CLINICAL INVESTIGATION

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 dehydrogenase (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* genotyping was performed in 535 healthy controls and 281 patients with ALD.

Results: The prevalence of the common form of the single nucleotide polymorphism 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 the heterozygous (glu/lys) genotype compared to 4.6% in those with ALD (odds ratio [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.

Key Indexing Terms: Aldehyde dehydrogenase; Gene polymorphism; Alcoholic liver disease; Risk. **[Am J Med Sci 2018;▮(▮):▮▮▮–▮▮▮.]**

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 multistep process consisting of a series of histopathologic 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 use due 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 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 Noninfectious Liver Disease between June 2013 and January 2015. These cases were age and sex-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, NY). The concentration of DNA was quantified by a Nanodrop 1000 UV-Vis spectrophotometer. All polymerase chain reactions were performed using 1 μL 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, CA) 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. Polymerase chain reaction conditions were set as follows: 94°C (30 seconds), 57°C (30 seconds) and 72°C (30 seconds) for a total of 40 cycles.

Statistical Analysis

Basic descriptive statistics, including mean, standard deviations 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 $P < 0.05$ was considered as statistical significance. All analyses were performed with SPSS 16.0 for Windows (SPSS Chicago, IL).

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