Association between Aldehyde Dehydrogenase 2 Glu504Lys Polymorphism and Alcoholic Liver Disease

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ABSTRACT

Background: Only a subset of patients with excessive alcohol use develop alcoholic liver disease (ALD); though the exact mechanism is not completely understood. Once ingested, alcohol is metabolized by 2 key oxidative enzymes, alcohol (ADH) and aldehyde dehydrogenase (ALDH). There are 2 major ALDH isoforms, cytosolic and mitochondrial, encoded by the aldehyde *ALDH1* and *ALDH2* genes, respectively. The *ALDH2* gene was hypothesized to alter genetic susceptibility to alcohol dependence and alcohol-induced liver diseases. The aim of this study is to determine the association between aldehyde dehydrogenase 2 (rs671) glu504lys polymorphism and ALD.

Methods: *ALDH2* genotype was performed in 535 healthy controls and 281 patients with ALD.

Results: The prevalence of the common form of the SNP rs671, 504glu (glu/glu) was significantly higher in patients with ALD (95.4%) compared to that of controls (73.7%, p<0.0001). Among controls, 23.7% had heterozygous (glu/lys) genotype when compared to 4.6% in those with ALD (OR 0.16, 95%CI 0.09-0.28). The allele frequency for 504lys allele in patients with ALD was 2.3%; compared to 14.5% in healthy controls (OR 0.13, 95%CI 0.07-0.24).

Conclusions: Patients with *ALDH2* 504lys variant were less associated with ALD compared to those with *ALDH2* 504glu using both genotypic and allelic analyses.

INTRODUCTION

Excessive alcohol drinking is one of the most significant risk factors for health problems such as injuries, liver diseases, and cancer[1]. Drinking becomes excessive when it causes or elevates the risk for alcohol-related problems or complicates the management of other health problems. According to the National Institute on Alcohol Abuse and Alcoholism (NIAAA), excessive drinking is defined as men who drink more than 4 standard drinks in a day (or more than 14 per week) and women who drink more than 3 drinks in a day (or more than 7 per week)[2]. Alcoholic liver disease (ALD) is a major adverse health event resulting from excessive drinking. Its pathogenesis is a multi-step process consisting of a series of histopathological changes[3]. More than 90% of drinkers develop alcoholic steatosis which is reversible upon abstinence[4]. However, if excessive alcohol use continues, the disease may progress to alcoholic hepatitis, advanced fibrosis, and alcoholic cirrhosis in up to 10-15% of heavy drinkers[3]. It is completely unknown why only a subset of excessive alcohol drinkers develops ALD.

Once ingested, more than 90% of alcohol is eliminated via metabolic degradation in the liver into acetaldehyde, mainly by alcohol dehydrogenase enzyme (ADH)[5]. Acetaldehyde is subsequently converted by aldehyde dehydrogenases (ALDH) to acetate, which is released from the liver and metabolized by the heart and muscle[5]. The rate of alcohol metabolism by ADH and ALDH is critical in determining its toxicity because the intermediate, acetaldehyde, is potentially toxic[5]. There are 2 major ALDH isoforms, cytosolic and mitochondrial, encoded by the aldehyde *ALDH1* and *ALDH2* genes, respectively. The *ALDH2* gene was hypothesized to alter genetic susceptibility to alcohol dependence and alcohol-induced liver diseases. Between both isoforms, mitochondrial ALDH2 plays the central role in human acetaldehyde metabolism because of its submicromolar K_m for acetaldehyde[5].

The *ALDH2* gene is polymorphic and the variants demonstrate the vital role of ALDH2 activity in alcohol oxidation. A single nucleotide polymorphism (SNP) at exon 12 predicts lysine at residue 504 instead of glutamic acid[6]. The common form of the SNP (rs671) (504glu) encodes the glu (G) allele (previously referred to as the ALDH2 *1 allele); the 504lys (A, formerly ALDH2 *2 and 487lys) allele produces a catalytically inactive isozyme and limits its activity to metabolize acetaldehyde[6, 7]. As a

result, subjects with the lys allele have a reduced capacity to eliminate acetaldehyde and typically have unpleasant side effects such as flushing, nausea, or vomiting after alcohol consumption[8, 9]. In fact, the peak blood acetaldehyde concentration after alcohol consumption is 6- and 19-fold higher in heterozygotes or homozygotes for 504lys allele than that in common allele individuals [10]. It is therefore plausible that those with this allele could have decreased risk of excessive alcohol usedue to adverse reactions from drinking, and subsequently, this allele could influence the risk of alcohol-related diseases such as ALD. To address this question, we performed a single center study in a well characterized cohort of Chinese patients to determine the association between ALDH2 variants and ALD.

METHODS

Human subject cohort

The study was performed at the Beijing 302 hospital; a large tertiary care center specialized in the treatment of liver diseases. The study was conducted in accordance with the guidelines set by the Declaration of Helsinki. Written informed consent was obtained from each participant and the study was approved by the Ethics Committee of the Beijing 302 hospital. Five hundred and thirty-five healthy men without history of excessive alcohol use or other causes of chronic liver diseases with normal hepatic panel seen at an outpatient clinic for routine health screening were enrolled. Two hundred and eighty one patients with alcoholic cirrhosis and no known history of hepatitis B or C infection were recruited from the Center for Diagnosis and Treatment of Non-infectious Liver Disease between June 2013 and January 2015. These cases were age and gender-matched to healthy controls. Alcoholic cirrhosis patients had history of alcohol consumption averaging at least 80 g per day (for men) or 50 g per day (for women), for at least 10 years [11]. The diagnosis of cirrhosis was made by radiographic imaging or clinical presentation of portal hypertension such as hepatic encephalopathy, ascites or the presence of esophageal varices on upper gastrointestinal endoscopy with exclusion of other known causes of chronic liver diseases such as hepatitis B or C and autoimmune liver diseases.

Data and biosample collection

All subjects completed self-administered questionnaires regarding history of alcohol consumption. Demographic data, medical history, and clinical characteristics were collected. Baseline laboratory tests were obtained and blood was collected from venipuncture and stored at -80°C until DNA extraction.

Extraction of genomic DNA

Genomic DNA was extracted using QIAamp DNA Blood Mini Kit (Qiagen, New York, USA). The concentration of DNA was quantified by a Nanodrop 1000 UV-Vis spectrophotometer. All PCRs were performed using 1µl of genomic DNA (1-10ng) in a final volume of 5 µl according to Custom Taqman® SNP genotyping assays kits for ALDH2 (ABI, Foster City, California, USA) with LightCycler® 480 Type II System (Roche, Basel, Switzerland). The forward and reverse primer sequence of *ALDH2* was 5'-TTTGGTGGCTAGAAGATGTC-3', and 5'-CACACT CACAGTTTTCTCTT-3', respectively. PCR conditions were set as follows: 94 °C (30 seconds), 57 °C (30 seconds), 72 °C (30 seconds) for a total of 40 cycles.

Statistical analysis

Basic descriptive statistics, including mean, standard deviations (SD), and percentages were used. Chi-square test and Student's t-test were used for comparison between groups for categorical and continuous variables, respectively. A *P* value of <0.05 was considered as statistical significance. All analyses were performed with SPSS 16.0 for Windows (SPSS Chicago, Illinois, USA).

RESULTS

Demographic and clinical characteristics of the study cohort

The detailed characteristics of subjects with ALD are summarized in **Table 1**. The mean age of patients with ALD was 49 years which was comparable to that of controls (48 years). The average duration of drinking was 21.5 years with daily drinking on the average of 95 grams.

The prevalence of ALDH2 variants in healthy controls and patients with ALD

We found that the prevalence of the common form of the SNP rs671, 504glu (glu/glu) was significantly higher in patients with ALD (95.4%) compared to that of controls (73.7%, p<0.0001). Among controls, 23.7% had heterozygous (glu/lys) genotype when compared to 4.6% in those with ALD (p<0.0001, OR 0.16, 95%CI 0.09-0.28). None of the patients with ALD had homozygous lys/lys genotype; when compared to 2.6% among controls (p=0.05). The allele frequency for 504lys allele in patients with ALD was 2.3%; compared to 14.5% in healthy controls (p<0.0001, OR 0.13, 95%CI 0.07-0.24, **Table 2**).

Clinical characteristics of ALD patients stratified by ALDH2 variants

We next determine the alcohol consumption history as well as clinical characteristics of ALD patients stratified by *ALDH2* variants (**Table 3**). ALD patients with heterozygous (glu/lys) genotype had shorter duration of drinking before the enrollment (17.3±9.3 years) than those with glu/glu genotype (22.5±10.6 years), though the difference was not statistical significance (p=0.09). However, that daily alcohol consumption was significantly lower in patients with heterozygous genotype (50.0±28.5 vs. 128.7±56.2 grams, p=0.001). While most laboratory tests were comparable between both groups, ALD patients with heterozygous (glu/lys) genotype had lower mean corpuscular volume (90.5±8.3 vs. 99.1±59.5 fl, p=0.04), lower total bilirubin (1.6±0.1 vs. 4.8±1.1 mg/dl, p=0.006), and lower alkaline phosphatase (101.6±55.9 vs. 155.2±112.4 U/L, p=0.02) than those with glu/glu genotype.

Clinical characteristics of ALD patients with similar quantity of alcohol consumption stratified by ALDH2 variants

The development of ALD depends on the quantity and quality of alcohol consumption. As shown in **Table 3**, the quantity of alcohol consumption was significantly lower in those with heterozygous genotype. To further assess clinical characteristics of ALD patients stratified by *ALDH2* variants adjusting for the quantity of alcohol consumption, we randomly selected patients with glu/glu genotype (n=66) with the duration and daily alcohol consumption matched to those with glu/lys genotype (n=11, **Table 4**). We found that patients with glu/lys genotype had lower total bilirubin level (1.6±0.9 vs. 3.9±3.8 mg/dl,

p=0.01) and alkaline phosphatase (96.6±53.4 vs. 163.0±100.6 U/l, p=0.03)

DISCUSSION

The major findings in our study is that patients with *ALDH2* 504lys variant were less associated with ALD compared to those with *ALDH2* 504glu using both genotypic and allelic analyses.

A single nucleotide polymorphism on *ALDH2* gene affects its enzymatic activity and its ability to metabolize acetaldehyde into acetate after alcohol ingestion[5, 6, 12]. The lys allele plays an important role in regulating the ALDH2 activity, for instance, the reduction in ALDH2 activity in patients with heterozygous (glu/lys) genotype is more than 100-fold compared with that of glu/glu homozygotes[5, 6]. Hepatic ALDH2 activity is almost non-detectable in heterozygous gly/lys and homozygous lys/lys patients[12]. Thus, the accumulation of acetaldehyde commonly occurs in patients with lys allele after alcohol consumption leading to vasodilation, facial flushing, tachycardia, nausea, and vomiting[8, 9]. It is postulated that this allele protects against excessive alcohol use or alcoholism because of unpleasant symptoms secondary to acetaldehyde accumulation. Several studies demonstrated a reduced frequency of the lys allele in alcoholics compared with to non-alcoholics[13-15]. Individuals with the lys allele have a 10-fold reduction in the risk of alcohol dependence[13], drink less alcohol, and have a lower prevalence of binge drinking[16, 17]. In our study, we also found that ALD patients with lys allele had significantly lower quantity of alcohol consumption compared to those with homozygous glu/glu genotype.

Previous studies have shown that the inactive ALDH2 504lys allele occurred mainly in Asian populations[5, 13]. Most alcoholics with inactive ALDH2 alleles were heterozygous while homozygous lys/lys was rarely found in the alcoholic subjects, although it was often observed in controls[6]. These findings are similar to what we observed in our study. The allele frequency for 504lys among patients with ALD is around 1.0%-11.6%, depending on the population being studied[6]. In this report, the overall frequency of this allele in our patients is 2.3%.

We found that patients with ALDH2 504lys are protected against alcoholic liver disease. Our findings are in accordance with previous reports[6, 18, 19]. It is plausible that this is the consequence of the reduction in risk of alcoholism among patients carrying this allele. However, when we carefully

analyzed alcohol drinking history, we found that these patients had a shorter duration (though notstatistocally significant) and less daily quantity of alcohol consumption when compared to those with ALDH2 504glu; suggesting the potential adverse effects of acetaldehyde accumulation secondary to lack of ALDH2 enzyme activity leading to ALD. We cannot directly test this hypothesis given the nature of our study design; however, in an animal study, *Aldh2*-/- mice were found to have hepatic inflammation and fibrosis after alcohol feeding when compared to wild type counterparts [20]; supporting our human observation. To further test the role of acetaldehyde-induced liver injury, we also performed additional analyses by randomly selecting patients with comparable alcohol consumption from those with glu/glu (n=66) and glu/lys (n=11) genotypes. We did not observe the differences in the levels of aspartate (AST) and alanine (ALT) aminotransferases between both groups (**Table 4**). However, there is a limitation regarding the sample size in this analysis. Lastly, we found that ALD patients with ALDH2 504glu had higher level of mean corpuscular volume compared to those with ALDH2 504lys. We believe that high corpuscular volume in these patients is likely due to higher quantity of alcohol consumption in this group[21, 22].

In conclusion, using both genotypic and allelic analyses, we found that the presence of the lys allele may be protected against ALD in a large cohort of Chinese patients.

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Table 1. Baseline demographic and clinical characteristics of the study cohort with ALD

Variables	ALD (N=281)
Age (years)	49.4±9.8
Alcohol consumption history	
Duration of drinking (years)	21.5±9.7
Estimated daily alcohol intake (grams)	94.8±55.8
Estimated lifetime alcohol consumption (kilograms)	765.5±611.0
Body mass index (kg/m^2)	24.8±3.8
White blood cells (10^3xcells/mm^3)	5.6±4.2
Hemoglobin (g/dl)	11.6±3.2
Platelet counts (10^3xcells/mm^3)	104±73
Total Bilirubin (mg/dL)	4.1±4.5
Aspartate aminotransferase (U/L)	97.7±103.4
Alanine aminotransferase (U/L)	95.1±145.2
Albumin (g/dL)	3.2±0.7
Alkaline phosphatase (U/L)	151.8±112.4
Gamma glutaryltransferase (U/L)	263.8±370.1

Table 2. The prevalence of ALDH2 Glu504Lys (rs671) variants in healthy controls and patients with ALD

	Controls (N=535)		ALD (N=281)		P-value	OR	95%CI
	n	%	n	%			
Genotype distribution							
Glu/Glu	394	73.7	268	95.4	<0.0001	7.38	4.09-13.3
Gly/Lys	127	23.7	13	4.6	<0.0001	0.16	0.09-0.28
Lys/Lys	14	2.6	0	0	0.05	0.06	0.004-1.07
Allele frequency							
Glu	915	85.5	549	97.7	<0.001	7.15	4.02-12.72
Lys	155	14.5	13	2.3	<0.0001	0.13	0.07-0.24

Table 3. Clinical characteristics of ALD patients stratified by ALDH2 variants

Clinical characteristics	ALDH2 Glu504Lys (rs671) variants		P-value
	Glu/Glu (N = 268)	Glu/Lys (N = 13)	
Alcohol consumption history			
Duration of drinking (years)	22.5±10.6	17.3±9.3	0.09
Estimated daily alcohol intake (grams)	128.7±56.2	50.0±28.5	0.001
Estimated lifetime alcohol consumption (kilograms)	897.1±714.9	317.9±243.8	0.0003
Body mass index (kg/m^2)	24.8±3.9	24.7±3.7	0.98
Platelet counts (10^9/L)	108.8±77.3	114.5±77.8	0.72
Mean corpuscular volume (fL)	99.1±59.5	90.5±8.3	0.04
Alanine aminotransferase (U/L)	82.5±132.9	58.4±48.2	0.65
Aspartate aminotransferase (U/L)	90.9±97.1	73.9±57.4	0.67
Total bilirubin（mg/dl）	4.8±1.1	1.6±0.1	0.006
Alkaline phosphatase (U/L)	155.2±112.4	101.6±55.9	0.02
Gamma gluteryltransferase (U/L)	256.9±370.2	165.0±242.7	0.18

Table 4. Clinical characteristics of ALD patients with similar quantity of alcohol consumption stratified by ALDH2 variants

Clinical characteristics	ALDH2 Glu504Lys (rs671) variants		P-value
	Glu/Glu (N = 66)	Glu/Lys (N = 11)	
Alcohol consumption history			
Duration of drinking (years)	18.1±6.6	18.6±8.6	0.87
Estimated daily alcohol intake (grams)	56.7±16.2	44.4±21.0	0.09
:Estimated lifetime alcohol consumption (kilograms)	359.1±129.4	294.7±174.4	0.26
Body mass index (Kg/m^2)	24.4±3.6	25.1±4.0	0.63
Platelet counts (10^9/L)	112.0±74.7	121.4±78.7	0.72
Mean corpuscular volume (fL)	95.7±13.8	90.7±8.9	0.13
Alanine aminotransferase (U/L)	79.1±67.3	55.4±48.3	0.26
Aspartate aminotransferase (U/L)	102.3±94.0	63.1±34.3	0.17
Total bilirubin（mg/dl）	3.9±3.8	1.6±0.9	0.01
Alkaline phosphatase (U/L)	163.0±100.6	96.6±53.4	0.03
Gamma gluteryltransferase (U/L)	295.3±412.9	165.3±256.8	0.31