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Allelic variations in alcohol metabolism genes (*ADH1B, ADH1C, CYP2E1*) and alcohol use disorder (AUD) in northeastern Brazil

Variações alélicas em genes do metabolismo do álcool (*ADH1B, ADH1C, CYP2E1*) e transtorno por uso de álcool (TUA) no nordeste do Brasil

Variaciones alélicas en los genes del metabolismo del alcohol (*ADH1B, ADH1C, CYP2E1*) y trastorno por consumo de alcohol en el noreste de Brasil

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Abstract
Alcohol use disorder (AUD) is a multifactorial disease caused by environmental and genetic factors. Genetic polymorphisms of the enzymes involved in alcohol metabolism influence the susceptibility to alcohol dependence. The distribution of the genetic variants varies depending on ethnicity. The aim of this study was to evaluate the effects of the polymorphisms of the three genes responsible for the degradation of ethanol, *ADH1B*, *ADH1C*, and *CYP2E1* to examine the influence of these mutations on the risk for alcohol use disorder in a population from northeastern Brazil. In addition, the allelic distribution of the northeastern population will be compared with that obtained for other populations. The allelic and genotypic frequencies were determined in 163 alcoholic patients and 182 control subjects. Genotyping was performed by PCR-RFLP. The allele frequencies in the northeastern population were similar to those reported in studies in Mexico but differed significantly from those reported in studies of a Chinese population. The polymorphic variants of *CYP2E1* were associated with a higher risk for alcohol use disorder [odds ratio (OR) = 2.80; 95% confidence interval (CI) = 1.35-5.83, p = 0.0072]. No significant result was obtained from the analyses of the *ADH1C* gene. A significant protective effect against alcohol dependence was observed in individuals carrying allelic and genotypic variations of the *ADH1B* gene, as determined through the combined analysis of homozygous and heterozygous variant forms of the gene in controls and alcoholics (P = 0.03). Furthermore, the combination of *ADH1B*2* with *ADH1C*1* and *CYP2E1* (c1/c1) may confer protection against alcohol use disorder.
Keywords: Alcohol use disorder (AUD); Genetic polymorphisms; Alcohol dehydrogenase; *CYP2E1*; Genetic susceptibility.

Resumo

O transtorno por uso de álcool (TUA) é uma doença multifatorial causada por fatores ambientais e genéticos. Polimorfismos genéticos das enzimas envolvidas no metabolismo do álcool influenciam a suscetibilidade à dependência do álcool. A distribuição das variantes genéticas varia dependendo da etnia. O objetivo deste estudo foi avaliar os efeitos dos polimorfismos dos três genes responsáveis pela degradação do etanol, *ADH1B*, *ADH1C* e *CYP2E1* para examinar a influência dessas mutações no risco de alcoolismo em uma população do nordeste do Brasil. Além disso, a distribuição alélica da população nordestina será comparada com a obtida para outras populações. As frequências alélicas e genotípicas foram determinadas em 163 pacientes alcoolistas e 182 controles. A genotipagem foi realizada por PCR-RFLP. As frequências alélicas na população do nordeste foram semelhantes às relatadas em estudos no México, mas diferiram significativamente daquelas relatadas em estudos de uma população chinesa. As variantes polimórficas de *CYP2E1* foram associadas a um maior risco de alcoolismo [odds ratio (OR) = 2,80; intervalo de confiança de 95% (CI) = 1,35-5,83, P = 0,0072]. Nenhum resultado significativo foi obtido das análises do gene *ADH1C*. Um efeito protetor significativo contra a dependência de álcool foi observado em indivíduos portadores de variações alélicas e genotípicas do gene *ADH1B*, determinado através da análise combinada de formas variantes homozigóticas e heterozigotas do gene em controles e alcoolistas (P = 0,03). Além disso, a combinação de *ADH1B*2* com *ADH1C*1* e *CYP2E1* (c1/c1) pode conferir proteção contra o transtorno por uso de álcool.

Palavras-chave: Transtorno por uso de álcool (TUA); Polimorfismos genéticos; Álcool desidrogenase; *CYP2E1*; Suscetibilidade genética.

Resumen

El trastorno por consumo de alcohol es una enfermedad multifactorial causada por factores ambientales y genéticos. Los polimorfismos genéticos de las enzimas involucradas en el metabolismo del alcohol influyen en la susceptibilidad a la dependencia del alcohol. La distribución de las variantes genéticas varía según la etnia. El objetivo de este estudio fue evaluar los efectos de los polimorfismos de los tres genes responsables de la degradación del etanol, *ADH1B*, *ADH1C* y *CYP2E1* para examinar la influencia de estas mutaciones en el riesgo de alcoholismo en una población del noreste de Brasil. Además, se comparará la distribución alélica de la población nororiental con la obtenida para otras poblaciones. Las frecuencias alélicas y genotípicas se determinaron en 163 pacientes alcohólicos y 182 sujetos control. El genotipado se realizó por PCR-RFLP. Las frecuencias alélicas en la población del noreste fueron similares a las reportadas en estudios en México pero difirieron significativamente de las reportadas en estudios de una población china. Las variantes polimórficas de *CYP2E1* se asociaron con un mayor riesgo de trastorno por consumo de alcohol [odds ratio (OR) = 2,80; Intervalo de confianza (IC) del 95% = 1,35-5,83, P = 0,0072]. No se obtuvo ningún resultado significativo de los análisis del gen *ADH1C*. Se observó un efecto protector significativo contra la dependencia del alcohol en individuos portadores de variaciones alélicas y genotípicas del gen ADH1B, según se determinó a través del análisis combinado de formas variantes homocigóticas y heterocigóticas del gen en controles y alcohólicos (P = 0,03). Además, la combinación de *ADH1B*2* con *ADH1C*1* y *CYP2E1* (c1/c1) puede conferir protección contra el trastorno por consumo de alcohol.

Palabras clave: Trastorno por consumo de alcohol; Polimorfismos genéticos; Alcohol deshidrogenasa; *CYP2E1*; Predisposición genética.

1. Introduction

Alcohol use disorder is a major public health problem that affects all aspects of human behavior. Alcoholism is a multifactorial disease that is inherited with different probabilities of expression in offspring (Ratsma et al., 2002). Socio-economic, cultural, behavioral, ethnic, and gender differences are among the major determinants for alcohol use disorder (Limosin et al., 2000). Epidemiological and clinical studies have shown that excessive alcohol use implies risks for the development of a variety of disorders, including neural and metabolic diseases. Alcohol abuse results in metabolic disorders and consequently affects the function of most organs. The digestive system (particularly the liver and pancreas) and nervous system are the two most frequently and severely affected areas of the body (Stickel & Österreicher, 2006; Kono et al., 1997).

The consumption of alcohol consumed in Brazil was evaluated in a survey that found that 11% of individuals drank alcohol every day and 28% three to four times a week. Another study carried out in 108 Brazilian cities stated that 69% of individuals had already used alcohol in their lifetime. The consumption of alcohol 3 or 4 times a week, including those who drink every day, detected this practice in 9% of men and 2% of women. According to the World Health Organization (WHO), AUD is prevalent in Brazil in 6% of the population.

In addition, frequent consumption of alcohol (from one to four times a week) varies between regions of the country,

with the following percentages being observed: 25% in the South, 21% in the Northeast, 18% in the Southeast, 18% in the Central- West and 10% in the North. On the other hand, very frequent consumption (drinking every day) was observed in 11% in the South, 6% in the Southeast, 6% in the Midwest, 4% in the North and 3% in the Northeast (Wolf et al., 2019).

The disease in question is influenced by physical, psychosocial, environmental, and genetic factors (Yin & Agarwal, 2001). Studies have shown a 50-65% index of heritability of the disease among twins (Heath et al., 1997; Kendler et al., 1992, 1997). The children of alcoholics are five times more likely to experience alcohol-related problems than the children of non-alcoholics (Edwards et al., 2005). Alcohol metabolism is one of the biological determinants that can influence an individual's alcohol consumption, the development of alcohol use disorder, and the organ damage induced by ethanol metabolism (Agarwal, 2001).

Susceptibility and resistance to alcohol dependence has been associated with the rate of ethanol metabolism, which is critical in determining alcohol toxicity (Brennan et al., 2004). Ethanol is completely absorbed through the membranes of the digestive tract, particularly the stomach and the proximal small intestine (Crabb et al., 2004). Only 5-15% of ethanol is excreted into the lungs, kidneys, sweat, and saliva. Most of the ethanol is metabolized in the liver at a rate of approximately 7 g of ethanol per hour (Hendriks & van Tol, 2005). In hepatocytes, alcohol is oxidized via three distinct pathways: the alcohol dehydrogenase pathway in the cell cytoplasm; the microsomal ethanol oxidizing system (MEOS) in the endoplasmic reticulum; or catalase in peroxisomes (Agarwal, 2001). Most of the ethanol is eliminated through oxidation to generate acetaldehyde, followed by the subsequent transformation of this product to acetic acid and water. These reactions are primarily conducted in the liver through the enzymes alcohol dehydrogenase (ADH) in the cytosol and aldehyde dehydrogenase (ALDH) in the mitochondria (Yao et al., 2011).

The MEOS involves the enzymes responsible for the metabolism of xenobiotics, such as cytochrome P450. The enzyme P450 is a major component of MEOS, which, along with ADH, participates in alcohol acetaldehyde metabolism and promotes the conversion of acetaldehyde into acetic acid and water by the enzyme ALDH (Garcia et al., 2010; Druesne-Pecollo et al., 2009).

All enzymes participating in the metabolism of ethanol exhibit a number of polymorphisms. These polymorphisms affect the metabolic action of these enzymes, particularly the degradation of ethanol and its byproducts, increasing alcohol resistance or facilitating alcohol dependence (Kayaaltı & Söylemezoğlu, 2010). Some isoforms of these enzymes mediate the accumulation of toxic metabolites, such as acetaldehyde, which cause DNA damage or negatively affect other cellular structures (Boffetta & Hashibe, 2006).

The genes *ADH1B*, *ADH1C*, and *CYP2E1* are responsible for the metabolism of ethanol in the liver, thereby providing an integrated response to alcohol. These three genes function in the conversion of ethanol to acetaldehyde. However, the *CYP2E1* gene is involved in the metabolism of a small amount of ethanol and displays a lower affinity for ethanol than ADH (Cerqueira, 2008).

ADH is a cytosolic protein encoded by seven genes (ADH1 - ADH7) in humans; single nucleotide polymorphisms (SNPs) are found in each ADH gene (Kayaaltı & Söylemezoğlu, 2010; Marichalar-Mendia et al., 2010).

The class I genes *ADH1B* and *ADH1C*, which are located on chromosome 4q22, encode isoenzyme β, the locus responsible for most of the ADH activity in the liver, and isoenzyme γ, respectively. The enzymes encoded by the genes *ADH1B* and *ADH1C* are abundant in the liver, suggesting the potential primary action on alcohol metabolism in this organ (CRABB et al., 2004). Class I enzymes are primarily expressed in the liver and contribute to the oxidation of approximately 70% of ingested ethanol. Based on this prevalence, most metabolic studies involve class I ADH genes, particularly *ADH1B* and *ADH1C*, which contain known functional polymorphisms in coding regions (Kuo, et al., 2008; Wolf, et al., 2019).

The well-characterized genetic polymorphisms in *ADH1B* (rs1229984) result from a change in the arginine (CGC)

(ADH1B*1) codon at position 48 to a histidine (CAC) (ADH1B*2) codon due to a G/A base transition in exon 3 (Matsuo et al., 2006; C.-F. Wu et al., 2005). Individuals expressing variations in *ADH1B* might exhibit different rates of alcohol elimination (Crabb et al., 2004). Although studies in Asian populations have been inconclusive, the *ADH1B*2* variant has been documented as a negative risk factor for the development of alcoholism, due to increased ADH activity associated with a low Km value, which produces intolerance to low doses of ethanol due to the rapid increase in acetaldehyde (Bosron et al., 1980; Hendershot et al., 2009; Takeshita et al., 1996; Higuchi et al., 1996). Alcohol dehydrogenase isoform 1B (ADH1B) is an important ethanol-oxidizing enzyme but is also involved in multiple molecular mechanisms and metabolic processes of several molecules such as fatty acids, acetone, epinephrine, glucose, and neurotransmitters (for instance serotonin and noradrenaline) (Legaki et.al, 2022).

The *ADH1C* gene contains a well-characterized polymorphism resulting from the mutation of isoleucine (A) to valine (G) at position 350 in exon 8 (rs698) (Garcia et al., 2010). This gene has two isoforms, γ1 and γ2. The γ1 gene contains isoleucine at position 350, while the γ2 gene contains valine. The γ1 (Ile.Ile) gene encodes an enzyme with a rate of alcohol metabolism two and a half times greater than the γ2 (Val.Val) form, suggesting the accumulation of acetaldehyde and a mechanism for "flushing" (facial redness), reducing the risk of alcohol dependence. Furthermore, individuals with reduced metabolism (γ2 Val.Val form) tend to consume more alcohol, extending the persistence of ethanol in the blood (Wall et al., 1996; Thomasson et al., 1993).

The *CYP2E1* gene is located on the long arm of chromosome 10 (10q 24.3) and is induced by a variety of compounds (alcohols, aldehydes, and aromatic ketones) present in food, organic solvents, tobacco, drugs, pesticides, and environmental pollutants (Canalle et al., 2004; Rossini et al., 2006).

The enzyme CYP2E1 is a member of the cytochrome P450E1 superfamily and a major alcohol-metabolizing enzyme in the liver. However, CYP2E1 is responsible for the metabolism of less than 10% of the ethanol ingested during high alcohol consumption (Asakage et al., 2007). This gene contains several polymorphisms that affect its expression. Polymorphisms in the 5' region of this gene, which are examined in the present study and have been studied previously (Kayaaltı & Söylemezoğlu, 2010; Celorrio et al., 2012; Gordillo-Bastidas et al., 2010), might promote an up to 10-fold increase in transcriptional activity compared with the common allele. The polymorphism resulting in a C for T substitution at position -1055 (rs2031920) results in the loss of the *RsaI* restriction site and gain of the *Pst*I restriction site, and the alleles were designated c2 (Hayashi et al., 1991) or is referred to as CYP2E1*5B (Wang et al., 2009).

CYP2E1 enzyme activity increases in the liver of chronic alcoholics when ethanol is present at high concentrations, increasing the release of acetaldehyde in the blood (Cerqueira, 2008; Lieber, 2001). The overexpression of CYP2E1 during ethanol oxidation involves the production of free radicals that contribute to liver damage. Alcohol consumption is the major cause of cirrhosis in Western countries (Cerqueira, 2008; Mincis & Mincis, 2006; D. Wu & Cederbaum, 2003).

The *CYP2E1* polymorphism is a potential indicator of cancer susceptibility (Liu et al., 2001). Changes in this gene are associated with increased risk for oral (Gattás et al., 2006), colorectal, and esophageal cancer (Howard et al., 2003).

The expression of these genes varies within the population. Given the practice of miscegenation in Brazil, this country is ideal for the analysis of the incidence of these polymorphisms (Rebello et al., 2011).

The incidence of alcohol use disorder is high in northeastern Brazil; the state of Piauí contains the second highest incidence of alcoholism in Brazil (Monteiro et al., 2011). To our knowledge, this is the first study of the relationship between these polymorphisms and alcoholism in a population from northeastern Brazil.

2. Materials and Methods

2.1 Individuals

This is a descriptive and analytical cross-sectional study (Pereira et al., 2018). The study protocol was approved by the Ethics Committee of the Federal University of Piauí (CAAE: 0234.0.045 - 00 010) in accordance with the guidelines established in resolution 196/96 of the National Council of Health. The study design was a case-control with 345 individuals, of which 182 were controls and 163 were alcoholics, from the city of Parnaíba, state of Piauí, Brazil participated in the study.

The 163 alcoholic patients participating in this study visited the Psychosocial Support Center for Alcohol and Drugs (CAPS-AD) in the city of Parnaíba, Piauí, Brazil, or were hospitalized at St. Hedwig's Hospital in Parnaíba. The alcoholics included 149 (91.41%) and 14 (8.59%), unrelated men and women, respectively, of ages 18 to 83 years.

The 182 control subjects and unrelated volunteers were over 18 years of age, were randomly recruited of the city of Parnaíba, PI, Brazil in the health services, Santa Edwiges Hospital, churches, and recreation center for old people, who reported no or minimal alcohol consumption. The control subjects included 81 men (44.5%) and 101 women (55.5%), ages 18-93 years (mean age, 64.72). The subjects were selected from March 2012 to November 2012.

The diagnostic evaluation of all individuals was performed through medical records and a questionnaire based on the Diagnostic and Statistical Manual of Mental Disorders - DSM-IV (American Psychiatric Association, 1994) and ICD-10 (International Classification of Diseases) (WHO - World Health Organization, 1993) for alcohol dependence (303.90) or alcohol abuse (305.00).

All participants were interviewed with a standard questionnaire to collect basic information, including socio-demographic characteristics (e.g.: age and education). Both experimental groups, alcoholics and controls, answered a questionnaire and provided written informed consent. Blood was obtained from the subjects and collected in tubes containing EDTA. The questionnaire contained questions about nationality, sex, tobacco use or other drug use, family history of alcoholism, and quantity and type of beverages consumed by each individual. We excluded individuals with diseases, such as hepatitis C, HIV, STDs, and cancer, that were not associated with alcohol consumption.

Education was measured according to the number of years studied. Items related to alcohol use (e.g.: average number of drinks per day, as 14 g of alcohol defined by the National Institute on Alcohol Abuse and Alcoholism), smoking and family background of alcoholism (qualitative measure), were analyzed aspreviously described by Vasconcelos et al. (2015).

2.2 Genotypic Analysis

2.2.1 DNA was extracted from peripheral blood

Whole blood samples were collected via venipuncture of alcoholic and control individuals. DNA was extracted from leukocytes with a DNA extraction kit (Wizard ® Genomic - Promega, Madison, WI, USA) according to the manufacturer's instructions and stored at -20 °C until use.

The isolated DNA was resuspended in Tris-EDTA, pH 8.0, and stored at-20ºC prior to use. The DNA concentration and purity were determined by electrophoresis on a 0.8% agarose gel stained with ethidium bromide and spectrophotometry at 260 and 280 nm on a Biospec-nano (Shimadzu Biotech). For SNP analysis, the DNA samples were diluted to a working concentration of 100 ng/µL in sterile water.

2.2.2 Polymorphism genotyping *ADH1B* Arg48His and *ADH1C* Ile350Val

The methods of Xu et al. (1988), with some modifications, were used to genotype the *ADH1B* Arg48His and *ADH1C* Ile350Val polymorphisms. The primers 5' - CTA ATT AAT TGT TTA ATT CAA GAA G - 3' (forward) and 5' - AAC ACT ACA GAA TTA CTG GAC - 3' (reverse) (Eurofins MWG Operon, Huntsville, Alabama, USA) were used to amplify the

ADH1B Arg48His gene in a total volume of 25 µL containing 2.5 µL PCR buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4), 2 mM $MgCl_2$, 2 mM dNTPs, 0.8 mM each primer, 1.5 U Taq DNA polymerase (Ludwig Biotechnology Ltd., Daybreak, RS, Brazil), and 200 ng of genomic DNA. The amplification parameters included 35 cycles of denaturation for 5 min at 94°C, annealing for 1 min at 55°C, and extension at 2 min at 72°C. The variant allele at position 48 of the *ADH1B* gene was detected after 12 h of digestion with 2.5 units *Msl I* (New England Biolabs, Ipswich, MA, USA) at 37°C. The fragments were analyzed by electrophoresis on a 2.0% agarose gel, stained with ethidium bromide, and visualized under UV light. The enzyme *Msl I* did not recognize the restriction site in samples from individuals homozygous for the wild-type *ADH1B* Arg/Arg 48 gene, generating fragments of 685 bp. In samples from individuals homozygous for the mutation, *ADH1B* His/His 48, fragments of 443 and 242 bp were formed. Samples from individuals heterozygous for the mutation yielded fragments of 685 bp, 443 bp, and 242 bp.

The primers used to amplify the gene polymorphism *ADH1C* Ile350Val were 5' - TTG TTT TTT TTT GAT ATC TGT TGT - 3' (forward) and 5' - TAC TGT CGT AGA AGC ATA CAA - 3' (reverse) (Eurofins MWG Operon). The amplification was performed in a total volume of 25 µL per PCR reaction containing 2.5 µL buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4), 2 mM $MgCl_2$, 2 mM dNTPs, 0.8 mM each primer, 1.5 U Taq DNA polymerase (Ludwig Biotechnology Inc.), and 200 ng genomic DNA. The amplification parameters included 30 cycles of 1 min at 94°C for denaturation, 1 min at 51°C for annealing and 30 seconds at 72°C for extension. The wild-type allele at position 350 of the *ADH1C* gene was detected after digestion for 12 h at 37°C with 1.25 units of *SspI* (New England Biolabs). The PCR fragments were analyzed by gel electrophoresis on a 2.0% agarose gel, stained with ethidium bromide, and visualized under UV light. The *SspI* restriction site was detected for individuals homozygous for the *ADH1C* Ile 350/Ile allele, generating fragments of 274 bp and 104 bp, while for individuals homozygous for the mutation, *ADH1C* 350 Val/Val, a fragment of 378 bp was detected. For individuals heterozygous for the *ADH1C* Ile 350/Val mutation, fragments of 378 bp, 274 bp and 104 bp were obtained.

2.2.3 *CYP2E1* polymorphism genotyping

The PCR-RFLP methods of Anwar et al. (1996) and Canalle et al. (2004) were implemented, with some modifications, to analyze the *CYP2E1* gene. The amplified PCR product corresponded to a 410-bp fragment comprising the region of DNA that regulates the transcription of *CYP2E1* and was detected with the restriction enzyme *PstI* (Ludwig Biotechnology Ltd.) (Hayashi et al., 1991). The 25 µL amplification reaction contained 100 ng genomic DNA, 2.5 µL PCR buffer (20 mM Tris-HCl, 50 mM KCl, pH 8.4), 2 mM dNTPs, 2 mM $MgCl_2$, 1.5 U Taq DNA polymerase, and 0.4 mM each primer $CYP2E1_1$ (5' – CCAGTCGAGTCTACATTGTCA – 3') and $CYP2E1_2$ (5' – TTCATTCTGTCTTCTAACTGG – 3'). The primers were generated by Eurofins MWG Operon. All other reagents in the PCR reaction were provided by Ludwig Biotechnology Ltda. The PCR reaction was performed with an initial denaturation step at 95°C for 1 min, followed by 35 cycles at 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, followed by 4 min at 72°C. A 10-µL aliquot of the PCR reaction was digested with 10 U *PstI* at 37°C for 12 h. The fragments were analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. The presence of the *PstI* restriction site indicates the detection of the mutant allele via the generation of two fragments of 290 and 120 bp.

2.3 Statistical Analysis

The allelic frequencies were determined by simple counting. The expected and observed genotype distributions were analyzed using the chi-square test to verify the Hardy-Weinberg equilibrium, with the aid of BioStat 5.3 software (Mamirauá Institute, Manaus, Brazil). The chi-square test was also used to compare the gene frequencies observed in this study with those obtained in other populations, and the analysis of these data was performed with SPSS 15.0. To determine the statistical

significance of the associations of the frequencies of each genotype and allele studied between alcoholics and the control group and among subgroups of alcoholics and control individuals, the Fisher exact test (two-tailed) was used. To analyze these data, we used Biostat 5.3 software. The odds ratio (OR) (likelihood ratios) and 95% confidence interval (CI) were calculated as an estimate of the relative risk and degree of association. A level of probability (*P*) of less than 0.05 was used as the criterion for significance.

3. Results

This study analyzed the distribution of genotypic and allelic frequencies of the genes *ADH1B* (polymorphism at codon 48), *ADH1C* (polymorphism at codon 350), and *CYP2E1* (polymorphism in the 5' regulatory gene) in 163 alcoholic patients and 182 unrelated controls with no history of alcohol consumption or binging (< 60 g/day). Table 1 shows the distribution of age, gender, and ethnicity for the patients with alcoholism and the controls. There was a prevalence of male patients. Tobacco use was more frequent in the group of alcoholics than in the controls ($P < 0.0001$). No significant differences in terms of the age of the individuals were observed among the groups ($P = 0.37$).

Table 1. Characteristics of alcoholic patients and controls in a population from northeastern Brazil.

Variables	Alcoholics n = 163 (%)	Controls n = 182 (%)	*P*- value*
Gender			
Male	149 (91.41)	81 (44.50)	
Female	14 (8.59)	101 (55.50)	< 0.0001
Age (years)	42.07 - 13.11	64.72 - 13.28	0.37
Smoker	98 (60.12)	50 (27.47)	
Non-smoker	65 (39.88)	132 (72.53)	< 0.0001

*Two-sided Fisher exact test or χ^2 test was used in the comparisons. Source: Authors.

The genotype distribution and allelic polymorphisms of *ADH1B*, *ADH1C*, and *CYP2E1* are illustrated in Table 2. All genotypic frequencies in the controls were distributed according to the Hardy-Weinberg equilibrium ($P > 0.05$). The genotype distribution of the *ADH1C* gene in alcoholics was not distributed according to the Hardy-Weinberg equilibrium ($P = 0.0027$).

The *ADH1B*1* allele (Arg) was significantly present in both the controls (93%) and patients (97%). The analysis of the distribution of this genotype revealed a prevalence of the heterozygous form *ADH1B*1/*2* (Arg/His) in controls (13.2%) compared with patients (6.7%). The results showed a trend toward significance ($P = 0.05$; Table 2), suggesting protection against alcohol use disorder in individuals with this variant (OR 0.47, 95% CI, 0.22 – 1.00). There was a significant difference in the association between the heterozygous form *ADH1B*1/*2* (Arg/His) and the homozygous polymorphic form *ADH1B*2/*2* (His/His) between the two groups ($P = 0.03$); these forms were the predominant forms observed in controls (13.7%) compared with alcoholics (6.7%). The results confirmed the protective effects of these genotypic variations against alcohol use disorder (OR 0.45, 95% CI: 0.21 to 0.95). There was a significant difference in allelic frequencies between the patients and controls ($P = 0.04$).

A similar comparison was made for the polymorphism in gene *ADH1C*, but no significant difference was observed between the controls and patients (Table 2). The *ADH1C*1* wild-type form (Ile) was predominant in both the control (67%) and the patient (66%) groups, with no significant difference between the two groups ($P = 0.93$). The association between *ADH1C*1/*2* (Ile/Val) and *ADH1C*2/*2* (Val/Val) was similar in the patient population (61.3%) and the controls (57.1%), with no significant distinctions between these groups ($P = 0.44$).

The wild-type allele of *CYP2E1* (c1) was predominant in both the control (89%) and patient (87%) groups (Table 2).

The difference in the frequencies of the alleles of the *CYP2E1* gene between the patients and controls was not statistically significant (*P* = 0.33), although the mutant allele was more common in patients (13%). There was a higher incidence of *CYP2E1* c1/c2 in alcoholics (26.5%) than in the controls (20%), although this difference was not significant (*P* = 0.19). Similar results were observed in the frequency of the association of two genetic variants (c1/c2 + c2/c2) in the patient and control groups. The combination of the frequencies of the heterozygous and homozygous mutant polymorphisms prevailed in alcoholics (26.5%) compared with controls (20.6%). However, the difference was not statistically significant (*P* = 0.24).

Table 2. Genotypes and allele frequencies of *ADH1B*, *ADH1C*, and *CYP2E1* polymorphisms and alcoholic patients and controls and OR of the genotypes associated with alcoholism.

Polymorphisms	Alcoholics (%)	Controls (%)	OR (95% CI)	*P*- value*
Codon 48 *ADH1B* (rs1229984)				
Arg/Arg	152/163 (93.3)	157/182 (86.3)	1.00 (reference)	
Arg/His	11/163 (6.7)	24/182 (13.2)	0.47 (0.22-1.00)	0.05
His/His	0/163 (0.0)	1/182 (0.5)	0.34 (0.01-8.52)	1.00
Arg/His +His/His	11/163 (6.7)	25/182 (13.7)	0.45 (0.21-0.95)	0.03*
Allele Frequency				
Arg	315 (97)	338 (93)		
His	11 (3)	26 (7)		0.04*
codon 350 *ADH1C* (rs689)				
Ile/Ile	63/163 (38.7)	78/182 (42.9)	1.00 (reference)	
Ile/Val	90/163 (55.2)	87/182 (47.8)	1.28 (0.82-1.99)	0.30
Val/Val	10/163 (6.1)	17/182 (9.3)	0.72 (0.31-1.70)	0.52
Ile/Val +Val/Val	100/163 (61.3)	104/182 (57.1)	1.19 (0.77-1.83)	0.44
Allele Frequency				
Ile	216 (66)	243 (67)		
Val	110 (34)	121 (33)		0.93
-1053C/T *CYP2E1* (rs2031920)				
c1/c1	119/162 (73.5)	131/165 (79.4)	1.00 (reference)	
c1/c2	43/162 (26.5)	33/165 (20.0)	1.43 (0.85-2.40)	0.19
c2/c2	0/162 (0.0)	1/165 (0.6)	1.39 (0.83-2.32)	0.24
c1/c2 +c2/c2	43/162 (26.5)	34/165 (20.6)	1.39 (0.83-2.32)	0.24
Allele Frequency				
c1	281 (87)	295 (89)		
c2	43 (13)	35 (11)		0.33

For technical reasons it was not possible to analyze all alcoholic patients and controls for all genotypes. The Arg, Ile, and c1 alleles are wild type alleles; the His, Val, and c2 alleles are mutant alleles. Arg/Arg, Ile/Ile, and c1/c1, homozygous for the wild-type allele; Arg/His, Ile/Val, and c1/c2, heterozygous; His/His, Val/Val, and c2/c2, homozygous for the mutant allele. OR, odds ratio, CI, confidence interval. *P* values were calculated by χ^2 test or Fisher exact test (two-tailed).*Statistical significance (*P* <0.05). Source: Authors.

To evaluate gene-gene interaction and the risk of alcohol use disorder, we performed a combined analysis of the polymorphisms of the three genes responsible for the first step of alcohol metabolism in the body: *ADH1B*, *ADH1C*, and *CYP2E1* (Table 3). The combination of the polymorphic form of *ADH1B* (48Arg/His + 48His/His) with the wild-type genotypes of *ADH1C* (350Ile/Ile) and *CYP2E1* (c1/c1) was predominant in the control group (7.9%) compared with patients (2.5%). This difference in genotypic frequencies between the patients and controls showed a trend toward significance (*P* = 0.05), suggesting a reduction in the risk of alcohol dependence in this population (OR 0.29; 95% CI: 0.08 to 0. 97).

Furthermore, in the present study, the association of wild-type forms of the genes *ADH1B* (Arg/Arg) and *CYP2E1* (c1/c1) with the polymorphic variants of *ADH1C* (350Ile/Val + 350Val/Val) was predominant in both groups, but no significant differences in the genotypic distribution of these genes relative to wild-type forms were observed between the

patients and controls (OR 0.95; 95% CI: 0.54 to 1.64, *P* = 0.88). (Table 3).

Table 3. Association between genotype combinations of *ADH1B*, *ADH1C*, and *CYP2E1* polymorphisms and the risk of alcohol use disorder in a population from northeastern Brazil.

codon 48 *ADH1B*	codon 350 *ADH1C*	-1053C/T *CYP2E1*	Alcoholics n = 162 (%)	Controls n = 163 (%)	OR (95% CI)	*p*-value*
Arg/Arg	Ile/Ile	c1/c1	41 (25.3)	39 (23.9)	1.00 (reference)	
Arg/His; His/His	Ile/Ile	c1/c1	4 (2.5)	13 (7.9)	0.29 (0.08-0.97)	0.05*
Arg/Arg	Ile/Val; Val/Val	c1/c1	71 (43.8)	71 (43.6)	0.95 (0.54-1.64)	0.88
Arg/Arg	Ile/Ile	c1/c2; c2/c2	16 (9.9)	15 (9.2)	1.01 (0.44-2.32)	1.00
Arg/His; His/His	Ile/Val; Val/Val	c1/c1	3 (1.9)	6 (3.7)	0.47 (0.11-2.03)	0.48
Arg/His; His/His	Ile/Ile	c1/c2; c2/c2	1 (0.6)	2 (1.2)	0.47 (0.04-5.45)	0.61
Arg/Arg	Ile/Val; Val/Val	c1/c2; c2/c2	23 (14.2)	14 (8.6)	1.56 (0.70-3.46)	0.32
Arg/His; His/His	Ile/Val; Val/Val	c1/c2; c2/c2	3 (1.8)	3 (1.9)	0.95 (0.18-4.99)	1.00

For technical reasons, it was not possible to analyze all patients and controls for all genotypes. Only samples with common genotypes were included in this analysis. OR, odds ratio; CI, confidence interval. Arg/Arg, Ile/Ile, and c1/c1, homozygous for the wild-type allele; Arg/His, Ile/Val, and c1/c2, heterozygous; His/His, Val/Val, and c2/c2, homozygous for the mutant allele. *P* values were calculated by χ^2 test or Fisher exact test (two-tailed).*Tending to statistical significance ($P < 0.05$). Source: Authors.

The *CYP2E1* gene is activated by ethanol and cigarette components. Table 4 shows a comparison of the frequencies of the *CYP2E1* polymorphism present in smokers and nonsmokers.

Table 4. Comparison of the frequency of the *CYP2E1* polymorphism in alcoholics and controls as a function of smoking.

Polymorphism	Smoking Alcoholics Number/Total (%)	Smoking Controls Number/Total (%)	OR (95% CI)	*P*-value*
CYP2E1				
c1/c1	75/97 (77.3)	31/48 (65.6)	1.00 (reference)	
c1/c2	22/97 (22.7)	16/48 (33.3)	0.56 (0.26-1.22)	**0.16**
c2/c2	0/97 (0.0)	1/48 (2.0)	0.13 (0.005-3.51)	**0.29**
c1/c2+c2/c2	22/97 (22.7)	17/48 (35.4)	0.53 (0.25-1.14)	**0.11**
Allele frequency				
c1	172 (89)	78 (81)		
c2	22 (11)	18 (19)		**0.10**
Polymorphism	**Non–smoking Alcoholics Number/Total (%)**	**Non–smoking Controls Number/Total (%)**	**OR (95% CI)**	***P*-value***
CYP2E1				
c1/c1	44/65 (67.7)	100/117 (85.5)	1.00 (reference)	
c1/c2	21/65 (32.3)	17/117 (14.5)	2.80 (1.35-5.83)	**0.0072***
c2/c2	0/65 (0.0)	0/117 (0.0)	2.25 (0.04-115.7)	**1.00**
c1/c2+c2/c2	21/65 (32.3)	17/117 (14.5)	2.80 (1.35-5.83)	**0.0072***
Allele frequency				
c1	109 (84)	217 (93)		
c2	21 (16)	17 (7)		**0.0115***

For technical reasons, it was not possible to analyze all patients and controls for all genotypes. OR, odds ratio; CI, confidence interval. c1/c1, homozygous for the wild-type allele; c1/c2, heterozygous; c2/c2, homozygous for the mutant allele. *P* values were calculated by χ^2 test or Fisher exact test (two-tailed).
* Statistical significance ($P < 0.05$). Source: Authors.

Comparing only the smokers, there was no significant difference in the distribution of the allelic and genotypic frequencies of the polymorphisms of *CYP2E1* between the patients and controls. Among non-smokers, an analysis of the alcoholics and controls revealed a significant difference between the frequencies of the heterozygous genotype (c1/c2) in the

two groups (P = 0.0072). This variant was more frequently observed among non-smoking alcoholics (32.3%) than non-smoker controls (14.5%). Individuals with this polymorphism might have a greater chance of developing alcohol dependence (OR 2.80, 95% CI: 1.35 to 5.83). The homozygous polymorphic genotype (c2/c2) was not observed in the groups of individuals analyzed. Moreover, the c2 allele frequency was significantly higher in alcoholics (16%) than in controls (7%) (P = 0.0115).

4. Discussion

As previously discussed, a 65% index of heritability of alcohol dependence from parents to children has been observed, prompting interest in the search for genes responsible for this predisposition. Some SNPs located in genes associated with alcohol metabolism have also been associated with alcoholism.

In the present study we analyzed three genes, *ADH1B*, *ADH1C*, and *CYP2E1*, which synthesize enzymes for the metabolism of ethanol to acetaldehyde, primarily in the liver. The enzyme CYP2E1 also metabolizes substrates other than alcohol (Hartley et al., 1995; Yang et al., 1994).

The *ADH1B* Arg48His polymorphism has been identified as a variation that protects individuals against alcoholism by accelerating ethanol processing and rapidly increasing the accumulation of acetaldehyde (Bosron et al., 1980). These results were obtained in Asian, African, Jewish, Japanese, and European populations (Carr et al., 2002; Ehlers et al., 2001; Whitfield, 2002; Borràs et al., 2000; Lorenzo et al., 2006; Sun et al., 2002). The analysis of the distribution of the *ADH1B*1/ADH1B*2* genotype in alcoholics and controls suggested that this allelic variation confers protection against alcohol use disorder (OR 0.47; 95% CI: 0.22 to 1; P = 0.05). The difference was significant for *ADH1B*1/ADH1B*2* + *ADH1B*2/ADH1B*2* (P = 0.03), confirming the previous hypothesis. In Japan, the additive effect of suppressing the *ADH1B*2* allele in alcohol consumption was detected in 95% of the participating male subjects (Sun et al., 2002). In Taiwan, Han Chinese male alcoholics had a significantly lower frequency of the *ADH1B*2* allele than controls (Thomasson et al., 1991). Among Spaniards, studies involving women showed that the allele variant protects against alcohol use disorder but increases vulnerability to liver disease (Lorenzo et al., 2006). The presence of the *ADH1B*2* allele also reduced the risk of alcohol use disorder in the European population (Borràs et al., 2000). Li et al. (2011) confirmed that the His allele greatly reduces the risk of addiction as well as diseases acquired through excessive alcohol consumption, particularly in Asian populations. The *ADH1B*1/ADH1B*1* genotype and the wild-type allele, which favor alcohol dependence, have been observed among Polish and Turkish populations (Aktas et al., 2012; Cichoż-Lach et al., 2010).

Variations in the *ADH1C* gene (*ADH1C*1* and *ADH1C*2*) yield different properties due to the substitution of a single amino acid. The wild-type form, *ADH1C*1*, is associated with twofold greater ethanol metabolism than that associated with the mutant *ADH1C*2*. Thus, there is an accumulation of acetaldehyde in individuals with wild-type *ADH1C*1*, which triggers a "flushing" reaction (facial redness), reducing the excessive consumption of alcohol (Wall et al., 1996; Thomasson et al., 1993).

The difference in the distribution of allelic and genotypic frequencies of *ADH1C* did not differ significantly between the controls and patients in southeastern Brazil (Rebello et al., 2011). The relationship between the *ADH1C*2* variant and increased susceptibility to alcohol use disorder was not verified in this study. Similarly, an association of the genotype (Val/Val) or its allele with alcohol use disorder was not observed in the Turkish population (Aktas et al., 2012). Rebello et al. (2011) suggested a possible association between *ADH1C*2* (form Ile/Val) and alcohol dependence in southeastern Brazil. An association between the *ADH1C* polymorphism and an increased risk of alcohol use disorder was detected in other studies in Trinidad and Tobago (Montane-Jaime et al., 2006) and China (Li et al., 2012). Contradictory results were obtained with Japanese and Polish populations. In Japanese individuals, the *ADH1C*2* allele was significantly more prevalent in non-alcoholic individuals (Cichoż-Lach et al., 2010; Matsuo et al., 2007).

The combination of the *ADH1B*2* (His) and *ADH1C*1* (Ile) genes, which are responsible for the synthesis of alcohol dehydrogenase, results in the rapid metabolism of ethanol to acetaldehyde, reducing the risk of addiction (Carr et al., 2002; Peters et al., 2005). Studies with Chinese and Thai populations confirmed an increase in the frequency of the *ADH1B*2* and *ADH1C*1* alleles in control subjects compared with alcoholics, suggesting protection against the disease (Chen et al., 2009).

In the northeastern Brazilian population, a possible relationship was detected between the combination *ADH1B*2* (His) + *ADH1C*1* (Ile) + *CYP2E1* (c1) that reduced the risk of alcohol dependence (OR 0.29; 95% CI: 0.08 to 0. 97) because the association was more predominantly observed in the control group (P = 0.05). Studies of Mexican Americans and other Chinese populations did not confirm this protective relationship (Konishi et al., 2004; Lee et al., 2001). Among Polish individuals, the genotype *ADH1C*1/1* and *ADH1B*1/1* favored alcohol dependence (Cichoż-Lach et al., 2010).

Haseba et al (2020) reported that the liver ADH activity and ADH1 protein increased even by feeding a liquid diet containing 4% ethanol to mice for 4 weeks, thereby enhancing the alcohol metabolism and inducing mild liver steatosis. The increase in liver ADH1 by chronic alcohol consumption (CAC) has been reported also at mRNA level as well as the protein and the activity levels in rats. Some other studies have demonstrated in rats that the ADH activity increased at early stages of CAC, but then decreased when fatty liver was developed by CAC. These findings suggest that ADH1 contributes to enhance alcohol metabolism during early stages of CAC; however, the contribution decreased by the decrease of the activity due to the development of alcoholic liver diseases.

After ADH, CYP2E1 is responsible for the second highest metabolism of alcohol in the livers in chronic patients (Agarwal, 2001). The enzyme CYP2E1 has low catalytic efficiency compared with ADH and is responsible for the metabolism of a small portion of ethanol. However, the expression of CYP2E1 is increased in the livers of alcoholic individuals (Kato et al., 2003).

The *CYP2E1* gene polymorphisms analyzed in this study enhance transcriptional activity, increasing the protein level and enzyme activity and resulting in the increased production of acetaldehyde after alcohol consumption (Hayashi et al. 1991; Pöschl and Seitz 2004).

The oxidation of alcohol by CYP2E1 in chronic alcoholics is induced up to 10-fold after alcohol consumption (Zavras et al., 2002). However, there was no significant difference in the allelic and genotype frequencies of *CYP2E1* between patients and controls. The wild-type allele was predominant in patients (87%) and controls (89%). The heterozygous variant prevailed in the patients (26.5%) compared with controls (20%), and the c2 allele was also observed in more alcoholics (13%) than in controls (11%).

Non-ADH1 pathway in Adh1−/− mice, which is responsible for the whole Alcohol elimination rate (AER) of this strain, was significantly increased by CAC, relating to the decline of high blood alcohol concentration (BAC). BACs 3 weeks after starting CAC. MEOS, whose main component is CYP2E1, has long been considered to represent the non-ADH1 pathway. However, under the current CAC condition, liver CYP2E1 protein was not increased despite the elevation of AER in all genotypes of mice. There was also reported no increase in MEOS activity in mice livers by CAC using 10% ethanol solution. Thus, CYP2E1 may not contribute to the elevation of AER by CAC in all ADH genotypes of mice (Haseba et al, 2020).

As previously reported, the transcription of *CYP2E1* can also be stimulated by substances present in cigarette smoke, such as nicotine (Schoedel & Tyndale, 2003). Therefore, an analysis of the *CYP2E1* polymorphism in smokers and non-smokers was performed (Table 4). In smokers, the differences in the allelic and genotypic frequencies of polymorphisms of *CYP2E1* in controls and alcoholics were not significant.

The comparison between non-smoking alcoholics and controls revealed significant differences in the genotype distribution (c1/c2) and c2 allele frequency between controls and alcoholics. In both cases, the frequency of the mutant was predominant in alcoholics (P = 0.0072 and P = 0.0115). Thus, the risk of developing alcohol use disorder might increase

almost threefold (OR 2.80; 95% CI: 1.35 to 5.83). The significant result among non-smokers suggests that the transcriptional activity of the enzyme CYP2E1 might be induced through the ingestion of alcohol. Thus, in alcoholics with allele c2, the production of acetaldehyde is increased, as determined by MOES analysis. This increased acetaldehyde is more difficult to metabolize and causes damage to organs such as the liver and pancreas (Maruyama et al., 1999). In a study of Japanese men, in 40% of the individuals, the c2 allele was associated with heavy alcohol consumption (Sun et al., 2002). Verlaan et al. (2004) demonstrated that there was no significant difference in the genotype distribution of *CYP2E1* c1/c2 between alcoholics and controls in Caucasian populations. Among Chinese populations, no association was observed between the alleles of the *CYP2E1* gene and alcohol use disorder (Carr et al., 1996).

Hepatocellular carcinoma (HCC) is the most common form of liver cancer characterized by a high recurrence rate and a poor prognosis. Most risk factors can lead to the formation of liver fibrosis and further development of fibrosis or cirrhosis, which is shown in between 80% and 90% of patients with HCC. Thus, the status of fibrosis liver tissue surrounding the tumor might be very important for the recurrence and the clinical outcome of these patients after surgical resection. In this study, the activities of ADH in 68 livers from HCC patients was determined based on liver tissues and showed substantial variation. The influence of gene polymorphisms and content on inter-individual variations in metabolic activities was systematically investigated. By studying the adjacent tumor liver tissue, risk factors that affect the development of HCC may be found (Gao et al., 2022). For example, Zhou et al (2016) and Gao et al (2018) have carried out a series of studies on adjacent tumor tissues in HCC patients and have found that CYP2E1 activity can affect the occurrence and prognosis of HCC. Secondly, compared with the whole liver, a tumor takes up a small proportion, so the adjacent tumor tissues can better reflect the overall condition of the metabolic enzyme in the liver. Lastly, enzyme activity is more representative of enzyme characteristics than enzyme content because the content is not the only factor that affects enzyme activity.

Moreover, the effects of ADH mutations on content were also analyzed. Interestingly, the study found that the polymorphism locus of ADH1C rs698, ADH1C rs2241894 significantly affected the content of the corresponding enzyme, and the extent of the effect on content was consistent with that on activity. This suggests that the effects of the above polymorphisms on activity were achieved by the effects on the content. While ADH1B rs1229984 only increased the activity, not content, this locus might directly affect enzyme activity (Gao et al, 2022).

Silva Junior et al (2020) evaluated the HTR2A and DRD4 genes related to the serotonergic and dopaminergic systems and their alterations in their sequences if they were associated with alcohol dependence, in Piauí, Brazil. A-1438G and T102C on the HTR2A gene, as well as duplication of 120 bp in the DRD4 gene, and the association of these mutations with alcohol abuse in a Brazilian male population. In this work, the genotypes GG and CC genotypes in HTR2A gene polymorphisms, as well as the AG+GG and TC+CC genotypes, associated with a positive history of alcohol dependence, may be more susceptible to the inheritance of the disease because they would be individuals with these mutations.

Thus, further studies are needed to better understand the role of the content and enzymatic activity of the polymorphic genes present in this work, ADH1B, ADH1C and CYP2E1, in alcohol dependence and in cancer. And further studies have to be done to understand the role of systems serotonergic and dopaminergic and their sequences changes with AUD.

5. Conclusion

Our study suggests a possible association between polymorphisms of the genes *ADH1B* (Arg48His), *ADH1C* (Ile350Val), and *CYP2E1* (*5*′ promoter region of gene regulation) with alcoholism in the population of northeastern Brazil. In conclusion, the *ADH1B* gene polymorphism (Arg48His) might reduce the risk of alcohol use disorder. By contrast, a mutation in the *Pst*I site in the *CYP2E1* gene was prevalent among alcoholics, suggesting a predisposition to alcohol use disorder in individuals carrying the polymorphism and the action of the c2 allele in alcohol metabolism when alcohol is present in high

concentrations in the blood. By contrast, the *ADH1C* gene did not exhibit a correlation with alcohol dependence. Additional studies with larger numbers of subjects, an analysis of other variables (diseases related to alcohol use disorder treatment response), and the inclusion of other regions of Brazil are needed to assess this correlation and enhance knowledge concerning this disease to facilitate more efficient prevention or treatment with respect to addiction and alcohol use disorder-related diseases.

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