Am J Cancer Res 2025;15(6):2631-2641
www.ajcr.us /ISSN:2156-6976/ajcr0165770

Original Article

The correlation between ALDH2 rs671 polymorphism and clinical prognosis in alcoholic liver disease-related hepatocellular carcinoma after curative resection

Chih-Jan Ko[1,2,21], Wen-Lung Wang[3,4], Hung-Yu Lin[4,5], Pei-Min Hsieh[6,7], Szu-Ying Chen[8,9,10], Steven Yu Lin[3,4,11], Kuang-Chun Hu[16,17], James Yu Lin[3,4,19], Li-Wei Chou[12,13,14*], Yaw-Sen Chen[6*], Yu-Wei Huang[3,10,15*], Chen-Ti Wang[18*], Wen-Chao Ho[1*], Chih-Wen Lin[3,4,20,21*]

[1]Department of Public Health, China Medical University, Taichung, Taiwan; [2]Department of General Surgery, China Medical University Hsinchu Hospital, Hsinchu, Taiwan; [3]Division of Gastroenterology and Hepatology, Department of Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [4]School of Medicine, College of Medicine, I-Shou University, Kaohsiung, Taiwan; [5]Deprtment of Surgery, E-Da Cancer Hospital, I-Shou University, Kaohsiung, Taiwan; [6]Department of Surgery, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [7]Department of Surgery, E-Da Dachang Hospital, I-Shou University, Kaohsiung, Taiwan; [8]Division of Occupational Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [9]Division of Surgical Intensive Care, Department of Critical Care Medicine, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [10]Department of Nursing, Fooyin University, Kaohsiung, Taiwan; [11]New York University, New York, New York, United States; [12]Department of Physical Medicine and Rehabilitation, China Medical University Hospital, Taichung, Taiwan; [13]Department of Physical Therapy and Graduate Institute of Rehabilitation Science, China Medical University, Taichung, Taiwan; [14]Department of Physical Medicine and Rehabilitation, Asia University Hospital, Asia University, Taichung, Taiwan; [15]Department of Anesthesiology, Emergency and Critical Care Center, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [16]Healthy Evaluation Center and Division of Gastroenterology, Department of Internal Medicine, MacKay Memorial Hospital, Taipei, Taiwan; [17]Mackay Junior College of Medicine, Nursing and Management, New Taipei, Taiwan; [18]Division of Endocrinology, Department of Metabolism, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan; [19]Wentworth Institute of Technology, Boston, Massachusetts, United States; [20]Lin Da Clinic, Lin Da Heath group, Kaohsiung, Taiwan; [21]School of Chinese Medicine, College of Chinese Medicine, and Research Center for Traditional Chinese Medicine, China Medical University, Taichung, Taiwan. [*]Equal contributors.

Received May 1, 2025; Accepted May 30, 2025; Epub June 15, 2025; Published June 30, 2025

Abstract: Whether the aldehyde dehydrogenase 2 (ALDH2) rs671 polymorphism predicts clinical prognosis in alcoholic liver disease (ALD)-related hepatocellular carcinoma (HCC) after hepatectomy remains unclear. Hence, this study aims to investigate the association between ALDH2 rs671 polymorphism and HCC recurrence and mortality in patients with ALD-related HCC who underwent hepatectomy. We also explored the risk factors for HCC recurrence and mortality in this population of patients. This retrospective cohort study enrolled 238 ALD-related HCC patients underwent hepatectomy from 2011 to 2022 at the E-Da Hospital, I-Shou University. Data analyses were finalized on October, 2023. Alcoholism was defined as consuming over 20 g of ethanol each day for at least 5 years. Patients with HBsAg-positive or/and HCV-positive status were excluded. ALDH2 rs671 polymorphism was analyzed. The endpoint was HCC recurrence and overall mortality. Of the 238 patients enrolled, 196 (82.4%) were men, and the mean (SD) age was 62.3 (10.2) years. HCC recurrence occurred in 70 patients, and 64 patients died. ALDH2 rs671 polymorphism was significantly associated with HCC recurrence and mortality. The 10-year cumulative HCC recurrence and mortality rates were significantly higher in patients with the ALDH2 rs671 genotype GA/AA relative to those with the ALDH2 rs671 genotype GG. In the Cox proportional analyses, the ALDH2 rs671 genotypes GA/AA (hazard ratio [HR]: 2.66, 95% confidence interval [CI]: 1.59-4.43, $P = 0.000$) and AST ≥ 40 IU/L (HR: 1.93, 95% CI: 1.18-3.17, $P = 0.009$) were significantly associated with increased HCC recurrence. Furthermore, the ALDH2 rs671 genotype GA/AA (HR: 2.02, 95% CI: 1.17-3.49, $P = 0.012$) and age ≥ 65 years (HR: 1.67, 95% CI: 1.01-2.78, $P = 0.048$) were significantly associated with increased mortality. In conclusion, the ALDH2 rs671 genotype GA/AA is significantly associated with unfavorable clinical prognosis in ALD-related HCC after hepatectomy.

Keywords: Hepatocellular carcinoma, ALDH2 rs671 polymorphism, predictors, recurrence, mortality, hepatectomy

https://doi.org/10.62347/EVBS5360

Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related mortality globally [1-5]. In Taiwan, HCC often results from viral- and alcohol-related cirrhosis [6, 7]. Moreover, early-stage HCC is difficult to diagnose, resulting in elevated HCC recurrence and mortality rates after surgical resection [8-13]. Despite the use of highly sensitive tumor markers to predict clinical outcomes after hepatectomy in patients with HCC, the result remains unsatisfactory [14]. Therefore, the identification of predictors for HCC recurrence and mortality could help improve the clinical prognosis of patients with HCC undergoing hepatectomy.

The aldehyde dehydrogenase 2 (ALDH2) polymorphism influences the development of HCC in alcoholic patients with or without viral hepatitis [15-18] and nonalcoholic patients [19, 20]. Several studies have demonstrated that the ALDH2 rs671 polymorphism is not associated with HCC in East Asians [21-24]. Our previous study revealed a significant increase in the risk of HCC development and mortality in HBV-related cirrhotic patients with heavy alcohol drinking and ALDH2 rs671 polymorphism [6]. Moreover, a previous study demonstrated that the ALDH2 rs671 genotype GG correlated significantly with shorter overall survival, but not with recurrence-free survival, particularly in patients with viral-related HCC who underwent surgical resection [25]. However, the effect of ALDH2 rs671 polymorphism on HCC recurrence and mortality in patients with alcoholic liver disease (ALD)-related HCC who underwent surgical resection remains unclear. Hence, we investigated the association between ALDH2 rs671 polymorphism and HCC recurrence and mortality in patients with ALD-related HCC who underwent hepatectomy. We also explored the risk factors for HCC recurrence and mortality in this population of patients.

Materials and methods

Patients and follow-up

This retrospective cohort study enrolled 238 patients with ALD-related HCC who underwent surgical resection from October 2011 to December 2022 at the E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. All patients underwent liver function tests, α-fetoprotein (AFP) tests, and imaging examinations, including ultrasonography, computed tomography, and magnetic resonance imaging examinations. These clinical investigations were conducted every 3-6 months or as deemed necessary for the detection of HCC. The data analyses were finalized on October 31, 2023. Patients with alcoholism were included in this study, and alcoholism was defined as consuming at least 20 g of ethanol each day for more than 5 years. However, patients with (1) hepatitis B virus infection; (2) hepatitis C virus infection; (3) alcohol intake of < 20 g/day and for < 5 years; and (4) HCC recurrence and mortality within 6 months after surgical resection were all excluded from the excluded. The presence of ALDH2 rs671 polymorphism was determined by using blood analysis. The primary endpoint was HCC recurrence whereas the secondary endpoint was overall mortality. The follow-up time was defined as the time from the date of inclusion to the date of death, the last follow-up, or the end of the study (October 31, 2023), whichever was earliest. The recurrence time was defined as the time from the date of inclusion to the date of HCC operation, the date of death, the last follow-up, or the end of the study (October 31, 2023), whichever was earliest. HCC recurrence was established based on histology or at least two typical HCC imaging methods, as outlined by the HCC guidelines of the American Association for the Study of Liver Disease [26].

The clinicopathological features of the patients, including demographic data and tumor characteristics, were recorded. This research was approved by the Institutional Review Board of E-DA Hospital (EMRP41111N). Additionally, the research was conducted per the guidelines of the International Conference on Harmonization for Good Clinical Practice. Notably, all participants were adults and provided written informed consent for study participation.

ALDH2 rs671 polymorphism

ALDH2 is a major enzyme involved in the elimination of acetaldehyde from the body. The ALDH2*2 allele variant is a single point mutation (G to A) in exon 12, resulting in a change from glutamine (Glu) to lysine (Lys) at codon 487 and the inactivation of ALDH2 enzyme activity in humans, causing deficiency. The presence of a single polymorphism (Glu to Lys, G to A, or *1 to *2) was evaluated using blood

samples. The ALDH2 rs671 polymorphism causes one of three genotypes: GG, AA, and GA. To evaluate ALDH2 deficiency, patients with the GA and AA genotypes were merged into a single GA/AA group.

Statistical analysis

Continuous data are expressed as the mean and standard deviation (SD). Categorical data are described using numbers and percentages. Normally distributed continuous variables were compared using the Student's *t*-test, and the Wilcoxon rank-sum test was applied for comparisons of two groups when continuous variables were not normally distributed. The chi-squared test was used to compare categorical variables. The cumulative HCC recurrence and mortality rates were evaluated using the Kaplan-Meier method. Recognizing that patients who died were no longer at risk for HCC recurrence, competing risk analyses were conducted. These analyses aimed to evaluate the cumulative HCC recurrence, with mortality considered a competing risk. Both univariable and multivariable analyses were used to evaluate the risk predictors associated with HCC recurrence and mortality. The multivariable analyses were conducted using Cox proportional regression modes for HCC. $P < 0.05$ indicated statistical significance. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 23.0; Chicago, IL).

Results

Baseline demographic data

The demographic and clinicopathological features of the study participants are presented in **Table 1**. Notably, 196 (82.4%) were men, and the mean (SD) age was 62.3 (10.2) years. HCC recurrence occurred in 70 patients, and 64 patients died. Additionally, one-fourth of the patients had liver cirrhosis. Regarding tumor stage, 15.5% and 14.7% of the patients were Barcelona clinic liver cancer (BCLC) stage B-C and TNM stage III-IV, respectively.

ALDH2 rs671 polymorphism is associated with advanced clinicopathological features

Notably, 44.6% (111) and 56.04% (127) of the 238 patients had ALDH2 rs671 genotype GG and genotype GA/AA, respectively (**Table 1**). The ALDH2 rs671 genotype was significantly correlated with advanced clinicopathological features, including Edmondson-Steiner Grades [105 (94.6) vs. 110 (86.6), $P = 0.038$], recurrence [22 (19.8) vs. 48 (37.8), $P < 0.001$], and mortality [23 (20.7) vs. 41 (32.3), $P = 0.003$].

Predictive factors associated with tumor recurrence in patients with HCC who underwent hepatectomy

HCC recurrence was observed in 70 patients after hepatectomy, with 1-, 3-, 5-, and 10-year cumulative incidence rates of 2.6%, 12.0%, 23.8%, and 37.7%, respectively (**Figure 1A**). The univariate analysis revealed that ALDH2 rs671 genotype GA/AA, AST ≥ 40 IU/L, and TNM stage III-IV were significantly correlated with increased HCC recurrence (**Table 2**). Furthermore, the multivariate Cox regression analysis revealed that the ALDH2 rs671 genotype GA/AA was significantly correlated with increased HCC recurrence (hazard ratio [HR]: 2.66, 95% confidence interval [CI]: 1.59-4.43, $P = 0.000$), followed by those with AST ≥ 40 IU/L (HR: 1.93, 95% CI: 1.18-3.17, $P = 0.009$) (**Table 2**).

The Kaplan-Meier analysis revealed that patients with ALDH2 rs671 genotype GA/AA had a significantly higher HCC recurrence rate than those with ALDH2 rs671 genotype GG. Additionally, for patients with ALDH2 rs671 genotype GA/AA, the 1-, 3-, 5- and 10-year cumulative HCC recurrence rates were 7.2%, 30.1%, 43.2%, and 59.0%, respectively. By contrast, those with genotype GG had 1-, 3-, 5- and 10-year cumulative HCC recurrence rates of 6.6%, 11.5%, 17.4%, and 23.8%, respectively (**Figure 1B**). In addition, patients with AST ≥ 40 IU/L had a significantly higher HCC recurrence rate than those with AST < 40 IU/L. For those AST ≥ 40 IU/L, the 1-, 3-, 5- and 10-year HCC recurrence rates were 8.9%, 28.9%, 39.5% and 53.2%, respectively. In contrast, those AST < 40 IU/L had 1-, 3-, 5- and 10-year mortality rates of 2.5%, 14.1%, 22.3%, and 29.1%, respectively (**Figure 1C**).

Furthermore, in the competing risk analysis, patients with ALDH2 rs671 genotype GA/AA still had a significantly higher HCC recurrence rate than those with ALDH2 rs671 genotype GG ($P = 0.000$, **Figure 2**). Additionally, for patients with ALDH2 rs671 genotype GA/AA, the 1-, 3-,

Table 1. Basic demographic data of all patients and correlations between ALDH2 polymorphisms and clinicopathologic features

Characteristics	All patients (n = 238)	ALDH2 rs671 genotype		*P*-value
		GG (n = 111)	GA/AA (n = 127)	
Gender				
Female	42 (17.6)	20 (18.0)	22 (17.3)	0.888
Male	196 (82.4)	91 (82.0)	105 (82.7)	
Age (years)	62.3 ± 10.2	62.8 ± 9.7	61.9 ± 11.1	0.510
BMI (kg/m^2)	24.8 ± 3.8	25.0 ± 3.5	24.7 ± 3.6	0.078
HTN	103 (43.3)	52 (46.8)	51 (40.2)	0.299
DM	59 (24.8)	30 (27.0)	29 (22.8)	0.455
Smoking	64 (26.9)	38 (34.2)	26 (20.5)	0.017
AST (IU/L)	48 ± 32	45 ± 26	50 ± 37	0.167
ALT (IU/L)	49 ± 45	50 ± 53	48 ± 37	0.780
Total Bilirubin (mg/dl)	0.9 ± 0.5	0.9 ± 0.6	0.9 ± 0.4	0.978
Albumin (g/dl)	4.2 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	0.918
Creatinine	1.3 ± 0.9	1.3 ± 1.0	1.3 ± 0.8	0.601
Platelet count (×10^3/ml)	188 ± 70	188 ± 68	188 ± 72	0.726
INR	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.428
AFP (ng/dl)	3295 ± 21189	4342 ± 26918	2380 ± 14497	0.477
ICG (%)	11.9 ± 6.7	12.1 ± 6.6	11.8 ± 6.9	0.732
Liver cirrhosis				
Absent	174 (73.1)	85 (76.6)	89 (70.1)	0.259
Present	64 (26.9)	26 (23.4)	38 (29.9)	
Child-Pugh class				
class A	236 (99.2)	109 (98.2)	127 (100)	0.419
class B	2 (0.8)	2 (1.8)	0 (0.0)	
Operative margin (> 1 cm)				
Absent	168 (70.6)	78 (70.2)	90 (70.8)	0.906
Present	70 (29.4)	33 (29.8)	37 (29.2)	
Edmondson-Steiner Grades				
I-II	23 (9.7)	6 (5.4)	17 (13.4)	0.038
III-IV	215 (90.3)	105 (94.6)	110 (86.6)	
Macrovascular invasion				
Absent	215 (90.3)	97 (87.4)	118 (92.9)	0.150
Present	23 (9.7)	14 (12.6)	9 (7.1)	
Microvascular invasion				
Absent	184 (77.3)	83 (74.8)	101 (79.5)	0.473
Present	54 (22.7)	28 (25.2)	26 (20.5)	
Lymph node invasion				
Absent	232 (97.5)	110 (99.1)	122 (96.1)	0.136
Present	6 (2.5)	1 (0.9)	5 (3.9)	
Tumor number				
Single	218 (91.6)	102 (91.9)	116 (91.3)	0.878
Multiple	20 (8.4)	9 (8.1)	11 (8.7)	
Tumor size (cm)	4.8 ± 2.9	4.8 ± 2.6	4.8 ± 3.1	0.912
Tumor size				
< 5 cm	145 (60.9)	61 (55.0)	84 (66.1)	0.078
≥ 5 cm	93 (39.1)	50 (45.0)	43 (33.9)	

TNM stage				
I-II	201 (84.5)	93 (83.8)	108 (85.0)	0.791
III-IV	37 (15.5)	18 (16.2)	19 (15.0)	
BCLC stage				
0-A	203 (85.3)	94 (84.7)	109 (85.8)	0.804
B-C	35 (14.7)	17 (15.3)	18 (14.2)	
Recurrence				
Absent	168 (70.6)	89 (80.2)	79 (62.2)	0.002
Present	70 (29.4)	22 (19.8)	48 (37.8)	
Recurrence time	4.6 ± 3.1	5.5 ± 3.2	3.8 ± 2.8	0.000
Mortality				
Absent	174 (73.1)	88 (79.3)	86 (67.7)	0.045
Present	64 (26.9)	23 (20.7)	41 (32.3)	
Follow up time	5.5 ± 3.1	6.2 ± 3.1	4.9 ± 3.0	0.003

Data shown as mean ± standard deviation or number (%). BMI: Body mass index; HTN: Hypertension; DM: Diabetes Mellitus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; AFP: Alpha-fetoprotein; ICG: Indocyanine green; BCLC stage: Barcelona clinic liver cancer.

5- and 10-year cumulative HCC recurrence rates were 9.7%, 34.7%, 48%, and 62.7%, respectively. By contrast, those with genotype GG had 1-, 3-, 5- and 10-year cumulative HCC recurrence rates of 3.1%, 13%, 19.3%, and 25.7%, respectively (**Figure 2**).

Predictive factors associated with mortality in patients with HCC who underwent hepatectomy

During the mean follow-up time of 5.5 years, 64 patients died. The cumulative mortality rates at 1, 3, 5, and 10 years after resection were 3.6%, 13.0%, 23.8%, and 37.7%, respectively (**Figure 3A**). The univariate analysis revealed that the following factors were significantly correlated with increased mortality: age ≥ 65 years, ALDH2 rs671 genotype GA/AA, AST ≥ 40 IU/L, TNM stage III-IV, platelet count < 100 K, multiple tumor number, Barcelona clinic liver cancer (BCLC) stage B-C, and HCC recurrence (**Table 3**). The multivariate Cox regression analysis revealed that patients with ALDH2 rs671 genotype GA/AA had the highest mortality rate (HR: 2.02, 95% CI: 1.17-3.49, P = 0.012), followed by those with age ≥ 65 years (HR: 1.67, 95% CI: 1.01-2.78, P = 0.048) (**Table 3**).

Patients with ALDH2 rs671 genotype GA/AA had a significantly higher mortality rate than those with ALDH2 rs671 genotype GG. For those with ALDH2 rs671 genotype GA/AA, the 1-, 3-, 5- and 10-year mortality rates were 3.2%, 17.9%, 29.8%, and 46.2%, respectively. In contrast, patients with genotype GG had 1-, 3-, 5- and 10-year mortality rates of 1.8%, 7.5%, 7.3%, and 28.7%, respectively (**Figure 3B**). In addition, patients with age ≥ 65 years had a significantly higher mortality rate than those with age < 65 years. For those aged ≥ 65 years, the 1-, 3-, 5- and 10-year mortality rates were 6.2%, 17.2%, 18.2% and 47.9%, respectively. In contrast, those aged < 65 years had 1-, 3-, 5- and 10-year mortality rates of 0%, 10%, 20.7%, and 31%, respectively (**Figure 3C**).

Discussion

This study, involving 238 patients with ALD-related HCC who underwent curative resection, aimed to identify predictive factors for HCC recurrence and mortality. Notably, the ALDH2 rs671 genotype GA/AA exhibited a remarkable correlation with higher rates of HCC recurrence and mortality relative to ALDH2 rs671 genotype GG. In addition, the multivariable Cox regression analysis revealed the significance of the ALDH2 rs671 genotype GA/AA (HR: 2.66, P = 0.000) and AST ≥ 40 IU/L (HR: 1.93, P = 0.009) as risk factors for HCC recurrence. Furthermore, the study revealed risk factors for mortality, with ALDH2 rs671 genotype GA/AA (HR: 2.02, P = 0.012) and age ≥ 65 years (HR: 1.67, P = 0.048) emerging as significant risk factors. These findings suggest that the ALDH2 rs671 genotype may serve as valuable predictors of HCC recurrence and mortality in ALD-related

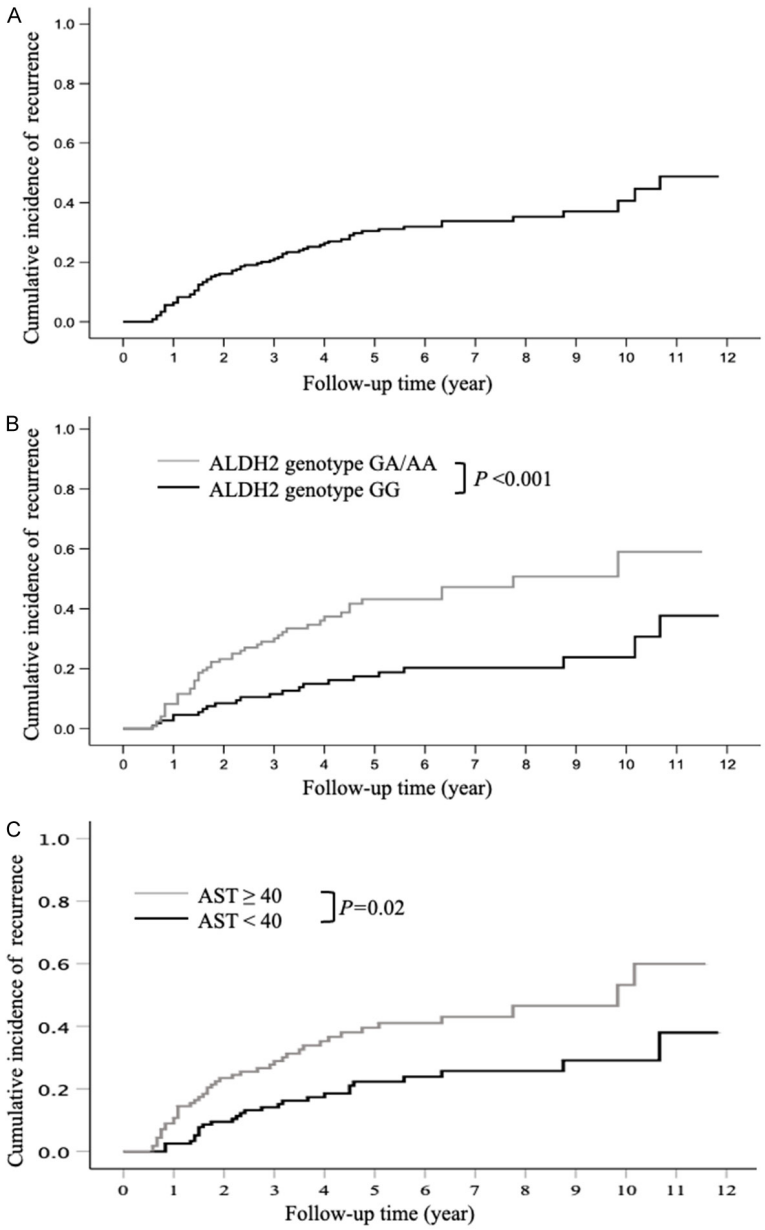

Figure 1. The cumulative incidences of hepatocellular carcinoma recurrence after surgical resection. The cumulative incidences of HCC recurrence in all patients (A). The cumulative incidences of HCC recurrence according to ALDH2 rs671 polymorphism. The pateints with GA/AA genotypes were significantly correlated with increased incidences of HCC recurrence compared with those with GG genotype (B). The patients with AST ≥ 40 IU/L were significantly correlated with increased incidences of HCC recurrence compared with those with AST < 40 IU/L (C).

HCC after hepatectomy. To the best of our knowledge, this study is the first to highlight the significant association between ALDH2 rs671 polymorphisms and HCC recurrence and mortality in ALD-related HCC after hepatectomy.

Several studies have revealed that ALDH2 polymorphism impacts HCC occurrence in alcoholic liver disease [16, 18]. However, some studies have reported that ALDH2 rs671 polymorphism is not correlated with HCC development [21, 24]. Our previous study revealed that ALDH2 rs671 polymorphism, along with heavy alcohol consumption, remarkably affects the development of HCC and mortality in patients with alcohol-related cirrhosis [6]. The ALDH2*2 allele variant is a single point mutation (G to A) that results in the inactivation of ALDH2 enzyme activity in humans, causing deficiency. Notably, the ALDH2 rs671 genotype GG effectively metabolizes alcohol and is thus less likely to cause the accumulation of carcinogenic acetaldehyde [6, 25]. Our study reveals that the ALDH2 rs671 genotype GA/AA increased the risk of HCC recurrence and mortality relative to ALDH2 rs671 genotype GG in ALD-related HCC following hepatectomy. Taken together, alcoholism with ALDH2 rs671 genotype GA/AA increased the incidence of HCC development and promoted the risk of HCC recurrence after hepatectomy. Therefore, clinicians should determine the presence of ALDH2 rs671 polymorphisms to predict HCC recurrence in ALD-related HCC after resection. Previous studies have reported that the ALDH2 rs671 genotype GA/AA is not correlated with recurrence-free survival in viral- and ALD-related HCC after resection. In that study, 96% of patients had HBV or HCV infection and only 25% had alcoholism [25]. However, the present study indicates that the ALDH2 rs671 genotype GA/AA is significantly associated with higher

Table 2. Univariate and multivariate analyses of factors associated with tumor recurrence of hepatocellular carcinoma patients who underwent curative resection

Characteristics	Univariate analyses		Multivariate analyses	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender, Female vs. Male	1.43 (0.73-2.80)	0.292		
Age (years), < 65 vs. ≥ 65	0.78 (0.48-1.29)	0.337		
Body Mass Index, (kg/m^2)	0.94 (0.59-1.50)	0.796		
Diabetes Mellitus, Absent vs. Presence	0.90 (0.51-1.6)	0.715		
Hypertension, Absent vs. Presence	0.86 (0.53-1.4)	0.863		
Smoking, Absent vs. Presence	1.02 (0.60-1.72)	0.946		
AST (IU/L), < 40 vs. ≥ 40	2.08 (1.29-3.37)	0.003	1.93 (1.18-3.17)	0.009
ALT (IU/L), < 40 vs. ≥ 40	1.39 (0.87-2.23)	0.167		
Total Bilirubin (mg/dl), < 1.2 vs. ≥ 1.2	1.10 (0.62-1.95)	0.740		
Albumin (g/dl), < 3.5 vs. ≥ 3.5	0.46 (1.7-1.27)	0.133		
Platelet count (×10^3/ml), < 100K vs. ≥ 100K	0.98 (0.36-2.68)	0.960		
INR, < 1.0 vs. ≥ 1.0	1.16 (0.65-2.05)	0.620		
AFP (ng/dl) < 200 vs. ≥ 200	0.95 (0.52-1.74)	0.877		
Liver cirrhosis, Absent vs. Presence	1.34 (0.81-2.21)	0.250		
Child-Pugh class, A vs. B	0.05 (0.01-131)	0.636		
Operative margin (cm), < 1.0 vs. ≥ 1.0	1.39 (0.84-2.31)	0.199		
Tumor number, Single vs. Multiple	1.47 (0.70-3.08)	0.306		
Tumor size (cm), < 5 vs. ≥ 5	1.06 (0.66-1.72)	0.806		
Edmondson-Steiner Grade I-II vs. III-IV				
Macrovascular invasion, Absent vs. Presence	1.12 (0.51-2.45)	0.778		
Microvascular invasion, Absent vs. Presence	1.05 (0.58-1.89)	0.868		
Lymph node invasion, Absent vs. Presence	1.69 (0.41-6.92)	0.464		
TNM stage, I-II vs. III-IV	1.89 (1.08-3.40)	0.026	1.62 (0.92-2.87)	0.098
BCLC stage, 0-A vs. B-C	1.72 (0.96-3.09)	0.070		
ALDH2 rs671 genotype, GG vs. GA/AA	2.63 (1.58-4.38)	0.000	2.66 (1.59-4.43)	0.000

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; AFP: Alpha-fetoprotein; BCLC stage: Barcelona clinic liver cancer.

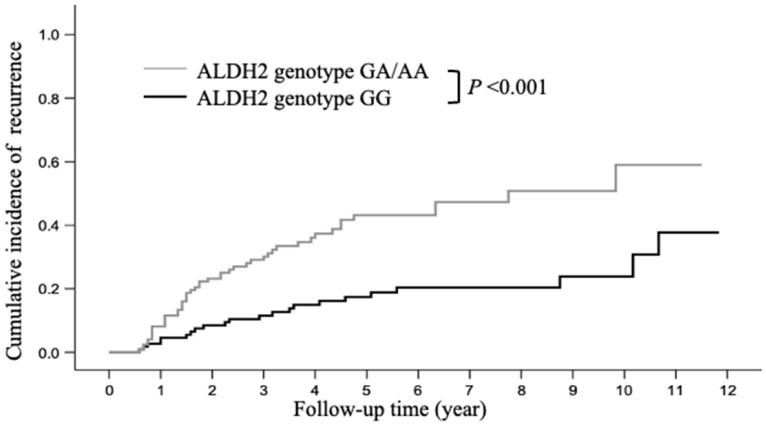

Figure 2. The cumulative incidences of hepatocellular carcinoma recurrence after surgical resection after competing risk analysis. The patients with GA/AA genotypes were significantly correlated with increased incidences of HCC recurrence compared with those with GG genotype after competing risk analysis.

HCC recurrence relative to the ALDH2 rs671 genotype GG in ALD-related HCC after hepatectomy. Notably, 100% of patients drank more than 20 g of ethanol each day for at least 5 years, and we excluded those with HBV or HCV infection. The findings in our study differ from those of a previous study that suggested no correlation between the ALDH2 rs671 genotype GA/AA and recurrence-free survival [25]. This disparity may be attributed to the fact that the previous study primarily included patients with viral-

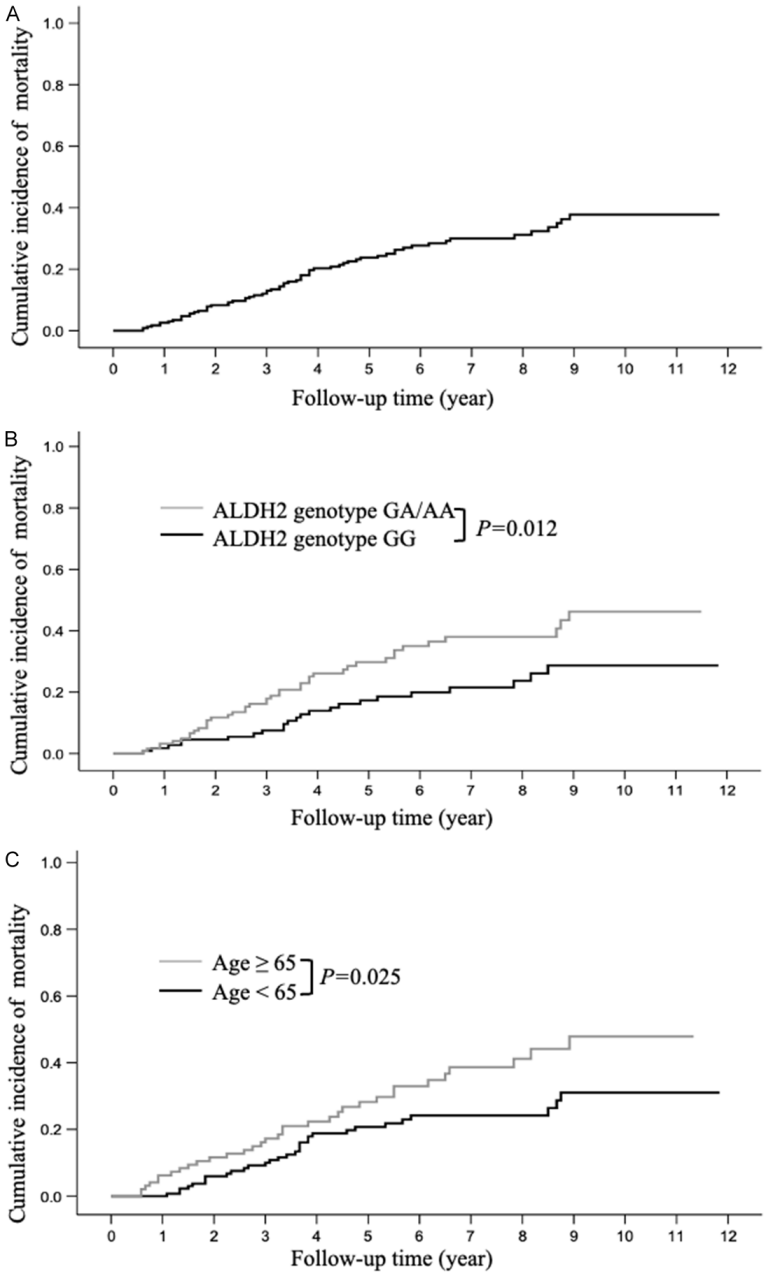

Figure 3. The cumulative incidences of mortality after surgical resection. The cumulative incidences of mortality in all patients (A). The cumulative incidences of mortality according to ALDH2 rs671 polymorphism. The pateints with GA/AA genotype were significantly correlated with increased incidences of mortality compared with those with GG genotype (B). The patients with age ≥ 65 years were significantly correlated with increased incidences of mortality compared with those with age < 65 years (C).

and ALD-related HCC, whereas our study included only patients who had alcoholism without viral hepatitis. Moreover, ALDH2 polymorphism is inconsistent with HCC development in viral-related HCC [6, 15, 17, 21-23]. However, alcoholism with ALDH2 polymorphism increased the incidence of HCC and the risk of HCC recurrence after hepatectomy.

Our previous study revealed that the ALDH2 rs671 genotype GA/AA is significantly associated with higher mortality relative to the ALDH2 rs671 genotype GG in ALD-related cirrhosis [6]. In our current study, we observed a similar trend in ALD-related HCC after hepatectomy. However, previous studies have revealed that the ALDH2 rs671 genotype GA/AA is highly correlated with longer overall survival in viral- and ALD-related HCC after surgical resection. In that study, 96% of patients had HBV or HCV infection and only 25% had alcoholism [25]. Our study shows that 100% of patients had alcohol drinking more than 20 g of ethanol each day for at least 5 years, and we excluded those with HBV or HCV infection. Our results differ from those of a previous study that suggested a significant correlation between the ALDH2 rs671 genotype GA/AA and overall survival [25]. This disparity may be because the previous study included only patients with viral-related HCC but our study included only patients who had alcoholism without viral hepatitis. In addition, alcoholism with ALDH2 polymorphism increased the incidence of mortality and promoted the risk of mortality after hepatectomy.

The ALDH2 enzyme plays a critical role in detoxifying acetaldehyde, a highly reactive and toxic byproduct of ethanol metabolism. In individuals with the rs671 GA/AA genotype, ALDH2 enzymatic activity is significantly reduced or nearly absent. First, this result causes acetaldehyde

Table 3. Univariate and multivariate analyses of factors associated with mortality of hepatocellular carcinoma patients who underwent curative resection

Characteristics	Univariate analyses		Multivariate analyses	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Gender, Female vs. Male	1.38 (0.68-2.79)	0.373		
Age (years), < 65 vs. ≥ 65	1.68 (1.03-2.75)	0.037	1.67 (1.01-2.78)	0.048
Body Mass Index (kg/m^2)	0.63 (0.38-1.04)	0.073		
Diabetes Mellitus, Absent vs. Presence	1.30 (0.76-2.25)	0.342		
Hypertension, Absent vs. Presence	1.45 (0.89-2.36)	0.139		
Smoking, Absent vs. Presence	1.17 (0.69-1.93)	0.551		
AST (IU/L), < 40 vs. ≥ 40	1.84 (1.12-3.05)	0.017	1.53 (0.88-2.66)	0.135
ALT (IU/L), < 40 vs. ≥ 40	1.01 (0.62-1.67)	0.959		
Total Bilirubin (mg/dl), < 1.2 vs. ≥ 1.2	1.36 (0.77-2.39)	0.291		
Albumin (g/dl), < 3.5 vs. ≥ 3.5	0.40 (0.14-1.09)	0.073		
Platelet count (×10^3/ml), < 100K vs. ≥ 100K	0.40 (0.19-0.84)	0.016	0.53 (0.25-1.14)	0.103
Prothrombin time, < 1.0 vs. ≥ 1.0	1.78 (0.90-3.5)	0.095		
AFP (ng/dl) < 200 vs. ≥ 200	1.17 (0.65-2.12)	0.596		
Liver cirrhosis, Absent vs. Presence	1.01 (0.58-1.75)	0.982		
Child-Pugh class, A vs. B	3.45 (0.47-25.2)	0.223		
Operative margin (cm), < 1.0 vs. ≥ 1.0	1.01 (0.59-1.74)	0.971		
Tumor number, Single vs. Multiple	2.29 (1.19-4.38)	0.013	1.70 (0.78-3.69)	0.179
Tumor size (cm), < 5 vs. ≥ 5	1.56 (0.96-2.55)	0.076		
Edmondson-Steiner Grade I-II vs. III-IV				
Macrovascular invasion, Absent vs. Presence	2.48 (1.35-4.57)	0.003	1.70 (0.81-3.56)	0.159
Microvascular invasion, Absent vs. Presence	1.73 (0.99-2.99)	0.052		
TNM stage, I-II vs. III-IV	2.99 (1.76-5.08)	0.000	1.89 (0.87-4.13)	0.108
BCLC stage, 0-A vs. B-C	2.56 (1.48-4.42)	0.001	0.94 (0.42-2.12)	0.881
Recurrence, Absent vs. Presence	1.66 (1.01-2.73)	0.044	1.30 (0.77-2.19)	0.326
ALDH2 rs671 genotype, GG vs. GA/AA	1.90 (1.14-3.16)	0.014	2.02 (1.17-3.49)	0.012

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; INR: International normalized ratio; AFP: Alpha-fetoprotein; BCLC stage: Barcelona clinic liver cancer.

accumulation. When alcohol is consumed, it is first metabolized by alcohol dehydrogenase into acetaldehyde. Normally, ALDH2 converts acetaldehyde into the less harmful acetate. However, in rs671 GA/AA carriers, impaired ALDH2 activity leads to sustained elevated levels of acetaldehyde in hepatic tissues [15]. Second, this result causes impaired DNA repair. ALDH2 deficiency is associated with decreased capacity for DNA repair mechanisms, enhancing the mutational burden in hepatocytes [15]. Third, this result causes genotoxic effects. Acetaldehyde is a potent DNA-damaging agent. It forms DNA adducts, which can result in replication errors and mutagenesis. These genetic alterations can drive tumor initiation and progression [27]. Fourth, this result causes epigenetic dysregulation. Acetaldehyde exposure has also been shown to alter DNA methylation patterns and histone modifications, further promoting oncogenic transformation and tumor aggressiveness. Fifth, this result causes synergism with Liver Injury and cirrhosis. In the background of alcohol-induced liver damage or metabolic dysfunction-associated steatotic liver disease, ALDH2 dysfunction exacerbates hepatic inflammation, fibrosis, and cirrhosis, creating a high-risk microenvironment for HCC recurrence after resection. Given this multifaceted role, ALDH2 rs671 polymorphism not only predisposes individuals to initial tumorigenesis but also creates a pro-recurrence molecular milieu following hepatic resection. This makes it a clinically relevant genetic marker for risk stratification and potentially a therapeutic target for personalized interventions.

This study has several limitations. First, it is a single-center design and retrospective nature, which might impact generalizability. Second, the relatively low number of patients may lead to type I error, which influences the results of univariate analyses. The limited number of certain events also made it difficult to perform robust multivariate analyses.

Conclusion

The ALDH2 rs671 genotype GA/AA is significantly associated with higher HCC recurrence and mortality relative to the ALDH2 rs671 genotype GG in ALD-related HCC after hepatectomy. This study is the first to demonstrate that the ALDH2 rs671 genotype GA/AA is significantly associated with unfavorable clinical prognosis in ALD-related HCC after hepatectomy. Therefore, clinicians should assess ALDH2 rs671 polymorphism to determine the risk of HCC recurrence and mortality in ALD-related HCC after surgical resection.

Acknowledgements

This study was partly supported by grants from the Ministry of Science and Technology (MOST 111-2314-B-214-008), E-Da Hospital (EDAHI114003, EDAHP114033, EDAHI113002, EDAHP113013, EDAHP112026, EDAHP112006, EDAHP109057, EDAHP108037, EDAHP107041, EDAHP104047, EDAHS111040, EDAHP111025, EDAHP110036, and EDAHP109044), National Health Research Institutes (NHRI-109BCC0-MF202016-03), CMUHCH-DMR-113-029, and CMUHCH-DMR-114-029, and the Liver Disease Prevention and Treatment Research Foundation, Taiwan.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Chih-Wen Lin, Division of Gastroenterology and Hepatology, Department of Internal Medicine, E-Da Hospital, I-Shou University, No. 1, Yida Road, Jiaosu Village, Yanchao District, Kaohsiung 82445, Taiwan. Tel: +886-7-6150011 Ext. 2981; Fax: +886-7-6150940; E-mail: lincw66@gmail.com; Dr. Wen-Chao Ho, Department of Public Health, China Medical University, Taichung, Taiwan. E-mail: whocmu@gmail.com; Dr. Li-Wei Chou, Department of Physical Medicine and Rehabilitation, China Medical University Hospital, Taichung, Taiwan. E-mail: chouliwe@gmail.com; Dr. Yaw-Sen Chen, Department of Surgery, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. E-mail: ed102489@edah.org.tw; Dr. Yu-Wei Huang, Department of Anesthesiology, Emergency and Critical Care Center, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. E-mail: yw.huang0211@gmail.com; Dr. Chen-Ti Wang, Division of Endocrinology, Department of Metabolism, E-Da Hospital, I-Shou University, Kaohsiung, Taiwan. E-mail: chentiwang@gmail.com

References

[1] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J and Finn RS. Hepatocellular carcinoma. Nat Rev Dis Primers 2021; 7: 6.

[2] McGlynn KA, Petrick JL and El-Serag HB. Epidemiology of hepatocellular carcinoma. Hepatology 2021; 73 Suppl 1: 4-13.

[3] Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR and Heimbach JK. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. Hepatology 2018; 68: 723-750.

[4] European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2018; 69: 182-236.

[5] Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W, Payawal DA, Ohki T, Ogasawara S, Chen PJ, Lesmana CRA, Lesmana LA, Gani RA, Obi S, Dokmeci AK and Sarin SK. Asia-Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int 2017; 11: 317-370.

[6] Tsai MC, Yang SS, Lin CC, Wang WL, Hsu YC, Chen YS, Hu JT, Lin JY, Yu ML and Lin CW. Association of heavy alcohol intake and ALDH2 rs671 polymorphism with hepatocellular carcinoma and mortality in patients with hepatitis B virus-related cirrhosis. JAMA Netw Open 2022; 5: e2223511.

[7] Lin CW, Lin CC, Mo LR, Chang CY, Perng DS, Hsu CC, Lo GH, Chen YS, Yen YC, Hu JT, Yu ML, Lee PH, Lin JT and Yang SS. Heavy alcohol consumption increases the incidence of hepatocellular carcinoma in hepatitis B virus-related cirrhosis. J Hepatol 2013; 58: 730-735.

[8] Roayaie S, Obeidat K, Sposito C, Mariani L, Bhoori S, Pellegrinelli A, Labow D, Llovet JM, Schwartz M and Mazzaferro V. Resection of hepatocellular cancer ≤2 cm: results from two Western centers. Hepatology 2013; 57: 1426-1435.

[9] Forner A, Vilana R, Ayuso C, Bianchi L, Sole M, Ayuso JR, Boix L, Sala M, Varela M, Llovet JM, Bru C and Bruix J. Diagnosis of hepatic nodules 20 mm or smaller in cirrhosis: prospective validation of the noninvasive diagnostic criteria for hepatocellular carcinoma. Hepatology 2008; 47: 97-104.

[10] Tabrizian P, Jibara G, Shrager B, Schwartz M and Roayaie S. Recurrence of hepatocellular cancer after resection: patterns, treatments, and prognosis. Ann Surg 2015; 261: 947-955.

[11] Lin CW, Chen YS, Lin CC, Lee PH, Lo GH, Hsu CC, Hsieh PM, Koh KW, Chou TC, Dai CY, Huang JF, Chuang WL, Chen YL and Yu ML. Autophagy-related gene LC3 expression in tumor and liver microenvironments significantly predicts recurrence of hepatocellular carcinoma after surgical resection. Clin Transl Gastroenterol 2018; 9: 166.

[12] Lin CW, Chen YS, Lin CC, Lee PH, Lo GH, Hsu CC, Hsieh PM, Koh KW, Bair MJ, Dai CY, Huang JF, Chuang WL, Chen YL and Yu ML. Significant predictors of overall survival in patients with hepatocellular carcinoma after surgical resection. PLoS One 2018; 13: e0202650.

[13] Hsu CC, Hsieh PM, Chen YS, Lo GH, Lin HY, Dai CY, Huang JF, Chuang WL, Chen YL, Yu ML and Lin CW. Axl and autophagy LC3 expression in tumors is strongly associated with clinical prognosis of hepatocellular carcinoma patients after curative resection. Cancer Med 2019; 8: 3453-3463.

[14] Toyoda H, Kumada T, Tada T, Ito T, Maeda A, Kaneoka Y, Kagebayashi C and Satomura S. Changes in highly sensitive alpha-fetoprotein for the prediction of the outcome in patients with hepatocellular carcinoma after hepatectomy. Cancer Med 2014; 3: 643-651.

[15] Seo W, Gao Y, He Y, Sun J, Xu H, Feng D, Park SH, Cho YE, Guillot A, Ren T, Wu R, Wang J, Kim SJ, Hwang S, Liangpunsakul S, Yang Y, Niu J and Gao B. ALDH2 deficiency promotes alcohol-associated liver cancer by activating oncogenic pathways via oxidized DNA-enriched extracellular vesicles. J Hepatol 2019; 71: 1000-1011.

[16] Abe H, Aida Y, Seki N, Sugita T, Tomita Y, Nagano T, Itagaki M, Sutoh S, Nagatsuma K, Itoh K, Matsuura T and Aizawa Y. Aldehyde dehydrogenase 2 polymorphism for development to hepatocellular carcinoma in East Asian alcoholic liver cirrhosis. J Gastroenterol Hepatol 2015; 30: 1376-1383.

[17] Liu J, Yang HI, Lee MH, Jen CL, Hu HH, Lu SN, Wang LY, You SL, Huang YT and Chen CJ. Alcohol drinking mediates the association between polymorphisms of ADH1B and ALDH2 and hepatitis B-related hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev 2016; 25: 693-699.

[18] Sakamoto T, Hara M, Higaki Y, Ichiba M, Horita M, Mizuta T, Eguchi Y, Yasutake T, Ozaki I, Yamamoto K, Onohara S, Kawazoe S, Shigematsu H, Koizumi S and Tanaka K. Influence of alcohol consumption and gene polymorphisms of ADH2 and ALDH2 on hepatocellular carcinoma in a Japanese population. Int J Cancer 2006; 118: 1501-1507.

[19] Ye X, Wang X, Shang L, Zhu G, Su H, Han C, Qin W, Li G and Peng T. Genetic variants of ALDH2-rs671 and CYP2E1-rs2031920 contributed to risk of hepatocellular carcinoma susceptibility in a Chinese population. Cancer Manag Res 2018; 10: 1037-1050.

[20] Tomoda T, Nouso K, Sakai A, Ouchida M, Kobayashi S, Miyahara K, Onishi H, Nakamura S, Yamamoto K and Shimizu K. Genetic risk of hepatocellular carcinoma in patients with hepatitis C virus: a case control study. J Gastroenterol Hepatol 2012; 27: 797-804.

[21] Chen J, Pan W, Chen Y, Wen L, Tu J and Liu K. Relationship of ALDH2 rs671 and CYP2E1 rs2031920 with hepatocellular carcinoma susceptibility in East Asians: a meta-analysis. World J Surg Oncol 2020; 18: 21.

[22] Koide T, Ohno T, Huang XE, Iijima Y, Sugihara K, Mizokami M, Xiang J and Tokudome S. HBV/HCV infection, alcohol, tobacco and genetic polymorphisms for hepatocellular carcinoma in Nagoya, Japan. Asian Pac J Cancer Prev 2000; 1: 237-243.

[23] Yu SZ, Huang XE, Koide T, Cheng G, Chen GC, Harada K, Ueno Y, Sueoka E, Oda H, Tashiro F, Mizokami M, Ohno T, Xiang J and Tokudome S. Hepatitis B and C viruses infection, lifestyle and genetic polymorphisms as risk factors for hepatocellular carcinoma in Haimen, China. Jpn J Cancer Res 2002; 93: 1287-1292.

[24] Takeshita T, Yang X, Inoue Y, Sato S and Morimoto K. Relationship between alcohol drinking, ADH2 and ALDH2 genotypes, and risk for hepatocellular carcinoma in Japanese. Cancer Lett 2000; 149: 69-76.

[25] Huang PH, Hu CC, Chien CH, Chen LW, Chien RN, Lin YS, Chao M, Lin CL and Yeh CT. The defective allele of aldehyde dehydrogenase 2 gene is associated with favorable postoperative prognosis in hepatocellular carcinoma. J Cancer 2019; 10: 5735-5743.

[26] Bruix J and Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. Hepatology 2005; 42: 1208-1236.

[27] Yamazaki K, Iguchi T, Kanoh Y, Takayasu K, Ngo TTT, Onuki A, Kawaji H, Oshima S, Kanda T, Masai H and Sasanuma H. Homologous recombination contributes to the repair of acetaldehyde-induced DNA damage. Cell Cycle 2024; 23: 369-384.