

# Aberrant methylation of UBE2Q1 promoter is associated with poor prognosis of acute-on-chronic hepatitis B pre-liver failure

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## Abstract

Acute-on-chronic hepatitis B liver failure (ACHBLF) is one severe liver disease with rapid progression and high mortality. Identification of specific markers for the prediction of ACHBLF has important clinical significance. We explored the feasibility of UBE2Q1 gene promoter methylation as an early prediction and prognosis biomarker of ACHBLF.

UBE2Q1 promoter methylation frequency was detected in 60 patients with acute-on-chronic hepatitis B pre-liver failure (Pre-ACHBLF), 40 patients with chronic hepatitis B and 20 cases of healthy control (HC). The UBE2Q1 mRNA was detected by quantitative real-time polymerase chain reaction.

The methylation frequency of the UBE2Q1 promoter in pre-ACHBLF patients was 38.33%, which was significantly lower than that in chronic hepatitis B patients (60.00%) and HCs (65.00%). The UBE2Q1 mRNA expression in pre-ACHBLF patients with UBE1Q1 non-methylation was significantly higher than that in patients with UBE1Q1 promoter methylation. Further analysis showed that hypomethylation of the UBE2Q1 promoter was positively correlated with total bilirubin and international normalized ratio levels in patients with pre-ACHBLF, but negatively correlated with PTA level. COX multivariate analysis showed that the model for end-stage liver disease score and UBE2Q1 promoter hypomethylation status were potential early warning factors that can predict the progression of pre-ACHBLF to ACHBLF. The sensitivity and specificity of UBE2Q1 promoter methylation status combined with the model for end-stage liver disease score for early diagnosis of ACHBLF were 92.9% and 75.0%, respectively. The area under the receiver-operating characteristic curve was 0.895.

The hypomethylation of UBE2Q1 promoter is associated with severity of Pre-ACHBLF, which could serve as a potential prognostic biomarker for pre-ACHBLF.

**Abbreviations:** ACHBLF = acute-on-chronic hepatitis B liver failure, ACLF = acute-on-chronic liver failure, AFP = alpha fetoprotein, ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, AUC = area under the ROC curve, CHB = chronic hepatitis B, GSTP1 = glutathione-S-transferase P1, HBeAg = Hepatitis B e antigen, HBsAg = hepatitis B surface antigen, HC = healthy control, INR = international normalized ratio, MELD = model for end-stage liver disease, PBMcs = peripheral blood mononuclear cells, Pre-ACHBLF = acute-on-chronic hepatitis B pre-liver failure, pre-ACLF = acute-on-chronic pre-liver failure, PTA = prothrombin time activity, ROC curves = receiver-operating characteristic curves, TBIL = total bilirubin.

**Keywords:** acute-on-chronic hepatitis B pre-liver failure, methylation, prognosis, UBE2Q1

## 1. Introduction

Liver failure is the inability of the liver to perform its normal synthetic and metabolic function as part of normal physiology. According to the characteristics of the onset and disease progression, liver failure can be divided into acute liver failure, subacute liver failure, acute-on-chronic liver failure (ACLF), and

chronic liver failure. ACLF is the most common type of liver failure in China. The main cause of liver failure in China is hepatitis B virus infection.<sup>[1–3]</sup>

ACLF is characterized by de-compensated cirrhosis, multiorgan failure, and early mortality. Compared with chronic hepatitis, ACLF features acute-onset and rapid disease progression, but its

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progression is slower than that of acute liver failure without chronic liver disease. Early diagnosis and timely treatment are particularly important in improving the survival rate of patients.<sup>[4]</sup> In fact, there is a time window from ACLF onset to the occurrence of liver failure, which called “acute-on-chronic pre-liver failure (pre-ACLF)”.<sup>[5]</sup> Therefore, identification of specific markers for the diagnosis of pre-ACLF has important clinical significances.

Studies showed that apoptosis, necrosis of hepatocytes, inflammatory cell infiltration, and ischemic liver damage are the key factors in the pathogenesis of liver failure. Both direct injury and immune-mediated liver injury are involved.<sup>[6–9]</sup> Moreover, hepatocyte apoptosis and necrosis occur throughout the whole pathological process.<sup>[10]</sup> The ubiquitin-binding enzyme UBE2Q1 is a putative E2 ubiquitin conjugating enzyme and has been shown to be over-expressed in various types of cancers.<sup>[11–12]</sup> It is also involved in hepatocellular carcinoma development and progression and therefore a potential therapeutic target in applicable hepatocellular carcinoma patients.<sup>[13]</sup> Recent studies also showed that hypomethylated UBE2Q1 gene promoter in the serum is a promising biomarker for hepatitis B virus-associated hepatocellular carcinoma.<sup>[14]</sup> Abnormal DNA methylation expression was also identified in peripheral blood mononuclear cells in patients with ACHBLF.<sup>[15–17]</sup> Our previous studies showed that the glutathione-S-transferase P1 (*GSTP1*) gene was aberrant methylated in Pre-ACHBLF.<sup>[18]</sup> Whether the methylation of the UBE2Q1 promoter is altered during the development of pre-ACHBLF is still unclear.

In this study, we will assess the feasibility of using UBE2Q1 promoter methylation as a biomarker to predict the early occurrence and prognosis of ACHBLF.

## 2. Materials and methods

### 2.1. Patients

Sixty patients with pre-ACHBLF, 40 patients with chronic hepatitis B (CHB), and 20 healthy controls (HC) were enrolled at Department of Hepatology, the Affiliated Hospital of Shandong Medical College from December 2016 to July 2019. The clinical diagnostic criteria of pre-ACHBLF were as follows: a history of chronic hepatitis with the presence of serum hepatitis B surface antigen (HBsAg)  $\geq 6$  months; acute worsening of CHB; extreme fatigue with severe digestive symptoms, such as obvious anorexia, abdominal distension, nausea, and vomiting; serum total bilirubin (TBIL) C 10-fold normal upper level  $>171 \mu\text{mol/L}$ ; prothrombin time activity (PTA)  $>40\%$ . The inclusion criteria for CHB were set by the 2015 *Chronic Hepatitis B Prevention and Treatment* guidelines issued by the Chinese Medical Association. In detail, HBsAg remains positive for  $>6$  months. Serum alanine aminotransferase increases continuously or repeatedly. The exclusion criteria were as follows: coinfection with hepatitis A, C, D, E; pregnancy, other liver diseases such as alcoholic liver disease, drug-induced hepatitis, Wilson disease, and autoimmune hepatitis; super infection with cytomegalovirus and human immunodeficiency virus or Epstein–Barr virus; malignant jaundice induced by obstructive or hemolytic jaundice; a history of diabetes or cardiac disease or nephrosis.

The healthy control group had no history of chronic disease, and the liver function was normal. There was no infection of hepatitis A, B, C, D, and E virus and Cytomegalovirus or Epstein–Barr virus. The study protocol was approved by Ethics Committee of the affiliated hospital of Shandong medical college.

### 2.2. Clinical data collection

The serum biochemical markers of liver function were performed using an automatic biochemical analyzer (Cobas c311, Roche Diagnostic Ltd., Germany), including alanine aminotransferase (ALT), aspartate aminotransferase (AST), TBIL, albumin (ALB), and creatinine (Cr). PTA and prothrombin time–international normalized ratio (INR) were quantified using ACL TOP 700 (Instrument Laboratory, Lexington, MA). Hepatitis B e antigen (HBeAg) and alpha fetoprotein were measured with an automatic biochemical analyzer (Roche Diagnostic Ltd., Germany). HBsAg and HBeAg were measured with an automatic biochemical analyzer (Roche Diagnostic Ltd., Germany). A model for end-stage liver disease (MELD) was used to assess the severity and possible prognosis of the disease. The calculation formula for MELD is as follows: MELD score =  $3.8 \times \ln(\text{bilirubin } [\mu\text{mol/L}] \times 0.058) + 11.2 \times \ln(\text{INR}) + 9.6 \times \ln(\text{creatinine } [\mu\text{mol}] \times 0.011) + 6.4 \times (0 \text{ or } 1)$  (0: cholestatic or alcoholic hepatitis; 1: other hepatitis).<sup>[19]</sup>

### 2.3. Isolation of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated using gradient centrifugation. In brief, 5 mL whole blood was centrifuged via Ficoll-Paque plus density gradient medium (Pharmacia Diagnostics, Uppsala, Sweden) according to the manufacturer’s protocol and harvested cells were stored at  $-20^\circ\text{C}$  for later use.

### 2.4. RNA extraction from and quantitative reverse transcription polymerase chain reaction

Total RNA was extracted from PBMCs by phenol chloroform isopropanol method according to the protocol recommended by the manufacturer. 2  $\mu\text{g}$  of total RNA were reverse transcribed into cDNA using first-strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania). The quantitative reverse transcription polymerase chain reaction (PCR) was carried out with Light cycler (Roche, Basel, Switzerland) using SYBR Green (Toyobo, Osaka, Japan), according to the following thermal profile: pre-denaturation at  $95^\circ\text{C}$  for 30 seconds, 40 cycles of denaturation at  $95^\circ\text{C}$  for 5 seconds, annealing at  $60^\circ\text{C}$  for 30 seconds, extension at  $72^\circ\text{C}$  for 30 seconds, and final extension at  $72^\circ\text{C}$  for 7 minutes. GAPDH was used as an internal control. The primers were shown as follows: UBE2Q1 Forward, 5'-CTGGCAAGGGTTGATCCAGC-3', UBE2Q1 Reverse 5'-TCCTGTCCGCATC-CAC TG

C A-3', GAPDH Forward, 5'-GACTCATGACCACAGTC-CATGC-3' and GAPDH Reverse,

5'-AGAGGCAGGGATGATGTTCTG-3'. The relative mRNA levels were determined using  $2^{-\Delta\Delta\text{Ct}}$  method.

### 2.5. Extraction of PBMC DNA and DNA sodium bisulfite modification

Genomic DNA was extracted from PBMCs using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). A total elution volume was 20  $\mu\text{L}$  and the extracted DNA was subjected to bisulfite modification treatment using an EZ DNA Methylation-Gold Kit<sup>TM</sup> (Zymo Research Corp, Orange, CA) to convert all unmethylated cytosines to thymines. A final volume of 20  $\mu\text{L}$  of modified DNA was stored at  $-20^\circ\text{C}$  for further analysis.

## 2.6. Methylation-specific PCR (MSP)

The methylation-specific polymerase chain reaction method was used to detect the methylation status of specific genes after bisulfite modification of DNA. The methylation-specific PCR forward and reverse primer sequences were 5'-GGGTAGTAGTAGT CCGGGTC-3' and 5'-GCTAACAAATACGAAAACGCT-3', respectively. The non-methylation-specific PCR forward and reverse primer sequences were 5'-TAGGGGTAGTAGTAGTTGGGGTT-3' and 5'-CACTAACAAATACAAAACACTCA-5', respectively. The PCR reaction mixture contained 9.5  $\mu$ L of RNase-free water, 0.5  $\mu$ L of each forward and reverse primer, 12.5  $\mu$ L of Taq Premix (Zymo Research, USA), and 2  $\mu$ L of DNA template. The mixture was thoroughly mixed and centrifuged for 5 seconds. The amplification conditions were as follows: pre-denaturation at 95°C for 5 minutes, 45 cycles of denaturation at 95°C for 45 seconds, annealing at 58°C for 45 seconds, extension at 72°C for 60 x 0.200A;seconds, and final extension at 72°C for 10 minutes followed by holding at 4°C. Agarose gel electrophoresis was performed using 5  $\mu$ L of each PCR product. The gel electrophoresis image analysis system was used for image acquisition, analysis, and documentation.

## 2.7. Statistical analysis

SPSS17.0 statistical software was used for statistical analysis (SPSS Inc, Chicago, IL). Numerical variables were expressed as median values, and quartiles and categorical variables were expressed as percentages. Comparisons between 2 sets of numerical variables were performed using the independent samples t-test or the Mann-Whitney *U* test. Categorical variables were compared using the  $\chi^2$  test. Spearman correlation coefficient analysis was used to determine correlations between the methylation status of the UBE2Q1 promoter and clinical parameters in patients with pre-ACHLF. Multiple logistic regression analysis was used to screen UBE2Q1 promoter methylation as an independent prediction factor. Univariate Cox proportional hazards model analysis was used to determine the clinical parameters for early warning of ACHBLF occurrence. The statistically significant parameters were selected for further analysis in the multivariate model. Receiver-operating characteristic curves (ROC curves) were used to examine reliability using the methylation of the UBE2Q1 promoter as a biological indicator for early warning of ACHBLF.  $P < .05$  was considered statistically significant.

**Table 1**

**Comparison of the baseline clinical data of the participants.**

	Pre-ACHBLF (n=60)	CHB (n=40)	HCs (n=20)	P
Sex (M/F)	46/14	26/14	11/9	.15
Age, y	45.5	40	41	.09
HBeAg+ (%)	24 (40%)	23 (57.5%)	NA	.09
Log <sub>10</sub> (HBV-DNA)	4.40	4.85	NA	.08
ALT, U/L	220	67.00	25.30	<.05
AST, U/L	201	47.5	23.08	<.05
TBIL, $\mu$ mol/L	293.65	20.95	13.45	<.05
ALB, g/L	33.70	40.95	45.66	<.05
INR	1.65	1.05	0.99	<.05
PTA	50.50	78.00	94.50	<.05
Cr, $\mu$ mol/L	73.50	80.50	71.00	<.05

ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHB = chronic hepatitis B, Cr = creatinine, HBV = hepatitis B virus, HC = healthy control, INR = international normalized ratio, NA = not available, Pre-ACHBLF = acute-on-chronic hepatitis B pre-liver failure, PTA = prothrombin time activity, TBIL = total bilirubin.

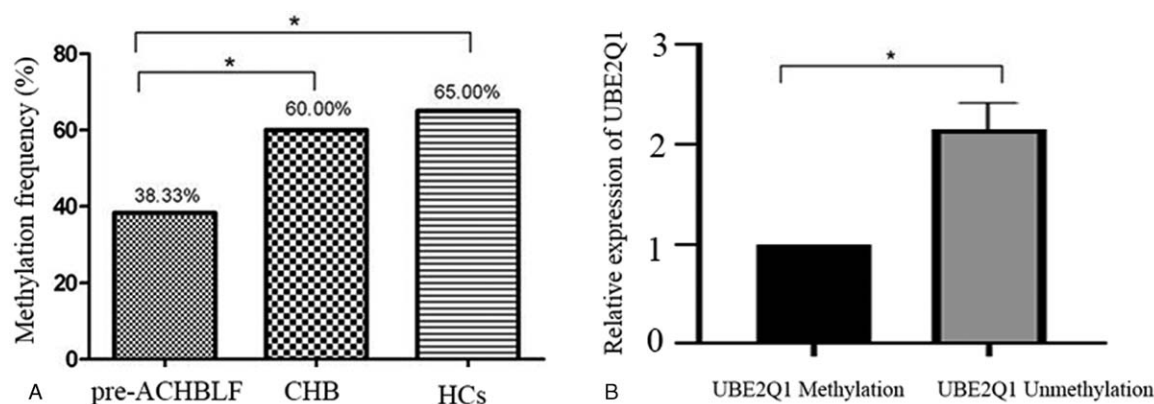
## 3. Results

### 3.1. Clinical data of the patients

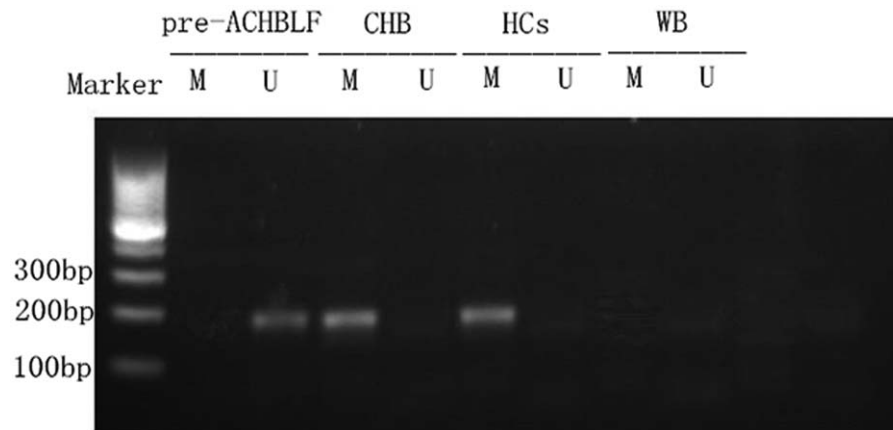
The clinical data of 60 patients with pre-ACHBLF, 40 patients with CHB and 20 HCs were shown in Table 1. There were no significant difference in age, sex and serum creatinine levels among the three groups ( $P > .05$ ). The level of ALT, AST, TBIL, ALB, INR, and PTA, which indicate the severity of liver failure, were significantly different. There was no significant difference in HBeAg and HBV-DNA levels between the ACHBLF and CHB groups ( $P > .05$ ).

### 3.2. Comparison of UBE2Q1 promoter methylation frequency and UBE2Q1 mRNA expression among the pre-ACHBLF, CHB, and HCs groups

The methylation-specific PCR assay was used to detect the methylation status of the UBE2Q1 gene promoter in the peripheral blood mononuclear cells. Figure 1 showed the typical bands obtained after electrophoresis of the amplified methylation-specific polymerase chain reaction products. The methylation rate of pre-ACHBLF group was 38.33%, significantly lower than that in CHB group (60.00%,  $P < .05$ ) and in HC group (65.00%,  $P < .05$ ). There was no significant difference in the methylation rate of the UBE2Q1 gene promoter in the CHB and HC groups ( $P > .05$  (Fig. 2A).



**Figure 1.** Representative electrophoresis results of UBE2Q1 promoter methylation status. CHB = chronic hepatitis B, HCs = healthy controls, M = methylated product band, Pre-ACHBLF = HBV-related pre-acute-on-chronic liver failure, U = unmethylated product band, WB = blank control group.



**Figure 2.** Comparison of UBE2Q1 promoter methylation frequency and UBE2Q1 mRNA expression level. (A) The methylation frequency of the UBE2Q1 promoter in different groups (B) UBE2Q1 mRNA expression in the UBE2Q1 methylation group and the non-methylation group. \* $P < .05$ .

DNA methylation often affects the transcriptional expression of genes. We further investigated the correlation between abnormal methylation status of the UBE2Q1 mRNA expression. Fluorescence-based real-time quantitative PCR assay was used to examine the UBE2Q1 mRNA level in patients with pre-ACHBLF. The results showed that the expression of UBE2Q1 mRNA in patients with UBE1Q1 nonmethylation was significantly higher than that in patients with UBE1Q1 gene promoter methylation (Fig. 2B).

### 3.3. Comparison between UBE2Q1 promoter methylation and clinical pathological characteristics of patients with pre-ACHBLF

The methylation status of the UBE1Q1 promoter may be related to the severity of the disease in patients with pre-ACHBLF. In this study, we analyzed the differences in the clinical parameters of patients with or without UBE2Q1 promoter methylation in the pre-ACHBLF group. The results showed there were no significant differences between the methylation and nonmethylation groups in age, sex, level of HBeAg, HBV-DNA viral load, ALT, AST,

ALB, or creatinine level ( $P > .05$ ). However, the parameters reflecting the severity of liver failure, including TBIL, INR, PTA, and MELD scores, TBIL, INR, and MELD scores were significantly higher in the non-methylation group than in the methylation group. PTA was significantly lower in the non-methylation group than in the methylation group ( $P < .05$ , Table 2). Spearman correlation analysis showed that hypomethylation of the UBE2Q1 promoter was positively correlated with TBIL and INR levels in patients with pre-ACHBLF ( $r_s = 0.545$  and  $0.505$ , respectively;  $P < .05$ ) but negatively correlated with PTA level ( $r_s = -0.441$ ,  $P < .05$ ). Multivariate logistic regression analysis was used to screen the independent prediction factors that affect serum UBE2Q1 gene promoter hypomethylation. The results showed that none of the clinical parameters of patients with pre-ACHBLF were independent prediction factors that reflect UBE2Q1 gene methylation status (Table 3).

### 3.4. Predictive values of UBE2Q1 promoter methylation for the incidence of ACHBLF in patients with pre-ACHBLF

Sixty patients with pre-ACHBLF were followed-up for 4 weeks. The ACHBLF was used as the end point of the observation. In our

**Table 2**  
Comparison between UBE2Q1 promoter methylation and clinical pathological characteristics of patients with pre-ACHBLF.

	UBE2Q1 unmethylation	UBE2Q1 methylation	P
Case	37	23	
Sex (M/F)	29/8	17/6	.69
Age, y	47	43	.10
HBeAg+(%)	14 (37.98%)	10 (43.48%)	.67
Log <sub>10</sub> (HBV-DNA)	4.21	4.47	.13
ALT, U/L	198	234	.19
AST, U/L	178	220	.11
TBIL, $\mu$ mol/L	312.30	222.60	<.05
ALB, g/L	32.60	35.10	.09
INR	1.68	1.59	<.05
PTA	47	55	<.05
Cr, $\mu$ mol/L	73	74	.95
MELD score	21.11	19.20	<.05

ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHB = chronic hepatitis B, Cr = creatinine, HBV = hepatitis B virus, INR = international normalized ratio, MELD = model for end-stage liver disease, Pre-ACHBLF = acute-on-chronic hepatitis B pre-liver failure, PTA = prothrombin time activity, TBIL = total bilirubin.

**Table 3**  
Analysis of UBE2Q1 promoter methylation status as an independent prediction factor.

	Coefficient	OR	95% CI	P
Sex	0.169	1.184	0.986–1.421	.07
Age	0.058	1.060	0.089–12.676	.96
HBeAg+	0.027	1.028	0.045–23.673	.99
Log <sub>10</sub> (HBV-DNA)	-2.222	0.108	0.012–1.017	.05
ALT	-0.006	0.994	0.971–1.017	.60
AST	-0.008	0.992	0.968–1.016	.52
TBIL	-0.030	0.971	0.863–1.092	.62
ALB	-0.006	0.994	0.760–1.301	.97
INR	-4.615	0.010	0.000–1.16E25	.89
PTA	-0.167	0.846	0.639–1.120	.24
Cr	-0.552	0.567	0.189–1.750	.33
MELD score	4.639	103.466	0.011–9.48E5	.32

ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHB = chronic hepatitis B, CI = confidence interval, Cr = creatinine, HBV = hepatitis B virus, INR = international normalized ratio, MELD = model for end-stage liver disease, OR = odds ratio, PTA = prothrombin time activity, TBIL = total bilirubin.

**Table 4****Analysis of the early warning factors for ACHBLF by means of the COX proportional hazards model.**

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Sex	0.854	0.346–2.015	.73			
Age	1.027	0.980–1.076	.27			
HBeAg+	0.685	0.325–1.441	.32			
Log <sub>10</sub> (HBV-DNA)	1.027	0.658–1.603	.91			
ALT	0.998	0.995–1.001	.24			
AST	0.998	0.993–1.002	.26			
TBIL	1.010	1.004–1.016	.00			
ALB	0.946	0.883–1.013	.11	0.999	0.991–1.007	.76
INR	7.7E3	29.272–20.1E5	.00	5.495	0.003–9.80E3	.65
PTA	0.932	0.873–0.995	.04	1.005	0.926–1.090	.91
Cr	1.024	0.993–1.055	.13	1.502	1.102–2.046	.01
MELD score	1.525	1.235–1.882	<.05	10.141	2.140–48.050	.00
UBE2Q1 (M)	13.069	3.083–55.414	<.05			

ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHB = chronic hepatitis B, CI = confidence interval, Cr = creatinine, HBV = hepatitis B virus, HR = hazard ratio, INR = international normalized ratio, MELD = model for end-stage liver disease, Pre-ACHBLF = acute-on-chronic hepatitis B pre-liver failure, PTA = prothrombin time activity, TBIL = total bilirubin.

study, 28 subjects developed from pre-ACHBLF to ACHBLF in 4 weeks. COX regression univariate analysis suggested that TBIL, PTA, INR, MELD score, and hypomethylation status of the UBE2Q1 promoter were early warning factors for ACHBLF ( $P < .05$ ). The above-mentioned parameters were included in the multivariate analysis, which showed that the MELD score and UBE2Q1 promoter hypomethylation status may predict the progression of pre-ACHBLF to ACHBLF ( $P < .05$ ) (Table 4).

The ROC curve analysis showed that the sensitivity and specificity for predictive values of ACHBLF using the methylation status of the UBE2Q1 promoter were 92.9% and 65.6%, respectively. The area under the ROC curve (AUC) was 0.792 (95% confidence interval [CI]: 0.675–0.910). The sensitivity and specificity for early diagnosis of ACHBLF using the MELD score were 96.4% and 62.5%, respectively. The AUC was 0.831 (95% CI: 0.729–0.934). The ROC indicated that the cutoff point for the MELD score was 19.97. The sensitivity and specificity of UBE2Q1

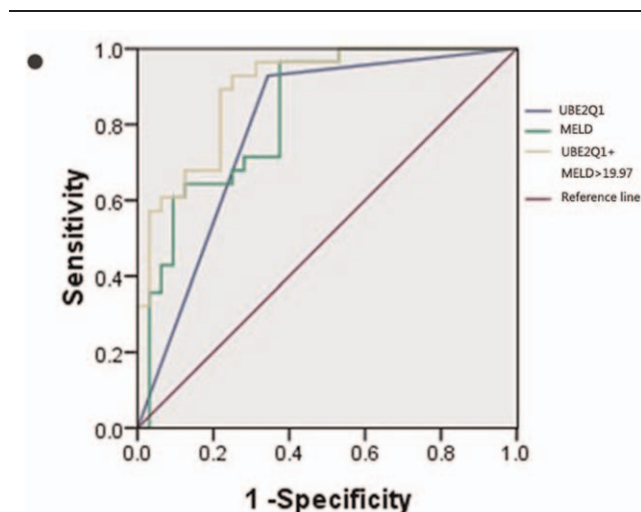
promoter methylation status combined with the MELD score for early diagnosis of ACHBLF were 92.9% and 75.0%, respectively. The AUC was 0.895 (95% CI: 0.818–0.973) (Fig. 3).

#### 4. Discussion and conclusions

ACLF is a disease with rapid progression and high mortality. Early screening and necessary intervention and treatment will have a significant impact on patient prognosis. In this study, we demonstrated that abnormal UBE2Q1 gene methylation status and MELD score can improve the sensitivity and specificity of the early diagnosis of ACHBLF.

UBE2Q1 is a ubiquitin-binding enzyme. The gene encoding UBE2Q1, which is located at site 21 on the long arm of chromosome 1, encodes the ubiquitin ligase E2 protein. E2 promotes the transfer of activated ubiquitin from ubiquitin-activating enzyme (E1) to ubiquitin ligase (E3).<sup>[20]</sup> The *UBE2Q1* gene is a common target in cancer research. The UBE2Q1 gene is highly expressed in breast cancer and colorectal tumor, and participates in the regulation of apoptosis in multiple myeloma cells through the p53 signaling pathway.<sup>[11–12]</sup> Recent studies also showed that hypomethylated UBE2Q1 gene promoter in the serum is a promising biomarker for Hepatitis B virus-associated hepatocellular carcinoma.<sup>[13–14]</sup> HBV-related ACLF is the most common type of liver failure in China. However, it is not clear whether the methylated status of UBE2Q1 gene promoter is associated with ACHBLF. Our data showed that the methylation rate of the UBE2Q1 promoter in patients with pre-ACHBLF was significantly lower than that in CHB and HCs groups. Combined with the finding in hepatocellular carcinoma, we infer that the hypomethylation rate of the UBE2Q1 promoter may be a bad indicator for chronic liver diseases related to the hepatitis B virus.

DNA methylation is one of the most important epigenetic modifications and is very common in most eukaryotes, which can influence the expression of genes. DNA methylation level is influenced by environment, disease, age, and sex. Various extents of DNA methylation may occur in different cells, tissues, or individuals. It is also different at different developmental stages of the same cell or individual.<sup>[21]</sup> In this study, we found that the UBE2Q1 gene was hypomethylated in pre-ACHBLF and that UBE2Q1 mRNA was highly expressed. The methylation status of the *UBE2Q1* gene was also correlated with important indexes



**Figure 3.** Predictive values of UBE2Q1 promoter methylation for the incidence of ACHBLF in patients with pre-ACHBLF. The areas under the curves of UBE2Q1 or MELD alone predicting ACHBLF were 0.792 and 0.831, respectively. The area under the curve of UBE2Q1 combined with MELD predicting ACHBLF was 0.895.

reflecting the severity of liver injury and necrosis, such as TBIL, INR, PTA. Studies showed that apoptosis, necrosis of hepatocytes, inflammatory cell infiltration and ischemic liver damage are the key factors in the pathogenesis of liver failure.<sup>[10]</sup> So, the abnormal hypomethylation of the *UBE2Q1* gene is associated with the pathology of pre-ACHBLF

The MELD-related scoring system has been regarded as an index of disease severity for patients with end-stage liver disease.<sup>[22]</sup> In our study, COX multivariate analysis showed that MELD score is indeed one early warning factor for the progression from pre-ACHBLF to ACHBLF. However, the MELD scoring system has some shortcomings: it is easily affected by diuretics and changes in circulating blood volume, it does not take HBV infection, ascites and hepatic encephalopathy into consideration.<sup>[23]</sup> We also found that the hypomethylation status of the *UBE2Q1* promoter is a biological indicator that can be used to determine the short-term survival status of patients with ACHBLF and that it can better predict the prognosis of patients with ACHBLF when combined with MELD score. We found that there are also some limitations of this study. We collected cases from 1 single center, so we need to validate the results in a multicenter cohort. In addition, gene sequencing will be helpful in further studies.

In conclusion, we firstly demonstrate that the *UBE2Q1* promoter is hypomethylated in peripheral blood mononuclear cells of patients with pre-ACHBLF. Hypomethylation of *UBE2Q1* gene is associated with severity of Pre-ACHBLF, which could serve as a potential prognostic biomarker for pre-ACHBLF, combined with MELD score.

## Author contributions

**Conceptualization:** Na Hu, Wei-dong Lai.

**Data curation:** Xian-ci Xie, Lin-lin Liu.

**Formal analysis:** Na Hu.

**Writing – original draft:** Na Hu.

**Writing – review & editing:** Na Hu.

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