RESEARCH ARTICLE

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Single Nucleotide Polymorphisms of *ADH1B, ADH1C* and *ALDH2* Genes in 235 People Living in Thai Nguyen Province of Vietnam

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Abstract

Objective: *ADH1B, ADH1C* and *ALDH2* genes are mainly responsible for alcohol metabolism in the body. Several single nucleotide polymorphisms (SNPs) of these genes have been reported to be associated with alcohol dependence and are considered risk factors for various human diseases. This study aims to identify the prevalence of three SNPs of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) in 235 unrelated individuals living in Thai Nguyen province, the northeast region of Vietnam. **Methods:** The target genotypes were identified by using PCR direct sequencing, and their frequencies were compared to previous reports. **Result:** Our data showed that allele frequencies of *ADH1B**2 (rs1229984), *ADH1C**2 C (rs698) and *ALDH2**2 (rs671) were 68.8%, 8.3% and 20.4%, respectively. The *ADH1B**2 and *ADH1C**2 frequencies were similar to those of the Kinh ethnic individuals living in the south region of Vietnam, while the *ALDH2**2 frequency was higher. Compared to data from other countries, *ADH1B**2 frequency is similar to the Philippines (60.5%) and Mongolia (62.9%) but significantly different from the other populations. The *ADH1C**2 frequency is not so different compared to Japanese (5.7%) and Chinese (7.1%) but is quite different in other populations. *ALDH2**2 frequency was lower than Japanese (29.3%), Indonesian (30%) and higher than other countries. Regarding the risk of alcoholism, the percentage of Vietnamese people in this study with genotypes related to alcohol dependence is 8.1%. In contrast, the carrier has genotypes protecting against alcoholism with high frequency, 91.9%. Among them, the individuals can cause high acetaldehyde accumulation accounting for 33.2%. **Conclusion:** This study helps to understand the genetic polymorphisms of alcohol metabolism genes in the community living in Thai Nguyen province, northeast of Vietnam, and provides valuable scientific data relating to alcohol consumption behavior as well as public health protection.

Keywords: *ADH1B- ADH1C- ALDH2-* genetic polymorphisms- Thai Nguyen-Vietnamese

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Introduction

Alcohol abuse is a global problem, constituting the seventh leading risk factor for death and disability (Degenhardt et al., 2018). According to the World Health Organization (2019), global cancer deaths causing alcohol consumption account for 4.2% of all cancers in 2016 (World Health Organization, 2019). In humans, the rate of alcohol elimination depends on various genetic and environmental factors such as sex, age, race, food, biological rhythms, exercise, medication and alcoholism (Cederbaum, 2012). Alcohol is eliminated mainly through pathways consisting of oxidative and non - oxidative metabolism, a small part of alcohol is excreted in the breath (0.7%), urine (0.3%) and sweat (0.1%). Many studies suggested that alcohol metabolism occurs mainly through oxidation in the liver with the participation of alcohol dehydrogenases (ADHs), aldehyde dehydrogenases (ALDHs) (Zakhari, 2006; Cederbaum, 2012). In human, *ADH1B* and *ADH1C* are enzymes that play a major function in alcohol metabolism. For first step, ADHs metabolize alcohol to acetaldehyde, a highly toxic and known as carcinogen. And then, acetaldehyde is broken down into a less toxic compound mainly by *ALDH2* enzyme metabolism (Zakhari, 2006; Cederbaum, 2012; Edenberg and McClintick, 2018).

It is known that polymorphisms of *ADH1B*, *ADH1C* and *ALDH2* genes have impacts on their coding enzyme activities. For instance, *ADH1B**2 allele (rs1229984) is associated a higher alcohol metabolizing activity, compared to the wild type *ADH1B**1 allele (Eng et al.,

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2007; Borinskaya et al., 2009). As the result, people carrying *ADH1B**2/*2 genotype showed elevated acetaldehyde in their blood (Bosron and Li, 1986). In contrast, *ADH1C**2 (rs698) is about 1.5 to 2-fold less active than the ancestor phenotype of *ADH1C**1 (Edenberg and McClintick, 2018). In the case of the *ALDH2* gene, the appearance of one or both *ALDH2**2 allele (rs671) can cause a severe decrease in the enzymatic activity down to 5-20% (Ye et al., 2018).

A large meta-analysis showed that carriers of the *ADH1B**1 and *ADH1C**2 alleles, the less active ethanol metabolizing alcohol dehydrogenase and the highly active *ALDH2**1 allele has increased risk of alcoholism (Cederbaum, 2012). This likely reflects low accumulation of acetaldehyde in these individuals. The association of *ADH1C* with alcohol dependence is less robust than that of *ADH1B*. Recent studies suggest that presence of a single *ADH1B**2 allele strongly reduced the risk alcohol dependence, and in those homozygous for *ADH1B**2, the risk was even further reduced. In addition, people carrying *ALDH2**2 against heavy drinking, alcohol dependence and have high blood acetaldehyde levels (Edenberg and McClintick, 2018). Acetaldehyde may stimulate carcinogenesis by disrupting DNA synthesis and repair, inhibiting DNA methylation by interacting with retinoid metabolism (Seitz and Stickel, 2007; Brooks and Zakhari, 2014). Genetic polymorphisms of genes related alcohol-metabolism pathway result in differences between individuals in exposure to acetaldehyde, leading to possible carcinogenic effects and some diseases in human (Druesne-Pecollo et al., 2009).

Using alcohol and alcoholic beverages is a habit imbued with traditional culture in many countries, including Vietnam. Alcohol is used in daily activities, holidays, festivals, associations, working communication. Alcohol abuse in Vietnam has caused many serious consequences for public health, family happiness, social safety and order. According to Luu and Nguyen (2018), Vietnam is currently assessed as a country with high alcohol consumption in Southeast Asia, only after Thailand. The search interviewed 5,200 people in 12 provinces/cities representing regions of Vietnam and showed that the average amount of alcohol used by Vietnamese person is relatively large, nearly 60% of surveyed people using alcohol, nearly 50% of men who are using alcohol at a moderate or more and 8% at the level of addiction or heavy addiction. In terms of perceived physical health, the majority of Vietnamese interviewed subjectively believed that drinking alcohol was healthier. In terms of perceived mental health, the notion that drinking alcohol helps relieve nervous tension is still common (Luu and Nguyen, 2018). Studies on gene encoding alcohol metabolism enzymes in Vietnamese are still limited. Iron et al (1992) studied *ADH1B* gene polymorphism (rs1229984) in 42 Vietnamese people and showed *ADH1B**1 and *ADH1B**2 accounted for 77.4% and 22.6%, respectively (Iron et al., 1992). Mutant allele frequencies of *ADH1B**2, *ADH1C**2 and *ALDH2**2 in 124 Kinh ethnic individuals living in Ho Chi Minh city, the south of Vietnam are 64.6%, 8.1% and 13.6%, respectively (Edenberg and McClintick, 2018). Thai Nguyen province belongs to the northeast of Vietnam, there are many ethnic minorities living here which using alcohol in daily activities and encouraging each other to drink in quantity. This study aimed to detect popularity of 3 SNPs *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) in Vietnamese group living in Thai Nguyen province, Vietnam. The obtained data would be oriented basis for screening genetic identifying individuals at risk of diseases to provide appropriate lifestyle and clinical advice.

Materials and Methods

Subjects

This study recruited 235 unrelated healthy volunteers from January to June of 2021 at Thai Nguyen General Hospital of Viet Nam (age from 20 to 77, 134 males and 101 females), including 107 young voluntary blood donors and 128 people for annual health checkups. The purpose of study was explained to all individuals and their informed consents were obtained before sample collection. 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.

DNA extraction

The peripheral blood of each volunteer (1ml) was obtained and placed in an EDTA tube and stored at -80 ^{o}C before DNA extraction. Then, genomic DNA was extracted from 200 µl subject's peripheral blood using ExgeneTM Blood SV mini Kit (GeneAll Biotechnology Co. LTD, Seoul, Korea) according to manufacturer's protocol and stored at -20^{o}C.

PCR and Sanger sequencing

Primers for PCR and sequencing of the *ADH1B* (exon 3), *ADH1C* (exon 8) and *ALDH2* (exon 12) were designed according to reference sequences in the GeneBank with accession NG_011435.1; NC_000004.12; NG_012250.2, respectively (Table 1). All primers were synthesized and provided by PHUSA Biochem, Can Tho, Vietnam. PCR reactions were carried out in a 25 µL final volume including 12.5µL Taq 2x Master mix (BioLabs, England); 0.5 µL of each PCR primer (10 µmol/L); 0.5 – 1.0 µL of template DNA (20 - 30 ng total genomic DNA); and deionized water. The thermo cycle was as following: denaturation at 95 °C for 3 min, following by 35 cycles of 95 °C for 45s, 56-60°Cfor 30-45s, 72°C for 30s and a final extension at 72 °C for 5 min. The obtained amplicons were purified using MultiScreen PCR filter plate (Merck Millipore, Darmstadt, Germany). The purified PCR products were sequenced using ABI Prism BigDye Terminator Cycle Sequencing Kit Version 3.1 (Applied Biosystems), on an ABI genetic analyzer 3500 (Applied Biosystems).

Sequence and statistical analysis

The sequencing data were analyzed by comparison with the standard sequences of the *ADH1B*, *ADH1C* and *ALDH2* gene (NG_011435.1; NC_000004.12; NG_012250.2) using BioEdit (Raleigh, NC) sequence alignment editor software.

Allele and genotype frequencies were computed using

gene counting methods. Chi Square ($\chi 2$) and Fisher-exact test were used to assess the Hardy–Weinberg equilibrium for each variant and the differences of allele frequencies between Vietnamese populations in this study and other populations, $p < 0.050$ was considered as statistically significance.

Results

*Genotype and allele frequencies of ADH1B*2 (rs1229984), ADH1C*2 (rs698) and ALDH2*2 (rs671)*

The genotypes of each SNPs were presented in the Figure 1 and their frequencies were calculated as showed in the Table 2. For *ADH1B* gene, rs1229984 has homozygous mutant genotype *2/*2 with the highest frequency 47.2% (n=111), heterozygous mutant genotype *1/*2 ranks second with 42.6% frequency (n=100), the homozygous wild-type genotype *1/*1 has the lowest frequency of 10.2% with 24 cases, the number of individuals carrying at least one mutant allele *ADH1B*2 account for 89.8%. Based on the genotype frequency of the study samples, we have determined the variant allele frequency of *ADH1B*2 is 68.5%, the variant *ADH1B*2 has higher twice frequency of *ADH1B*1 (31.5%). For *ADH1C* gene, rs678 polymorphism has homozygous wild-type genotype *1/*1 with the highest frequency 83.8% (n=197), the homozygous mutant genotype *2/*2 has the lowest frequency of 0.4% (n= 1). However, variant allele frequency of *ADH1C*2 is 8.3% because heterozygous mutant genotype *1/*2 accounts 15.8% (n=37). Therefore, *ADH1C*2 allele is eleven times lower than *ADH1C*1 (91.7%). For *ALDH2* gene, homozygous wild-type genotype *1/*1 with the highest frequency 63.8% (n=150), heterozygous mutant genotype *1/*2 ranks second with 31.5% frequency (n=74), the homozygous mutant genotype *2/*2 has the lowest rate of 4.7% with 11 cases, the number of individuals carrying at least one mutant allele *ALDH2*2 account for 36.2%, variant allele frequency of *ALDH2*2 is 20.4%, *ALDH2*1 allele frequency is nearly four times higher than *ALDH2*2 allele (79.6%). Besides, allele and genotype frequencies of *ADH1B*, *ADH1C* and *ALDH2* did not deviate the Hardy-Weinberg equilibrium ($p>0.050$).

*Allele frequencies of ADH1B*2, ADH1C*2 and ALDH2*2 in comparison with other populations*

Mutant alleles of *ADH1B*2, *ADH1C*2, and *ALDH2*2 identified in the study population were compared with reported data from different geographical areas. As shown in Table 3, the frequency of the *ADH1B*2 allele was relatively high, ranking 4th out of 39 compared to other populations. This result was comparable to the Philippines (60.5%), Mongolia (62.9%), and the Vietnamese Kinh ethnic group (64.6%) with a p-value >0.050 and significantly higher than other populations with a p-value <0.001, except Malays with 0.010< p-value <0.050). The

Table 1. Primers Used for *ADH1B* (exon 3), *ADH1C* (exon 8) and *ALDH2* (exon 12) Amplification and Sequencing.

Gene region	Forward primer (5′–3′)[a]	Reverse primer (5′–3′)[b]	Fragment size (bp)
ADH1B exon 3	GGCTTTAGACTGAATAACCTTGGG	GGGAAAGAGGAAACTCCTGAAGTC	458
ADH1C exon 8	TTATCCTCAGTTATAGCAGTCTGG	CATCCTTCCACCTTTTCATTCTC	364
ALDH2 exon 12	CCAAGAGTGATTTCTGCAATCTCG	CAGGTCCTGAACTTCCAGCAG	312

[a,b], These primers used for sequencing

Table 2. Allele and Genotype Frequencies of *ADH1B* (rs1229984), *ADH1C* (rs698) and ALDH2 (rs671)

Gene	Polymorphism	Nucleotide change	Protein change	Genotypes and alleles	N	Frequencies		HWP value	$\chi 2$
						Observed frequency (%)	Expected frequency (%)		
ADH1B exon 3	rs1229984	c.254G>A	p.Arg48His	*1/*1	24	10.2	10.1	0.989	0.027
				*1/*2	100	42.6	43.3		
				*2/*2	111	47.2	46.6		
				*1	148	31.5			
				*2	322	68.5			
ADH1C exon 8	rs698	c.1418A>G	p.Ile350Val	*1/*1	197	83.8	84.1	0.914	0.179
				*1/*2	37	15.8	15.2		
				*2/*2	1	0.4	0.7		
				*1	431	91.7			
				*2	39	8.3			
ALDH2 exon 12	rs671	c.1606G>A	p.Glu487Lys	*1/*1	150	63.8	63.3	0.946	0.112
				*1/*2	74	31.5	32.5		
				*2/*2	11	4.7	4.2		
				*1	374	79.6			
				*2	96	20.4			

Table 3. Comparison of *ADH1B**2, *ADH1C**2 and *ALDH2**2 Frequencies in Vietnamese Individuals and Previous Study Populations.

Population	*ADH1B**2		*ADH1C**2		*ALDH2**2		References
	N	%	N	%	N	%	
Asian							
Vietnamese	235	68.5	235	8.3	235	20.4	present study
	42	22.6***	-	-	-	-	(Iron et al., 1992)
Vietnamese (Kinh)	124	64.6	124	8.1	124	13.6*	(Coriell Institute for medical research, 2008; Edenberg and McClintick, 2018)
Filipinos	57	60.5	-	-	86	0.6***	(Goedde et al., 1992)
Malays	65	59.2*	-	-	73	3.4***	
Thais	111	33.3***	-	-	111	5.0***	
Laos	1050	33.7***	-	-	-	-	(Li et al., 2007)
Indonesian	-	-	-	-	100	30.0**	(Septelia, 2002)
Chinese	480	77.5***	-	-	-	-	(Guo et al., 2008)
	-	-	105	7.1	-	-	(Chao et al., 2000)
Chinese (Han, Hui, Tibetan, Mongolian, Uygur)	584	38.6***	584	12.4*	584	9.2***	(Wei et al., 2018)
Japanese	2299	78.2***	-	-	-	-	(Matsuo et al., 2006)
	-	-	465	5.7	-	-	(Shimizu et al., 2013)
	-	-	-	-	1944	29.3***	(Yoshimasu et al., 2014)
Koreans	177	80.5***	-	-	-	-	(Goedde et al., 1992)
	-	-	-	-	481	15.9*	(Lee et al., 1997)
	-	-	100	13.5*	-	-	(Kim et al., 2004)
Mongolian	35	62.9	-	-	35	8.6***	(Shen et al., 1997)
Uzbeks	161	28.6***	-	-	161	1.6***	(Ahn et al., 2009)
Kirghizia	102	33.3***	-	-	-	-	(Borinskaya et al., 2009)
Tadjikistan	90	28.4***	-	-	-	-	
Kazakhstan	64	19.5***	-	-	-	-	
Indians	167	9.9***	-	-	179	2.0***	(Goedde et al., 1992)
	-	-	100	33***	-	-	(Singh et al., 2015)
Sri Lankan	128	5.0***	-	-	-	-	(Coriell Institute for medical research, 2008; Edenberg and McClintick, 2018)
Pakistan	81	15.9***	-	-	-	-	(Li et al., 2007)
Armenia	39	10.3***	-	-	-	-	(Borinskaya et al., 2009)
Iranians	41	24.4***	-	-	-	-	
European							
Turkism	211	16.1***	-	-	211	0.0***	(Kayaalti and Soylemezoglu, 2010)
	-	-	80	55***	-	-	(Vatansever et al., 2015)
Finnish	85	1.2***	-	-	85	0***	(Goedde et al., 1992)
Hungarians	115	5.2***	-	-	117	1.3***	
Germans	233	4.1***	-	-	233	0***	
Swedish	90	0.6***	-	-	90	0***	
Polish	54	5.6***	-	-	54	0***	(Cichoż-Lach et al., 2006)
	172	5.5***	172	59.3***	-	-	(Cichoż-Lach et al., 2010)
Spanish	255	7.6***	-	-	-	-	(García-Martín et al., 2010)
	-	-	255	44.7***	255	0***	(Vidal et al., 2004)
Russians	23	8.7***	-	-	23	0***	(Ahn et al., 2009)
Crezch Gypsies/Roma	285	7.4***	-	-	-	-	(Hubáček et al., 2018)
Czech/Slavic	286	5.9***	-	-	-	-	
Caucasians	-	-	131	45***	-	-	(Foley et al., 2004)
American							
Mexican American	104	4.3***	104	50***	104	0***	(Konishi et al., 2003)
Mexican Huichols	97	0***	-	-	97	0.5***	(Gordillo-Bastidas et al., 2010)
Mexican Mestizo	218	3.4***	-	-	227	0***	

Table 3. Continued

Population	*ADH1B**2		*ADH1C**2		*ALDH2**2		References
	N	%	N	%	N	%	
American							
Colombian	148	8.4***	148	27.7***	148	0***	(Méndez and Rey, 2015)
African							
Africans	37	0***	-	-	49	0***	(Goedde et al., 1992)
Niger	45	12.2***	-	-	-	-	(Iron et al., 1992)
African-Trinidadian	-	-	45	12.2	-	-	(Montane-Jaime et al., 2006)
Other							
Australia	-	-	-	-	37	0***	(Goedde et al., 1992)
Jewish	108	18.5***	-	-	-	-	(Neumark et al., 2004)
American Jewish	-	-	-	-	129	8.1***	(Fischer et al., 2007)

N, number of subjects; *p < 0.05; *p < 0.01; ***p < 0.001

frequency of the *ADH1C**2 allele ranked 11th of 14 in the comparison populations. There was no significant difference compared to the Japanese (5.7%), Chinese (7.1%), and Kinh ethnic group of Vietnam frequency (8.1%) with p-value>0.050 but significant difference in other populations with p-value <0.001). Lastly, the *ALDH2**2 allele with a high frequency (20.4%) ranked 3rd out of 27 comparison populations, which was statistically higher than those observed from the remaining population except Japanese (29.3%) and Indonesian (30%) with

Table 4. Genotypes Frequency of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) Polymorphisms in Combination.

Genotype			N	(%)	Phenotype	
ADH1B (rs1229984)	*ADH1C* (rs698)	*ALDH2 (rs671)*	235	100	Likely phenotype	%
*1/*1	*1/*1	*1/*1	14	6	Increasing risk of alcoholism/risk for alchohol dependence	8.1
*1/*1	*1/*2	*1/*1	5	2.1		
*1/*1	*2/*2	*1/*1	0	0		
*1/*1	*1/*1	*1/*2	3	1.3	Reducing the risk for alcoholism/protecting against alcoholism	58.7
*1/*1	*1/*2	*1/*2	2	0.9		
*1/*1	*2/*2	*1/*2	0	0		
*1/*2	*1/*1	*1/*1	43	18.2		
*1/*2	*1/*2	*1/*1	16	6.8		
*1/*2	*2/*2	*1/*1	1	0.4		
*2/*2	*1/*1	*1/*1	71	30.2		
*2/*2	*1/*2	*1/*1	2	0.9		
*2/*2	*2/*2	*1/*1	0	0		
*1/*1	*1/*1	*2/*2	0	0	Reducing the risk for alcoholism/protecting against alcoholism/ higher acetaldehyde accumulation	33.2
*1/*1	*1/*2	*2/*2	0	0		
*1/*1	*2/*2	*2/*2	0	0		
*1/*2	*1/*1	*1/*2	23	9.8		
*1/*2	*1/*1	*2/*2	5	2.1		
*1/*2	*1/*2	*1/*2	9	3.8		
*1/*2	*1/*2	*2/*2	3	1.3		
*1/*2	*2/*2	*1/*2	0	0		
*1/*2	*2/*2	*2/*2	0	0		
*2/*2	*1/*1	*1/*2	35	14.9		
*2/*2	*1/*1	*2/*2	3	1.3		
*2/*2	*1/*2	*1/*2	0	0		
*2/*2	*1/*2	*2/*2	0	0		
*2/*2	*2/*2	*1/*2	0	0		
*2/*2	*2/*2	*1/*2	0	0		

N, number of subjects

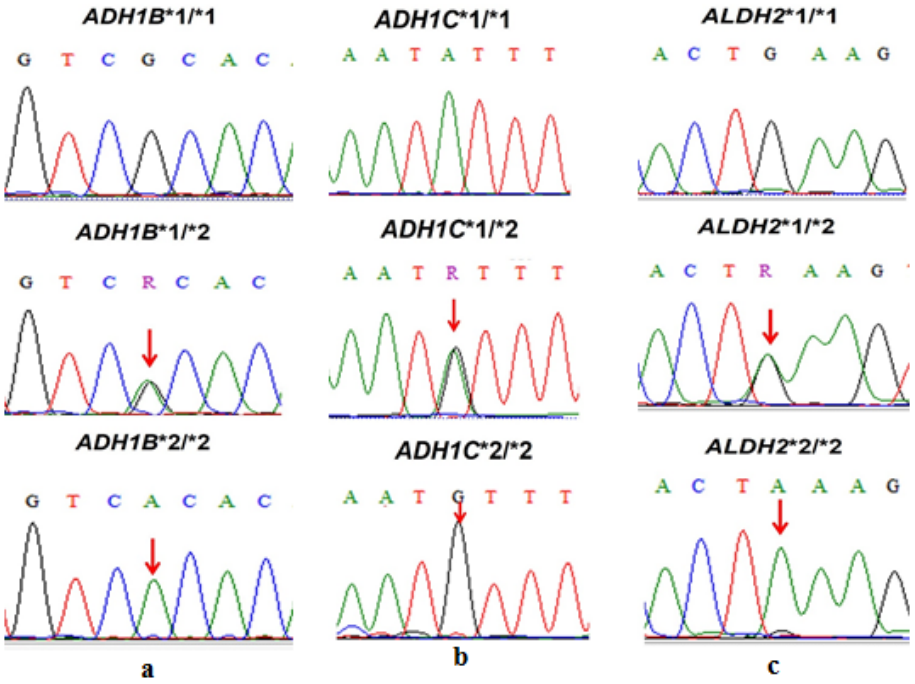

Figure 1. Sequence of 3 SNPs rs1229984 *(ADH1B)*, rs698 *(ADH1C)* and rs671 *(ALDH2)* (a) rs1229984 (*ADH1B**1/*1: homozygous for the *ADH1B* p.Arg48; *ADH1B**1/*2: heterozygous for the *ADH1B* p.Arg48His; *ADH1B**2/*2: homozygous for the *ADH1B* p.His48); (b), rs698 (*ADH1C**1/*1: homozygous for the *ADH1C* p.Ile350; *ADH1C**1/*2: heterozygous for the *ADH1B* p.Ile350Val; *ADH1C**2/*2: homozygous for the *ADH1C* p.Val48); (c), rs671 (*ALDH2**1/*1: homozygous for the ALDH2 p.Glu487; ALDH2*1/*2: heterozygous for the ALDH2 p.Glu487Lys; *ALDH2**2/*2: homozygous for the ALDH2 p.Lys487)

p-value < 0.050, p-value < 0.010 and p-value < 0.001, respectively.

Combination of ADH1B, ADH1C and ALDH2 genotypes in Vietnamese individuals

Data analysis reports of Cederbaum (2012) and Edenberg (2018) showed that individuals with different alleles of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms express likely phenotype such as increacing or reducing risk for alcholism and exposuring to acetaldehyde, leading to possible carcinogenic effects and some diseases in human (Cederbaum, 2012; Edenberg and McClintick, 2018). Individuals in this study carry *ADH1C* genotype without any *ADH1B**2 and *ALDH2**2 relating to drinking habits that lead to risk for alcoholism with low frequency (8.1%). In contrast, people carry genotypes at least an allele *ADH1B**2 or *ALDH2**2 protecting against alcoholism accounts for high frequency, 91.9%. Individuals protect against alcoholism can cause high acetaldehyde accumulation accounting for 33.2%. Some genotype combinations of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms have not been appeared in this studying population (Table 4).

Discussion

Polymorphism of alcohol metabolizing genes in Vietnamese was interested since 1992, in a report about the frequency of *ADH1B**2 (rs1229984) in Vietnamese Orientals (Iron et al., 1992). Later, a study on the frequencies of *ADH1B**2, *ADH1C**2, and *ALDH2**2 alleles in the Kinh individuals living in Ho Chi Minh City was reported (Edenberg and McClintick, 2018). However, neither data of people living in the north of Vietnam nor northeastern areas like Thai Nguyen were shown so far. This study identified the prevalence of three SNPs relating to alcohol metabolism activity in Thai Nguyen residents. With the identified frequencies of *ADH1B**2, *ADH1C**2 and *ALDH2**2, all genotypes and alleles examined in this study were in Hardy-Weinberg equilibrium. This proves that those alleles' frequency has been inherited stably in the population and the study subjects represent Vietnamese people. The frequency of *ADH1B**2 was similar to data reported by Edenberg (Edenberg and McClintick, 2018), but significantly higher than that found by Iron (p<0.001) (Iron et al., 1992). This difference is possibly due to the small number of subjects investigated. Besides that, there is a significant difference in *ALDH2**2 frequency in our study in comparison with Edenberg's analysis (Edenberg and McClintick, 2018) (p<0.050). Subjects in our study were people living in Thai Nguyen province. In addition to the Kinh which is the major ethnic group of Vietnam, there are also other ethnic minorities co-living here, such as Tay, Mong, Nung and Dao...(United Nations Population Fund in viet nam, 2011). Therefore, we suggest that the difference in *ALDH2**2 allele frequency in our study compared with the Kinh group may be due to the mixed ethnic background of the current study subjects. The similar figure was previously found when comparing allelic frequencies of *ADH1B**2, *ADH1C**1 and *ALDH2**2 among different Chinese ethnic groups as shown in the study of Wei et al in Chinese people (Wei et al., 2018). Given the diversity of ethnicity in Vietnam, it

is necessary to expand the study of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms not only in Kinh people but also in other ethnic minority groups in Vietnam. The comparing result showed that *ADH1B**2, *ADH1C**2 and *ALDH2**2 allele frequency exhibit differences between populations in Asia, Europe, Africa and other continents. However, these mutant variants have significant differences between regions of each continent, even between countries in each area. *ADH1B**2 allele frequency was shown to be common in parts of Asia comparing other continents, especially, selection distribution in East Asia and following in Southeast Asia. And *ADH1C**2 has a significantly lower frequency in Asia and Africa, higher in West Asia, America and Europe. Whereas, *ALDH2**2 was detected only in Asian and Jewish populations, almost absent in the rest of the continents (Table 3). Our comparing analysis is also similar to results from previous reviews (Cederbaum, 2012; He et al., 2015; Vatansever et al., 2015; Yokoyama et al., 2015; Edenberg and McClintick, 2018).

Many studies around the world have shown that people carrying *ADH1B**2 strongly reduce the risk of alcoholism, even further decrease in those homozygous for *ADH1B**2 and independent of that of *ALDH2**2. The presence of unique *ALDH2**2 was suggested protective against heavy drinking and alcoholism. Meanwhile, *ADH1C**2 with lower alcohol metabolism activity compared with *ADH1C**1, which tends to be inherited together with *ADH1B**2 and has a protective role against alcoholism (Edenberg and McClintick, 2018). Genetic variants of *ADH1B**2, *ADH1C**1 and *ALDH2**2 were not only involved in alcoholism and behavioral disorders but also associated with alcohol relating diseases. In fact, these alleles protect some people from alcoholism but can cause alcohol-related health problems (Zakhari, 2006; National Institute on Alcohol Abuse and Alcoholism, 2007; Cederbaum, 2012; Hurley and Edenberg, 2012). In total, two combined genotypes that were previously reported to associate with alcohol dependence and protection against alcoholism were 8.1% and 91.9%. Individuals carry *ADH1C* genotype with at least one *ADH1B**2 and *ALDH2**2 causing high acetaldehyde accumulation accounting for 33.2% (Table 4).

Many reports have been conducted to elucidate the relationship between these SNPs and human diseases. A meta-analysis demonstrated that rs1229984 polymorphism may be an important protective factor for alcoholic liver cirrhosis, especially for Asians (He et al., 2015). The *ADH1B**2/*2 genotype was significantly more prevalent in the alcohol-related group than in the alcohol-unrelated group in patients with symptomatic Brugada syndrome (Wu et al., 2019) and significantly decreased the odds of lung cancer and developing hemorrhagic stroke than other genotypes (Govind et al., 2020; Lin et al., 2021). For the *ADH1C* gene, *ADH1C**1/*1 individuals consuming alcohol are at higher risk for alcoholic pancreatitis than *ADH1C**1/*2 and *ADH1C**2/*2 genotypes (Vatansever et al., 2015). The *ALDH2**2 was related to ischemic stroke risk and may be a risk factor for this condition. The *ALDH2**1/*1 genotype was more frequently in an alcoholic population and patients with liver diseases, meanwhile *ALDH2**1/*2 was associated with a decreased risk of alcoholic liver cirrhosis comparing to *ALDH2**1/*1 (Wang et al., 2020). With the relatively high prevalence of *ADH1B**2/*2, *ADH1C**1/*1, *ALDH2**1/*2 and *ALDH2**2/*2 in study subjects, they are predicted to be at increased risk of diseases if they abuse alcohol. According to Micu et al., (2019), in addition to ethanol-metabolizing enzymes involved in alcohol abuse leading to alcohol dependence, there are also psychological and social factors. Vietnam is currently assessed as a country with high alcohol consumption, especially among men. Social factors greatly influence the alcohol abuse of Vietnamese because drinking alcoholic beverages has become a cultural feature of Vietnamese used in daily life and public relations. On the other hand, Luu and Nguyen, (2018) showed that Vietnameses have misconceptions when using alcoholic beverages. Most Vietnamese interviewed subjectively believe that drinking alcohol is healthier, but the concept of drinking alcohol to relieve nervous tension is still common. From the research and analysis results, we suggested that Vietnamese will have a very high risk of accumulating acetaldehyde, causing serious health and behavioral problems if they abuse alcohol. Therefore, it is necessary to change lifestyle to avoid drinking too much alcohol and further study these genetic polymorphisms in alcohol-related diseases in Vietnamese, contributing to elucidating the influence of these polymorphisms.

This is the first study on the polymorphism of *ADH1B*, *ADH1C* and *ALDH2* (rs1229984, rs689, rs671) in Vietnamese people living in Thai Nguyen, the northeast region of Vietnam. Our result supports useful scientific information for genetic screening strategies in identifying individuals at risk of diseases and giving appropriate lifestyle as well as clinical advice.

Author Contribution Statement

Conceptualization: Ha Hai Nguyen, Yen Hoang Thi Thu. Data curation: Yen Hoang Thi Thu, Nhung Phuong Vu, Huong Bui Thi Thu, Yen Thi Nguyen, Hai Dinh Nguyen, Anh Thi Phuong Le. Formal analysis: Yen Hoang Thi Thu, Nhung Phuong Vu. Investigation: Yen Hoang Thi Thu, Huong Bui Thi Thu, Yen Thi Nguyen, Hai Dinh Nguyen, Anh Thi Phuong Le. Methodology: Ha Hai Nguyen, Yen Hoang Thi Thu. Supervision: Ha Hai Nguyen. Validation: Nhung Phuong Vu, Huong Bui Thi Thu. Writing of original manuscript: Yen Hoang Thi Thu. Review and editing of manuscript: Ha Hai Nguyen, Nhung Phuong Vu, Yen Hoang Thi Thu.

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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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