RESEARCH ARTICLE

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Association of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 with Alcohol abuse and Alcoholic Cirrhosis in People Living in Northeast Vietnam

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Abstract

Objective: Alcohol abuse can cause developing cirrhosis, even liver cancer. Several single nucleotide polymorphisms (SNPs) of *ADH1B*, *ADH1C*, and *ALDH2* genes have been reported to be associated with alcohol abuse and alcoholic cirrhosis (ALC). This study investigated the association between three SNPs of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 with alcohol abuse and ALC in people living in the Northeast region of Vietnam. **Methods:** 306 male participants were recruited including 206 alcoholics (106 ALC, 100 without ALC) and 100 healthy non-alcoholics. Clinical characteristics were collected by clinicians. Genotypes were identified by Sanger sequencing. Chi-Square ($\chi 2$) and Fisher-exact tests were used to assess the differences in age and clinical characteristics, Child-Pugh score, frequencies of alleles and genotypes. **Result:** Our data showed that the frequency of *ALDH2**1 was significantly higher in alcoholics (88.59%) and ALC groups (93.40%) than that of healthy non-alcoholics (78.50%) with p=0.0009 and non-ALC group (83.50%) with p=0.002, respectively. We detected opposite results when examined *ALDH2**2. Frequency of combined genotypes with high acetaldehyde accumulation were significantly lower in alcoholics and ALC group than those of control groups with p=0.005 and p=0.008, respectively. Meanwhile, the proportion of combined genotypes with non-acetaldehyde accumulation were significantly two times higher in the ALC group (19.98%) than those of the non-ALC group (8%) with p=0.035. These combined genotypes showed a decreasing trend in the Child-Pugh score from likely phenotype causing risk for non-acetaldehyde accumulation to high acetaldehyde accumulation. **Conclusion:** The *ALDH2**1 allele was found as a risk factor for alcohol abuse and ALC, and combined genotypes of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 with non-acetaldehyde accumulation increase ALC risk. In contrast, *ALDH2**2 and the genotype combinations related to high acetaldehyde accumulation were protective factors against alcohol abuse and ALC.

Keywords: Alcohol metabolism genes- alcoholics- alcohol cirrhosis (ALC)- genetic polymorphism/variant

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Introduction

In humans, alcohol is metabolized mainly through oxidation with the participation of alcohol dehydrogenases (ADHs) and aldehyde dehydrogenases (ALDHs) (Zakhari, 2006; Cederbaum, 2012). Alcohol dehydrogenases *ADH1B*, *ADH1C*, and aldehyde dehydrogenase *ALDH2* are enzymes that play a major role in the alcohol metabolism pathway. The *ADH1B*, *ADH1C*, and *ALDH2* genes are located on chromosomes 4, and 12, respectively. Three most well-known single nucleotide polymorphisms (SNPs) which affect alcohol metabolism are *ADH1B**2 (rs1229984: c.254G>A, p.Arg48His), *ADH1C**2 (rs698: c.1418A>G, p.Ile350Val) and *ALDH2**2 (rs671: c.1606G>A, p.Glu487Lys). The *ADH1B**2 allele is associated with a higher alcohol metabolizing activity compared to the wild-type *ADH1B**1 allele. In contrast, *ADH1C**2 is about 1.5 to 2-fold less active than the ancestor phenotype of *ADH1C**1. The appearance of one or both *ALDH2**2 alleles can cause a severe decrease in their enzymatic activity to 5-20% (Edenberg and McClintick, 2018). These SNPs were not only involved in alcoholism

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and behavioral disorders but also resulted in differences between individuals in exposure to acetaldehyde, leading to possible carcinogenic effects and various diseases in humans (Zakhari, 2006; National Institute on Alcohol Abuse and Alcoholism, 2007; Cederbaum, 2012; Hurley and Edenberg, 2012).

The association of three SNPs (rs1229984, rs698, and rs671) with alcohol abuse and alcoholic cirrhosis (ALC) has been reported in different populations worldwide. A study in male Korean patients has shown that *ALDH2**2 protected against alcoholism but was not associated with cirrhosis (Lee et al., 2001). Although, Frenzer et al. suggested that the *ADH1C**2 allele is common in ALC Caucasian patients (Frenzer et al., 2002) in a study previously, He et al. found that *ADH1C**2 allele might increase the risk of ALC in Asians but be a protective factor for ALC among Caucasians (He et al., 2015a). A meta-analysis demonstrated that the *ADH1B**2 allele might protect people from alcoholic cirrhosis, especially for Asians (He et al., 2015b), while *ADH1B**2 and *ALDH2**2 alleles protect against alcoholism (Edenberg and McClintick, 2018). In Japanese and Chinese, the *ALDH2**1/*1 genotype increased the risk of ALC, and *ALDH2**1/*2 decreased the risk of ALC. In additional, recent research showed that the *ALDH2**2 allele (*ALDH2**1/*2 + *ALDH2**2/*2) increased the risk of developing ALC (Zeng et al., 2021) and *ALDH2**1/*2 genotype is a potential risk factor for the development of ALC among Hakka people in the South of China (Chen et al., 2022).

Alcohol abuse is a global problem, constituting the seventh leading risk factor for death and disabilities (Degenhardt et al., 2018). More than 90% of people abusing alcohol develop fatty liver disease, the first stage of liver disadvantage, followed by inflammation, apoptosis, fibrosis, cirrhosis, and even liver cancer (Osna et al., 2017). Currently, Vietnam is supposed to be a country with high alcohol consumption, especially among men. An investigation suggested that nearly 60% of surveyed people use alcohol, about 50% of men using alcohol at a moderate or more, and 8% are at the level of addiction or heavy addiction. In additional, the Vietnamese interviewed subjectively believed that drinking alcohol was healthier and helped relieve nervous tension (Luu and Nguyen, 2018). People living in the Thai Nguyen province of the Northeast region of Vietnam are estimated to be at increased risk of diseases if they abuse alcohol (Hoang et al., 2022). According to the published reports in the world, the relationship between SNPs of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 and ALC are inconsistent. This study aims to examine the association between these SNPs and ALC risk as well as contribute to elucidating the relationship of genetic factors to alcohol abuse and ALC in Vietnamese people living in the Northeast region of Vietnam.

Materials and Methods

Subjects

There were 306 male individuals enrolled from January to December of 2021 at Thai Nguyen General Hospital of Viet Nam (ages 27 to 87), including 100 healthy non-alcoholic individuals and 206 alcoholics. The alcoholics consisted of 106 ALC patients and 100 non-ALC individuals. The ALC subjects was recruited from patients in Department of Internal Gastroenterology, healthy non-alcoholic individuals and non-ALC group are recruited during annual health checkups. All subjects were diagnosed by the clinician at Thai Nguyen General Hospital of Viet Nam according to etiology, history, clinical manifestations, complications, examinations, imaging, and histology (Smith et al., 2019). Alcoholism were determined according to International Classification of Diseases, 10th Revision. ALC patients were negative for the hepatitis B surface antigen and the antibody hepatitis C virus by enzyme- linked immunosorbent assay. The severity of liver dysfunction in ALC patients was assessed by the Child-Pugh score (Peng et al., 2016). The aim of this study was explained to all individuals, and informed consents were obtained from participants whose privacy was protected carefully. The study had been approved by the Human Ethics Committee of the Thai Nguyen General Hospital, Ministry of Health of Viet Nam.

Chemical analysis

*Serum Liver Enzymes and Serum Lipid Measurement*s

Peripheral blood of each participant (2mL) was taken into tube containing heparine as anticoagulant, and plasma was isolated and tested promptly. Plasma samples were evaluated using the Olympus AU5800 system (Beckman Coulter, USA) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), NH3, total bilirubin (Tbil), direct bilirubin (Dbil), total serum protein (TP), serum globulin (GLB), albumin (ALB), total cholesterol (TC), triglyceride (TG). NH3, ALT, AST and GGT analyses were carried out with the kinetic method; Tbil and Dbil was determined by chemical oxidation method; total serum protein (TP) concentration was determined by biuret method; GLB and ALB concentration were carried out with bromocresol green method; TC, TG, analyses were carried out using cholesterol esterase/peroxidase (CHOD/PAP) enzymatic method. These standard operating procedures (SOP) accorde to guiding from Ministry of Health of Viet Nam.

Routine blood analysis

Approximately 2 mL of venous blood from each subject was taken into tube containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Blood cells correlative indices were detected by Celltac F blood analyzer (Nihon Kohden, Japan) like red blood cells (RBC), Hemoglobin (HGB), white blood cells (WBC), Platelets (PLT), Prothrombin time (PT), international normalized ratio (INR) according to the standard operating procedures (SOP) in Thai Nguyen General Hospital, Ministry of Health of Viet Nam.

DNA extraction, PCR direct sequencing and genotype analysis

The obtained peripheral blood of each participant (2 ml) was collected in EDTA containing tube and stored at -80ºC. Total genomic DNA was later extracted using

ExgeneTM Blood SV mini Kit (GeneAll Biotechnology Co. LTD, Seoul, Korea) and subsequently preserved at -20°C.

PCR and Sanger sequencing methods of the *ADH1B* (exon 3), *ADH1C* (exon 8), and *ALDH2* (exon 12) had been performed according to previous report (Hoang et al., 2022).

The genotypes of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 were detected by using BioEdit (Raleigh, NC) sequence alignment editor software and checked from a database of human genomic structural variation (dbvar) of NCBI data.

Statistical analysis

Frequencies of alleles and genotypes, clinical testing results had computed using counting methods. All statistical analyses were performed using SPSS software ver 22. Chi-Square (χ^2) and Fisher-exact tests were used to assess the Hardy–Weinberg equilibrium for each variant compare the categorical variables between groups. Difference in mean value of Child-Pugh score was examined by one-way ANOVA test. P value < 0.05 was considered as statistical significance. The relationship between an allele/genotype and possibility of an event occurrence was calculated by odd ratio (OR) and 95% confidence interval (95% CI).

Results

Age and clinical characteristics of study subjects

The characteristics of 306 male participants living in the Northeast region of Vietnam, including 100 healthy non-alcoholic individuals and 206 alcoholics consisting of 100 individuals with non-ALC and 106 ALC patients, had shown in Table 1. The ALC patients' average age was 55.79±9.61 years, with 42.96±877 years for non-ALC group and 56.78±15.89 years for healthy non-alcoholics group. There was a significant difference in average age comparing between non-ALC and ALC group (p<0.001). Analysis data showed a difference in the levels of clinical characteristics in study groups comparing to normal range. The levels of AST, ALT, GGT, and NH3 in healthy non-alcoholics, non-ALC, and ALC groups showed an increasing trend. The levels of RBC, HBG, TP, ALB, and A/G in healthy non-alcoholics, non-ALC, and ALC showed a decreasing trend, while the levels of WBC, PLT, Tbil, GLB, and INR showed an increasing trend.

Genotype and allele frequencies of ADH1B rs1229984, ADH1C rs698, and ALDH2 rs671 polymorphisms

The allele and genotype frequencies of three interested SNPs in study subjects were identified and presented in the Table 2. Allele and genotype frequencies of each SNP did not violate Hardy-Weinberg equilibrium in studied population with p>0.05.

Compared to non-alcoholics group, the allele frequencies of *ADH1B* rs1229984, *ADH1C* rs698 in the alcoholics group were not significantly different (p>0.05), but the frequency of rs671 *ALDH2**2 allele was significantly lower (p<0.05). The frequencies of *ALDH2**1 and *ALDH2**2 allele in alcoholics group was

Table 1. Age and Clinical Characteristics of Healthy Non-Alcoholics, Non-ALC and ALC Groups

Characteristics	Healthy non-alcoholics	Alcoholics		Normal rank
		Non-ALC	ALC	
Age	56.78±15.89	42.96±877	55.79±9.61	-
RBC, 1012/L	4.21±0.61	4.12±0.49	3.36±0.94	4.2-6.3
HGB, g/L	135.13±16.88	125.3±17.93	99.07±32.11	12-18
WBC, 109/L	6.34±1.79	6.84±2.45	8.72±4.25	4.4-10.9
PLT, 1012/L	134.64±31.06	142.76±53.66	151.83±109.50	140-440
PT, s	12.74±9.59	11.98±1.18	19.57±7.74	10-14
INR	1.36±0.25	1.45±0.32	1.58±0.66	0.8-1.2
TP, g/L	71.58±5.98	74.13±5.54	66.39±10.32	60 – 80
ALB, g/L	42.82±3.46	42,79±2.93	29.21±6.29	38 – 54
GLB, g/L	28.76±3.72	31.33±3.58	37.18±8.85	26 – 42
A/G	1.52±0.30	1.38±0.15	0.84±0.28	1-1.5
Tbil, µmol/L	13.71±3.52	10.33±4.67	58.18±54.94	3.4-17.1
Dbil, µmol/L	2.85±1.20	2.47±1.78	24.67±27.83	<7
AST, U/L	28.39±10.15	39.29±33.27	225.04±564.65	20-40
ALT, U/L	25.36±7.45	36.50±33.99	69.21±87.74	7-56
GGT, U/L	43.51±85.79	119.30±84.69	442.49±442.23	7-60
NH3, µmol/L	33.97±13.58	37.73±17.95	65.17±45.65	11-32
TC, mmol/L	4.08±2.09	5.21±1.57	4.02±1.91	<5.2
TG, mmol/L	1.76±0.52	4.59±6.21	3.29±2.10	<1.7

Abbreviations: RBC, red blood cells; HBG, Hemoglobin; WBC, white blood cells; PLT, Platelets; PT, Prothrombin time; INR, international normalized ratio; TP, total serum protein; GLB, serum globulin; ALB, albumin; A/G, serum albumin/globulin ratio; Tbil, total bilirubin; Dbil, direct bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; TC, total cholesterol; TG, triglyceride.

Table 2. Genotype and Allele Frequencies of *ADH1B* rs1229984, *ADH1C* rs698 and *ALDH2* rs671 in Study Groups

Gene polymorphism	Genotypes and alleles	Healthy non-alcoholics	Alcoholics N=206 (%)	P values, OR	Non-ALC	ALC	P values, OR
ADH1B rs1229984	*1/*1	11 (11.00)	30 (14.56)	0.391	10 (10.00)	20 (18.87)	0.071
	*1/*2	43 (43.00)	84 (40.78)	0.711	45 (45.00)	39 (36.79)	0.231
	*2/*2	46 (46.00)	92 (44.66)	0.825	45 (45.00)	47 (4.34)	0.924
	*1/*2+*2/*2	89 (89.00)	176 (85.44)	0.391	90 (90.00)	86 (81.13)	0.071
	*1	65 (32.50)	144 (34.95)	0.549	65 (32.50)	79 (37.26)	0.311
	*2	135 (67.50)	268 (65.05)		135 (67.50)	133 (62.74)	
	HWE		$\chi2$ =0.969; p=0.616				
ADH1C rs698	*1/*1	78 (78.00)	166 (80.58)	0.598	82 (82.00)	84 (79.24)	0.617
	*1/*2	22 (22.00)	38 (18.45)	0.463	18 (18.00)	20 (18.87)	0.872
	*2/*2	0 (0)	2 (0.97)	0.323	0 (0)	2 (1.89)	0.167
	*1/*2+*2/*2	22 (22.00)	40 (19.42)	0.598	18 (18.00)	22 (20.75)	0.617
	*1	178 (89.00)	370 (89.81)	0.76	182 (91.00)	188 (88.68)	0.437
	*2	22 (11.00)	42 (10.19)		18 (18.00)	24 (11.32)	
	HWE		$\chi2$=0.482; p=0.786				
ALDH2 rs671	*1/*1	63 (63.00)	163 (79.13)	0.003, 2.23 (1.31-3.77)	71 (71.00)	92 (86.79)	0.005, 2.68 (1.32-5.45)
	*1/*2	31 (31.00)	39 (18.93)	0.018, 0.52 (0.3-0.9)	25 (25.00)	14 (13.21)	0.031, 0.46 (0.22-0.94)
	*2/*2	6 (6.00)	4 (1.94)	0.061	4 (4.00)	0 (0)	0.038, 0.23 (0.03-2.08)
	*1/*2+*2/*2	37 (37.00)	43 (20.87)	0.003, 0.45 (0.27-0.76)	29 (29.00)	14 (13.21)	0.005, 0.37 (0.18-0.76)
	*1	157 (78.50)	365 (88.59)	0.0009, 2.13 (1.35-3.35)	167 (83.50)	198 (93.40)	0.002, 1.35 (0.79-2.31)
	*2	43 (21.50%)	47 (11.41%)		33 (16.50)	14 (6.60)	
	HWE		$\chi2$=1.093; p=0.579				

Abbreviations: N, number of subjects; SD, standard deviation; OR, odds ratio; CI, confidence interval; P< 0.05 was considered statistically significant; The OR between events was calculated based on comparing the odd of genotype of interest and odd of the remaining genotypes in the studied population.

88.59% and 11.41%; compared to 78.50% and 21.50% in non-alcoholics group, respectively, there was statistically significant difference with p=0.0009 (OR=2.13, 95%CI=1.35-3.35). The *ALDH2**1/*1 frequency was significantly higher in the alcoholics group (79.13%) than that of the healthy non-alcoholics group (63%) with p=0.003 (OR=1.92, 95% CI=1.11-3.33). Though the frequency of *ALDH2**2/*2 was not clearly differed between the non-alcoholics and alcoholics groups (6% and 1.94%) with p=0.061, the proportion of the *ALDH2**1/*2 and *ALDH2**1/*2+*ALDH2**2/*2 was significantly lower in alcoholics group than those of non-alcoholics group (18.93% and 20.97% comparing 31.00% and 37.00%, respectively) with p=0.018 (OR = 0.52, 95%CI = 0.3-0.9) and p=0.003 (OR = 0.45, 95%CI = 0.27-0.76, respectively.

Similarly, when comparing between non-ALC and ALC group, the data showed only a significant difference in *ALDH2* rs671 polymorphism. The frequency of *ALDH2**1/*1 in ALC group (86.79%) was significantly higher than that found in non-ALC group (71%) with p=0.005 (OR=2.68, 95%CI=1.32-5.45). Additionally, the proportion of the *ALDH2**1/*2 and *ALDH2**2/*2 in ALC group was significantly lower than those detected in non-ALC group (13.21% and 0% comparing 25% and 4%, respectively) with p=0.031 (OR=0.46, 95%CI=0.22-0.94) and p=0.038 (OR=0.23, 95%CI=0.03-2.08), respectively. Therefore, frequency of *ALDH2**2 allele carriers (*ALDH2**1/*2+*ALDH2**/*2) was significantly different between ALC and non-ALC group (13.21% and 29%) with p=0.005 (OR=0.37, 95%CI=0.18-0.76). From that result, there was statistically significant difference between frequency of *ALDH2**1 and *ALDH2**2 alleles in ALC group (93.40% and 6.60%) and non-ALC group (83.50% and 16.50%) with p=0.002 (OR=1.35, 95%CI=0.79-2.31).

Combination of ADH1B rs1229984, ADH1C rs698 and ALDH2 rs671 genotypes

Besides evaluating the genotype effect of single genes, the impact of three genes in combination was further examined (Table 3). In total, there were 16 genotype combinations from three polymorphisms of three genes. Based on likely phenotypes, those 16 genotype combinations could be classified into three groups: increasing risk for alcoholism/non-acetaldehyde accumulation, reducing the risk for alcoholism/low acetaldehyde accumulation, and reducing the risk for alcoholism/high acetaldehyde accumulation.

For comparing healthy non-alcoholics and alcoholics group, there was no significant difference in three combined genotypes increasing risk for alcoholism with non-acetaldehyde accumulation and six combined genotypes reducing risk for alcoholism with low acetaldehyde accumulation (p>0.05) (Table 3). However, a significant difference was found in the seven combined genotypes that lessen the risk for alcoholism due to high acetaldehyde accumulation. In fact, the proportion of the genotypes the risk for alcoholism with high acetaldehyde accumulation in alcoholics group (19.42%) was significantly lower than that of healthy non-alcoholics group (34%) with p=0.005 (OR=0.47, 95%CI=0.27-0.8).

Table 3. Combination of *ADH1B* rs1229984, *ADH1C* rs698and *ALDH2* rs671 Genotypes in Sample Groups

Genotype			Likely phenotype	Healthy non-alcoholics	Alcoholics N=206 (2)	1/2 (%)	P values, OR	Non-ALC N=100 (3)	ALC N=106 (4)	3/4 (%)	P values, OR
ADH1B (rs1229984)	*ADH1C* (rs698)	*ALDH2* (rs671)									
*1/*1	*1/*1	*1/*1	Increasing risk for alcoholism/non-acetaldehyde accumulation	5	14	8/12.62	0.228	5	10	8/16.98	0.035, 2.51 (1.05-6.03)
*1/*1	*1/*2	*1/*1		3	10			3	7		
*1/*1	*2/*2	*1/*1		0	2			0	2		
*1/*1	*1/*1	*1/*2	Reducing the risk for alcoholism/low acetaldehyde accumulation	0	3	58/67.96	0.087	2	1	65/70.75	0.461
*1/*1	*1/*2	*1/*2		2	0			0	0		
*1/*2	*1/*1	*1/*1		17	44			22	22		
*1/*2	*1/*2	*1/*1		9	15			6	9		
*2/*2	*1/*1	*1/*1		26	75			34	40		
*2/*2	*1/*2	*1/*1		4	3			1	2		
*1/*2	*1/*1	*1/*2	Reducing the risk for alcoholism/ highacetaldehyde accumulation	11	15	34/19.42	0.005, 0.47 (0.27-0.8)	9	9	27/12.26	0.008, 0.48 (0.24-0.96)
*1/*2	*1/*1	*2/*2		2	2			2	0		
*1/*2	*1/*2	*1/*2		5	8			6	2		
*2/*2	*1/*1	*1/*2		12	12			7	5		
*2/*2	*1/*1	*2/*2		4	1			1	0		
*2/*2	*1/*2	*1/*2		0	1			1	0		
*2/*2	*1/*2	*2/*2		0	1			1	0		

Abbreviations: N, number of subjects; SD, standard deviation; OR, odds ratio; CI, confidence interval; P< 0.05 was considered statistically significant; The OR between groups was calculated based on comparing the odd of combined genotype of interest and odd of remaining genotypes. OR values indicate the risk of alcoholics or ALC compared with reference groups while individuals carrying the certain combined genotypes.

Table 4. Association of *ADH1B* rs1229984, *ADH1C* rs698 and *ALDH2* rs671 Genotypes with ALC Status in ALC Patients

Gen polymorphism	Genotypes	N	Child-Pugh Score	
			Mean value ± SD	P value
ADH1B rs1229984	*1/*1	20	8.40 ± 2.062	0.566
	*1/*2	39	8.38 ± 2.571	
	*2/*2	47	7.89 ± 2.324	
ADH1C rs698	*1/*1	84	8.14 ± 2.355	0.546
	*1/*2	20	8.10 ± 2.511	
	*2/*2	2	10.0 ± 0.0	
ALDH2 rs671	*1/*1	92	8.2 ± 2.392	0.775
	*1/*2	14	8.0 ± 2.253	
	*2/*2	0	-	

Abbreviations: N, number of subjects; SD, standard deviation; P< 0.05 was considered statistically significant.

For comparing non-ALC and ALC group, there are no significant difference in combined genotypes with low acetaldehyde accumulation (p>0.05) but statistically significant differences appeared in combined genotypes with non and high acetaldehyde accumulation. The proportion of the phenotype with high acetaldehyde accumulation was significantly lower in ALC group (12.26%) than that of non-ALC group (27%) with p=0.008 (OR= 0.48, 95%CI=0.24-0.968). Meanwhile, the proportion of the phenotype with non-acetaldehyde accumulation was significantly two times higher in ALC group (19.98%) than that of non-ALC group (8%) with p=0.035 (OR=2.51, 95%CI=1.05-6.03).

Association between genotypes of ADH1B rs1229984, ADH1C rs698 and ALDH2 rs671 with ALC status

The Child-Pugh score is a system for assessing the prognosis to provide treatment solutions and the necessity of liver transplant in chronic liver disease and cirrhosis patients. Based on the Child-Pugh score, cirrhosis patients are classified into three categories: Child-Pugh A, 5 to 6 points, good hepatic function; Child-Pugh B, 7 to 9 points, moderately impaired hepatic function; and Child-Pugh C, 10 to 15 points, advanced hepatic dysfunction (Peng et al., 2016). Therefore, the Child-Pugh score was used to assess liver dysfunction severity in the ALC patients. We analyzed the association of genotypes of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 with Child-Pugh scores. Table 4 showed that all these genotypes belong to Child-Pugh B, and there was no significant difference among genotypes (p>0.05). There was also no significant difference in each genotype combination with p>0.05 (Table 5). However, these combined genotypes showed a decreasing trend in the Child-Pugh score from likely phenotype causing risk for non-acetaldehyde accumulation (8.32±2.0280) to risk for high acetaldehyde accumulation (7.85 ± 2.178).

Discussion

This is the first study to access the association between SNPs of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) with alcohol abuse and clinical characteristics in alcoholics and ALC patients living in the Northeast region of Vietnam. In our study, sample groups were recruited randomly, the obtained result showed that there was a

Table 5. Combination of *ADH1B* rs1229984, *ADH1C* rs698 and *ALDH2* rs671 Genotypes with ALC Status

Genotype			N	Child-Pugh Score for combined genotype		Likely phenotype	Likely Phenotype ratio (%)	Child-Pugh Score for likely phenotype	
ADH1B (rs1229984)	*ADH1C* (rs698)	*ALDH2* (rs671)		mean value ± SD	P value			mean value ± SD	P value
*1/*1	*1/*1	*1/*1	10	8.2 ± 2.390	0.499	Increasing risk for alcoholism / non-acetaldehyde accumulation	17.93	8.32 ± 2.028	0.854
*1/*1	*1/*2	*1/*1	7	8.0 ± 1.826					
*1/*1	*2/*2	*1/*1	2	10.0 ± 0.0					
*1/*1	*1/*1	*1/*2	1	10.0 ± 0.0	0.545	Reducing the risk for alcoholism/ low acetaldehyde accumulation	69.81	8.19 ± 2.453	
*1/*2	*1/*1	*1/*1	22	8.14 ± 2.356					
*1/*2	*1/*2	*1/*1	9	9.0 ± 3.123					
*2/*2	*1/*1	*1/*1	40	8.10 ± 2.437					
*2/*2	*1/*2	*1/*1	2	6.0 ± 0.0					
*1/*2	*1/*1	*1/*2	6	9.0 ± 2.898	0.243	Reducing the risk for alcoholism/ high acetaldehyde accumulation	12.26	7.85 ± 2.178	
*1/*2	*1/*2	*1/*2	2	6.50 ± 0.707					
*2/*2	*1/*1	*1/*2	5	7.0 ± 1.00					

Abbreviations: N, number of subjects; SD, standard deviation; P< 0.05 was considered statistically significant.

significant difference in average age comparing between non-ALC and ALC group (p<0.001). Based on this characteristic, we suggest that long-term alcohol abuse may lead to risk for high cirrhosis. Additionally, the levels of AST, ALT, GGT, NH3, WBC, PLT, Tbil, GLB and INR showed an increasing trend, while the levels of RBC, HBG, TP, ALB and A/G showed a decreasing trend in healthy non-alcoholics, non-ALC and ALC groups (Table 1). The clinical testing results suggested the severity of liver lesions in the ALC group comparing non-ALC and healthy non alcoholics group (Dias et al., 2009; Gowda et al., 2009; Xie et al., 2016; Deutsch-Link et al., 2022).

Alcohol abuse is thought to be affected by genes encoding ethanol-metabolizing enzymes, psychological and social factors (Micu et al., 2019). With the identified allele and genotype frequencies of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 showed that these SNPs were in Hardy-Weinberg equilibrium and have been inherited stably in the Vietnamese population living in Northeast region of Vietnam (p>0.05) (Table 2). The prevalence of *ADH1B**2 and *ADH1C**2 alleles is similar to healthy Vietnamese living in the Thai Nguyen province belonging to the Northeast region of Vietnam (p>0.05). In contrast, the *ALDH2**2 allele appeared to have a significant difference with p=0.013 (Hoang et al., 2022). Subjects in this study were people living in the Northeast region of Vietnam, where are also other ethnic minorities co-living here, such as Tay, Mong, Nung and Dao... like as Thai Nguyen province (United Nations Population Fund in viet nam, 2011). Therefore, we suggest that the significant difference in *ALDH2**2 allele frequency in this study compared with our previous report may be due to obtained sample population, in addition to healthy non-alcoholics, there are also alcoholic subjects, including non-ALC and ALC patients. This study showed no significant difference of allele and genotype frequencies of *ADH1B* rs1229984 and *ADH1C* rs698 between the study groups. We found that the allele and genotype frequencies of *ALDH2* rs671 may be related to alcohol abuse and ALC in people living in the Northeast region of Vietnam (p<0.05). In fact, the proportion of the *ALDH2**1/*1 was significantly higher in alcoholics and ALC group (79.13% and 86.79%) than that of healthy non-alcoholics group (63%) with p=0.003 (OR=1.92, 95% CI=1.11-3.33) and non-ALC group (71%) with p=0.005 (OR=2.68, 95%CI=1.32-5.45). So, people carrying the *ALDH2**1/*1 genotype were at higher risk of alcoholism and ALC. The proportion of the *ALDH2**1/*2 was significantly lower in the alcoholics group (18.93%) than those of the healthy non-alcoholics group (31.00%) with p=0.018 OR = 0.52, 95%CI 0.3-0.9 while the frequency of *ALDH2**2/*2 were comparable between healthy non-alcoholics and alcoholics group (6% and 1.94%) with p=0.061. The proportion of the *ALDH2**1/*2 and *ALDH2**2/*2 was significantly lower in the ALC group than those of the non-ALC group (13.21% and 0% comparing 25% and 4%, respectively) with p=0.031 (OR=0.46 95%CI=0.22-0.94) and p=0.038 (OR=0.23 95%CI=0.03-2.08), respectively. Therefore, we suggest that the *ALDH2**2 allele could play a protective role in alcoholism and ALC. A similar analysis showed that *ALDH2**1 was a risk factor and *ALDH2**2 was a protecting factor for alcoholism and ALC. Our result was similar to previously studies (Yokoyama et al., 2013; Edenberg and McClintick, 2018; Wang et al., 2020). However, this result isn't inconsistent, the *ALDH2**2 allele frequency was significantly lower in alcoholics of Korean, Japanese, Taiwan, Han - China and Vietnamese this study comparing healthy non-alcoholics group (Chen et al., 1999; Feng et al., 2008; Yokoyama et al., 2013; Jiang et al., 2017) except Indians and Hakka - China (Rao et al., 2017; Arunachalam et al., 2022; Chen et al., 2022). This allele could play a protective role against alcoholism in Korean, Japanese, Taiwanese, Han - China and Vietnamese but be a risk factor for alcoholism in Indians and Hakka - China. In additional, the *ALDH2**1 allele is a risk factor for ALC in Japanese (Yokoyama et al., 2013) and Vietnamese in this study, but the *ALDH2**2 allele suggested that increasing the risk of developing ALC among Hakka people in southern China. It reported that was no significant difference in the *ALDH2**2 frequency in ALC Korean, possibly due to the small number of subjects investigated (Lee et al., 2001). In additional, our research also obtained the proportion of individuals with genotype *ALDH2**2/*2 in the non-ALC group (~2%). This can be explained that psychological and social factors also have influence on drinking habits because adult Vietnamese are encouraged or challenged to drink more alcohol at social events, and many Vietnamese believed that drinking alcohol was healthier and helped relieve nervous tension (Luu and Nguyen, 2018).

Chen's report suggested that the *ALDH2**1/*2 genotype increased the risk of developing ALC in Hakka people in the south of China (Chen et al., 2022). This has been explained as people carrying the *ALDH2**1/*2 genotype metabolized alcohol in the middle level, which lead to the accumulation of acetaldehyde in the body (Sakamoto et al., 2006; Seitz and Stickel, 2006). Moreover, according to Mello et al, alcohol abuse inhibits the activity of *ALDH2*, and even promotes the oxidation of ethanol to acetaldehyde, causing to a significant increase in acetaldehyde accumulation (Mello et al., 2008). Genetic polymorphisms of genes related alcohol-metabolism pathway result in differences between individuals in exposure to acetaldehyde, leading to possible carcinogenic effects and several human diseases (Druesne-Pecollo et al., 2009). Up to now, the role of acetaldehyde in cirrhosis development is still not clear. According to Zakhaki (2006), a fast ADH or a slow ALDH is expected to elevate acetaldehyde levels and thus reduce alcohol drinking (Zakhari, 2006). Then, Cederbaum suggested that the capacity of ALDH to remove acetaldehyde exceeds the capacity of acetaldehyde generation by various pathways of alcohol oxidation. Therefore, circulation levels of acetaldehyde are usually very low (Cederbaum, 2012). In additional, analysis reported by Edenberg (2018) have shown that the *ADH1B**2 allele (rs1229984) is associated with a higher alcohol metabolizing activity, compared to the wild-type *ADH1B**1 allele. For this reason, people carrying the *ADH1B**2/*2 genotype showed elevated acetaldehyde in their blood and strongly reduced risk of alcoholism. Meanwhile, *ADH1C**2 showed lower alcohol metabolism activity compared with *ADH1C**1,

which tends to be inherited together with *ADH1B**2 and has a protective role against alcoholism. People carrying *ALDH2**2 can cause a severe decrease in enzymatic activity and be suggested protective against heavy drinking, alcohol dependence and have high blood acetaldehyde levels. Allele *ADH1B**2 and *ALDH2**2 were shown to be protective against alcoholism (Edenberg and McClintick, 2018). From these data, we suggested three likely phenotypes, including phenotypes for increasing the risk of alcoholism (non-acetaldehyde accumulation), reducing the risk of alcoholism with low acetaldehyde accumulation, and reducing the risk of alcoholism with high acetaldehyde accumulation. Likely phenotype for increasing risk for alcoholism include slow *ADH1B* and fast *ALDH2* (combined genotypes of *ALDH2**1/*1, *ADH1B**1/*1, and genotypes of *ADH1C* rs698). For phenotype reducing the risk for alcoholism with low acetaldehyde accumulation, including fast *ALDH2* and fast *ADH1B* (combinations of *ALDH2**1/*1 with *ADH1B**1/*2 or *ADH1B**1/*2 and genotypes of *ADH1C* rs698) or slow *ALDH2* and slow *ADH1B* (*ALDH2**1/*2, *ADH1B**1/*1 and genotypes of *ADH1C* rs698). And for phenotype reducing the risk for alcoholism with high acetaldehyde accumulation, including remaining combined genotypes (*ALDH2**2/*2 combined *ADH1B**1/*1 and genotypes of *ADH1C* rs698 or *ALDH2**1/*2 and *ALDH2**2/*2 combined *ADH1B**1/*2 or *ADH1B**2/*2 and genotypes of *ADH1C* rs698). In our previous study, these combined genotypes were reported to be able to associate with alcohol dependence and protection against alcoholism in people living in the Thai Nguyen province. People living in the Thai Nguyen province, belong to the Northeast region of Vietnam, are evaluated to be at increased risk of diseases if they abuse alcohol (Hoang et al., 2022). In this study, there was no significant difference in combined genotypes of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 polymorphism causing non-acetaldehyde accumulation and low acetaldehyde accumulation. In contrast, a significant difference in combined genotypes causing high acetaldehyde accumulation was found between alcoholics and healthy non-alcoholics groups with p=0.005 (OR=0.47, 95%CI=0.27-0.8). This means that high acetaldehyde accumulation is a protective factor from alcoholism. Our analysis is similar to the results from the previous review (Edenberg and McClintick, 2018). Moreover, a significant difference in combined genotypes between individuals causing high acetaldehyde accumulation is found in non-ALC and ALC groups with p=0.008 (OR=0.48, 95%CI=0.24-0.96). This demonstrated that high acetaldehyde accumulation could be a protective factor from ALC. This result is differ from Chen's report (Chen et al., 2022). Interestingly, there was a significant difference between individuals carrying combined genotypes that do not cause acetaldehyde accumulation when comparing non-ALC and ALC groups with p=0.035 (OR=2.51, 95%CI=1.05-6.03). These combined genotypes causing not acetaldehyde accumulation means slow ethanol and fast acetaldehyde metabolism (Table 3). Based on this result, we suggest that high acetaldehyde accumulation could play a protective role and ethanol exploration may be a risk factor for ALC.

The Child-Pugh score is a system for assessing the prognosis of cirrhosis patients (Peng et al., 2016). We further analyzed the association between genotypes of *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 in ALC patients and Child-Pugh scores. The results showed ALC patients had a severity of cirrhosis status with Child-Push B and no significant difference obtained in each genotype (Table 4). However, combined genotypes showed a decreasing trend in Child-Pugh score from likely phenotype causing non-acetaldehyde accumulation to risk for high acetaldehyde accumulation (Table 5). This means that likely phenotype with high acetaldehyde accumulation had a lower Child-Pugh score, and slow ethanol metabolism or long-term-ethanol exploration caused more severity of cirrhosis status. This result may open the research on ADH polymorphisms' role in causing slow ethanol metabolism.

There are limitations in this study. First, all genotype-phenotype correlations were interpreted due to statistical power and the exact enzyme activity of certain genotypes as well as plasma alcohol level should be investigated in further study. Second, more comprehensive research on larger sample size and diverse ethnicities would substantially provide genetic rick factors of alcohol abuse, which is representative for Vietnamese populations.

In summary, this is the first study on the association between *ADH1B* rs1229984, *ADH1C* rs698, and *ALDH2* rs671 polymorphism with clinical results in alcoholics and ALC patients living in the Northeast region of Vietnam. This study suggested that unique *ALDH2* rs671 and some combined genotypes are associated with alcohol abuse and ALC, *ALDH2**2 allele and acetaldehyde accumulation could play a protecting role while *ALDH2**1 and long term-ethanol exploration could be a risk for causing ALC. The likely phenotype causing high acetaldehyde accumulation had a lower Child-Pugh score which has a lower severity of cirrhosis status. Our result supports useful scientific information that elucidates the association of genetic factors with alcoholism and ALC.

Author Contribution Statement

Conceptualization: Ha Hai Nguyen, Yen Thi Thu Hoang; Data curation: Yen Thi Thu Hoang, Huong Thi Thu Bui, Yen Thi Nguyen, Lan Thi Vu, Quang Viet Nguyen; Formal analysis: Yen Thi Thu Hoang, Huong Thi Thu Bui, Quang Viet Nguyen; Investigation: Yen Thi Thu Hoang, Huong Thi Thu Bui, Yen Thi Nguyen, Lan Thi Vu; Methodology: Ha Hai Nguyen, Huong Thi Thu Bui, Nhung Phuong Vu, Yen Thi Thu Hoang; Supervision: Ha Hai Nguyen; Validation: Ha Hai Nguyen, Ton Dang Nguyen, Huong Thi Thu Bui; Writing of original manuscript: Yen Thi Thu Hoang; Review and editing of manuscript: Ha Hai Nguyen, Nhung Phuong Vu, Ton Dang Nguyen, Yen Thi Thu Hoang.

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General

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Ethical Declaration

This study was approved by ethics committees of the Thai Nguyen General Hospital, Ministry of Health of Viet Nam with the ID 59/HĐĐĐ-BVTWTN

Conflict of Interest

The authors declare no conflict of interest.

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