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Inter-correlation of lncRNA THRIL with microRNA-34a and microRNA-125b and their relationship with childhood asthma risk, severity, and inflammation

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Abstract
Background: Long noncoding RNA (lncRNA) THRIL targets microRNA (miR)-34a and miR-125b to modify immunity, inflammation, and respiratory injury. The current study aimed to determine the inter-correlation of lncRNA THRIL with miR-34a and miR-125b and their relationship with childhood asthma risk, severity, and inflammation.
Methods: Exacerbated asthma children (N=65), remissive asthma children (N=65), and healthy controls (N=65) were enrolled in this case-control study. LncRNA THRIL, miR-34a, and miR-125b in peripheral blood mononuclear cells, as well as inflammatory cytokines in serum, were detected by reverse transcription-quantitative polymerase chain reaction and enzyme-linked immunosorbent assay, respectively.
Results: LncRNA THRIL was highest in exacerbated asthma children, then in remissive asthma children, and lowest in healthy controls (P<0.001); reversely, miR-34a (P<0.001) and miR-125b (P=0.004) exhibited the opposite treads. LncRNA THRIL (area under curve (AUC)=0.686) and miR-34a (AUC=0.614) could predict exacerbation risk of asthma, while miR-125b failed. Interestingly, lncRNA THRIL was negatively related to miR-34a and miR-125b in exacerbated asthma children and remissive asthma children (all P<0.05) but not in healthy controls (both P>0.05). Specifically, in exacerbated asthma children: lncRNA THRIL is related to increased eosinophil count (P=0.013), immunoglobulin E (P=0.020), tumor necrosis factor-α (P=0.002), interleukin-1ß (P=0.004), interleukin-6 (P=0.012), interleukin-17 (P=0.004) and exacerbated severity (P=0.030); Meanwhile, miR-34a and miR-125b linked with decreased levels of most of the above indexes (most P<0.05).
Conclusion: LncRNA THRIL negatively relates to miR-34a and miR-125b, correlate with inflammatory cytokines, and exacerbated the risk and severity of childhood asthma, indicating their potential as biomarkers for childhood asthma management.

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Introduction

Asthma is a chronic respiratory disease characterized by allergy-mediated inflammation in the lower respiratory tract, mainly affecting children.[1,2] In China, the current prevalence of pediatric asthma reaches 4.4% to 5.3%, with a high proportion of boys and 3-6 years children.[3-5] Although the frequency of hospitalization for asthmatic attacks and the percentage of patients with asthma have decreased owing to improved childhood asthma management, some still experience impaired pulmonary function, which reduces their quality of life and long-term prognosis.[6-8] Therefore, monitoring disease progression and identifying high-risk patients to prevent the occurrence of asthmatic exacerbation is necessary. Based on previous studies, predicting asthmatic exacerbation by serum biomarkers is one promising direction to improve long-term outcomes in childhood asthma patients.[9,10]

Long noncoding RNA (lncRNA) THRIL, as a recently identified RNA, is highly involved in innate immunity and inflammatory response by regulating multiple inflammatory cytokine expressions (such as tumor necrosis factor-α (TNF-α)).[11-13] For instance, lncRNA THRIL targets microRNA (miR)-424 to suppress Rho-associated coiled-coil containing protein kinase 2 (ROCK2) to promote inflammation in the cell model of lung injury.[14] In the clinical field, lncRNA THRIL expression relates to higher systemic inflammation and organ injuries of sepsis patients.[15] also linked with T help (Th) 1/Th2 imbalance and symptom degree of allergic rhinitis patients.[11] Besides, lncRNA THRIL targets miR-34a and miR-125b to release inflammatory cytokines in HK-2 and ATDC5 cells under lipopolysaccharide treatment, respectively.[16,17] Despite the heavy investigation of lncRNA THRIL, miR-34a, and miR-125b separately, fewer studies report their clinical interaction in asthma.

Hence, the study set out to determine the inter-correlation of lncRNA THRIL with miR-34a and miR-125b and their relationship with childhood asthma risk, severity, and inflammation.

Materials and Methods

Participants

The current case-control study enrolls exacerbated asthma children (N=65), remissive asthma children (N=65), and healthy controls (N=65). The inclusion and exclusion criteria and the definition of asthma exacerbation or remission were the same as in our previous study.[18] The protocol of the study was permitted by our Ethics Committee. The informed consent was provided by the guardian, along with the agreement of the participant if aged above 10 years.

Clinical features and samples

Basic clinical features of each participant were documented, including age, gender, height, weight, family history of asthma, forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1 (%predicted), eosinophil count, and immune globulin E (IgE) level. Meanwhile, exacerbated severity was assessed according to the method in our previous study.[18] Peripheral blood mononuclear cell (PBMC) and serum were obtained from each participant to quantify RNA (lncRNA THRIL, miR-34a, miR-125b) and inflammatory cytokines, respectively.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA) assays

PBMC lncRNA THRIL, miR-34a, and miR-125b were quantified using RT-qPCR with the method described in our previous study.[18] The primers are shown in Table S1. Besides, inflammatory cytokines such as TNF-α, interleukin (IL)-1β, IL-6, and IL-17, were detected using ELISA with the method described in our previous study.[18] TNF-α, IL-1β, IL- 6, and IL-17 are common pro-inflammatory cytokines. Therefore, they were chosen for our study.

Statistics

SPSS 23.0 software (IBM, USA) and GraphPad Prism 7.02 software (GraphPad, USA) were applied for data processing. One-way analysis of variance, Kruskal–Wallis, or Chi-square test was used for comparative analysis among three groups. Spearman test was applied for correlation analysis. The receiver operating characteristic (ROC) curves were drawn to determine the index's ability to distinguish different subjects. *P* less than 0.05 was concluded to be statistically significant.

Results

Clinical features

Detailed information on clinical features among exacerbated asthma children, remissive asthma children, and healthy controls are presented in Table 1. In brief, age, gender, height, weight, and family history of asthma were not different among the three groups (all $P>0.05$); . At the same time, eosinophils, IgE, FEV_1/FVC, and FEV_1 (% predicted) were different among them (all $P<0.001$).

SD, standard deviation; IQR, interquartile range; IgE, immunoglobulin E; FEV_1, forced expiratory volume in 1 second; FVC: forced vital capacity; FEV_1 (%predicted): the percentage of the tested FEV_1 value against the predicted normal FEV_1 value.

LncRNA THRIL, miR-34a, and miR-125b levels

LncRNA THRIL was highest in exacerbated asthma children, then in remissive asthma children, and lowest in healthy controls ($P<0.001$, Figure 1A); reversely, miR-34a ($P<0.001$, Figure 1B) and miR-125b ($P=0.004$, Figure 1C) exhibited the opposite treads. Subsequent analyses revealed that lncRNA THRIL (area under curve (AUC) (95%CI): 0.686 (0.595-0.778), Figure 2A) and miR-34a (AUC (95%CI): 0.614 (0.517-0.712), Figure 2B) could predict exacerbation risk of asthma to

Table 1 Clinical characteristics.

Items	Healthy controls (N=65)	Remissive asthma children (N=65)	Exacerbated asthma children (N=65)	P value
Age (years), mean±SD	6.6±2.8	6.5±2.6	6.3±2.7	0.848
Gender, No. (%)				0.675
Female	27 (41.5)	30 (46.2)	32 (49.2)	
Male	38 (58.5)	35 (53.8)	33 (50.8)	
Height (cm), median (IQR)	121.6±18.6	117.0±16.0	116.7±16.6	0.184
Weight (kg), median (IQR)	24.9±9.6	23.1±7.6	22.9±7.5	0.301
Family history of asthma, No. (%)	11 (16.9)	12 (18.5)	18 (27.7)	0.265
Eosinophil count (X10^9/L), median (IQR)	0.1 (0.1-0.1)	0.2 (0.1-0.3)	0.4 (0.4-0.7)	<0.001
IgE (IU/mL), median (IQR)	34.3 (20.0 -51.8)	78.5 (56.6-121.1)	219.8 (149.2-365.9)	<0.001
FEV_1/FVC (%), median (IQR)	82.8 (81.6-85.4)	78.3 (75.5-80.7)	65.0 (60.2-69.6)	<0.001
FEV_1 (% predicted), median (IQR)	98.6 (94.6-104.4)	82.4 (79.9-84.8)	74.4 (70.6-78.7)	<0.001

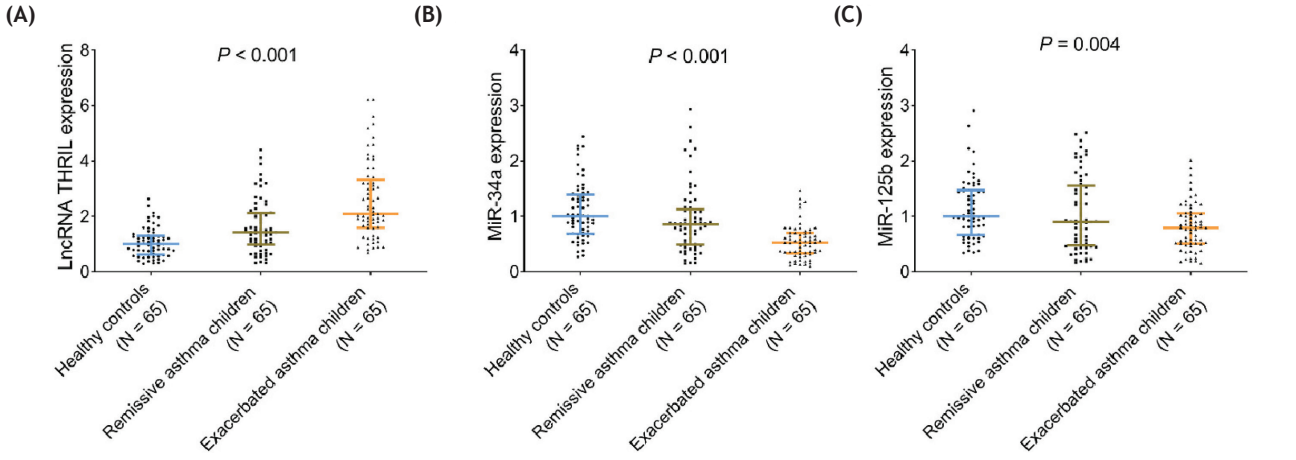

Figure 1 Dysregulated lncRNA THRIL, miR-34a, and miR-125b. Comparison of lncRNA THRIL (A), miR-34a (B), and miR-125b (C) expressions among exacerbated asthma children, remissive asthma children, and healthy controls. The median line meant median value, and the top and bottom lines meant interquartile range.

some extent, while miR-125b failed to predict exacerbation risk with AUC (95%CI) of 0.573 (0.473-0.674) (Figure 2C). Furthermore, the combination of lncRNA THRIL, miR-34a, and miR-125b presented with a better prediction for asthma exacerbation risk with an AUC (95%CI) of 0.769 (0.687-0.851) (Figure S1).

Relation of lncRNA THRIL with miR-34a and miR-125b

LncRNA THRIL is negatively related to miR-34a (P<0.001, Figure 3A) and miR-125b (P=0.004, Figure 3B) in exacerbated asthma children. Meanwhile, lncRNA THRIL is inversely linked with miR-34a (P=0.009, Figure 3C) and miR-125b (P=0.030, Figure 3D) in remissive asthma children. In healthy controls, lncRNA THRIL was not associated with miR-34a (P=0.098, Figure 3E) and miR-125b (P=0.058, Figure 3F).

Linkage of lncRNA THRIL, miR-34a, and miR-125b with biochemical and respiratory indexes

LncRNA THRIL is positively related to eosinophil count (P=0.013) and IgE (P=0.020) in exacerbated asthma children, and it is positively connected to eosinophil count (P=0.017) in remissive asthma children (Table 2). MiR-125b is only negatively associated with eosinophil count in exacerbated asthma children (P=0.006). Besides, miR-34a did not relate to any above indexes in the three groups.

Relation of lncRNA THRIL, miR-34a, and miR-125b to inflammatory cytokines

In exacerbated asthma children, lncRNA THRIL was positively linked with TNF-α (P=0.002), IL-1ß (P=0.004), IL-6 (P=0.012), and IL-17 (P=0.004) (Table 3). Meanwhile, miR-34a inversely connected to TNF-α (P=0.016), IL-1ß

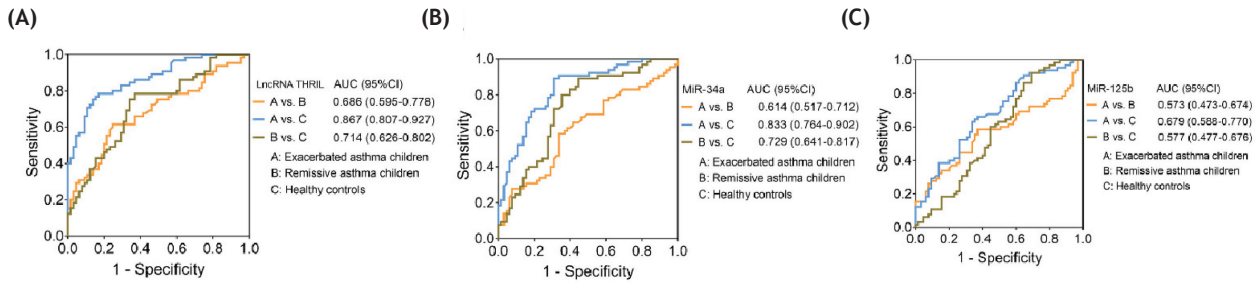

Figure 2 LncRNA THRIL, miR-34a, and miR-125b are linked with asthma exacerbated risk. ROC curve analyses of lncRNA THRIL (A), miR-34a (B), and miR-125b (C) to distinguish exacerbated asthma children, remissive asthma children, and healthy controls from each other.

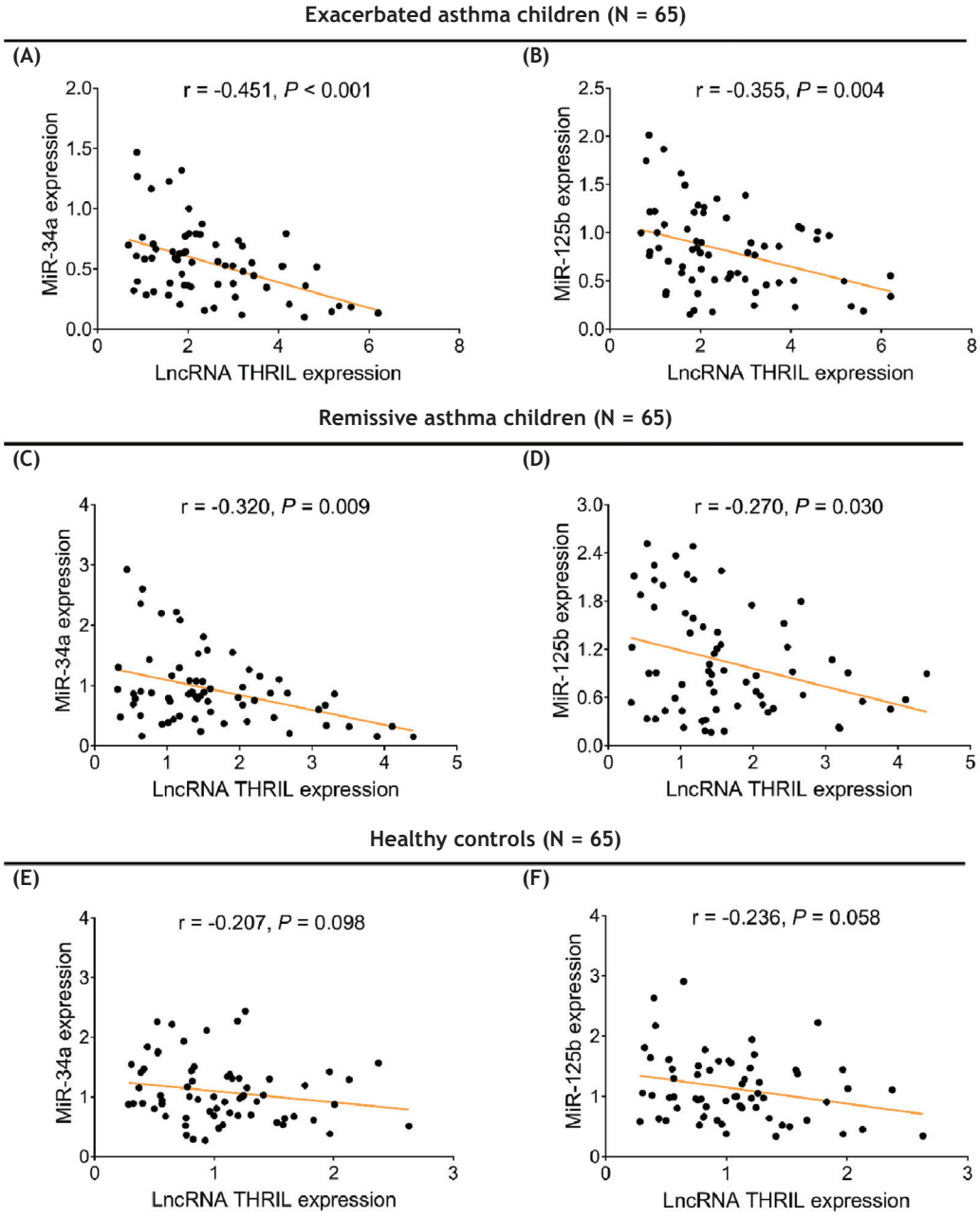

Figure 3 LncRNA THRIL is negatively connected with miR-34a and miR-125b in asthma children. Association of lncRNA THRIL with miR-34a (A) and miR-125b (B) in exacerbated asthma children (A-B), in remissive asthma children (C-D), and healthy controls (E-F).

Table 2 The relation of lncRNA THRIL, miR-34a, and miR-125b with biochemical and respiratory function indexes.

Items	LncRNA THRIL		MiR-34a		MiR-125b	
	r	*P* value	r	*P* value	r	*P* value
Exacerbated asthma children						
Eosinophil count	0.306	0.013	-0.200	0.110	-0.335	0.006
IgE	0.289	0.020	-0.208	0.097	-0.221	0.077
FEV_1/FVC	-0.110	0.384	0.173	0.168	0.206	0.099
FEV_1 (% predicted)	-0.128	0.308	0.082	0.516	0.150	0.232
Remissive asthma children						
Eosinophil count	0.296	0.017	0.022	0.860	-0.099	0.432
IgE	0.209	0.094	0.002	0.987	-0.077	0.542
FEV_1/FVC	0.158	0.208	0.044	0.727	-0.058	0.645
FEV_1 (% predicted)	-0.140	0.266	0.098	0.436	0.163	0.194
Healthy controls						
Eosinophil count	-0.012	0.923	-0.141	0.262	-0.139	0.268
IgE	0.242	0.052	-0.202	0.106	0.104	0.409
FEV_1/FVC	-0.040	0.751	0.185	0.139	0.087	0.493
FEV_1 (% predicted)	-0.025	0.845	0.070	0.582	-0.041	0.749

IgE, immunoglobulin E; FEV1, forced expiratory volume in 1 second; FVC: forced vital capacity; FEV1 (%predicted): the percentage of the tested FEV1 value against the predicted normal FEV1 value.

Table 3 Linkage of lncRNA THRIL, miR-34a, miR-125b with inflammatory cytokines.

Items	LncRNA THRIL		MiR-34a		MiR-125b	
	r	*P* value	r	*P* value	r	*P* value
Exacerbated asthma children						
TNF-α	0.375	0.002	-0.299	0.016	-0.281	0.023
IL-1ß	0.357	0.004	-0.351	0.004	-0.311	0.012
IL-6	0.311	0.012	-0.276	0.026	-0.141	0.263
IL-17	0.350	0.004	-0.306	0.013	-0.296	0.017
Remissive asthma children						
TNF-α	0.260	0.037	-0.279	0.025	-0.236	0.058
IL-1ß	0.249	0.046	-0.312	0.011	-0.215	0.085
IL-6	0.284	0.022	-0.350	0.004	-0.265	0.033
IL-17	0.237	0.057	-0.237	0.057	-0.140	0.265
Healthy controls						
TNF-α	0.127	0.312	-0.340	0.006	-0.103	0.415
IL-1ß	0.093	0.459	-0.177	0.158	-0.078	0.537
IL-6	0.164	0.191	-0.032	0.802	-0.256	0.040
IL-17	0.128	0.309	-0.241	0.054	0.020	0.872

TNF-α, tumor necrosis factor-α; IL-1ß, interleukin-1 ß; IL-6, interleukin- 6; IL-17, interleukin-17.

(*P*=0.004), IL-6 (*P*=0.026) and IL-17 (*P*=0.013). Besides, miR-125b also reversely associated with TNF-α (*P*=0.023), IL-1ß (*P*=0.012) and IL-17 (*P*=0.017).

In remissive asthma children, lncRNA THRIL is linked with increased TNF-α (*P*=0.037), IL-1ß (*P*=0.046), and IL-6 (*P*=0.022). Besides, miR-34a correlated with decreased TNF-α (*P*=0.025), IL-1ß (*P*=0.011), and IL-6 (*P*=0.004). Furthermore, miR-125b is only related to reduced IL-6 (*P*=0.033).

In healthy controls, only miR-125b showed a negative correlation with IL-6 (*P*=0.040). Besides, lncRNA THRIL and miR-34a were not related to any inflammatory cytokines.

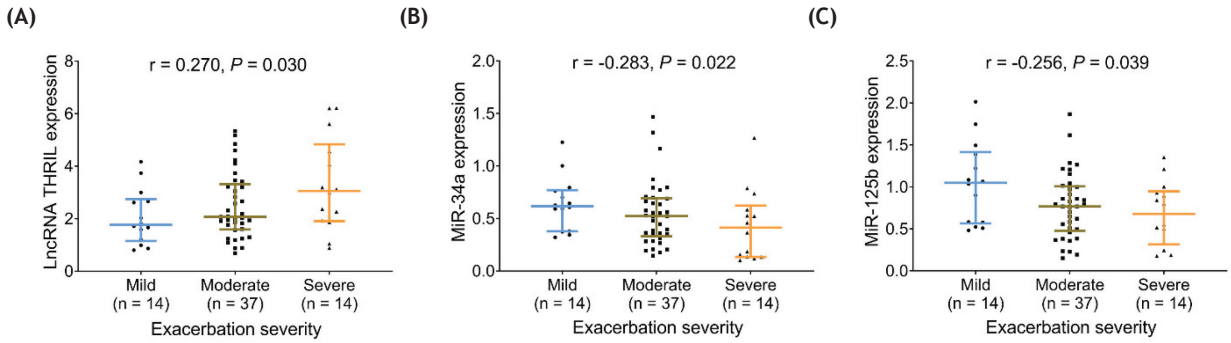

Figure 4 Relation to exacerbated severity. Association of lncRNA THRIL (A), miR-34a (B), and miR-125b (C) with exacerbated severity. The median line meant median value, and the top and bottom lines meant interquartile range.

LncRNA THRIL, miR-34a, miR-125b and exacerbated severity

LncRNA THRIL was linked with high exacerbated severity (P=0.030, Figure 4A); but miR-34a (P=0.022, Figure 4B) and miR-125b (P=0.039, Figure 4C) inversely correlated with exacerbated severity.

Discussion

LncRNA THRIL affects immunity and inflammation, and its clinical involvement in immune/inflammation-related disease was discovered in 2014.[11,15,19,20] For instance, lncRNA THRIL is elevated and linked with inflammation outbreaks and multiple organ dysfunctions in sepsis patients,[15,19] it has a high level in pediatric immune thrombocytopenia (ITP) patients and relates to elevated ITP risk.[20] Besides, and-THRIL discloses a positive association with Th1/Th2 imbalance and disease severity of allergic rhinitis patients.[11] However, no study reports its clinical relevance to childhood asthma yet. The present study firstly observed that lncRNA THRIL relates to higher asthmatic exacerbated risk and severity, also linked with elevated levels of inflammatory cytokines. The possible explanations were: (1) LncRNA THRIL related to dysregulated Th1/Th2 cell balance, to increase the risks of asthma and its exacerbated risk.[11,21] (2) LncRNA THRIL targets several anti-inflammation miRNAs (such as miR-19a and miR-424) to enhance the secretion of inflammatory cytokines, resulting in higher exacerbated risk.[14,19] (3) Higher inflammatory cytokines might suggest impaired immune function and increased respiratory injury, thus leading to elevated exacerbated risk and severity in childhood asthma patients.[22]

Previous studies indicate that lncRNA THRIL regulates miR-34a and miR-125b during the inflammatory process. Also, they are reported to inhibit inflammation individually.[16,17,23,24] Therefore, we suspected that lncRNA THRIL might regulate these two miRNAs in childhood asthma patients. The current study found that lncRNA THRIL was negatively related to miR-34a and miR-125b in exacerbated asthma children and remissive asthma children; the latter two were inversely linked with inflammatory cytokines and exacerbated severity in exacerbated asthma children, which may result from (1) lncRNA THRIL directly bound to miR-34a and miR-125b via sequence connection.[16,17] therefore, they were reversely inter-correlated; (2) MiR-34a modulated Th1 differentiation to regulate allergy, to increase the risks of asthma and its exacerbated risk; meanwhile, it directly regulated inflammatory cytokines.[25,26] (3) MiR-125b regulated Th1/Th2 dysregulation and inflammation in multiple ways, leading to an elevated risk of asthma and its exacerbation.[11,27]

Some points could be mentioned in our study: Firstly, multiple target miRNAs (not only miR-125b but also miR-34a) instead of one were detected in the study; Secondly, childhood patients were enrolled instead of adult ones. Some limitations did occur in the present study. Firstly, the findings needed to be validated in a large population-based study in the future. Secondly, further *in vivo* and *in vitro* experiments were necessary to investigate the detailed interacting mechanisms among lncRNA THRIL, miR-34a, and miR-125b in asthma. Thirdly, determining lncRNA THRIL at various visit points during treatment could be performed to further evaluate its value for asthma monitor. Fourthly, Th1 and Th2 cells were not detected in the current study; therefore, the direct correlation of lncRNA THRIL with Th1/Th2 imbalance in asthma was not determined in the study. Finally, lncRNA THRIL was also linked with other miRNAs such as miR-424 and miR-24-3p that also participated in inflammation regulation, while they were not detected in the current study, which could be explored in the future.

Conclusion

Conclusively, lncRNA THRIL negatively correlates with miR-34a and miR-125b, and they may relate to inflammatory cytokines, exacerbated risk, and severity of childhood asthma, while a further large-scale study is needed for validation. These findings may provide evidence for the potency of lncRNA THRIL, miR-34a, and miR-125b biomarkers for childhood asthma management.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary

Table S1 Primers.

Items	Forward (5'→3')	Reverse (5'→3')
LncRNA THRIL	AACTTCACAGGAACACTACACAAGA	TAGGCAACAGAGCAAGACTTCATC
MiR-34a	TGGCAGTGTCTTAGCT	TGGTGTCGTGGAGTCG
MiR-125b	ACACTCCAGCTGGGTCCCTGAGACCCTAACTT	TGTCGTGGAGTCGGCAATTC
GAPDH	GAGTCCACTGGCGTCTTCAC	ATCTTGAGGCTGTTGTCATACTTCT
U6	CTCGCTTCGGCAGCACATATACTA	ACGAATTTGCGTGTCATCCTTGC

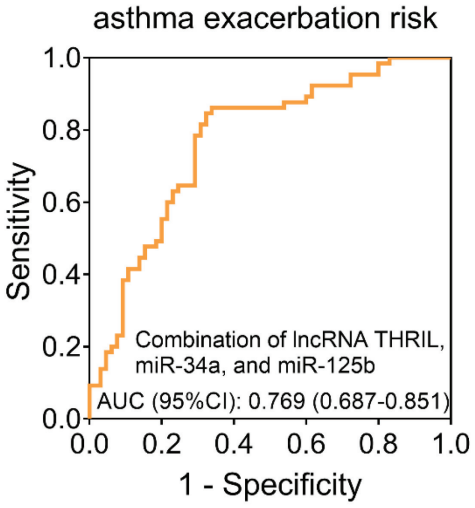

Figure S1 Relating the combination of lncRNA THRIL, miR-34a, and miR-125b with asthma exacerbation risk.