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Correlation of *METTL4* genetic variants and severe pneumonia pediatric patients in Southern China

Liuheyi Ma[1], Xiaoyu Zuo[2], Bingtai Lu[3,4*] and Yuxia Zhang[1,3*]

Abstract

Background Pneumonia is a major cause of mortality and health burden in children under five, yet its genetic etiology remains poorly understood. Methyltransferase 4, N6-adenosine (METTL4), is a methyltransferase enzyme responsible for RNA and DNA methylation and is known to be activated under hypoxic conditions. However, its potential link to susceptibility to pneumonia has not been evaluated. This study aimed to explore candidate regulatory single nucleotide polymorphisms (SNPs) within the *METTL4* gene and their association with the development of severe pneumonia.

Results In this study, we recruited a cohort of 1034 children with severe pneumonia and 8426 healthy controls. We investigated the associations of candidate regulatory single nucleotide polymorphisms (SNPs) within *METTL4* polymorphisms with severe pneumonia. Our results indicated that the C allele of rs9989554 ($P=0.00023$, $OR=1.21$, 95% CI: 1.09–1.34) and the G allele of rs16943442 ($P=0.0026$, OR = 1.22, 95% CI: 1.07–1.38) were significantly associated with an increased risk of severe pneumonia. The regulatory potential of these two SNPs in the lung was investigated using tools such as expression quantitative trait loci (eQTLs), RegulomeDB, and FORGEdb.

Conclusions This study represents the first investigation elucidating the role of genetic variations in the *METTL4* gene and their influence on susceptibility to severe pneumonia in pediatric populations. *METTL4* is identified as a novel predisposing gene for severe pneumonia and a potential therapeutic target. Further research is warranted to validate this correlation and to comprehensively elucidate the biological role of the *METTL4* gene in severe pneumonia.

Keywords Methyltransferase4, N6-adenosine (*METTL4*), Single nucleotide polymorphisms (SNPs), Severe pneumonia, Genetic susceptibility

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Background

Pneumonia is the most common lower respiratory tract infection in children and poses a significant threat to their health [1]. In 2015, community-acquired pneumonia (CAP) was responsible for 15% of deaths in children under five years old worldwide, resulting in a total of 922,000 fatalities among children of all ages globally [2]. Infections originating in the lung are the primary cause of severe pneumonia and childhood death [3]. *Influenza viruses*, *Respiratory syncytial virus*, *Adenovirus*, *Mycoplasma*, *Chlamydophila*, *Staphylococcus aureus*, etc., are the most frequently identified pathogens in severe pneumonia [4–6]. Pathogen invasion triggers host responses that stimulate the secretion of inflammatory cytokines such as TNF-a, IL-1b and IL-6. These cytokines impair the alveolar epithelial barrier and enhance epithelial permeability. An excessive inflammatory response is a hallmark of severe pneumonia, characterized by the presence of pulmonary edema and interstitial transparent membranes in the alveolar interstitium, which subsequently leads to clinical symptoms of fever, cough, chest pain, and dyspnea [7, 8]. Severe pneumonia may lead to the development of life-threatening syndromes, including acute respiratory distress syndrome (ARDS) [9] or respiratory failure [10]. Treatments for severe pneumonia mainly rely on antibiotics or corticosteroids, which are sometimes ineffective.

Methyltransferase 4, N6-adenosine (*METTL4*), belongs to the methyltransferase-like (METTL) family and methylates nucleotides, proteins, and small molecules [11]. *METTL4* catalyzes the methylation of RNA (m6A) and DNA (6 mA), including internal *N*6-methylation of cap-adjacent *N*6,2′-*O*-dimethyladenosine of U2 small nuclear RNA (snRNA), microRNA and mammalian mitochondrial DNA [12, 13]. A recent study showed that *METTL4* promotes ferroptosis by increasing *BECN1* mRNA m6A modification and autophagy in hepatic stellate cells [14]. Hsu et al. showed that in mammalian tumor cells, hypoxia results in increased 6 mA levels through *METTL4* via the activation of multiple metastasis-inducing genes [15]. Interestingly, in a human fibroblast cell line under hypoxic conditions, the methylated levels of the vasoactive microRNA m6A were significantly increased. Moreover, m6A modification is also present in viruses such as adenovirus [16] and SARS-CoV-2 [17]. In soft tissue sarcomas (STS), copy number variation (CNV) of the *METTL4* gene could serve as a prognostic biomarker by potentially influencing mast cell infiltration and DNA methylation [18].

Given that *METTL4* is activated under hypoxic conditions and that widespread m6A modification occurs during viral replication [19, 20], alterations in the *METTL4* gene may correlate with the risk of developing severe pneumonia. In this study, we assembled a cohort including 1034 severe pneumonia patients and 8624 controls from a South Chinese population aged 0–15 years. First, the associations between *METTL4* SNPs and severe pneumonia susceptibility were investigated. Then, haplotype-association and subtype-specific association analyses of the SNPs were conducted.

Table 1 Cohort characteristics

	Severe pneumonia (*n* = 1034)	**Controls (*n* = 8426)**
Age, median (range), years	0.6 (2days-15 years)	6 (1 month-20 years)
Gender, n (%)		
Male	642 (62.1)	4735 (56.2)
Female	392 (37.9)	3691 (43.8)
Days in hospital, median (range), days	16 (3–329)	N/A
Diagnosed as primary severe pneumonia	719 (69.5)	N/A
Diagnosed as secondary severe pneumonia	315 (30.5)	N/A
Pathogens, n (%)		
Viral	721 (69.7)	N/A
Bacterial	625 (60.4)	N/A
Fugus	108 (10.4)	N/A
Comorbid conditions, n (%)	978 (94.6)	N/A
Complications		
Acute respiratory failure	344 (33.3)	N/A
Acute respiratory distress syndrome (ARDS)	17 (1.6)	N/A
Sepsis	70 (6.8)	N/A
Mechanical ventilation, n (%)	776 (75.0)	N/A
Clinical outcomes, n (%)		
Discharge	979 (94.7)	N/A
Mortality	55 (5.3)	N/A

N/A not applicable

Methods

Study subjects

This work was conducted with the approval of the Medical Ethics Committees of Guangzhou Women and Children's Medical Center (No: 2016111853), with 1034 children who were diagnosed with severe pneumonia and a group of 8624 children without a history of pneumonia involved in previous studies [21]. Written informed consent to participate was obtained from the legal guardians of all participants. Data on demographic characteristics and disease severity were extracted from the electronic medical records of the patients.

Characteristics of the study participants

The clinical characteristics of the study participants were shown in Table 1. We included a severe pneumonia cohort with 1,034 patients and 8,426 healthy controls. The ages of the participants ranged from 0 to 180 months,

with 37.9% being female. The control group consisted of individuals aged between 1 month and 240 months. Among the pneumonia patients, 719 (69.5%) were diagnosed with primary severe pneumonia, while 315 (31.5%) were diagnosed with secondary severe pneumonia.

Criteria for severe pneumonia, primary and secondary severe pneumonia

Criteria for severe pneumonia: (a) invasive mechanical ventilation; (b) fluid refractory shock; (c) acute need for non-invasive positive-pressure ventilation; and (d) hypoxemia requiring fractional inspired oxygen (FiO2) > inspired concentration or flow feasible in the general-care area. Primary pneumonia was defined as severe pneumonia with initially diagnosed with pathogens infections. Secondary pneumonia was defined as severe pneumonia in connection with other disease such as cardiovascular disease, acute renal failure, or gastrointestinal dysfunction. The pathogen information was retrieved from the electronic medical records of the participants. Pathogen detection was performed at the central hospital diagnostic laboratories using blood, throat swab, sputum, or BAL samples.

Polymorphism selection and genotyping

The procedure for extracting and amplifying genomic DNA from venous blood samples of the included cases, following the selection criteria outlined in our published report, has been previously described [21]. The SNPs were genotyped using a MassARRAY iPLEX Gold system (Sequenom). HWE tests were performed using the goodness-of-fit χ^2 test, with $P < 0.05$ indicating a deviation from HWE. The raw genotyping data supporting this analysis are provided in Supplementary Material 2.

SNP- and haplotype-based association analysis

All common SNPs within ± 5 kb flanking *METTL4* were retrieved from the "dbSnp153Common" database by using UCSC hgTable and filtered by the minor allele frequency (MAF > 0.05) in the East Asian (EAS) population (CHS + CHB + JPT in 1000 Genomes Data). The pairwise linkage disequilibrium (LD) between SNPs was assessed in the 1000 Genome EAS population and visualized as an LD heatmap by using the R package "LDheatmap". A $R^2 > 0.8$ was considered to indicate high LD. Tag-SNPs were selected as the minimal set of independent SNPs that represent all SNPs in each LD block. Finally, 17 SNPs were selected for subsequent analyses. Additionally, a haplotype-specific competitive test was conducted, which compares each haplotype against all others. A *P* value less than 0.0045 was considered to indicate statistical significance.

Statistical analysis

The allelic association between *METTL4* polymorphisms and severe pneumonia was assessed by comparing allele frequencies in patients and controls using the PLINK program (v1.9b). Age and sex were adjusted for in the multivariate logistic regression. The 95% confidence intervals (CIs) and odds ratios [22] were used to estimate the effect sizes of the SNPs. Bonferroni correction was applied to control for type 1 errors caused by multiple testing. A *P* value < 0.0045 was considered to indicate statistical significance. The GTEx portal was used to assess the expression quantitative trait locus (eQTL) effects of the SNPs (https://gtexportal.org/home/). The LD between SNPs was calculated by LDmatrix (https://ldlink.nci.nih.gov/?tab=home) and visualized by the R packages "LDlinkR" and "gaston".

Potential regulatory SNPs in *METTL4*

The potential regulatory SNPs were subjected to comprehensive scoring analysis using the FORGEdb (https://forge2.altiusinstitute.org/files/forgedb.html) and Regulome DB (https://regulomedb.org/regulome-search) databases. High-confidence regulatory SNPs with a RegulomeDB score less than 4 and a FORGEdb score greater than 5 were identified.

Results

Correlations of *METTL4* genetic variants and the occurrences of severe pneumonia

The UCSC platform was utilized to examine common SNPs located within 5 kb upstream and downstream of the *METTL4* gene. Totally 119 candidate SNPs were identified based on linkage disequilibrium (LD) patterns with an r^2 threshold greater than 0.8. From the gene, 11 SNPs (rs9989554, rs16943442, rs80010836, rs2138848, rs9948895, rs17534687, rs12457106, rs72857010, rs66873847, rs2644175, and rs11663148) within the *METTL4* gene from 17 selected tag SNPs based on the 1000 Genomes database were screened for genotyping (Fig. 1 and Table 2). Among the selected SNPs, rs9989554 and rs16943442 showed statistical significance after Bonferroni correction for multiple comparisons when compared to control subjects. Specifically, results shown that the minor C allele frequency of rs9989554 (0.30 vs. 0.27, OR = 1.21, 95% CI = 1.09–1.34, *P* = 0.00023) and the G allele frequency of rs16943442 (0.16 vs. 0.14, OR = 1.22, 95% CI = 1.09–1.34, *P* = 0.0026) were significantly associated with an increased risk of severe pneumonia (Table 2).

Further haplotype-specific analyses, using the TA haplotype as a reference, showed that the CG haplotype of rs9989554 and rs16943442 is associated with a significantly increased risk of severe pneumonia (OR = 1.25, 95% CI: 1.10–1.42, *P* = 0.0007), while the CA haplotype

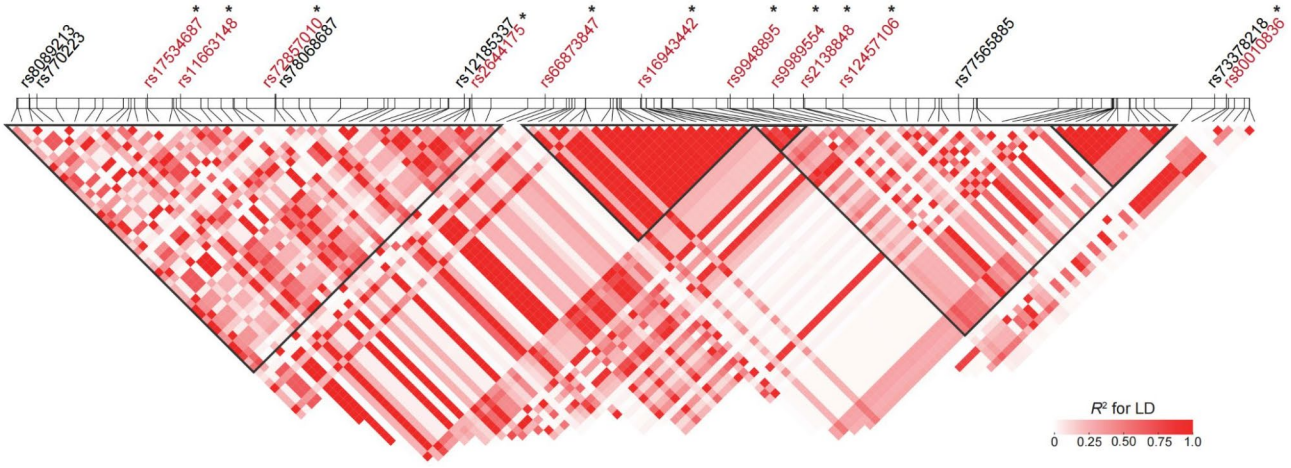

Fig. 1 Overview of Linkage Disequilibrium (LD) within a 5-kilobase region upstream and downstream of the *METTL4*. The schematic representation of the physical positions of the investigated SNPs are shown in the top panel, while LD between SNPs was calculated by LDmatrix, and the LD plots were generated from the R software packages"LDlinkR" and "gaston"

Table 2 Association between *METTL4* polymorphisms and the risk of severe pneumonia

CHR	BP	dbSNP	Major allele	Minor allele	HWE	Minor Allele Frequency in Cases	Minor Allele Frequency in Controls	Consequences of the variants	OR (95% CI)	*p*-value
18	2559434	rs9989554	T	C	0.98	0.30	0.27	Intronic	1.21 (1.09–1.34)	**0.00025**
18	2554836	rs16943442	A	G	0.23	0.16	0.14	Synonymous	1.22 (1.09–1.34)	**0.0026**
18	2575199	rs80010836	A	C	0.18	0.16	0.14	Upstream, intronic	1.19 (1.05–1.35)	0.0077
18	2560519	rs2138848	A	C	0.92	0.34	0.32	Intronic	1.13 (1.02–1.24)	0.016
18	2557926	rs9948895	C	G	0.69	0.36	0.35	Intronic	1.08 (0.98–1.20)	0.096
18	2537639	rs17534687	G	A	0.79	0.14	0.13	UTR3	1.15 (1.00–1.31)	0.040
18	2561851	rs12457106	T	A	0.39	0.08	0.09	Intronic	0.86 (0.72–1.02)	0.091
18	2542078	rs72857010	G	C	0.36	0.06	0.05	Intronic	1.11 (0.91–1.36)	0.29
18	2551772	rs66873847	G	A	0.45	0.10	0.10	Intronic	1.06 (0.91–1.23)	0.46
18	2548845	rs2644175	A	G	0.44	0.18	0.18	Intronic	1.04 (0.92–1.17)	0.52
18	2538788	rs11663148	C	T	0.09	0.21	0.21	UTR3	0.98 (0.88–1.10)	0.78

Abbreviations: *CHR* chromosome, *BP* base pair (where the SNP is located), *SNP* single-nucleotide polymorphism, *HWE* Hardy–Weinberg equilibrium, *OR* odds ratio, *CI* Confidence interval

P-values were adjusted by gender and age. The nominal *p*-values were showed. Calculation of the OR was also based on the risk allele of each SNP

demonstrated nominal significance ($P = 0.0263$). Thus, our findings suggest that the CG haplotype confers susceptibility to severe pneumonia (Table S2).

Associations of *METTL4* SNPs with subtypes of severe pneumonia

To further investigate the correlations between *METTL4* polymorphisms and subtypes of severe pneumonia, we divided our cohort into two groups: those with a primary diagnosis of severe pneumonia (mainly caused by pathogens, $n = 719$) and those with a secondary diagnosis of severe pneumonia (mainly caused by other diseases, $n = 315$). As shown in Table 3, only the minor A allele of the *METTL4* rs12457106 SNP showed a statistically significant difference ($p = 0.0129$) between the two subtypes. No significant differences were observed for the other SNPs.

Expression quantitative trait loci (eQTLs) of *METTL4* genetic variants

Next, we performed expression quantitative trait locus (eQTL) analysis to assess the regulatory effects of the *METTL4* genetic variants. The results indicated that 4 SNPs were linked to elevated METTL4 mRNA expression levels in multiple organs (Table 4). Specifically, the rs9989554 and rs9948895 SNPs appear to be associated with increased *METTL4* mRNA expression (indicated by effect size > 0) across different tissues. Conversely, rs2138848 and rs16943442 were shown to be associated with decreased *METTL4* mRNA expression (indicated by effect size < 0) in various tissues.

Table 3 Subgroup-specific association between *METTL4* polymorphism s and the risk of severe pneumonia

				Primary pneumonia			Secondary pneumonia		
CHR	**BP**	**dbSNP**	**Minor Allele**	**MAF**	**OR(95%CI)**	***p*-value**	**MAF**	**OR(95%CI)**	***p*-value**
18	2561851	rs12457106	A	0.07	0.76(0.61–0.94)	0.0110	0.10	1.16(0.88–1.55)	0.2929
18	2542078	rs72857010	C	0.06	1.08(0.85–1.37)	0.5071	0.05	1.31(0.93–1.83)	0.1217
18	2559434	rs9989554	C	0.30	1.18(1.04–1.33)	0.0083	0.32	1.29(1.07–1.54)	0.0061
18	2575199	rs80010836	C	0.16	1.16(1.00–1.35)	0.0532	0.17	1.30(1.04–1.63)	0.0237
18	2537639	rs17534687	A	0.14	1.10(0.94–1.28)	0.2501	0.15	1.23(0.97–1.55)	0.0837
18	2557926	rs9948895	G	0.36	1.06(0.95–1.19)	0.2841	0.38	1.15(0.97–1.37)	0.1153
18	2554836	rs16943442	G	0.16	1.20(1.03–1.39)	0.0181	0.17	1.30(1.04–1.63)	0.0236
18	2538788	rs11663148	T	0.20	0.97(0.84–1.11)	0.6172	0.21	1.02(0.83–1.24)	0.8840
18	2560519	rs2138848	C	0.34	1.12(1.00–1.26)	0.0603	0.35	1.16(0.97–1.39)	0.0978
18	2551772	rs66873847	A	0.11	1.08(0.91–1.29)	0.3743	0.10	1.06(0.80–1.39)	0.6963
18	2548845	rs2644175	G	0.19	1.04(0.90–1.20)	0.5830	0.18	1.03(0.83–1.27)	0.8212

Abbreviations: *CHR* chromosome, *BP* base pair (where the SNP is located), *SNP* single-nucleotide polymorphism, *MAF* minor allele frequency in patients, *OR* odds ratio, *CI* Confidence interval

P-values were adjusted by gender and age. Calculation of the OR was also based on the risk allele of each SNP

Potential regulatory effects of *METTL4* SNPs on pediatric severe pneumonia

Additionally, the regulatory potential of *METTL4* SNPs on severe pneumonia was assessed using the RegulomeDB and FORGEdb scoring system. Our results indicated that 5 SNPs of the *METTL4* gene (rs9989554, rs16943442, rs9948895, rs2138848, rs80010836) received scores ranging from 9 to 5 in the FORGEdb system and from 3a to 4 in the RegulomeDB system (Table 5). Among these SNPs, rs9989554 and rs16943442 have relatively high FORGEdb scores, suggesting that these 2 SNPs may have a stronger regulatory potential on the *METTL4* gene compared to the other 3 SNPs.

FORGEdb scores suggests that the SNPs are more likely to fall within regulatory regions. We found that the *METTL4* rs9989554 and rs16943442 had relatively high FORGEdb scores of 9. In addition, the FORGEdb database predicts that rs9989554 may impact H3K36me3 binding in fetal lung cells and fetal lung fibroblast lines. Collectively, these findings suggest that rs9989554 and rs16943442 may play a role in regulating the expression of the *METTL4* gene.

Discussion

In this study, we identified a correlation between *METTL4* gene polymorphisms and susceptibility to severe pneumonia. Specifically, the rs9989554 C allele and rs16943442 G allele of the *METTL4* gene were significantly associated with an increased risk of severe pneumonia (Table 2). Additionally, the minor A allele of the rs12457106 SNP showed nominal significance when comparing patients with primary versus secondary severe pneumonia (Table 3). Furthermore, eQTL analysis of the rs9989554 C and rs16943442 G alleles suggested that these variants may influence the regulation of *METTL4* mRNA expression (Table 4). Moreover, results from the FORGEdb and RegulomeDB analyses indicated that the rs9989554 C and rs16943442 G alleles might be SNPs with regulatory potentials of *METTL4* gene (Table 5).

Severe pneumonia is a major cause of childhood mortality [23]. Throughout the COVID-19 pandemic, numerous studies have indicated that host genetic risk factors may influence disease severity [24, 25]. Nonetheless, there is a scarcity of data specifically addressing genetic risk factors for childhood pneumonia that can result in adverse outcomes, such as life-threatening symptoms, clinical deterioration, and the development of complications [26]. To address this gap, we conducted a MassARRAY Genotyping to investigate genetic risk factors for childhood pneumonia in a cohort comprising 1,034 children with severe pneumonia and 8,426 healthy controls. Here, we aim to further investigate whether *METTL4* polymorphisms are associated with severe pneumonia primarily caused by pathogens (primary diagnosis) or following other diseases (secondary diagnosis). As no significant difference was observed between these two groups, this suggests that the *METTL4* gene may be associated with factors beyond just infections.

METTL4 mediates N^6-adenosine methylation in both eukaryotic DNA and RNA [27, 28], as well as RNA splicing [12]. Recent studies have demonstrated that hypoxia induces 6 mA modification through *METTL4*. The activation of *METTL4* promotes tumor metastasis by activating multiple metastasis-associated genes [15]. Hao et al. found that hypoxic stress leads to the accumulation of *METTL4* in mitochondria, resulting in increased 6 mA methylation [13]. It is important to note that hypoxia is a common feature in all types of severe pneumonia, including COVID-19 [29], adenovirus infection [30], acute lung injury (ALI), and acute respiratory distress syndrome (ARDS) [9, 31]. Additionally, Shen et al. (2021) reported that exposure to ferroptosis-inducing compounds elevates *METTL4* levels and m6A modification, which in turn trigger autophagy and enhance ferroptosis

Table 4 eQTL analysis of potential regulatory SNPs in *METTL4* among different tissue

SNP	Tissue	Effect Size	*p*-value
rs9989554	Whole Blood	0.13	1.85E-07
	Adipose - Visceral (Omentum)	0.15	1.49E-06
	Adipose - Subcutaneous	0.15	9.47E-06
rs16943442	Adipose - Visceral (Omentum)	-0.33	1.38E-08
	Adipose - Subcutaneous	-0.41	3.77E-09
rs9948895	Cells - Cultured fibroblasts	0.18	5.08E-11
	Adrenal Gland	0.32	5.81E-07
	Heart - Left Ventricle	0.25	2.86E-06
	Brain - Cerebellar Hemisphere	0.28	2.15E-05
	Esophagus - Gastroesophageal Junction	0.19	1.66E-05
	Muscle - Skeletal	0.24	4.30E-13
	Esophagus - Muscularis	0.26	6.82E-10
	Artery - Aorta	0.22	1.61E-07
	Brain - Amygdala	0.39	1.21E-05
	Skin - Sun Exposed (Lower leg)	0.12	7.69E-05
rs2138848	Cells - Cultured fibroblasts	-0.19	1.42E-12
	Thyroid	-0.13	3.40E-05
	Testis	-0.22	3.11E-06
	Adrenal Gland	-0.37	8.96E-09
	Brain - Frontal Cortex (BA9)	-0.31	1.48E-05
	Heart - Left Ventricle	-0.24	3.64E-06
	Brain - Cerebellar Hemisphere	-0.30	5.00E-06
	Esophagus - Gastroesophageal Junction	-0.22	1.45E-06
	Muscle - Skeletal	-0.26	2.07E-14
	Esophagus - Muscularis	-0.28	3.65E-11
	Artery - Aorta	-0.22	2.41E-07
	Skin - Not Sun Exposed (Suprapubic)	-0.13	6.61E-05
	Artery - Tibial	-0.14	6.08E-05
	Brain - Amygdala	-0.44	1.42E-06
	Skin - Sun Exposed (Lower leg)	-0.13	8.12E-06

Functional relevance of SNP on gene expression in GTEx database

eQTL expression quantitative trait loci; Effect size, the degree to which an SNP influences the expression of genes in a particular tissue

in human hematopoietic stem cells (HSCs) [14]. Also, the autophagy pathway has been shown to play a critical role during severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection [32]. Notably, a recent study showed that *METTL4* promotes ferroptosis in alveolar epithelial cells during sepsis-induced lung injury through N6-methyladenosine modification of ferroptosis-related genes [33], indicating a strong potential link between pulmonary dysfunction and *METTL4* gene. Therefore, it is plausible that *METTL4* polymorphisms influence pneumonia susceptibility by affecting the hypoxia response or regulating autophagy pathways.

It should be also noted that there are some limitations in our study. First, more clinical and microbiologic data, such as information on patients who were hospitalized more than once, should be included. Additionally, a larger sample size is needed to enhance statistical power. Second, including diverse ethnic groups would provide a more comprehensive assessment of genotype distributions. Third, the samples were heterogeneous in terms of age and putative phenotypes, especially with regard to secondary pneumonia phenotypes. Fourth, further research is needed to investigate how these SNPs affect the expressions and functions of *METTL4*, in order to understand how genetic variations influence the onset and progression of severe pneumonia.

Conclusions

In summary, we demonstrated that the C allele of rs9989554 and the G allele of rs16943442 are significantly associated with an increased risk of severe pneumonia. However, most SNPs of the *METTL4* gene did not show significant differences between primary and secondary severe pneumonia. To our knowledge, this is the first study to establish a link between genetic variations in the *METTL4* gene and susceptibility to severe pneumonia in children. These genetic variations may play a pivotal role in the development of severe pneumonia related to *METTL4* dysfunction, potentially aiding in the identification of effective treatments and the development of

Table 5 Candidate regulatory SNPs in *METTL4* by forgedb and RegulomeDB resources

SNP	Position	Alleles	MAF	Distance	Dprime	FORGEdb[a]	RegulomeDB[b]
rs9989554	**chr18:2571846**	**(A/G)**	**0.23**	**12,412**	**0.98**	**9**	**4**
rs16943442	**chr18:2535245**	**(C/T)**	**0.12**	**-19,591**	**1**	**9**	**3a**
rs9948895	chr18:2555922	(A/G)	0.36	-2004	0.99	8	3a
rs2138848	chr18:2560519	(C/A)	0.30	0	1	8	4
rs80010836	chr18:2569985	(T/A)	0.11	-5214	1	5	3a

5 SNPs with high regulatory potentials were selected by securing FORGEdb and RegulomeDB. SNPs with potential regulatory effects were marked in bold

[a]FORGEdb scores indicated the regulatory potentials for SNPs (https://forge2.altiusinstitute.org/files/forgedb.html). FORGEdb scores of from 0 to 10 and higher scores indicate greater likelihood that the SNP is a regulatory variant

[b]RegulomeDB scores indicated the regulatory potentials for SNPs (https://regulomedb.org/regulome-search). RegulomeDB scores of 1–6 indicate most to least likely to affect binding and expression of target gene

Abbreviations: *MAF* Minor Allele Frequency, MAF was presented as (MAF in cases)/(MAF in controls); Dprime, SNP haplotype frequency; R2, SNP correlation, Correlated alleles refer to alleles that are correlated if linkage disequilibrium is present (R2 > 0.1). rs3918251 was used as reference SNP

targeted therapies. Further research is needed to elucidate the functional contributions of *METTL4* gene to pneumonia development and to explore its potential for risk assessment or therapeutic intervention.

In this study, we demonstrated that *METTL4* gene is a susceptibility factor for severe pneumonia. Specifically, analysis performed by eQTLs, RegulomeDB, and FORGEdb tools indicated that the representative SNPs of *METTL4*, rs9989554 and rs16943442, exhibit high regulatory potential and are associated with an increased risk of severe pneumonia.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12863-025-01306-5.

Supplementary Material 1.

Supplementary Material 2.

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Authors' contributions
Y.Z. conceived the ideas and supervised the project. B.L. assembled the pneumonia cohort. L.M. and B.L. performed the experiments. X.Z. performed bioinformatics analysis. L.M. and B.L. wrote the manuscript with significant input from Y.Z. and X.Z., All authors discussed and approved the manuscript.

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Data availability
The datasets generated during and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Medical Ethics Committees of Guangzhou Women and Children's Medical Center (2016111853). Written informed consent to participate was obtained from the legal guardians of all participants.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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