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ORIGINAL RESEARCH

ALDH2 Polymorphism rs671 *1/*2 Genotype is a Risk Factor for the Development of Alcoholic Liver Cirrhosis in Hakka Alcoholics

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Background: Alcoholics are prone to alcoholic cirrhosis (ALC). Aldehyde dehydrogenase 2 (ALDH2) is involved in alcohol metabolism. Herein, the relationship between *ALDH2* genotypes and ALC was analyzed among Hakka alcoholics in southern China.
Methods: A total of 213 alcoholics and 214 non-alcoholics were included in the study. The *ALDH2* gene rs671 polymorphism was analyzed, life history, disease history, and auxiliary examination results of these participants were collected.
Results: The alcoholics had higher level of total serum protein, and serum globulin, lower level of serum albumin, serum albumin/globulin ratio, serum prealbumin, neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR) than non-alcoholics. In the 213 alcoholics, 180 developed ALC. There were 206 non-ALC persons in the 214 non-alcoholics. The proportion of the *ALDH2* rs671 G/G homozygous (*1/*1) was significantly lower in ALC patients (83.3%) than that of other groups (100.0% in non-ALC in alcoholics, 95.6% in non-ALC in non-alcoholics), while the proportion of the G/A heterozygous (*1/*2) was significantly higher in ALC patients (16.7%) than that of other groups (0% in non-ALC in alcoholics, 4.4% in non-ALC in non-alcoholics). Logistic regression analysis indicated that participants with low level of NLR (adjusted OR 5.543, 95% CI 2.964–10.368, *P*<0.001), LMR (adjusted OR 9.256, 95% CI 4.740–18.076, *P*<0.001), and PLR (adjusted OR 6.047, 95% CI 3.372–10.845, *P*<0.001), and *ALDH2* G/A genotype (adjusted OR 6.323, 95% CI 2.477–16.140, *P*<0.001) had a significantly higher risk of ALC.
Conclusion: *ALDH2* polymorphism rs671 *1/*2 genotype is a potential risk factor for the development of ALC among Hakka alcoholics.
Keywords: ALDH2, alcoholics, alcoholic liver cirrhosis, gene polymorphism, Hakka

Introduction

Alcohol consumption is linked to about hundreds of diseases and injury-related health conditions. Alcohol abuse is an important public health problem and causes a relatively large social burden.[1] Alcoholic liver disease (ALD) is one of the main causes of chronic liver injury. It is a group of diseases caused by alcoholic steatohepatitis, alcoholic hepatitis, liver fibrosis, cirrhosis, and eventually liver cancer due to long-term heavy drinking.[2,3] The prevalence rate of ALD is about 4.5% in China and is estimated to affect at least 62 million people.[4] ALD has become one of the major health problems in the world today.[5] Alcoholic liver cirrhosis (ALC) is the most serious ALD due to long-term heavy drinking, without timely control and effective treatment of the disease.[6] Among heavy drinkers, 10–15% of patients may progress to alcoholic hepatitis, advanced fibrosis, and alcoholic cirrhosis.[7] It is not entirely clear why ALD occurs only in a subset of

4067

excessive alcohol drinkers. In addition to alcohol consumption, it is closely related to environmental factors, lifestyle habits and genetic factors.[8,9]

Alcohol metabolism is one of the biological determinants that can significantly influence the drinking behavior and the development of alcoholic disease. Most ethanol elimination occurs by oxidation to acetaldehyde and acetate, catalyzed principally by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Aldehyde dehydrogenase 2 (ALDH2) is one of the important enzymes in alcohol metabolism in human body, is an important rate-limiting enzyme in acetaldehyde metabolism.[10] When the human body ingests excessive ethanol, the high concentration of ethanol in the blood cannot be completely metabolized, and more acetaldehyde will accumulate in the body. The activity of ALDH2 directly affects the level of acetaldehyde in vivo. Studies have found that acetaldehyde has cytotoxic and carcinogenic effects, so excessive accumulation of acetaldehyde in the body may promote the occurrence of alcoholic liver disease and even liver cancer.[11,12]

ALDH2 activity level in vivo is closely related to the *ALDH2* gene polymorphisms.[13] The human *ALDH2* gene is located on chromosome 12q24.2 and contains 13 exons.[14] At present, some single nucleotide polymorphisms (SNPs) have been identified in this gene, and the most important is Glu504Lys polymorphism (SNP rs671, G>A, GAA>AAA, with the G corresponding to *1 allele, and A corresponding to *2 allele). Glu504Lys polymorphism can lead to the decreased activity of ALDH2.[15] The enzyme activity is close to 0% and 17–38% of normal activity among persons carrying ALDH2 Lys/Lys and Glu/Lys, respectively. A sharp decrease in the activity of this enzyme leads to the accumulation of acetaldehyde in the circulation.[16]

Some studies have reported that *ALDH2* polymorphism may be related to ALC susceptibility.[10,17–19] However, other studies have shown that *ALDH2* polymorphism is not an independent factor influencing ALC.[20–23] Genomic studies have showed that genetic variants often vary in frequency across ancestral populations. Genetic differences in race or ethnicity may be related to the cause, severity, or course of disease.[24] The Hakka is a Han ethnic group with a unique genetic background formed by the Hakka ancestors from the Han nationality in central China, who migrated southward for many times and fused with the ancient Yue residents in Guangdong, Fujian and Jiangxi.[25] Meizhou is a city located in the northeast of Guangdong Province, is overwhelmingly populated by Hakka people. The purpose of this study was to study the relationship between *ALDH2* genotypes and ALC among Hakka alcoholics.

Materials and Methods
Subjects

A total of 427 individuals were recruited from the inpatients of Meizhou People's Hospital (Huangtang Hospital), from January 2016 to August 2020. The subjects consisted of 213 alcoholics and 214 individuals with non-chronic alcohol exposure (non-alcoholics) as controls. Patients with severe liver and kidney insufficiency, cardiovascular and cerebro-vascular diseases, and malignant tumors were excluded. Information was recorded including age, sex, history of smoking, and risk factors for ALC. All control subjects were randomly selected from the Physical Examination Center of the Meizhou People's Hospital during the same period. This case-control study was approved by the Human Ethics Committees of Meizhou People's Hospital. Informed consent was obtained from the patients or their families, and participants' privacy was carefully protected.

ALC was diagnosed by the clinician considering the etiology, history, clinical manifestations, complications, examinations, imaging, and histology.[26,27]

Serum Liver Enzymes and Serum Lipid Measurements

Approximately 3 mL of venous blood from each subject was taken into tube containing no anticoagulant, and serum was isolated and tested promptly. Serum samples were evaluated using the Olympus AU5400 system (Olympus Corporation, Tokyo, Japan) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bile acid (TBA), total bilirubin (Tbil), direct bilirubin (Dbil), total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), apolipoprotein B (Apo-B) and apolipoprotein A1 (Apo-A1). ALT, AST, ALP, and GGT analyses were carried out with

the kinetic method,[28] TBA with circulating enzymatic method,[29] Tbil and Dbil with chemical oxidation method,[30] respectively. TC, TG, LDL-C, HDL-C, Apo-A1/Apo-B analyses were carried out using cholesterol esterase/peroxidase (CHOD/PAP) enzymatic method,[31] Glycerophosphate oxidase/peroxidase (GPO-PAP) enzymatic method,[32] direct sur-factant removal method,[33] direct immunoinhibition method,[34] and immunoturbidimetry method,[35] respectively. Total serum protein concentration was determined by biuret method and serum albumin concentration by bromocresol green method.

Routine Blood Analysis

The blood samples were collected at admission and 2–3 days before treatment. 2 mL blood sample was taken via venipuncture of an antecubital vein from each subject and collected in tube with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Blood cells correlative indices were detected by Sysmex XE-2100 blood analyzer (Sysmex Corporation, Japan) according to the standard operating procedures (SOP).

Blood routine results were calculated the relevant index according to the following formula: neutrophil-to-lymphocyte ratio (NLR)=neutrophil count/lymphocyte count, platelet-to-lymphocyte ratio (PLR)=platelet count /lymphocyte count, lymphocyte-to-monocyte ratio (LMR)= lymphocyte count /monocyte count.

DNA Extraction and Genotyping Assay

About 2 mL of venous blood from each subject was stored into tube containing ethylenediaminetetraacetic acid (EDTA), genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini Kit (Qiagen GmbH, North Rhine-Westphalia, Germany). The DNA concentration was measured using a Nanodrop 2000^{TM} Spectrophotometer (ThermoFisher Scientific, Massachusetts, USA). *ALDH2* was genotyped by polymerase chain reaction (PCR)-gene chip method. PCR was performed according to the following protocol: denaturation at 94°C for 5 min; amplification of 35 cycles (94°C for 25 sec, 56°C for 25 sec, and 72°C for 25 sec); final elongation at 72°C for 5 min. The specific hybridization reaction was carried out between the amplification product and the detection probe fixed on the chip, and the color of the specific hybridization signal was made by enzymatic chromogenic reaction (BaiO Technology Co, Ltd., Shanghai, China). Positive control, negative control, and blank control were used for quality control. When the positive control, negative control, and blank control were controlled, the test results of this batch of samples are reliable.

Statistical Analysis

Data analysis was performed using SPSS statistical software version 21.0 (IBM Inc., USA). Student's *t*-test or the Mann–Whitney *U*-test was used for continuous data analysis. The Hardy-Weinberg equilibrium (HWE) of *ALDH2* genotypes in both non-chronic alcohol exposure group and chronic alcohol exposure group were assessed using the chi-square test. Genotype composition ratios and allele frequencies between groups were analyzed by the chi-square test. Logistic regression analysis was applied to assess the interactions between *ALDH2* polymorphisms and various factors in ALC. *P*< 0.05 was considered statistically significant.

Results
Characteristics of Participants

The study included 427 participants, including 213 alcoholics (205 males and 8 females) and 214 individuals with non-alcoholics (207 males and 7 females) as controls. The alcoholics' average age was 54.43±10.69 years, with 54.42±18.09 years in controls. The alcoholics group had higher percentage of smoking history (46.5% vs 17.8%, *P*<0.001), and lower proportion of hypertension (9.4% vs 23.4%, *P*<0.001), higher level of ALT (65.54±75.47 vs 33.78±43.12 U/L, *P*<0.001), AST (139.57±167.37 vs 38.09±26.59 U/L, *P*<0.001), ALP (175.63±126.31 vs 81.90±30.38 U/L, *P*<0.001), GGT (399.11 ±454.47 vs 48.60±83.69 U/L, *P*<0.001), TBA (61.30±69.97 vs 7.14±18.83 μmol/L, *P*<0.001), Tbil (76.20±98.51 vs 21.48 ±40.91 μmol/L, *P*<0.001), Dbil (43.47±64.95 vs 8.37±25.63 μmol/L, *P*<0.001), total serum protein (TP) (65.79±10.93 vs 63.27±7.68 g/L, *P*<0.001), and serum globulin (GLB) (34.17±8.93 vs 25.98±5.77 g/L, *P*<0.001) than non-alcoholics group. It suggests that the liver function of the alcoholic group was relatively poor overall. The alcoholics group had lower level of

HDL-C (1.02±0.59 vs 1.24±0.42 mmol/L, *P*<0.001), Apo-A1 (0.82±0.43 vs 1.03±0.31 g/L, *P*<0.001), serum albumin (ALB) (31.62±7.15 vs 37.29±5.34 g/L, *P*<0.001), serum albumin/globulin ratio (A/G) (0.99±0.36 vs 1.50±0.36, *P*<0.001), serum prealbumin (PAB) (120.19±75.31 vs 186.05±68.62 mg/L, *P*<0.001) than non-alcoholics group. It suggests that the adverse changes in lipid levels were more pronounced in the alcoholics group. The alcoholics group had lower level of NLR (4.93 ±7.23 vs 7.09±5.74, *P*=0.001), LMR (2.50±1.55 vs 3.70±5.25, *P*=0.002), and PLR (129.58±107.58 vs 167.82±123.15, *P*=0.001) than non-alcoholics group. NLR, LMR and PLR reflect the immune response of the body, and the lower the level of these, the higher the degree of liver function injury. There were no statistically significant differences in the ratio of gender (*P*=0.800), percentage of diabetes (*P*=0.575), and the level of TC (*P*=0.441) (Table 1).

Clinical Characteristics of ALC Group, Non-ALC in Alcoholics Group and Non-ALC in Non-Alcoholics Group

In the 213 chronic alcohol exposure patients, 180 (84.5%) patients developed ALC and 33 (15.5%) patients did not. There were 206 (96.3%) non-ALC persons in the 214 non-chronic alcohol exposure persons. Among ALC group, non-ALC in chronic alcohol exposure group and non-ALC in non-chronic alcohol exposure group, there were significant

Table 1 Clinical Characteristics of Alcoholics Group and Non-Alcoholics Group

	Total (n=427)	Non-Alcoholics Group (n=214)	Alcoholics Group (n=213)	*P* values
Age, y	54.42±14.85	54.42±18.09	54.43±10.69	0.528
Gender				
Male, n(%)	412(96.5%)	207(96.7%)	205(96.2%)	0.800
Female, n(%)	15(3.5%)	7(3.3%)	8(3.8%)	
History of smoking, n(%)	137(32.1%)	38(17.8%)	99(46.5%)	<0.001
Hypertension, n(%)	70(16.4%)	50(23.4%)	20(9.4%)	<0.001
Diabetes, n(%)	59(13.8%)	32(15.0%)	27(12.7%)	0.575
ALT, U/L	49.62±63.38	33.78±43.12	65.54±75.47	<0.001
AST, U/L	88.71±129.90	38.09±26.59	139.57±167.37	<0.001
ALP, U/L	128.66±102.97	81.90±30.38	175.63±126.31	<0.001
GGT, U/L	223.44±370.24	48.60±83.69	399.11±454.47	<0.001
TBA, μmol/L	34.16±57.87	7.14±18.83	61.30±69.97	<0.001
Tbil, μmol/L	48.78±80.10	21.48±40.91	76.20±98.51	<0.001
Dbil, μmol/L	25.88±52.31	8.37±25.63	43.47±64.95	<0.001
TG, mmol/L	1.73±2.24	1.60±2.09	1.85±2.38	0.236
TC, mmol/L	4.30±1.61	4.24±1.22	4.36±1.93	0.441
HDL-C, mmol/L	1.13±0.52	1.24±0.42	1.02±0.59	<0.001
LDL-C, mmol/L	2.42±1.03	2.31±0.88	2.52±1.15	0.031
Apo-A1, g/L	0.92±0.39	1.03±0.31	0.82±0.43	<0.001
Apo-B, g/L	0.83±0.38	0.74±0.28	0.93±0.43	<0.001
TP, g/L	64.53±9.51	63.27±7.68	65.79±10.93	0.006
ALB, g/L	34.46±6.91	37.29±5.34	31.62±7.15	<0.001
GLB, g/L	30.07±8.55	25.98±5.77	34.17±8.93	<0.001
A/G	1.25±0.44	1.50±0.36	0.99±0.36	<0.001
PAB, mg/L	153.20±79.14	186.05±68.62	120.19±75.31	<0.001
NLR	6.01±6.61	7.09±5.74	4.93±7.23	0.001
LMR	3.10±3.91	3.70±5.25	2.50±1.55	0.002
PLR	148.74±117.09	167.82±123.15	129.58±107.58	0.001

Notes: Values for age expressed as mean±SD. *P*< 0.05 was considered statistically significant.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBA, total bile acid; Tbil, total bilirubin; Dbil, direct bilirubin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TP, total serum protein; ALB, serum albumin; GLB, serum globulin; A/G, serum albumin/globulin ratio; PAB, serum prealbumin; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio.

differences in percentage of smoking history and hypertension ($P<0.001$), and the levels of ALT, AST, ALP, GGT, TBA, Tbil, Dbil, HDL-C, Apo-A1, Apo-B, total serum protein, ALB, GLB, A/G, PAB, NLR, LMR, PLR (all $P<0.05$) (Table 2). The levels of ALT, AST, ALP, GGT, and TBA in ALC group, non-ALC in chronic alcohol exposure group and non-ALC in non-chronic alcohol exposure group showed a decreasing trend. It suggests that the liver function of ALC group is worse than that of non-ALC in chronic alcohol exposure group and non-ALC in non-chronic alcohol exposure group. The liver function of non-ALC persons in chronic alcohol exposure group was worse than that of non-ALC in non-chronic alcohol exposure group. The levels of GLB in ALC group, non-ALC in chronic alcohol exposure group and non-ALC in non-chronic alcohol exposure group showed a decreasing trend, while the levels of ALB and ALB/GLB (A/G) showed an increasing trend. These indicators reflect the severity of liver lesions in the ALC group.

The Distribution of *ALDH2* rs671 Genotypes and Alleles Among ALC, Non-ALC in Alcoholics, and Non-ALC in Non-Alcoholics Groups

In this study, the percent of *ALDH2* *1/*1, *1/*2, *2/*2 genotype was 90.2%, 9.6% and 0.2% in all subjects, respectively. The genotype distributions in both alcoholics and non-alcoholics group were consistent with Hardy-Weinberg

Table 2 Clinical Characteristics of ALC Group, Non-ALC in Alcoholics Group and Non-ALC in Non-Alcoholics Group

	ALC (n=180)	Non-ALC in Alcoholics Group (n=33)	Non-ALC in Non-Alcoholics Group (n=206)	*P* values
Age, y	53.60±10.03	58.94±13.02	54.23±18.20	0.163
Gender				
Male, n(%)	172(95.6%)	33(100.0%)	199(96.6%)	0.613
Female, n(%)	8(4.4%)	0(0%)	7(3.4%)	
History of smoking, n(%)	73(40.6%)	26(78.8%)	38(18.4%)	<0.001
Hypertension, n(%)	12(6.7%)	8(24.2%)	50(24.3%)	<0.001
Diabetes, n(%)	24(13.3%)	3(9.1%)	31(15.0%)	0.729
ALT, U/L	70.67±80.04	37.58±30.62	31.15±23.17	<0.001
AST, U/L	158.07±175.06	38.67±41.54	37.52±26.53	<0.001
ALP, U/L	187.42±132.70	111.36±46.42	80.41±28.73	<0.001
GGT, U/L	455.90±470.88	89.33±104.88	47.85±85.04	<0.001
TBA, μmol/L	70.75±71.16	9.76±29.42	4.89±9.27	<0.001
Tbil, μmol/L	87.85±102.95	12.66±8.68	17.11±12.30	<0.001
Dbil, μmol/L	50.63±68.26	4.38±4.32	5.68±5.39	<0.001
TG, mmol/L	1.91±2.54	1.54±1.21	1.59±2.12	0.329
TC, mmol/L	4.35±2.03	4.40±1.25	4.22±1.18	0.684
HDL-C, mmol/L	0.99±0.61	1.18±0.39	1.24±0.42	<0.001
LDL-C, mmol/L	2.53±1.19	2.51±0.87	2.31±0.86	0.087
Apo-A1, g/L	0.77±0.43	1.06±0.32	1.03±0.31	<0.001
Apo-B, g/L	0.94±0.44	0.89±0.42	0.72±0.26	<0.001
TP, g/L	65.75±11.32	66.00±8.61	63.19±7.67	0.020
ALB, g/L	30.60±6.89	37.18±5.99	37.38±5.03	<0.001
GLB, g/L	35.15±8.88	28.82±7.22	25.81±5.72	<0.001
A/G	0.93±0.32	1.36±0.36	1.51±0.35	<0.001
PAB, mg/L	105.42±62.54	200.80±88.17	188.10±65.86	<0.001
NLR	4.84±7.28	5.45±7.01	7.17±5.80	0.002
LMR	2.34±1.39	3.39±2.05	3.74±5.33	0.002
PLR	116.22±95.43	202.47±138.73	170.15±124.23	<0.001

Notes: Values for age expressed as mean±SD. $P<0.05$ was considered statistically significant.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyltransferase; TBA, total bile acid; Tbil, total bilirubin; Dbil, direct bilirubin; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; Apo-A1, apolipoprotein A1; Apo-B, apolipoprotein B; TP, total serum protein; ALB, serum albumin; GLB, serum globulin; A/G, serum albumin/globulin ratio; PAB, serum prealbumin; NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio.

equilibrium (χ^2 = 1.222, P = 0.269 and χ^2 = 3.470, P = 0.062, respectively). The proportion of the *ALDH2* rs671 G/G homozygous was significantly lower in ALC patients (83.3%) than that of other groups (100.0% in non-ALC in alcoholics group, 95.6% in non-ALC in non-alcoholics group) (P<0.001), while the proportion of the G/A heterozygous was significantly higher in ALC patients (16.7%) than that of other groups (0% in non-ALC in alcoholics group, 4.4% in non-ALC in non-alcoholics group) (P<0.001). The frequencies of G and A allele in ALC patients was 91.7% and 8.3%; compared to 97.8% and 2.2% in non-ALC in non-alcoholics group, respectively, there was statistically significant differences (P<0.001) (Table 3). It indicated that there were significant differences in the distribution of *ALDH2* rs671 genotypes among ALC, non-ALC in alcoholics, and non-ALC in non-alcoholics groups.

Logistic Regression Analysis of Risk Factors Associated with ALC

The optimal cut-off value for the diagnosis or risk prediction was determined by receiver operating characteristic (ROC) curve analysis. When ALC was taken as the endpoint of NLR, LMR, and PLR, the critical value of NLR was 4.295 (sensitivity 67.2%, specificity 61.9%), the LMR cutoff value was 2.415 (sensitivity 63.9%, specificity 56.9%), and the PLR cutoff value was 96.585 (sensitivity 56.7%, specificity 77.8%) (Figure 1).

Logistic regression analysis was performed to determine independent predictors for ALC. Univariate regression analysis indicated that there was significantly high risk of ALC in the presence of smoking history (P=0.003), low level of NLR ($\leq$4.295), LMR ($\leq$2.415), and PLR ($\leq$96.585) (P<0.001), and low risk of ALC in the presence of hypertension (P<0.001). The *ALDH2* G/A genotype (OR 5.111, 95% CI 2.360–11.070, P<0.001) was significant risk factor for ALC. Multiple logistic regression analysis indicated that participants with smoking history (adjusted OR 1.759, 95% CI 1.047–2.954, P=0.033), low level of NLR ($\leq$4.295) (adjusted OR 5.543, 95% CI 2.964–10.368, P<0.001), LMR ($\leq$2.415) (adjusted OR 9.256, 95% CI 4.740–18.076, P<0.001), and PLR ($\leq$96.585) (adjusted OR 6.047, 95% CI 3.372–10.845, P<0.001), and *ALDH2* G/A genotype (adjusted OR 6.323, 95% CI 2.477–16.140, P<0.001) had a significantly higher risk of ALC (Table 4).

Table 3 The Distribution of *ALDH2* rs671 Genotypes and Alleles Among ALC, Non-ALC in Alcoholics, and Non-ALC in Non-Alcoholics Groups

| Genotypes	Total (n=427)	Non-Alcoholics Group (n=214)	Alcoholics Group (n=213)	
G/G	385(90.2%)	202(94.4%)	183(85.9%)	0.002
G/A	41(9.6%)	11(5.1%)	30(14.1%)	
A/A	1(0.2%)	1(0.5%)	0(0)	
G/G + G/A	426(99.8%)	213(99.5%)	213(100.0%)	1.000
G/A + A/A	42(9.8%)	12(5.6%)	30(14.1%)	0.003
Allele				
G	811(95.0%)	415(97.0%)	396(93.0%)	0.008
A	43(5.0%)	13(3.0%)	30(7.0%)	
HWE		χ^2=3.470, P=0.062	χ^2=1.222, P=0.269	
Genotypes	**ALC (n=180)**	**Non-ALC in alcoholics group (n=33)**	**Non-ALC in non-alcoholics group (n=206)**	**P value**
G/G	150(83.3%)	33(100.0%)	197(95.6%)	<0.001
G/A	30(16.7%)	0(0)	9(4.4%)	
A/A	0(0)	0(0)	0(0)	
G/G + G/A	180(100.0%)	33(100.0%)	206(100.0%)	-
G/A + A/A	30(16.7%)	0(0%)	9(4.4%)	<0.001
Allele				
G	330(91.7%)	66(100.0%)	403(97.8%)	<0.001
A	30(8.3%)	0(0%)	9(2.2%)	
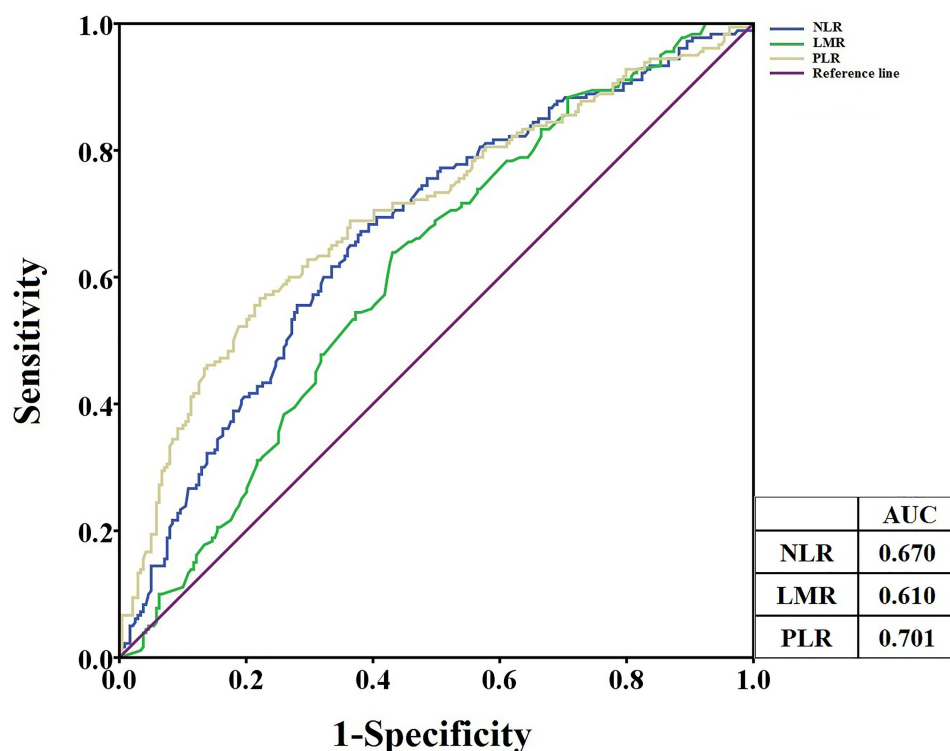
Figure 1 Optimal cut-off value of NLR, LMR, and PLR for the diagnosis or risk prediction was determined by receiver operating characteristic (ROC) curve analysis.

Discussion

Alcohol abuse has always been considered as a risk factor for chronic liver disease.[36] Long-term alcoholism significantly inhibits the activity of mitochondrial ALDH, and even promotes the oxidation of ethanol to acetaldehyde, leading to a significant increase in acetaldehyde levels in tissues and plasma.[37] Alcoholics develop steatosis in the liver first, and

Table 4 Logistic Regression Analysis of Risk Factors Associated with ALC

Variables	Genotypes	Unadjusted Values		Adjusted Values	
		OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
History of smoking		1.866(1.234–2.819)	0.003	1.759(1.047–2.954)	0.033
Hypertension		0.223(0.116–0.430)	<0.001	0.272(0.129–0.577)	0.001
Diabetes		0.928(0.529–1.628)	0.793	1.287(0.617–2.682)	0.501
NLR					
>4.295		1.000(reference)			
≤4.295		3.335(2.222–5.007)	<0.001	5.543(2.964–10.368)	<0.001
LMR					
>2.415		1.000(reference)			
≤2.415		2.489(1.665–3.721)	<0.001	9.256(4.740–18.076)	<0.001
PLR					
>96.585		1.000(reference)			
≤96.585		4.589(3.002–7.015)	<0.001	6.047(3.372–10.845)	<0.001
Genetic model of *ALDH2* gene					
	G/G	1.000(reference)			
	G/A	5.111(2.360–11.070)	<0.001	6.323(2.477–16.140)	<0.001

Note: *P*< 0.05 was considered statistically significant.
Abbreviations: NLR, neutrophil to lymphocyte ratio; LMR, lymphocyte to monocyte ratio; PLR, platelet to lymphocyte ratio; OR, odds ratio; CI, confidence interval.

develop alcoholic hepatitis when inflammation and damage to liver cells increase. In the cases of long-term alcohol intake, the accumulation of extracellular collagen and other matrix proteins leads to liver fibrosis, which gradually develops into cirrhosis and even hepatocellular carcinoma.[38] Liver cirrhosis is a pathological stage characterized by diffuse fibrous pseudolobules forming intrahepatic and extrahepatic vascular proliferation. In recent years, the proportion of ALC in the etiological composition of cirrhosis has shown a significant increasing.[39] ALC is the final stage of ALD caused by long-term heavy drinking.[40,41]

All subjects in this study were tested for the *ALDH2* rs671 polymorphism. Analysis of the distribution of *ALDH2* rs671 genotypes in different ethnic groups showed that it's different in distinct regions and ethnic groups. In European and American countries, *ALDH2* *1/*1 (G/G) is the most dominant genotype, and very few people contain *ALDH2* *2 (A) allele.[42] *ALDH2* *1/*2 (G/A) with an incidence of 35–57% in different East Asian subpopulations.[43] There was no significant difference in the *ALDH2* polymorphic frequencies between alcoholics and controls in the Arcadian population of central India.[44] In this study, the percent of *ALDH2* *1/*1, *1/*2, *2/*2 genotype was 85.9%, 14.1% and 0% in alcoholics group, respectively. According to previous report, the frequency of the *ALDH2* genotype *1/*1, *1/*2 and *2/*2 was 52.03%, 39.67%, and 8.30% in the general population in this area, respectively.[45] People carrying *ALDH2* *1/*2 and *2/*2 genotypes have lower speed of alcohol metabolism in vivo and are prone to various adverse reactions after drinking alcohol. There were significant differences in *ALDH2* genotypes distribution between alcohol-dependent and general populations. It suggests that people with the *ALDH2* *1 allele have a faster conversion rate of acetaldehyde and are more likely to consume more alcohol and develop alcohol dependence.

In addition, 15.5% (33/213) of the chronic alcoholics in the study did not develop cirrhosis. Study has shown that colonic mucosal permeability can be improved for some reasons. Acetaldehyde accumulates in intestinal mucosa in large quantities, damaging intestinal mucosal barrier function, resulting in tight connection of intestinal mucosal epithelial cells and barrier dysfunction.[46] Alcohol consumption leads to increased intestinal permeability and migration of bacterial products. This protective pathway may partly explain why some alcoholics do not develop severe alcoholic liver injury.[47] Whether the changes of gut microbiota play an important role in the formation of ALC, and the regulation of gut-liver axis may need further study.[48] In addition, there are also interactions between immune regulation and alcohol metabolism, which may also be related to the formation of ALC.[49]

In this study, the proportion of the *1/*2 genotype was significantly higher in ALC patients than that of non-ALC patients in chronic alcoholics group and non-ALC patients in non-alcoholics group. Logistic regression analysis showed that *ALDH2* SNP rs671 *1/*2 genotype was the risk factor for ALC. This study suggests that *ALDH2* SNP rs671 *1/*2 genotype is a susceptibility factor for ALC in the population of this area. Zeng et al analyzed the relationship between *ALDH2* genotypes, serum liver enzymes, serum lipid levels, serum protein levels and ALC in Hakka population, and logistic regression analysis showed that *ALDH2* rs671 A allele increased the risk of developing ALC after adjustment for smoking and alcohol consumption.[50] Our results are consistent with the study. The alcohol intake and ALDH2 enzyme activity of individuals with *1/*2 heterozygotes are in the middle level, which leads to the accumulation of acetaldehyde in the body and makes them more susceptible to alcoholic diseases.[10,51] A study has showed that *ALDH2* rs671 G/A and A/A genotypes are strong with ALC in East-Asians,[52] however, another study showed no association between *ALDH2* rs671 and ALC in East Asian males.[22] *ALDH2* *1/*1 genotype increased risk for ALC among Japanese alcoholic men.[19] Results from a Korean population showed that ALC development was not associated with *ALDH2* rs671 polymorphism.[21] Different sample sizes and different populations may be responsible for the inconsistent results of the relationship between *ALDH2* genotypes and ALC.

Although different genotypes of *ALDH2* play a role in the development of ALC, other risk factors also play an important role. At present, many inflammatory and immune response markers such as neutrophils, lymphocytes, NLR, PLR, and LMR have been found to be associated with liver disease. The decrease of LMR indicates the increase of neutrophils and monocytes or the relative or absolute decrease of lymphocyte count. LMR is a diagnostic or prognostic marker for some liver diseases, such as bacterial infection in patients with liver cirrhosis,[53] liver transplantation for hepatocellular carcinoma[54] and hepatitis B virus (HBV)-related liver cirrhosis.[55] PLR as a predictive biomarker of liver fibrosis in patients with hepatitis C virus (HCV)-related liver disease,[56] and was associated with HBV-related liver fibrosis.[57] NLR is simple and robust predictors of 30-day mortality in alcoholic cirrhosis patients,[58] independently

predicts survival in patients with liver cirrhosis.[59] In this study, participants with low level of NLR, LMR, and PLR had a significantly higher risk of ALC. It supports the conclusion that NLR, LMR and PLR can be used as predictive biomarkers for ALC.

There are some shortcomings in this study. First, all the subjects in this study came from one hospital, which may have selection bias. Second, the history of alcoholism of the subjects involved in this study was determined based on the descriptions of patients and their families, and the actual situation may differ from the description. The last, due to the small number of subjects in this study, there may be some deviations in the results. Next, it is necessary to increase the sample size of the study and cover more relevant gene polymorphisms.

Conclusion

In the present study, *ALDH2* polymorphism rs671 *1/*2 genotype might be a potential risk factor for the development of ALC among Hakka alcoholics. The results need to be confirmed by further studies with large samples. The results should enrich the relevant data and provide valuable information for the future related research.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval and Consent to Participate

The study was approved by the Ethics Committee of Medicine, Meizhou People's Hospital (Huangtang Hospital), Meizhou Academy of Medical Sciences. All participants signed informed consent in accordance with the Declaration of Helsinki.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests in this work.

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