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Clinical significance of serum ATP8B1 in children with *Mycoplasma pneumoniae* infection complicated with myocardial injury

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Abstract

Introduction and Objective. *Mycoplasma pneumoniae* (MP) infection can lead to various extrapulmonary complications, including myocardial injury (MI). However, the expression levels of ATPase phospholipid transporting 8B1 (ATP8B1) in MP-infected individuals with MI and its potential therapeutic role remain elusive. The aim of the study is to evaluate ATP8B1 as a therapeutic target for MP-induced MI.

Materials and Method. The study quantified ATP8B1 expression in patient serum via RT-qPCR and analyzed its diagnostic value for MP and MP+MI using receiver operating characteristic (ROC) curves. Binary logistic regression assessed its association with MP+MI. Serum interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) levels were measured by ELISA and correlated with ATP8B1. In a dual-cell model (MP-infected BEAS-2B cells and AC16 cells exposed to their supernatant), ATP8B1's effects on inflammation, proliferation, reactive oxygen species (ROS), and cardiac injury markers were evaluated via RT-qPCR, CCK-8, and ELISA.

Results. The study revealed that serum ATP8B1 levels were significantly reduced in the MP group and further decreased in the MP+MI group. ATP8B1 expression levels could distinguish MP patients from MP+MI patients. Serum IL-6 and TNF-α levels were significantly higher in the MP+MI group than in the MP group, and ATP8B1 was negatively correlated with IL-6 and TNF-α. *In vitro,* ATP8B1 was downregulated in both cell lines. Functionally, over-expression of ATP8B1 effectively attenuated inflammation, ROS production, deficits in cell proliferation, and myocardial injury.

Conclusions. ATP8B1 was expressed lowly in the serum of MP+MI patients. Functional experiments demonstrated that ATP8B1 upregulation significantly attenuated inflammation, ROS generation, proliferation deficits, and myocardial injury.

Key words

Mycoplasma pneumoniae, diagnosis, inflammation, myocardial injury, ATP8B1

INTRODUCTION

Mycoplasma pneumoniae (MP), a unique wall-less bacterium classified among structurally small prokaryotic cells, exhibiting independent viability [1]. This pathogen demonstrates broad clinical manifestations, encompassing not only upper respiratory tract infections and pneumonia, but also diverse extra-pulmonary complications [2]. In respiratory contexts, MP induces both upper and lower respiratory tract infections, causing diseases such as *Mycoplasma pneumoniae* pneumonia (MPP) and tracheobronchitis. Characteristic symptoms include cephalalgia, pyrexia, myalgia, and persistent cough. Notably, MPP has emerged as a predominant form of community-acquired pneumonia (CAP) in paediatric populations, accounting for 20–40% of childhood pneumonia cases globally [3], thereby posing substantial health risks to children. MP infections exhibit seasonal prevalence peaks during summer and early autumn, with an incubation period of 2–3 weeks and the highest incidence among children and adolescents [4]. Significantly, September 2023 witnessed a marked surge in MP cases accompanied by heightened clinical severity, with infected children presenting protracted cough, elevated body temperature, hoarseness, and tension-type headaches [3].

In addition to respiratory and pneumonic manifestations, MP infection is associated with various extra-pulmonary complications, which occur in up to 25% of cases and involve multiple systems such as vasculitis, myocarditis, and thrombosis. Although ventricular wall thrombus is relatively uncommon, most reported cases have been described in paediatric patients [5]. Growing evidence suggests a reciprocal relationship between pulmonary disease and extra-pulmonary organs. For instance, cardiac abnormalities have been observed in nearly half of the patients at an average of 16 months after MP infection [6]. Furthermore, in children with Kawasaki disease (KD), MP-positive cases showed higher acute-phase myocardial enzyme levels, and a greater incidence of coronary artery aneurysms compared to MP-negative cases, indicating MP may act as a significant co-factor in cardiac complications of KD [7]. Given that patients with myocardial injury (MI) often present with more complicated conditions and poorer prognosis, there is an urgent need to identify reliable biomarkers for early diagnosis and targeted treatment, which may offer potential therapeutic strategies for MP-associated MI.

ATPase phospholipid transporting 8B1 (ATP8B1), a member of the P4-ATPase family, is broadly expressed in multiple organs and tissues, including the lungs and pancreas. Its

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primary role involves the translocation of phospholipids, such as phosphatidylserine and phosphatidylethanolamine from the outer to the inner leaflet of the plasma membrane, thereby preserving phospholipid asymmetry. Research indicates that dysfunction of intestinal ATP8B1 contributes to hepatic choline deficiency and steatohepatitis [8]. Additionally, ATP8B1 plays a significant role in colorectal cancer [9]. In lung squamous cell carcinoma (LUSC), the expression levels of ATP8B1 are closely associated with patient prognosis [10]. On the other hand, within pulmonary physiological processes, ATP8B1 exerts a protective effect on the lungs and maintains normal pulmonary function [11]. Despite these advances, the function of ATP8B1 in MP infection remains elusive and warrants further investigation.

The study centres on elucidating the impact of the ATP8B1 gene on MP complicated with MI. Investigation of ATP8B1's role in this pathological process may facilitate early identification of paediatric patients developing concurrent MI complications, enabling timely clinical interventions to improve prognostic outcomes.

MATERIALS AND METHOD

Bioinformatic screening for candidate genes. The NCBI GEO database (www.ncbi.nlm.nih.gov/geo/) was searched using the key words '*Mycoplasma pneumoniae* infection' and 'Myocardial injury', identifying datasets GSE179051 and GSE240053, respectively. Differential expression analysis was performed on both datasets using the GEO2R tool. Statistically significantly differentially expressed genes (DEGs) were defined as those with an absolute $\log_2$ fold change ($|\log_2 FC| > 1$ and an adjusted p-value < 0.05. From these, an intersection was taken between genes with $|\log_2 FC| > 2$ in GSE179051 and $|\log_2 FC| > 1.5$ in GSE240053.

Participant recruitment and sample collection. This study enrolled a total of 100 paediatric patients with MP infection, who were categorized into two subgroups based on the presence of MI: 62 patients without MI (MP group) and 38 patients with MI (MP+MI group). Additionally, 100 age- and gender-matched healthy children were recruited as controls. Inclusion criteria for the MP group included: a positive PCR result from a nasopharyngeal swab or a ≥4-fold increase in MP-specific IgG titers in paired serum samples, patients adhering well to the standard treatment plan, and individuals with complete clinical records. Exclusion criteria included: those with congenital heart defects, pulmonary hypoplasia, or immunodeficiency; patients with recurrent upper respiratory infections or other relevant pulmonary diseases; individuals with severe liver/kidney dysfunction or malignant tumors; participants who used immunosuppressants or anticoagulants in the past three months; and patients with other acute/chronic pathogen infections. MI was defined as an elevated serum creatine kinase-MB isoenzyme (CK-MB) level >25 U/L and/or the presence of electrocardiographic abnormalities, such as arrhythmia, ST-segment changes, or pathological Q waves. Patients who met at least one of these criteria were assigned to the MP+MI subgroup, whereas those without were assigned to the MP subgroup. Written informed consent was obtained from parents/legal guardians before enrollment, and the study was approved by the Ethics Committee of Tangshan Central Hospital (Approval No.: No. TZYL/K/2025–03). Fasting peripheral venous blood samples were collected into tubes, centrifuged at 1,500g for 10 minutes within 30 minutes, and the serum was frozen at -80 °C after being divided into sterile EP tubes.

Cell culture. The human bronchial epithelial cell line BEAS-2B (Delf Biotech, Delf-10130) was cultured in its dedicated medium (Delf-14709) and infected with MP strain ATCC 15531 (Huiying Biotechnology, ATCC15531) to establish a pulmonary infection model. Subsequently, the conditioned medium from these infected cultures was collected and applied to the human cardiomyocyte line AC16 (Sigma-Aldrich) to induce an *in vitro* model of MI [12]. AC16 cells were maintained in AC16 cell complete medium, and all cultures were incubated at 37 °C in a 5% CO_2 atmosphere.

ATP8B1 transfection experiments. In this study, we transfected BEAS-2B and AC16 cells with the ATP8B1 knockdown plasmid (si-ATP8B1, RiboBio) and the ATP8B1 over-expression plasmid (pcDNA-ATP8B1, Shanghai GenePharma) using Lipofectamine 3000 transfection reagent (Invitrogen). The experiments were divided into three groups: a blank control group (un-transfected), a negative control group (transfected with empty vector), and an experimental group (transfected with si-ATP8B1 or pcDNA-ATP8B1). Forty-eight hours post-transfection, subsequent experiments were conducted to evaluate the effects of ATP8B1 knockdown or over-expression on the cells. Each experiment was independently repeated three times.

Reverse transcription-quantitative PCR (RT-qPCR). Based on the SYBR Green detection methodology, RT-qPCR analysis was performed using the Luna Universal One-Step RT-qPCR Kit (New England Biolabs). The reaction setup incorporated 1 μL of extracted RNA in a reduced reaction volume of 10 μL. Amplification and detection were carried out on a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories). Threshold cycle (Ct) values were automatically determined using CFX Maestro software, with thermal cycling conditions executed according to the manufacturer's protocol. β-Actin mRNA expression served as the endogenous control for normalization. The following primer sequences were used for amplification: ATP8B1 (forward: 5'-GCTACAGGATGGAGTTCCAG-3', reverse: 5'-ATTCCAACGCCAATGTGGGC-3') and β-actin (forward: 5'-GAGCGGGAAATCGTGCGTGACATT-3', reverse: 5'-GAAGGTAGTTTCGTGGATGCC-3').

Enzyme-linked immunosorbent assay (ELISA). The levels of Interleukin-6 (IL-6) and Tumour Necrosis Factor-α (TNF-α) were determined using the Human IL-6 ELISA Kit (Ayns Biotechnology, CB10373) and Human TNF-α ELISA Kit (Sigma-Aldrich, T6674), respectively. Reactive oxygen species (ROS) levels were assessed with the Reactive Oxygen Species Assay Kit (Beyotime Biotech, S0033S). The concentration of creatine kinase MB isoenzyme (CK-MB) was measured with a human ELISA kit (Shanghai Keaibo Biology, CB10733-Hu). Absorbance at 450 nm was measured for ELISA assays using a microplate reader (Thermo Fisher Scientific).

Cell counting kit-8 (CCK-8). First, 10 μL of CCK-8 solution (Sigma-Aldrich, 96992) was added to each well, followed by

incubation at 37 °C for 2 hours in the dark. The absorbance of each well was measured at a wavelength of 450 nm using a microplate reader (Thermo Scientific, 1410101).

Statistical analysis. Data were presented as mean ± standard deviation (SD) from ≥3 independent experiments with technical replicates. Statistical analyses were conducted using GraphPad Prism 9.3.1, employing independent samples t-tests for two-group comparisons and one-way analysis of variance (ANOVA) for multi-group analyses. MedCalc and IBM SPSS Statistics 23 were utilized for receiver operating characteristic (ROC) curve analysis and binary logistic regression, respectively. Statistical significance was defined as $p < 0.05$.

RESULTS

Serum ATP8B1 exhibits potential diagnostic value for MP+MI. The intersection of the two datasets yielded two candidate genes: HKDC1 and ATP8B1. Considering both the statistical significance of differential expression and biological relevance, ATP8B1 was selected for further investigation. This decision was based not only on its more pronounced statistical significance in expression changes, but more importantly, on existing research support for its role in pulmonary pathologies: studies have demonstrated that low ATP8B1 expression is significantly associated with poor prognosis in patients with LUSC [10]. These findings provide important theoretical support for investigating the role of ATP8B1 in the mechanisms underlying MPP complicated by MI (Fig. 1A).

Serum ATP8B1 expression was significantly reduced in patients with MP, with a further pronounced decrease in those complicated by MI (Tab. 1, Fig. 1B), which indicates a correlation between ATP8B1 levels and disease severity, implicating it in the pathogenesis of cardiac involvement. ROC analysis demonstrated the diagnostic capacity of ATP8B1, with significant discrimination observed between healthy controls and MP patients (AUC (area under the curve) = 0.8191, 95% CI (confidence interval): 0.7541–0.8838;

Table 1. Comparison of clinical data indicators in patients with MP versus MP+MI group

Parameters	MP (n = 62)	MP+MI (n = 38)	p
ATP8B1	1.09±0.36	0.67±0.28	<0.001***
Gender (Male/Female)	35/27	17/21	0.199
Age (years)	8.08±2.83	7.86±2.85	0.718
BMI (kg/m²)	16.96±2.00	17.12±1.40	0.665
Blood sugar (mmol/L)	4.73±0.75	4.96±0.77	0.139

MP – *Mycoplasma pneumoniae*; MP+MI – *Mycoplasma pneumoniae* + myocardial injury; BMI – body mass index. The quantification of ATP8B1 expression was performed using qPCR, and the relative expression levels of the target genes were determined by the $2^{-\Delta\Delta Ct}$ method

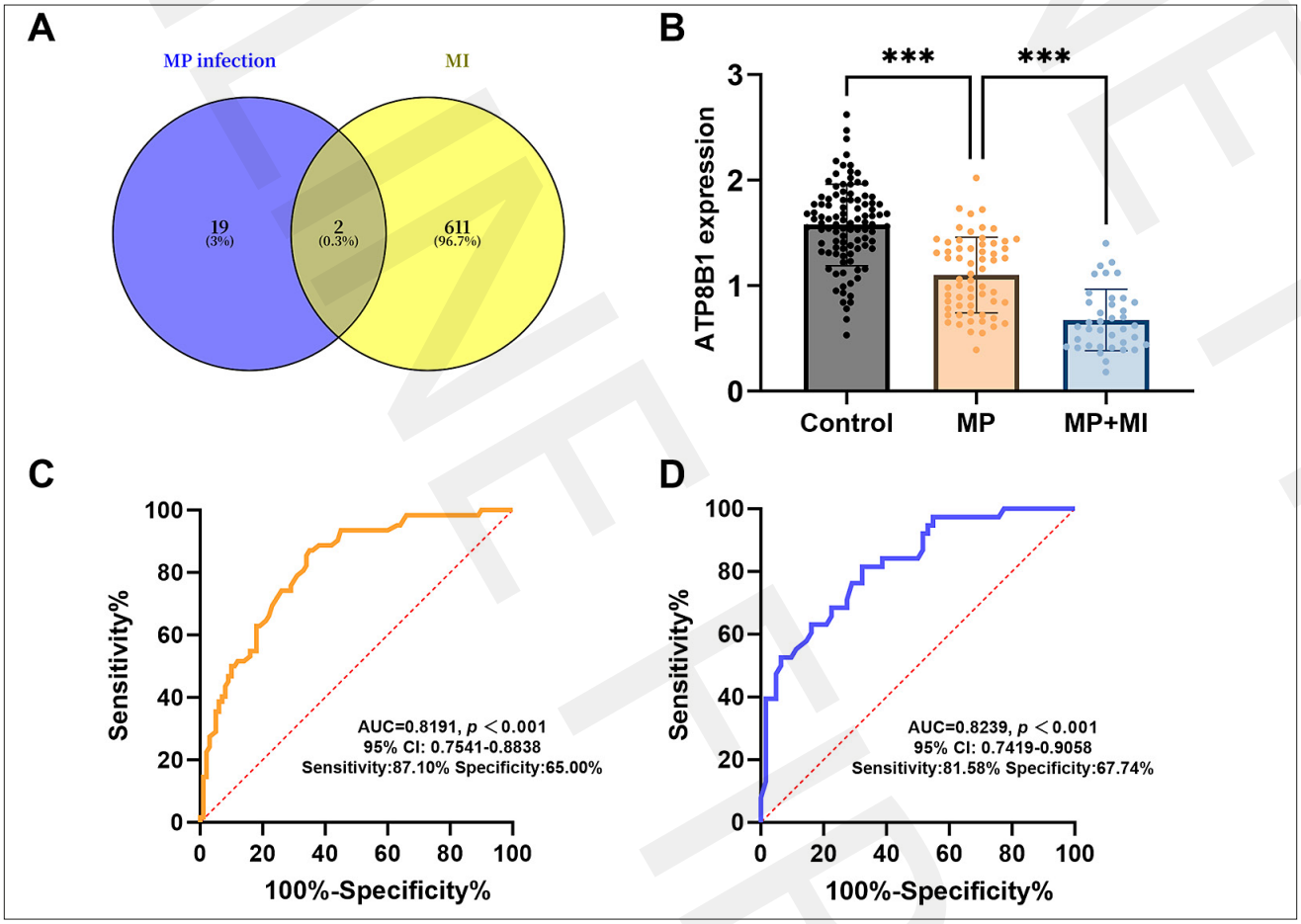

Figure 1. Analysis of serum ATP8B1 expression and diagnostic utility. A. Screening of candidate genes common to MP infection and MI. B. Serum ATP8B1 expression levels across control, MP, and MP+MI cohorts. C. ROC curve analysis evaluating ATP8B1's capacity for discriminating against control versus MP patients. D. ROC curve analysis assessing ATP8B1's efficacy in differentiating MP patients from MP+MI patients.
***$p < 0.001$

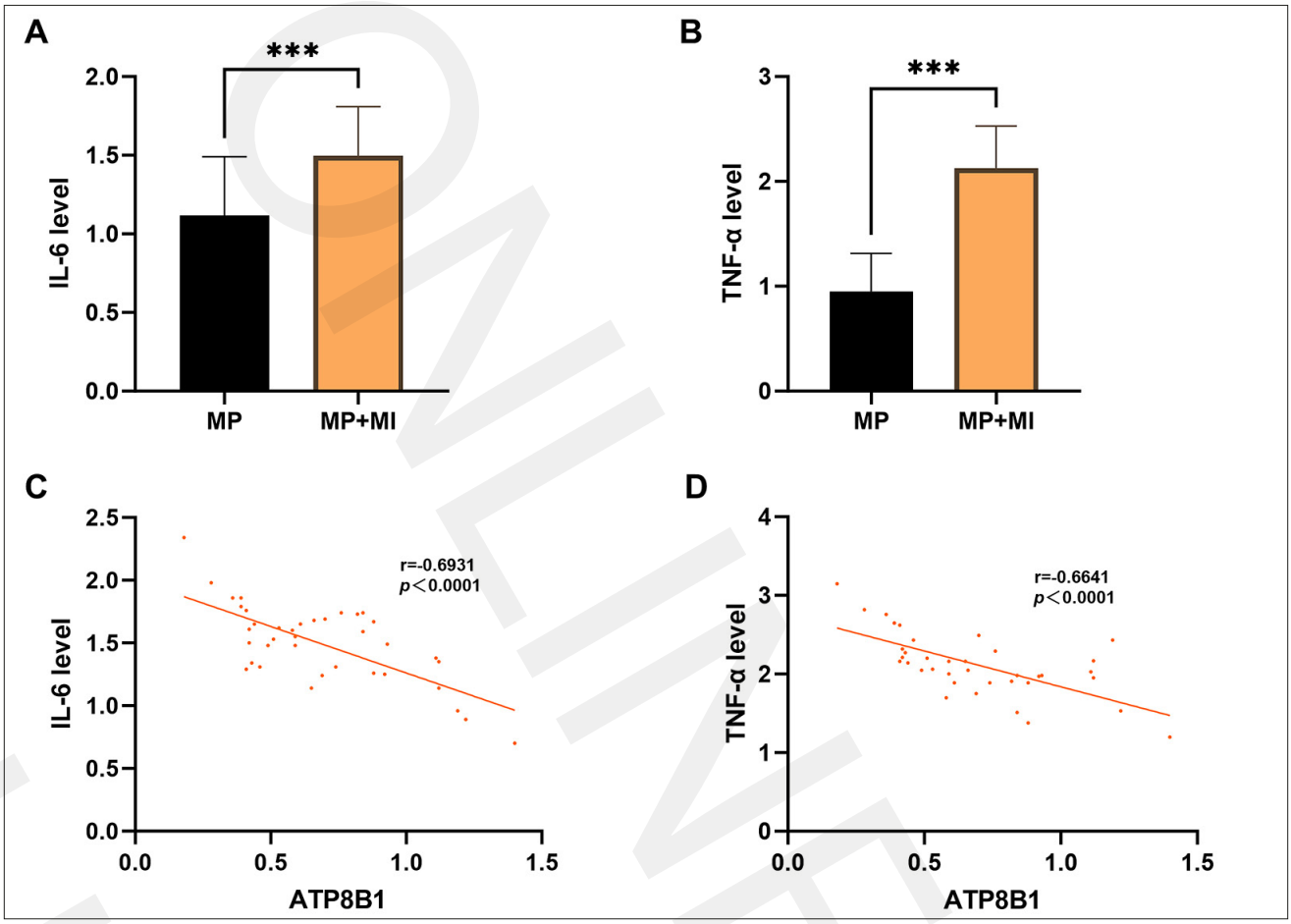

Figure 2. Analysis of serum IL-6 and TNF-α levels in relation to ATP8B1. A. Comparative IL-6 expression levels in MP versus MP+MI groups. B. TNF-α expression levels in MP versus MP+MI groups. C. Correlation of serum ATP8B1 levels with IL-6 in MP+MI patients. D. Correlation of serum ATP8B1 levels with TNF-α in MP+MI patients. ***$p < 0.001$

$p < 0.001$; sensitivity 87.10%, specificity 65.00%) (Fig. 1C). The biomarker also effectively distinguished MP patients from those with MP-related MI (AUC = 0.8239, 95% CI: 0.7419–0.9058; $p < 0.001$; sensitivity 81.58%, specificity 67.74%) (Fig. 1D). Furthermore, binary logistic regression analysis revealed that ATP8B1 expression was significantly associated with the occurrence of MI in MP-infected patients (Tab. 2, odds ratio [OR] = 0.029, 95% CI: 0.006–0.146, p < 0.001). Patients with low ATP8B1 expression had a significantly higher risk of developing MI than those with high expression, indicating a statistically significant link between ATP8B1 and the risk of MP-related MI. These findings support the potential of ATP8B1 as a diagnostic marker for different stages of MP infection.

ATP8B1 demonstrates an inverse relationship with the pro-inflammatory cytokines IL-6 and TNF-α. The results showed that compared with the MP group, the levels of IL-6 (Fig. 2A) and TNF-α (Fig. 2B) in the serum of the MP+MI group were significantly increased, indicating that the levels of inflammatory factors were higher when there was MI based on MP infection. Then, Pearson correlation analysis was used to analyze the correlations between ATP8B1 and IL-6 and TNF-α. The results showed that in the correlation analysis with IL-6, r was -0.6931 and $p < 0.0001$ (Fig. 2C), indicating a significant negative correlation between ATP8B1 and IL-6. In the correlation analysis with TNF-α, r was -0.6641 and $p < 0.0001$ (Fig. 2D), indicating that ATP8B1 also showed a significant negative correlation with TNF-α. Collectively, these correlation results suggested that ATP8B1 may contribute to the pathology of MP-related MI by modulating the inflammatory response.

ATP8B1 alleviates MP-induced inflammation, oxidative stress, and proliferation impairment in BEAS-2B cells. To investigate the functional role of ATP8B1 in MP-infected airway epithelium, BEAS-2B cells treated with MP-conditioned medium were utilized. In this model, ATP8B1 expression was significantly down-regulated (Fig. 3A). Following validation of transfection efficiency using siRNA and overexpression plasmids (Fig. 3B), we observed that ATP8B1 knockdown markedly enhanced the secretion of IL-6 and TNF-α, whereas ATP8B1 over-expression suppressed their release (Fig. 3C-D). Similarly, ATP8B1 silencing increased intracellular ROS levels, while its over-expression alleviated MP-induced oxidative stress (Fig. 3E). Functional assays further demonstrated that ATP8B1 depletion exacerbated the loss of cell viability, and its restoration improved proliferative capacity (Fig. 3F). Together, these findings identified ATP8B1 as a protective regulator in MP-induced bronchial epithelial injury, attenuating inflammatory responses, reducing oxidative damage, and supporting cell proliferation.

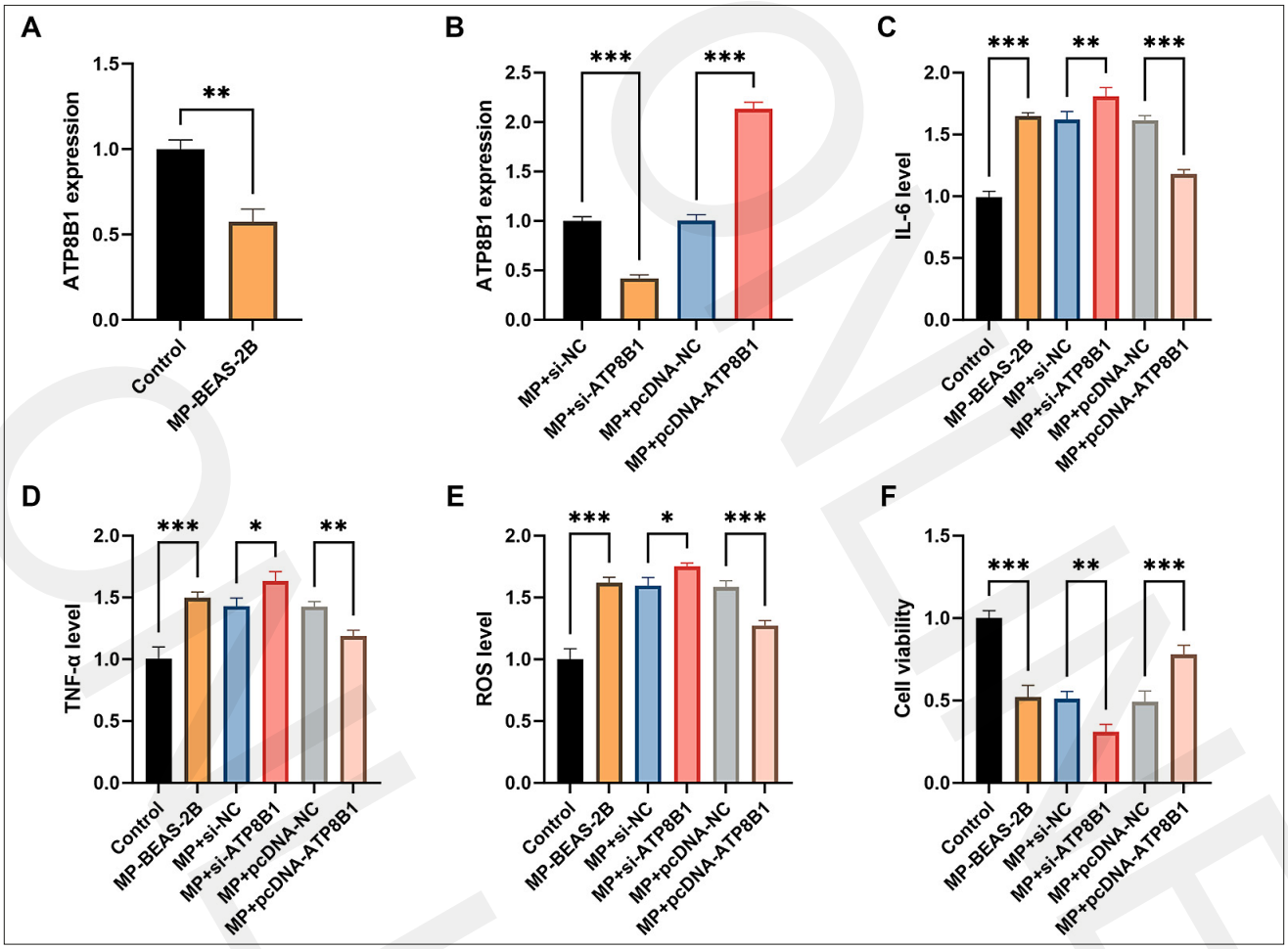

Figure 3. Effects of ATP8B1 expression on MP-stimulated BEAS-2B cells. A. Relative ATP8B1 expression in MP-stimulated BEAS-2B cells. B. Validation of ATP8B1 overexpression/knockdown by transfection. C. Impact of ATP8B1 modulation on IL-6 release in MP-stimulated BEAS-2B cells. D. Impact of ATP8B1 modulation on TNF-α release in MP-stimulated BEAS-2B cells. E. Impact of ATP8B1 modulation on intracellular ROS levels in MP-stimulated BEAS-2B cells. F. Impact of ATP8B1 modulation on the proliferative capacity of MP-stimulated BEAS-2B cells.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

ATP8B1 alleviates MP-CM-induced cardiac injury markers and proliferation impairment in AC16 cells. ATP8B1 expression was significantly down-regulated in AC16 cells following MP-CM stimulation (Fig. 4A). Genetic manipulation through siRNA knockdown and plasmid over-expression verified the transfection efficiency (Fig. 4B). Notably, ATP8B1 knockdown exacerbated MP-CM-induced injury by increasing creatine kinase MB (CK-MB) release, while its over-expression demonstrated protective effects by reducing this cardiac injury marker (Fig. 4C). Similarly, ATP8B1 depletion impaired cell viability, whereas its restoration attenuated the proliferation suppression caused by MP-CM (Fig. 4D). These findings collectively demonstrate that ATP8B1 exerted a protective role in the MP-CM-induced AC16 cell injury model by mitigating cardiac injury markers and promoting cell proliferation, suggesting its potential as a therapeutic target for preventing myocardial complications in mycoplasma pneumonia.

DISCUSSION

Among 16 human mycoplasma species, six exhibited pathogenicity, with MP representing the most clinically significant causative agent [13]. Individuals remain susceptible to MP re-infection following initial exposure [4]. As a predominant pathogen in paediatric respiratory infections, MP demonstrates peak prevalence during seasonal outbreaks among school-aged children. Antibiotic therapy remains the primary intervention for paediatric MPP. However, with the wide application of antibiotics, the number of macrolide-resistant *Mycoplasma pneumoniae* (MRMP) strains has gradually increased, which, to some extent, has weakened the therapeutic effect of antibiotics [3]. Infections caused by MRMP complicate clinical management. Ineffective antimicrobial treatment potentiates pulmonary deterioration and increases extra-pulmonary complications. MRMP-infected patients exhibit prolonged febrile periods, extended hospitalization, heightened oxygen requirements, and prolonged antibiotic courses. Notably, MRMP pneumonia elevates paediatric intensive care unit admission risk fivefold. This pathogen demonstrates efficient transmission within schools, households, and communities through close contact, imposing socio-economic burdens [14].

Substantial evidence indicates that MP infection alters the levels of inflammatory cytokines and ROS [15, 16]. For instance, elevated levels of cytokines such as IL-1β, TNF-α, IL-6, and IL-8 have been detected in bronchoalveolar lavage fluid (BALF) in patients with ARDS. This aberrant inflammatory

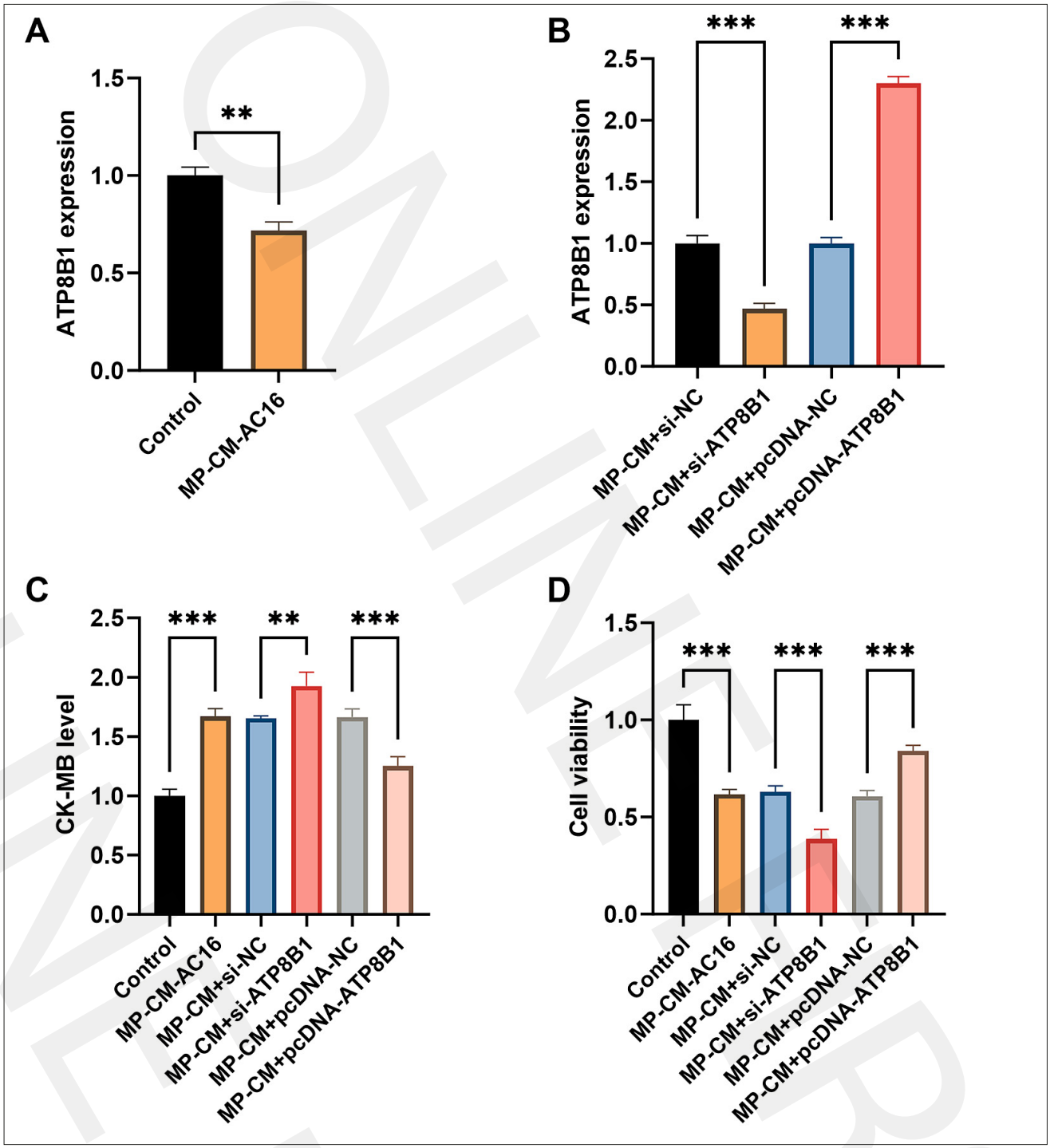

Figure 4. Effects of ATP8B1 expression on MP-CM-stimulated AC16 cells. A. Relative ATP8B1 expression in MP-CM-stimulated AC16 cells. B. Validation of ATP8B1 overexpression/knockdown by transfection. C. Impact of ATP8B1 modulation on CK-MB levels in MP-CM-stimulated AC16 cells. D. Impact of ATP8B1 modulation on the proliferative capacity of MP-CM-stimulated AC16 cells.
$^{**}p < 0.01$; $^{***}p < 0.001$

response is a key driver of increased short- and long-term morbidity and mortality in this patient population [17, 18]. Additionally, one study reported that MP infection increased intracellular ROS levels by 2.4-fold after 24 hours [19]. The role of ATP8B1 in regulating inflammatory and oxidative processes in other diseases has been established. During the progression of chronic pancreatitis, ATP8B1 expression is progressively down-regulated, and acinar ATP8B1 facilitates macrophage activation and inflammation resolution, thereby influencing disease pathogenesis [20]. In prostate cancer research, ATP8B1 was consistently down-regulated in two cell lines [21]. In LUSC, reduced ATP8B1 expression is associated with poor patient prognosis. Experimental models indicate that ATP8B1 knockdown exacerbates LUSC tumourigenesis and progression by elevating ROS production and disrupting intracellular redox homeostasis [10]. Separately, deficiency in ATP8B1 renders alveolar epithelial cells more susceptible to oxidative stress, leading to increased cell death [11].

Table 2. A binary logistics analysis was conducted to assess the significance of ATP8B1 in predicting MP+MI in children

Parameters	OR	95%CI	p
ATP8B1	0.029	0.006–0.146	<0.001***
Sex (Male/Female)	1.527	0.568–4.102	0.401
Age (years)	1.074	0.846–1.363	0.558
BMI (kg/m^2)	1.051	0.784–1.408	0.739
Blood sugar (mmol/L)	1.441	0.753–2.758	0.270

BMI – body mass index.

Supplementary Table 1. The amplification efficiency of primers

Parameters	R2	k	E (%)
ATP8B1	0.9915	-3.12	101.3
β-Actin	0.9956	-3.35	96.1
IL-6	0.9971	-3.25	97.8
TNF-α	0.9924	-3.38	98.2

R2 – Correlation coefficient; k – Slope; E – Amplification efficiency, E=$10^{-1/k}$-1.

Consistent with these reports, *in vitro* findings in the current study position ATP8B1 as a central regulator in the inflammatory cascade triggered by MP infection. The anti-inflammatory effect of ATP8B1 over-expression, evidenced by suppressed IL-6 and TNF-α release, likely occurs through the stabilization of plasma membrane asymmetry, which may interfere with early pro-inflammatory signalling events. Furthermore, its ability to curb ROS generation points to a role in mitigating oxidative stress, a key driver of cellular damage in both pulmonary and cardiac contexts. The concomitant enhancement of cell proliferation underscores the functional consequence of this reduced inflammatory and oxidative burden, indicating that ATP8B1 not only protects cells from damage, but also facilitates recovery processes. Collectively, these data propose a model wherein ATP8B1 acts at the interface of inflammation and oxidative stress to exert its protective effects.

The translational relevance of these mechanistic insights is strongly supported by the clinical data in the current study. The progressive down-regulation of serum ATP8B1 from MP-only to MP+MI patients suggests a potential exhaustion of this endogenous protective pathway with worsening disease severity. This loss of ATP8B1 function could create a permissive environment for uncontrolled inflammation, as evidenced by the significant inverse correlation between ATP8B1 levels and the pro-inflammatory cytokines IL-6 and TNF-α. This consistency across experimental and clinical models underscores ATP8B1 deficiency as a key event in disease progression, supporting its potential as both a diagnostic biomarker and a therapeutic target.

Limitations of the study. This study has several limitations. First, all clinical specimens were obtained from a single medical institution, resulting in a relatively homogeneous study population, and a limited sample size that may have affected the generalizability of the findings. Second, while the cell models employed provide certain advantages in simulating MP-related MI with convenience and reproducibility, they also present inherent limitations: BEAS-2B immortalized bronchial epithelial cells differ in physiological characteristics from primary pulmonary epithelial cells [22]. AC16 human embryonic cardiomyocytes exhibit differentiation and maturation levels distinct from adult cardiomyocytes [23]. Additionally, the current models cannot fully replicate the complexity of the *in vivo* microenvironment where pulmonary inflammation triggered by MP infection affects myocardial function through multiple pathways, including circulatory and neural reflexes.

Furthermore, experimental validation in this study was primarily conducted at the cellular level. Although the results obtained clearly demonstrated the functional role of ATP8B1 *in vitro*, its *in vivo* relevance requires further verification through animal experiments. This limitation stems from practical considerations, including research timeframe, experimental costs, and technical challenges associated with maintaining stable animal models, indicating that the physiological relevance and translational potential of these findings need further confirmation in more complex biological systems.

CONCLUSION

In conclusion, ATP8B1 demonstrates low expression in the serum of MP+MI patients. Its upregulation significantly inhibited inflammation, ROS generation, and cardiac injury markers, and rescued the impairment of cell proliferation, which may alleviate MP-associated MI.

Future investigations should establish MP infection animal models combined with multi-centre clinical cohorts to systematically elucidate the regulatory mechanism of ATP8B1 in MP-associated MI, thereby providing a more substantial theoretical foundation for clinical therapeutic strategies.

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