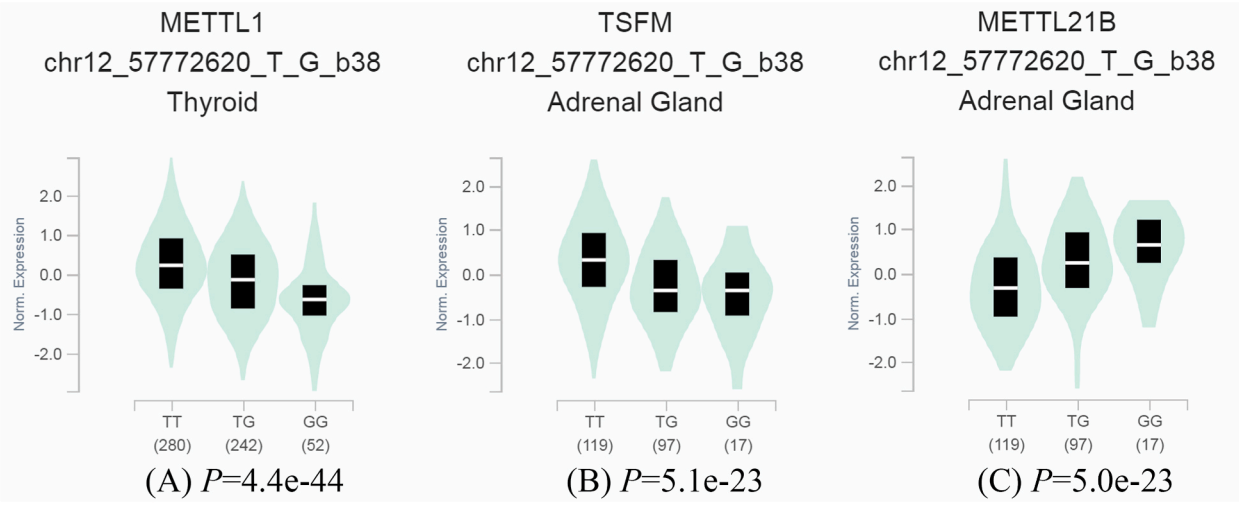

Fig. 1. eQTL analysis for *METTL1* rs2291617 G > T. *METTL1* rs2291617 G > T genotype-based mRNA expression alteration of *METTL1* in thyroid (**A**), *TSFM* in adrenal gland (**B**), and *METTL21B* in adrenal gland (**C**).

rs15736 G > A) in the *WDR4* gene are associated with neuroblastoma susceptibility significantly. Our findings may be conducive to identifying the high-risk population of neuroblastoma and practicing early intervention, which will be prospective to improve the cure rate.

Neuroblastoma is a multifaceted disorder with a complicated etiology that involves the interaction of environmental and genetic factors. Although extensive studies have been conducted, little is known about the genetic etiology of neuroblastoma. However, accumulating evidence showed that genetic factors play a crucial role in the occurrence of neuroblastoma. Plenty of studies revealed that SNPs in some tumor-related genes are associated with increased or decreased neuroblastoma risk significantly, such as DNA repair [36] and N6-methyladenosine (m6A) [37] mediated genes. SNPs may modify the expression or activity of these genes, thus affecting the capability of DNA repair and m6A, which may cause disturbances in cell functions and downstream genes. For example, Vodicka et al. demonstrated that SNP rs1052133 G > C modified the DNA repair capacity of *hOGG1*, rs1052133 CC genotype contributed to 2-fold higher DNA damage repair capacity of *hOGG1* than rs1052133 GG genotype [38].

M7G is one of the most important epigenetic modifications of RNAs, which determines the fate of various RNAs, including the regulation of tRNA stability [39], enhancement of mRNA translation efficiency [40], promotion of mi-RNA maturation [27], Therefore, m7G modifications have the potential to regulate the target RNAs and downstream signal pathways. Numerous studies have revealed that m7G modification is involved in the cancer-related genes and signal pathways, influencing cancer progress. For example, *METTL1* knockdown augments the phosphorylation of eIF2α, which inhibits mRNA translation of downstream genes involved in the cell cycle and EGFR signal pathways, therefore causing the suppression of intrahepatic cholangiocarcinoma [41]. Chen et al. demonstrated that aberrant METTL1/WDR4-mediated tRNA m7G modification led to the abnormal translation of *PIK3CA*, *PTEN*, and *EGFR*, which drives the carcinogenesis and progression of head and neck squamous cell carcinoma by activating the PI3K/AKT/mTOR pathway [42]. Chen et al. also found that in nasopharyngeal carcinoma, m7G modification was involved in tumorigenesis and chemoresistance through the WNT/β-catenin signal pathway [43]. SNP is the most common genetic variation in humans, which has specific effects on gene expression, structure, and activity depending on its position in the gene. Similarly, SNP in *METTL1* and *WDR4* may cause the abnormity in gene expression, structure, and activity of the host genes, eventually leading to aberrant m7G modification and oncogenesis. However, few studies explored the associations between SNPs in the *METTL1/WDR4* genes and disease, and only several studies found that the SNPs in *METTL1/WDR4* genes were disease-causing. One SNP rs703842 A > C located in 3' UTR of *METTL1* gene was identified as associated with multiple sclerosis susceptibility by a GWAS performed in 2009 [44]. Furthermore, one more recent study conducted by Wang et al.showed that *WDR4* rs465663 T > C was related to male fertility. Further GTEx analysis showed that the rs465663 TT/TC genotype was associated with a lower expression level of *WDR4* than the CC genotype [45]. In addition, Our research group recently evaluated the association between the SNPs in *METTL1/WDR4* genes and hepatoblastoma susceptibility. Although no significant association was found between the single SNP and hepatoblastoma risk, the combined effect of several risk genotypes was shown to confer significantly increased hepatoblastoma risk [46,47].

However, no study has reported the correlation between SNP in m7G-meditated genes and neuroblastoma susceptibility. In this present study, we comprehensively evaluated the effects of SNPs in *METTL1/WDR4* genes on neuroblastoma risk. Our results revealed that *METTL1* rs2291617 G > T was associated with an increased risk of neuroblastoma and *WDR4* rs2156316 C > G, rs6586250 C > T, and rs15736 G > A related to a decreased risk of neuroblastoma. Stratification analysis showed that the risk effect of rs2291617 G > T and the protective effects of rs2156316 C > G, rs6586250 C > T, and rs15736 G > A were strengthened in the above different subgroups. Thus, the effects of SNP of m7G-meditated *METTL1/WDR4* genes on neuroblastoma risk may be context-dependent on age, gender, sites of origin, and clinical stages.

To further reveal the potential effects of the associated SNPs on gene expression and explore the possible mechanisms by which the associated SNPs affect the neuroblastoma risk, we conducted the eQTL analysis. The results showed that the *METTL1* rs2291617 T allele was related to a higher mRNA level of *METTL1*, *TSFM*, and *METTL21B* genes when compared to the rs2291617 G allele. Maybe

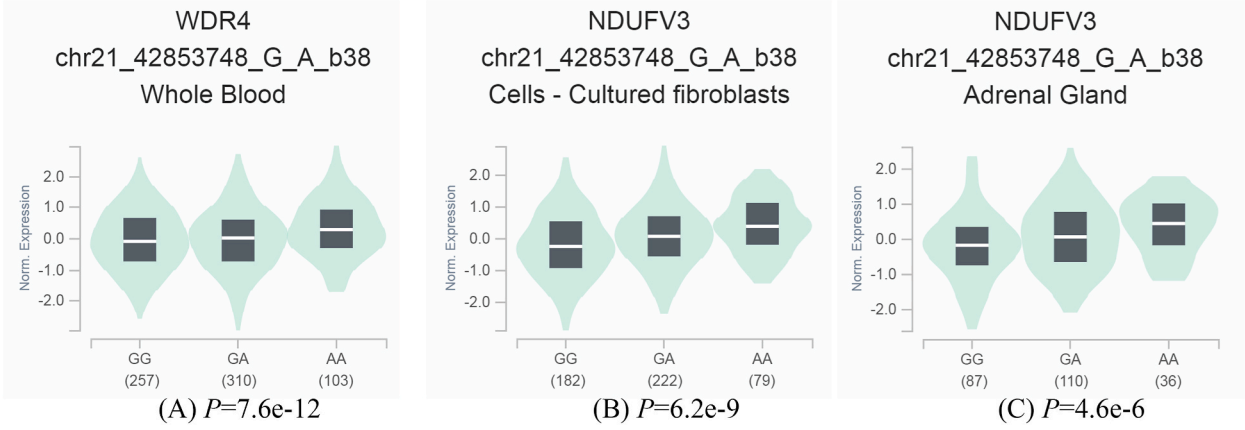

Fig. 2. eQTL analysis for *WDR4* rs15736 G > A. *WDR4* rs15736 G > A genotype-based mRNA expression alteration of *WDR4* in whole blood(**A**), *NDUFV3* in cells-cultured fibroblasts (**B**) and in adrenal gland (**C**).

the rs2291617 TT genotype increased neuroblastoma risk that was due to this genotype-base up-regulated of *METTL1*. Previous studies also indicated the oncogenicity of *METTL1* and increased expression levels of *METTL1* in numerous cancers. However, the associations of *TSFM* and *METTL21B* with neuroblastoma risk and the mechanism that rs2291617 G > T polymorphism affects the mRNA expression of *TSFM* and *METTL21B* remain to be further explored. However, *WDR4* rs6586250 T and rs15736 A alleles were associated with higher mRNA of *WDR4*, although these two alleles were shown to be related to reduced risk of neuroblastoma. Numerous studies have shown the tumorigenicity and increased expression of *WDR4* in various cancers, which seems to contradict our result. Maybe the role of *WDR4* in oncogenesis differs based on the context. In addition, *WDR4* rs6586250 C > T also related to the expressions of *CBS,* and rs15736 G > An also affected the expressions of *NDUFV3*. These SNP-base differential expressions of neighboring genes may contribute to rs6586250 C > T and rs15736 G > A genotype-base neuroblastoma risk. However, the results need further verification by well-designed studies and elucidate the potential mechanisms. Here, we offer new insights into how SNPs in m7G-mediated genes modify neuroblastoma susceptibility.

Several accompanying shortcomings in this case-control study should be mentioned. First, the study subjects were collected from a single center, with poor representativeness, and the sample size was comparatively small, especially for stratification analysis. Second, other potential functional SNPs in the *METTL1/WDR4* genes should be evaluated. Third, in addition to genetic analysis, environmental factors should be included because of neuroblastoma's complicated etiology, which involves complex interactions between multiple genetic and environmental factors. Fourth, functional experiments should be designed to reveal the underlying mechanism that SNPs in *METTL1/WDR4* genes modify the neuroblastoma susceptibility.

5. Conclusion

In summary, as the first case-control study to comprehensively assess the associations between SNPs in m7G-mediated *METTL1/WDR4* genes and neuroblastoma susceptibility, we identified 4 SNPs in *METTL1/WDR4* genes that modified the neuroblastoma susceptibility significantly in eastern Chinese children. However, well-designed research with more samples collected from multiple centers should be performed to verify the conclusion. Moreover, a series of mechanism studies should be performed to elucidate the potential mechanisms by which *METTL1/WDR4* genetic variants modify neuroblastoma susceptibility.

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Disclosure of conflict of interest

None.

Human/animal ethics approval declaration

The research scheme was approved by the institutional review board of Children's Hospital of Nanjing Medical University (Approval No: 202112141–1). In accordance with the guidelines of the Declaration of Helsinki, written informed consent was signed by each participant.

Data availability statement

Data will be made available on request from the correspondence author (Jing He).

CRediT authorship contribution statement

Jiabin Liu: Writing - review & editing, Writing - original draft, Investigation. **Changmi Deng:** Investigation, Formal analysis, Data curation. **Huiran Lin:** Writing - review & editing, Investigation, Formal analysis. **Xinxin Zhang:** Writing - original draft, Investigation. **Jinhong Zhu:** Writing - review & editing, Investigation. **Chunlei Zhou:** Writing - review & editing, Resources, Investigation, Funding acquisition, Data curation. **Haiyan Wu:** Writing - review & editing, Resources, Investigation, Conceptualization. **Jing He:** Writing - review & editing, Supervision, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23658.

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