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ORIGINAL RESEARCH

Genetic Variants in *METTL14* are Associated with the Risk of Acute Lymphoblastic Leukemia in Southern Chinese Children: A Five-Center Case-Control Study

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Background/Aim: Acute lymphoblastic leukemia (ALL) is the most common form of pediatric cancer. *METTL14*, an N^6-methyladenosine (m^6A) modification protein, plays several roles in cancer development and is involved in the pathogenesis of various types of cancers. However, the role of *METTL14* gene single nucleotide polymorphisms (SNPs) in pediatric ALL susceptibility remains to be investigated.

Methods: A case-control design and multinomial logistic regression were used to develop models to estimate the overall risk for pediatric ALL and three *METTL14* gene SNPs (rs298982 G/A, rs298981 A/G and rs1064034 T/A) in 808 cases and 1340 controls, which were genotyped using a TaqMan assay. The associations were estimated by odds ratios (ORs) with their 95% confidence intervals (CIs). Furthermore, stratified analysis was performed to explore associations of rs298982 and rs1064034 with pediatric ALL susceptibility in terms of age, sex, immunophenotype, minimal residual disease (MRD), and other clinical characteristics.

Results: Among the three analyzed SNPs, rs298982 G/A and rs1064034 T/A exhibited a significant association with decreased childhood ALL risk, while rs298981 A/G exhibited no difference. In stratified analysis, rs298982 GA/AA and rs1064034 TA/AA had a protective effect in children <120 months of age and males, common B ALL, TEL-AML, non gene fusion, normal diploid, and high WBC. However, the rs1064034 TA/AA genotype was associated with an increased risk of mixed immunophenotyping. Compared with the reference haplotype GAT, haplotypes CAA, CGT and CGA were significantly associated with elevated ALL risk, while haplotype GGT was significantly associated decreased ALL risk. Moreover, subjects carrying rs298982 A or rs1064034 A exhibited less minimal MRD after induced chemotherapy. Functional annotations revealed that *METTL14* gene SNPs rs298982 G/A and rs1064034 T/A could be potential functional variants.

Conclusion: In conclusion, *METTL14* gene polymorphisms influence the risk of ALL in southern Chinese children and might be potential biomarkers for pediatric ALL susceptibility and chemotherapeutics.

Keywords: ALL, *METTL14*, SNP, susceptibility, Chinese children

Introduction

Acute lymphoblastic leukemia (ALL) is the most common form of pediatric cancer, accounting for approximately 25% of childhood cancers and nearly 80% of all leukemia cases.[1,2] ALL primarily leads to changes in the lymphocyte composition in the blood, resulting in the replacement of mature functional lymphocytes with

immature lymphocytes.[3] ALL arises from hematopoietic cells in either the B-cell precursor (B-ALL) or T-cell lineages (T-ALL).[4,5] Bone marrow transplantation and targeted drugs are preferred for ALL treatment, but the economic and human resource cost is enormous.[3] Investigating the pathogenesis of ALL may facilitate the complete cure of the disease to some extent. Genetic changes play a key role in the childhood prevalence of ALL.[6] Gene polymorphisms were found to be closely associated with ALL sensitivity. Mosaad et al found that *ARID5B* rs10821936 and the CA haplotype can be considered susceptibility risk factors for the development of pediatric ALL and adult ALL in the studied cohort of Egyptian patients.[1] Yang et al revealed that *FOXO3* gene polymorphisms influence the risk of ALL in children and might be a potential biomarker for ALL susceptibility.[7]

N^6-methyladenosine (m^6A) is the most abundant internal modification in mRNAs and lncRNAs in eukaryotic cells. The m^6A modification process is accomplished by a series of proteins, which include methyltransferases (writers), demethylases (erasers) and binding proteins (readers). M^6A modification has been reported to play multiple roles in cancer development and to be involved in the pathogenesis of various types of cancers.[8–10] However, there were few studies about the role of METTL14 in leukemia. METTL14, an m6A "writer", was found a high expression level in normal hematopoietic stem/progenitor cells (HSPCs) and in AMLs, when its depletion could promote differentiation of HSPCs and AML cells.[11] And to date, only one studies have characterized *METTL14* in childhood patients with ALL. Sun et al found that the expression levels of *METTL3* and *METTL14* in E/R-positive ALL patients were lower than those in controls, which suggested that *METTL3* and *METTL14* expression may be a new prognostic factor and indicate specific treatment intensification in possible E/R-positive relapse patients.[12] Previously, we reported that multiple m^6A-modified enzyme-encoding genes, *METTL3, METTL14, WTAP, FTO*, and *ALKBH5*, are closely related to glioma susceptibility. *METTL14* polymorphisms have been reported to increase susceptibility to neuroblastoma.[13] Based on these findings, we hypothesized that polymorphisms of *METTL14* gene are associated with the risk of pediatric ALL.

To identify novel ALL susceptibility single nucleotide polymorphisms (SNPs) of *METTL14* gene, an m^6A modification key regulator, we conducted a five-center hospital-based case-control study. We addressed the associations between three *METTL14* gene SNPs and ALL risk in a population of Chinese children.

Materials and Methods

Patients and Healthy Controls

In this study, participants were divided into 2 groups: childhood ALL patients and healthy controls. A total of 808 patients and 1340 controls, with an age range of 0.5 to 17 years old, were recruited from Guangzhou Women and Children's Center, Sun Yat-sen Memory Hospital Affiliated Sun Yat-sen University (SYSU), Nanfang Hospital Affiliated Southern Medical University, The First Hospital Affiliated SYSU and Zhujiang Hospital Affiliated Southern Medical University. There were no statistically significant differences in age, sex, or other general clinical data between the ALL group and the control group ($P>0.05$) as described previously.[14] Informed consent was obtained from the parents of children with ALL. All patients were diagnosed according to standard methods, including cytomorphologic, cytochemical, and immunophenotyping methods. ALL patients without previous therapy were included in the study. Control subjects, who were recruited concurrently with ALL patient subjects, were randomly selected from the volunteers visiting the hospital and matched according to the expected age and gender distribution of patients. The markers of CD38/CD81, CD66c/CD34/CD10/CD19/CD45 were used for B-ALL; and markers of CD3/CD99/CD19, CD33/HLA-DR/CD5/CD7/CD45, CD10/CD19, CD33/HLA-DR/CD5/CD7/CD45 were used to for T-ALL to detect MRD by flow cytometry. The institutional review board of Guangzhou Women and Children's Medical Center gave approval for the current study.

SNP Selection and Genotyping

DNA was extracted from EDTA blood using the TIANamp DNA Kit (Tiangen, Beijing, China) according to the standard instructions of the kit. We used NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) and SNPinfo (http://snpinfo.niehs.nih.gov/snpfunc.htm) to select the candidate SNPs of METTL14 which is potentially functional, which follows the previously described criteria: located in the 5′ untranslated region, 3′ untranslated region, 5′ flanking region, and exon of *METTL14* gene.[15] Three *METTL14* gene SNPs, rs298982 G/A, rs298981 A/G and rs1064034 T/A, were chosen. TaqMan genotyping kits (Tiangen, Beijing, China) were used for

SNP genotyping on an ABI 7900 (Applied Biosystem, Foster City, CA, USA) as described previously.[16–18] Genotyping results were confirmed by randomly assaying 10% of the original specimens for replication to exclude genotyping errors. There were no discrepancies between genotypes determined in duplicate. Further functional annotation of the significant SNPs was performed using Genotype-Tissue Expression (GTEx) (https://gtexportal.org). All tests for statistical significance used a two-sided alpha of 0.05. All statistical analyses were conducted using SAS v10.0 (SAS Institute, Cary, NC, USA).

Statistical Analyses

The compliance of genotypes with HWE among controls was appraised by a χ^2 test. Differences in demographic characteristics between the cases and the controls were evaluated by the χ2 test. The age- and sex-adjusted ORs and 95% CIs for the relationships between the SNPs and ALL risk were determined by multivariate logistic regression analysis. Logistic regression analyses were adopted to estimation haplotype frequencies and their effect on ALL risk, with the adjustment for gender and age.[19,20] To determine whether the significant findings were "noteworthy," false-positive report probability (FPRP) analysis was performed for each significant finding as described previously.[21] A prior probability of 0.1 was adopted to detect odds ratios (ORs) of 1.50 and 0.67 for risk and protective effects, respectively. An association with an FPRP value < 0.2 was considered noteworthy. All statistical analyses were conducted using SAS v10.0 (SAS Institute, Cary, NC, USA). In the present study, all *P* values were two-sided, and values of $P<0.05$ were considered statistically significant.

Results

Association of *METTL14* Gene SNPs on Pediatric ALL Risk

Three *METTL14* gene SNPs (rs298982 G/A, rs298981 A/G and rs1064034 T/A) were successfully genotyped in 808 cases and 1340 controls. Table 1 shows the association of these three SNPs with pediatric ALL risk. All SNPs were in HWE in the two groups ($P>0.05$). Of the three SNPs, in a single–locus analysis, carriers of the rs298982 (GA versus GG: adjusted odds ratio [OR]=0.66, 95% confidence interval [CI]=0.52–0.84, $P=0.001$; AA/GA versus GG: adjusted OR=0.69, 95% CI=0.55–0.87, $P=0.002$) and rs1064034 (TA versus TT: adjusted OR=0.70, 95% CI=0.58–0.86, $P=0.001$; AA/TA versus TT: adjusted OR=0.72, 95% CI=0.60–0.87, $P=0.001$) variant alleles showed decreased susceptibility to ALL. However, the remaining genotype, rs298981 A/G, was not associated with ALL risk. We then defined rs298982 GG/GA and rs1064034 TT/TA as risk genotypes.

Stratification Analysis of rs298982 and rs1064034 with ALL Susceptibility

Subgroup analyses by age, sex, immunophenotyping, gene infusion, karyotype, WBC, MRD and other clinical information to evaluate the effect of rs298982 G/A and rs1064034 T/A is shown in Table 2. We found that rs298982 GA/AA had a protective effect in children <120 months of age (adjusted OR=0.70, 95% CI=0.55–0.90, $P=0.004$) and males (adjusted OR=0.69, 95% CI=0.51–0.92, $P=0.013$). We also found that rs298982 GA/AA decreased the ALL risk in patients with common B ALL (adjusted OR=0.36, 95% CI=0.24–0.56, $P=0.001$), TEL-AML (adjusted OR=0.56, 95% CI=0.33–0.93, $P=0.025$), non gene fusion (adjusted OR=0.75, 95% CI=0.58–0.98, $P=0.031$), normal diploid (adjusted OR=0.67, 95% CI=0.51–0.89, $P=0.005$), primitive/naïve lymphocytes in marrow <5% (adjusted OR=0.71, 95% CI=0.54–0.93, $P=0.013$), high WBC (adjusted OR=0.59, 95% CI=0.41–0.83, $P=0.003$) and with MRD ≥0.01 on Day 33 after induced therapy (adjusted OR=0.40, 95% CI=0.25–0.64, $P=0.001$).

Rs1064034 TA/AA had a protective effect in children <120 months of age (adjusted OR=0.74, 95% CI=0.61–0.90, $P=0.003$) and males (adjusted OR=0.71; 95% CI=0.56–0.90, $P=0.004$). Compared to the TT genotype, the rs1064034 TA/AA genotype was associated with a decreased risk of common B ALL (adjusted OR=0.58, 95% CI=0.44–0.78, $P=0.001$), pre B ALL (adjusted OR=0.71; 95% CI=0.50–0.99, $P=0.046$), T ALL (adjusted OR=0.54; 95% CI=0.30–0.95, $P=0.032$), MLL (adjusted OR=0.26; 95% CI=0.07–0.94, $P=0.040$), non gene fusion type (adjusted OR=0.74; 95% CI=0.60–0.91, $P=0.005$), high WBC (adjusted OR=0.64, 95% CI=0.49–0.84, P=0.002) and normal diploid (adjusted OR=0.76; 95% CI=0.61–0.94, $P=0.011$). In addition, rs1064034 T/A decreased ALL risk in patients with primitive/naïve lymphocytes in marrow <5% on Day 33 (adjusted OR=0.67; 95% CI=0.54–0.84, $P=0.001$) and Week 12 (adjusted OR=0.77; 95% CI=0.60–0.99, $P=0.040$), MRD ≥0.01

Table 1 Logistic Regression Analysis of Associations Between *METTL14* Polymorphisms and ALL Susceptibility

Genotype	Cases (N=808)	Controls (N=1340)	P^a	Crude OR (95% CI)	*P*	Adjusted OR (95% CI) b	P^b
rs298982 (HWE=0.8164)							
GG	611 (82.79)	1027 (76.99)		1.00		1.00	
GA	113 (15.31)	286 (21.44)		0.66 (0.52–0.84)	0.001	0.66 (0.52–0.84)	0.001
AA	14 (1.90)	21 (1.57)		1.12 (0.57–2.22)	0.744	1.13 (0.57–2.23)	0.735
Additive			0.009	0.76 (0.62–0.94)	0.009	0.76 (0.62–0.93)	0.009
Dominant	127 (17.21)	307 (23.01)	0.002	0.69 (0.55–0.87)	0.002	0.69 (0.55–0.87)	0.002
Recessive	724 (98.10)	1313 (98.43)	0.585	1.21 (0.61–2.39)	0.586	1.21 (0.61–2.40)	0.577
rs298981 (HWE=0.6453)							
AA	201 (26.34)	311 (23.52)		1.00		1.00	
AG	379 (49.67)	669 (50.61)		0.87 (0.70–1.09)	0.235	0.87 (0.70–1.08)	0.215
GG	183 (23.98)	342 (25.87)		0.83 (0.64–1.07)	0.143	0.82 (0.64–1.05)	0.121
Additive			0.143	0.91 (0.80–1.03)	0.143	0.90 (0.80–1.03)	0.121
Dominant	562 (73.66)	1011 (76.48)	0.150	0.86 (0.70–1.06)	0.150	0.85 (0.70–1.05)	0.131
Recessive	580 (76.02)	980 (74.13)	0.339	0.90 (0.74–1.11)	0.339	0.90 (0.73–1.10)	0.306
rs1064034 (HWE=0.6798)							
TT	431 (60.20)	685 (52.09)		1.00		1.00	
TA	231 (32.26)	524 (39.85)		0.70 (0.58–0.85)	0.001	0.70 (0.58–0.86)	0.001
AA	54 (7.54)	106 (8.06)		0.81 (0.57–1.15)	0.236	0.82 (0.58–1.16)	0.267
Additive			0.004	0.81 (0.70–0.93)	0.004	0.81 (0.70–0.94)	0.005
Dominant	285 (39.80)	630 (47.91)	0.001	0.72 (0.60–0.87)	0.001	0.72 (0.60–0.87)	0.001
Recessive	662 (92.46)	1209 (91.94)	0.678	0.93 (0.66–1.31)	0.678	0.94 (0.67–1.33)	0.729

Notes: [a]χ^2 test for genotype distributions between ALL cases and cancer-free controls. [b]Adjusted for age and gender.

on Day 33 (adjusted OR=0.42; 95% CI=0.30–0.60, *P*=0.001) and on Week 12 (adjusted OR=0.05; 95% CI=0.007–0.39, *P*=0.004). However, the rs1064034 TA/AA genotype was associated with an increased risk of mixed immunophenotyping (adjusted OR=1.88; 95% CI=1.08–3.27, *P*=0.026).

Haplotype Analysis of Three *METTL14* Gene SNPs Correlated with ALL Susceptibility

We further determined whether the haplotypes of the three METTL14 gene SNPs are linked to ALL risk. As shown in Table 3, the wildtype allele GAT was defined as the reference group. Compared with the reference haplotype GAT, the following haplotypes were significantly associated with elevated ALL risk: CAA (adjusted OR=5.27, 95% CI=4.25–6.53, *P*<0.001), CGT (adjusted OR=1.64, 95% CI=1.24–2.17, *P*=0.001) and CGA (adjusted OR=6.83, 95% CI=5.59–8.34, p<0.001). While haplotype GGT (adjusted OR=0.12, 95% CI=0.08–0.16, *P*<0.001) was significantly associated decreased ALL risk.

The Influence of Identified SNPs on Sensitivity to Different Treatment Strategies Based on MRD Levels

We further assessed the MRD in the marrow of patients with different alleles after treatment with Chinese Children Cancer Group chemotherapeutics (CCCGs) or South China Children Leukemia Group chemotherapeutics (SCCLGs) (Tables 4 and 5). For rs298982, GG alleles had a harmful effect on MRD in marrow ≥0.01% at 33 d (adjusted OR=3.00, 95% CI=1.66–5.44, *P*=0.001) after CCCG treatment. For rs1064034, TT alleles had a harmful effect on MRD in marrow ≥0.01% at 33 d (adjusted OR=2.32, 95% CI=1.45–3.69, *P*=0.001) after CCCG treatment. These results indicated that southern Chinese pediatric patients with rs298982 GA/AA alleles or rs1064034 TA/AA alleles may have better outcomes after receiving CCCG treatment.

Expression Quantitative Trait Loci (eQTL) Analyses

We further assessed the putative functional relevance of METTL14 rs298982 and rs1064034 using released data

Table 2 Stratification Analysis of *METTL14* Polymorphisms with ALL Susceptibility

Variables	rs298982 (Cases/Controls)		Adjusted OR[a]	*P*[a]	rs1064034 (Cases/Controls)		Adjusted OR[a]	*P*[a]
	GG	GA/AA	(95% CI)		TT	TA/AA	(95% CI)	
Age, month								
<120	532/926	111/276	0.70 (0.55–0.90)	0.004	375/619	251/565	0.74 (0.61–0.90)	0.003
≥120	79/101	16/31	0.66 (0.34–1.29)	0.225	56/66	34/65	0.62 (0.36–1.07)	0.083
Gender								
Females	244/374	51/111	0.71 (0.49–1.02)	0.064	180/262	109/213	0.75 (0.56–1.01)	0.059
Males	367/653	76/196	0.69 (0.51–0.92)	0.013	251/423	176/417	0.71 (0.56–0.90)	0.004
Immunophenotyping								
Pro B	167/1027	48/307	0.96 (0.68–1.36)	0.814	124/685	89/630	0.78 (0.58–1.05)	0.095
Common B	230/1027	25/307	0.36 (0.24–0.56)	0.001	153/685	81/630	0.58 (0.44–0.78)	0.001
Pre B	124/1027	34/307	0.91 (0.61–1.36)	0.656	93/685	60/630	0.71 (0.50–0.99)	0.046
Mature B	3/1027	0/307	0.001 (0.00–999)	0.960	2/685	1/630	0.54 (0.05–6.00)	0.616
T ALL	50/1027	8/307	0.55 (0.26–1.19)	0.128	38/685	19/630	0.54 (0.30–0.95)	0.032
Mix	37/1027	12/307	1.10 (0.57–2.15)	0.772	21/685	35/630	1.88 (1.08–3.27)	0.026
Gene fusion type								
BCR-ABL	19/1027	7/307	1.29 (0.52–3.17)	0.584	13/685	13/630	1.08 (0.49–2.38)	0.858
TEL-AML	108/1027	18/307	0.56 (0.33–0.93)	0.025	73/685	43/630	0.63 (0.42–0.94)	0.023
E2A-PBX	15/1027	7/307	1.55 (0.62–3.84)	0.347	12/685	10/630	0.92 (0.39–2.15)	0.843
SIL-TAL	6/1027	1/307	0.57 (0.07–4.71)	0.597	4/685	2/630	0.54 (0.10–2.97)	0.479
MLL	14/1027	1/307	0.24 (0.03–1.81)	0.165	12/685	3/630	0.26 (0.07–0.94)	0.040
Others	24/1027	0/307	0.001 (0.00–999)	0.943	17/685	3/630	0.20 (0.06–0.68)	0.010
Non	409/1027	92/307	0.75 (0.58–0.98)	0.031	293/685	197/630	0.74 (0.60–0.91)	0.005
Karyotype								
Hypo-diploid	14/1027	6/307	1.43 (0.54–3.77)	0.467	9/685	10/630	1.18 (0.47–2.92)	0.726
Normal diploid	391/1027	79/307	0.67 (0.51–0.89)	0.005	270/685	179/630	0.72 (0.58–0.90)	0.004
Abnormal diploid	24/1027	13/307	1.85 (0.93–3.68)	0.080	21/685	16/630	0.84 (0.44–1.64)	0.615
Low hyperdiploid	22/1027	4/307	0.62 (0.21–1.81)	0.381	14/685	10/630	0.54 (0.10–2.97)	0.480
High hyperdiploid	46/1027	14/307	1.02 (0.55–1.88)	0.960	34/685	25/630	0.79 (0.35–1.79)	0.565
WBC								
Low (<4)	171/1027	44/307	0.86 (0.60–1.23)	0.411	126/685	81/630	0.71 (0.52–0.96)	0.024
High (>10)	246/1027	43/307	0.59 (0.41–0.83)	0.003	171/685	101/630	0.64 (0.49–0.84)	0.002
Normal (4–10)	123/1027	26/307	0.70 (0.45–1.09)	0.111	92/685	57/630	0.67 (0.47–0.95)	0.024
Primitive /naïve lymphocytes in marrow (%, 33d)								
<5	381/1027	81/307	0.71 (0.54–0.93)	0.013	274/685	170/630	0.67 (0.54–0.84)	0.001
≥5	20/1027	7/307	1.16 (0.49–2.77)	0.741	15/685	13/630	0.95 (0.45–2.01)	0.883
MRD in marrow (%, 33d)								
<0.01	216/1027	66/307	1.02 (0.75–1.38)	0.896	160/685	122/630	0.83 (0.64–1.08)	0.162
≥0.01	175/1027	21/307	0.40 (0.25–0.64)	0.001	125/685	49/630	0.42 (0.30–0.60)	0.001
Primitive /naïve lymphocytes in marrow (%, 12w)								
<5	244/1027	75/307	1.02 (0.77–1.37)	0.877	186/685	132/630	0.77 (0.60–0.99)	0.040
≥5	9/1027	4/307	1.47 (0.45–4.85)	0.525	6/685	7/630	1.32 (0.44–4.00)	0.618

(*Continued*)

Table 2 (Continued).

Variables	rs298982 (Cases/Controls)		Adjusted OR[a]	P[a]	rs1064034 (Cases/Controls)		Adjusted OR[a]	P[a]
	GG	GA/AA	(95% CI)		TT	TA/AA	(95% CI)	
MRD in marrow (%, 12w)								
<0.01	226/1027	78/307	1.15 (0.86–1.53)	0.351	164/685	137/630	0.91 (0.71–1.17)	0.459
≥0.01	24/1027	0/307	0.001 (0.01–999)	0.943	21/685	1/630	0.05 (0.007–0.39)	0.004
Relapse								
–	422/1027	110/307	0.87 (0.68–1.11)	0.267	304/685	230/630	0.83 (0.68–1.02)	0.075
+	21/1027	3/307	0.49 (0.14–1.62)	0.235	13/685	10/630	0.83 (0.36–1.91)	0.660

Note: [a]Adjusted for age and gender.

Table 3 Association Between Inferred Haplotypes of the *METTL14* Gene and ALL Risk

Haplotypes[a]	Cases (n=808)	Controls (n=1340)	Crude OR (95% CI)	P	Adjusted OR[b] (95% CI)	P[b]
	No.%	No.%				
GAT	300 (20.35)	881 (35.5)	1.00		1.00	
GAA	42 (2.85)	0	>999.999 (<0.001, >999.999)	0.961	>999.99 (<0.001, >999.999)	0.96
CAT	39 (2.65)	143 (5.76)	0.69 (0.473–1.000)	0.050	0.69 (0.47–1.00)	0.052
CAA	380 (25.78)	186 (7.49)	5.15 (4.164–6.373)	<0.001	5.27 (4.25–6.53)	<0.001
GGT	42 (2.85)	909 (36.62)	0.12 (0.084–0.162)	<0.001	0.12 (0.08–0.16)	<0.001
GGA	8 (0.54)	0	>999.999 (<0.001, >999.999)	0.983	>999.999 (<0.001, >999.999)	0.983
CGT	100 (6.78)	154 (6.20)	1.64 (1.239–2.164)	0.001	1.64 (1.24–2.17)	0.001
CGA	563 (38.2)	209 (8.42)	6.79 (5.566–8.290)	<0.001	6.83 (5.59–8.34)	<0.001

Note: [a]The haplotypes order was rs298982, rs298981 and rs1064034. [b]Obtained in logistic regression models with adjustment for age and gender.

from GTEx. Analysis revealed that the rs298982 A genotype was associated with significantly increased RP11-3846.6 expression level in spleen (Figure 1). In addition to the *METTL14* rs298982 A genotype, samples with the rs1064034 T genotype had a higher lncSNHG8 (long noncoding RNA SNHG8) expression level in the cultured fibroblasts than the A allele (Figure 2A). Our cis-eQTL analysis also detected an association between the rs1064034 A genotype and increased expression of the RP11-3846.6 gene in whole blood and cultured fibroblasts (Figure 2B and C).

Discussion

In the present case-control study, we investigated the potential association of *METTL14* polymorphisms with ALL risk between 808 ALL patients and 1340 healthy controls from a population of southern Chinese children. We showed that two of the three selected SNPs were associated with ALL risk: both rs298982 G/A and rs1064034 T/A were related to decreased ALL risk. To the best of our knowledge, this is the first study on the association between the *METTL14* rs298982 and rs1064034 polymorphisms and ALL risk.

METTL14, methyltransferase like 14, a methyltransferase, has been reported to act as an oncogene by affecting RNA stability and degradation through pre-RNA splicing, protein translation, and miRNA processing in a variety of tumors.[11,22,23] Sun et al reported that *METTL14* was significantly upregulated in breast cancer and that a novel LNC942-*METTL14*-CXCR4/CYP1B1 signaling axis was involved in BRCA prevention and treatment.[24] Chen et al detected a lower expression level of *METTL14* in colorectal cancer tissues and cell lines by modulating the N6-methyladenosine level of SOX4 mRNA, which was significantly associated with poor overall survival.[23] Yi et al identified the potential effect of *METTL14* on the miRNA expression profile and the effect of hsa-miR-146a-5p on the migration and invasion of breast cancer cells. Weng et al found that *METTL14* was highly expressed in acute myeloid leukemia cells and played an oncogenic role.[11] However, there are few studies about the

Table 4 The Influence of METTL14 Polymorphisms (rs298982, GA/AA vs GG) on Sensitivity to Different Treatment Strategies Based on MRD Levels

Variables		MRD in Marrow (%, 19d)				MRD in Marrow (%, 33d)				MRD in Marrow (%, 12w)			
		Case (%)		P^a	Adjusted OR[a] (95% CI)	Case (%)		P^a	Adjusted OR[a] (95% CI)	Case (%)		P^a	Adjusted OR[a] (95% CI)
		<0.01	≥0.01			<0.01	≥0.01			<0.01	≥0.01		
CCCG-ALL -2015	GG	8 (2.78)	280 (97.22)			152 (51.01)	146 (48.99)			181 (90.50)	19 (9.50)		
	GA	0 (0.00)	47 (100.0)			49 (76.56)	15 (23.44)			61 (100.0)	0 (0.00)		
	AA	2 (25.00)	6 (95.31)			4 (66.67)	2 (33.33)			5 (100.0)	0 (0.00)		
	GA/AA	2 (3.64)	53 (96.36)			53 (75.71)	17 (24.29)			66 (100.0)	0 (0.00)		
	GG	8 (2.78)	280 (97.22)	0.653	1.44 (0.29–7.04)	152 (51.01)	146 (48.99)	0.001	3.00 (1.66–5.44)	181 (90.50)	19 (9.50)	0.968	999 (0.00–999)
SCCLG-ALL -2016	GG	15 (31.91)	32 (68.09)			34 (69.39)	15 (30.61)			15 (93.75)	1 (6.25)		
	GA	0 (0.00)	7 (100.0)			8 (80.00)	2 (20.00)			11 (84.62)	2 (15.38)		
	AA	1 (100.0)	0 (0.00)			1 (100.0)	0 (0.00)			9 (90.00)	1 (10.00)		
	GA/AA	1 (12.50)	7 (87.50)			9 (81.82)	2 (18.18)			26 (89.66)	3 (10.34)		
	GG	15 (31.91)	32 (68.09)	0.378	0.37 (0.04–3.41)	34 (69.39)	15 (30.61)	0.321	2.43 (0.42–14.04)	9 (90.00)	1 (10.00)	0.644	1.85 (0.14–25.4)
NA	GG	21 (38.89)	33 (61.11)			30 (68.18)	14 (31.82)			20 (95.24)	1 (4.76)		
	GA	0 (0.00)	2 (100.0)			4 (80.00)	1 (20.00)			3 (100.0)	0 (0.00)		
	AA	0 (0.00)	2 (100.0)			0 (0.00)	1 (100.0)			0 (0.00)	0 (0.00)		
	GA/AA	0 (0.00)	4 (100.0)			4 (66.67)	2 (33.33)			3 (100.0)	0 (0.00)		
	GG	21 (38.89)	33 (61.11)	0.952	0.00 (0.00–999)	30 (68.18)	14 (31.82)	0.984	1.02 (0.16–6.38)	20 (95.24)	1 (4.76)	0.980	999 (0.00–999)

Note: [a]Adjusted for age and gender.

Abbreviations: CCCG, Chinese Children Cancer Group; SCCLG, South China Children Leukemia Group; NA, not available.

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Table 5 The Influence of *METTL14* Polymorphisms (rs1064034, TA/AA vs TT) on Sensitivity to Different Treatment Strategies Based on MRD Levels

Variables		MRD in Marrow (%, 19d)				MRD in Marrow (%, 33d)				MRD in Marrow (%, 12w)			
		Case (%)		*P*[a]	Adjusted OR[a] (95% CI)	Case (%)		*P*[a]	Adjusted OR[a] (95% CI)	Case (%)		*P*[a]	Adjusted OR[a] (95% CI)
		<0.01	≥0.01			<0.01	≥0.01			<0.01	≥0.01		
CCCG-ALL -2015	TT	6 (2.93)	199 (97.07)			113 (52.07)	104 (47.93)			136 (89.47)	16 (10.53)		
	TA	3 (3.41)	85 (96.59)			79 (77.45)	23 (22.55)			89 (98.89)	1 (1.11)		
	AA	0 (25.00)	26 (100.0)			14 (50.00)	14 (50.00)			20 (100.0)	0 (0.00)		
	TA/AA	3 (2.63)	111 (97.37)			93 (71.54)	37 (28.46)			109 (99.09)	1 (0.91)		
	TT	6 (2.93)	199 (97.07)	0.924	0.93 (0.22–3.83)	113 (52.07)	104 (47.93)	0.001	2.32 (1.45–3.69)	136 (89.47)	16 (10.53)	0.017	11.9 (1.55–91.5)
SCCLG-ALL -2016	TT	10 (29.41)	24 (70.59)			24 (70.59)	10 (29.41)			15 (78.95)	4 (21.0)		
	TA	6 (31.58)	13 (68.42)			17 (70.83)	7 (29.17)			17 (100.0)	0 (0.00)		
	AA	0 (0.00)	1 (100.0)			1 (100.0)	0 (0.00)			1 (100.0)	0 (0.00)		
	TA/AA	6 (30.00)	70.00			18 (72.00)	7 (28.00)			18 (100.0)	3 (0.00)		
	TT	10 (29.41)	24 (70.59)	0.970	1.02 (0.29–3.53)	24 (70.59)	10 (29.41)	0.911	1.07 (0.33–3.43)	15 (78.95)	4 (21.0)	0.971	999 (0.00–999)
NA	TT	14 (38.89)	22 (61.11)			23 (67.65)	11 (32.35)			13 (92.86)	1 (7.14)		
	TA	3 (21.43)	11 (78.57)			8 (66.67)	4 (33.33)			8 (100.0)	0 (0.00)		
	AA	0 (0.00)	4 (100.0)			3 (75.00)	1 (25.00)			2 (0.00)	0 (0.00)		
	TA/AA	3 (16.67)	15 (83.33)			11 (68.75)	5 (31.25)			10 (100.0)	0 (0.00)		
	TT	14 (38.89)	22 (61.11)	0.101	0.30 (0.07–1.27)	23 (67.65)	11 (32.35)	0.885	1.10 (0.30–4.04)	13 (92.86)	1 (7.14)	0.979	999 (0.00–999)

Note: [a]Adjusted for age and gender.

Abbreviations: CCCG, Chinese Children Cancer Group; SCCLG, South China Children Leukemia Group; NA, not available.

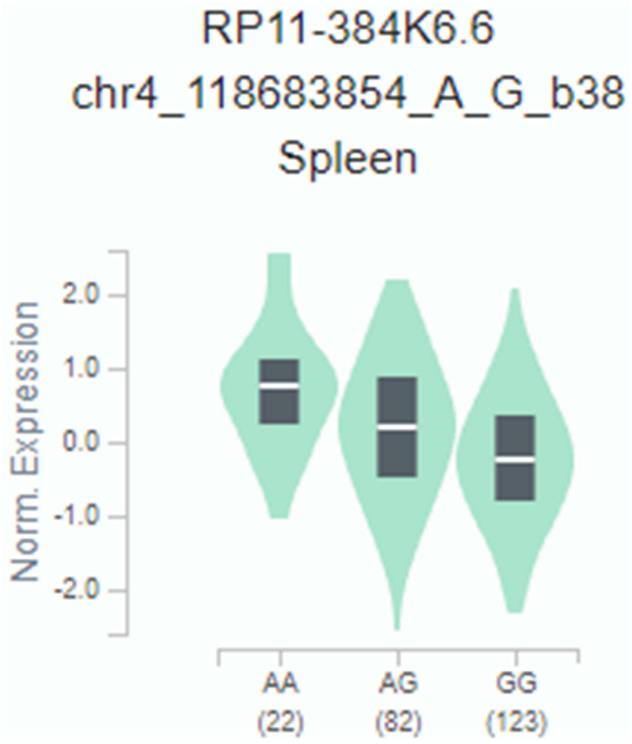

Figure 1 Functional implication between the *METTL14* gene rs298982 polymorphism and the expression of its neighboring gene *RP11-384K6.6* in spleen based on the public database GTEx portal (P=8.70×10^{-8}).

association of *METTL14* genetic variants with cancer risk. Zhou et al investigated four *METTL14* gene SNPs (rs298982 G/A, rs62328061 A/G, rs9884978 G/A, and rs4834698 T/C) associated with neuroblastoma susceptibility.[13] In our previous research, we reported that multiple m^{6}A-modified enzyme-encoding genes are closely related to glioma susceptibility, and none of the four studied *METTL14* gene SNPs (rs1064034, rs298981, rs62328061, and rs9884978) were associated with glioma risk in Chinese children.[15]

In the present work, we performed genotyping of three SNPs of *METTL14*, rs298982, rs298981 and rs1064034 and demonstrated that rs298982 G/A and rs1064034 T/A were associated with pediatric ALL risk. Our results suggested that the rs298982 A allele and rs1064034 A allele decreased the ALL risk in a population of southern Chinese children. Both rs298982 and rs1064034 play a role in decreased ALL risk predominantly in children < 10 years and males and in patients with common B immunophenotyping, normal diploid or positive MRD in CCCGs, rather in SCCLG. Moreover, the effects of rs1064034 on ALL risk were also predominant in patients with T-ALL and MLL fusion types. These results suggested that rs298982 G/A and rs1064034 T/A may provide a reference for the diagnosis and therapy of this disease. Compared with single SNPs, multiple markers association studies based on haplotypes significantly improve the power of mapping and characterizing disease-causing genes.[25] We explored whether various haplotypes consisting of the *METTL14* gene polymorphisms rs298982, rs298981 and rs1064034 are associated with ALL risk. These results suggest that these variants may interact with each other to modify the risk of ALL. We further attempted to interpret the possible mechanism of *METTL14* gene SNP-mediated ALL risk. Furthermore eQTL evidence suggested that the A allele in rs298982 are associated with increased *RP11-384K6.6* levels in spleen. We also revealed that the higher expression of lncSNHG8 caused by the T allele in rs1064034 in cultured fibroblasts but decreased expression of *RP11-384K6.6* in

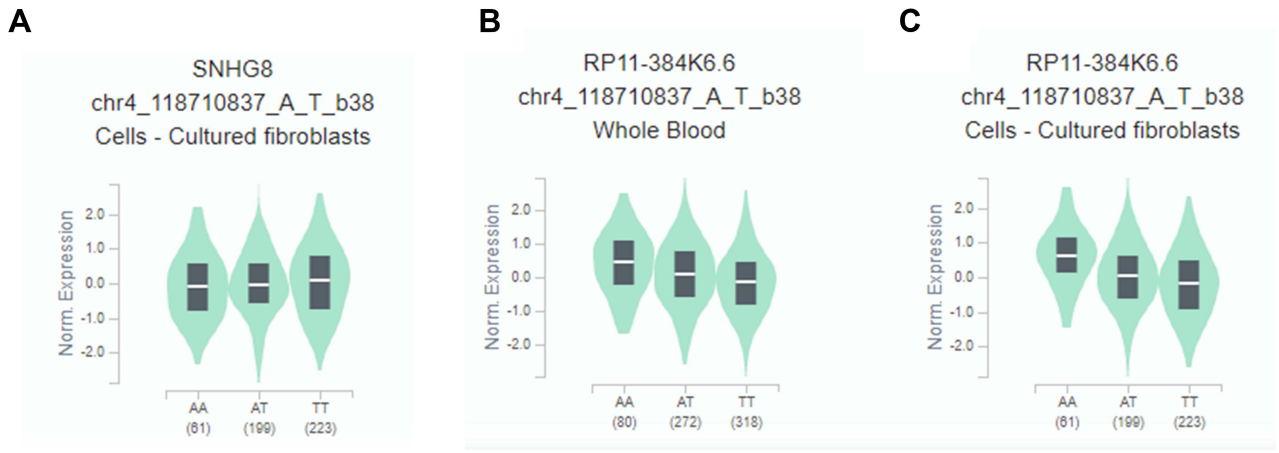

Figure 2 Functional implication of the *METLL14* gene rs1064034 polymorphism based on the public database GTEx portal. The genotype of rs1064034 and expression of its neighboring gene (**A**) lncSNHG8 in cultured fibroblasts (P=1.80×10^{-5}) and RP11-384K6.6 in (**B**) whole blood (P=9.90×10^{-14}) and (**C**) cultured fibroblasts (P=3.50×10^{-12}).

whole blood and c cultured fibroblasts. *LncSNHG8* was found to be abnormally expressed in cancers and to play important roles in several kinds of cancers.[26–28] Tian et al found *LncSNHG8* higher expression level in nasopharyngeal carcinoma tissues and cells, as well as it regulates nasopharyngeal carcinoma cell proliferation, colony formation, migration, and invasion via regulating miR-656-3p.[27] Miao et al demonstrated that lncSNHG8 expression was elevated in ovarian carcinoma cells, and revealed that silencing of it impedes cell proliferation and promote cell apoptosis.[29] Furthermore, Dong et al found that *lncSNHG8* expression was increased in human hepatocellular carcinoma tissues which promoted cell migration and invasion.[30] But there are no research about *LncSNHG8* in ALL and *RP11-384K6.6* in cancers, We propose that the higher expression of *lncSNHG8* caused by the A allele in rs298982 and the T allele in rs1064034 may facilitate the development of ALL. On the other hand, with increases in the rs1064034 A genotype, the average expression of *RP11-384K6.6* gradually increased. This conclusion requires further interpretation because the role of *RP11-384K6.6* in cancers remains to be revealed. In conclusion, more functional experiments are needed to support this possible mechanism.

Although this study explored *METTL14* polymorphisms and ALL susceptibility in a novel and relatively large sample size of pediatric acute lymphoblastic leukemia cases and included multiple-center participants in a single Chinese population, several limitations should be noted. First, the study population involved only Chinese subjects and was limited to volunteers. Larger-scale studies with more samples and SNP sites are encouraged to confirm the roles of *METTL14* in ALL risk. Finally, this study only analyzed genetic factors, and other environmental factors were not available for ALL susceptibility investigation. The functions of *METTL14* and these SNPs in the progression of ALL should also be further investigated in the future.

In conclusion, our five-center case-control study, for the first time, showed that *METTL14* rs298982 G/A and rs1064034 T/A decreased the risk of ALL in southern Chinese children and suggested that the *METTL14* polymorphism might be a potential biomarker for pediatric ALL susceptibility and chemotherapeutic choice. Nevertheless, these SNPs in the *METTL14* gene are intriguing loci for further studies, and the underlying biological mechanisms should be revealed.

Abbreviations

METTL14, methyltransferase like 14; ALL, acute lymphoblastic leukemia; m^6A, N6-methyladenosine; SNPs, single nucleotide polymorphisms; ORs, odds ratios; CIs confidence intervals; MRD, minimal residual disease; B-ALL, B-cell precursor acute lymphoblastic leukemia; T-ALL, T-cell lineages acute lymphoblastic leukemia; ARID5B, AT-rich interaction domain 5B; USP1, ubiquitin specific peptidase 1; METTL3, methyltransferase 3; WTAP, WT1 associated protein; FTO, fat mass and obesity-associated protein; ALKBH5, α-ketoglutarate-dependent dioxygenase homolog 5; SYSU, Sun Yat-sen University; GTEx, the Genotype-Tissue Expression; FPRP, false-positive report probability; AML, acute myelocytic leukemia; CCCG, Chinese Children Cancer Group chemotherapeutics; SCCLG, South China Children Leukemia Group chemotherapeutics; eQTL, expression quantitative trait loci; mRNA, messenger RNA; lncRNA, long noncoding RNA; SNHG8, small nucleolar RNA host gene 8.

Ethics Approval and Consent to Participate

The experiments were approved by the Ethics Committee of the Guangzhou Women and Children's Medical Center. Written informed consent was obtained from all patients. The experiments were carried out following the Declaration of Helsinki.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, agreed to the submitted journal, and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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