

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

**The effect of alcohol dehydrogenase gene
polymorphisms on alcohol consumption and chronic liver
diseases in Hungary**

By Reka Toth

Supervisor: Roza Adany, MD, Ph.D., D.Sc.



UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF HEALTH SCIENCES

Debrecen, 2011

Introduction

Epidemiology of liver diseases

Although standardized death rates have recently declined, premature mortality from chronic liver disease and cirrhosis remains markedly higher in the countries of Central and Eastern Europe – especially in Hungary – than in their Western European neighbours. The mortality from chronic liver diseases (CLDs) and liver cirrhosis is particularly high among Hungarian males; at its peak in 1994, it was over five times higher than the rate seen in Western European countries at any time in the previous decades.

Alcohol is the major cause of liver cirrhosis in the Western world. According to the European Detailed Mortality Database, among Hungarian males, the alcoholic liver disease (ALD) was responsible for more than 80% of the deaths from liver diseases. At least 80 % of heavy drinkers develop fatty liver, 10-35% develop alcoholic hepatitis and only 10% develop cirrhosis.

Despite that 87.2% of the Hungarian males consuming alcohol and more than 20 % of drinkers have heavy drinking episodes, the high death rates for CLDs observed in Hungary cannot simply be explained by elevated alcohol consumption rates because among males, the per capita consumption is not remarkably higher than in the Western European countries. According to these data, it has long been suspected that, aside from the alcohol, other factors may also contribute in the high chronic liver disease mortality in Hungary and other Central-Eastern European (CEE) countries. Some evidence shows that locally produced alcohol beverages that are consumed widely are rich in highly hepatotoxic aliphatic alcohols. There has, however, been no research so far on the contribution of genetic factors in Hungary.

Effects of alcohol consumption on ALD

Chronic alcohol abuse can cause liver disease which progresses from simple steatosis through steatohepatitis, fibrosis and cirrhosis to liver disease. To understand the molecular mechanisms underlying the pathogenesis, the pathway of ethanol degradation have to be reviewed.

The liver is the main organ responsible for ethanol metabolism. The major route of alcohol degradation is the oxidation of ethanol to acetaldehyde. The dominant enzymes involved in this step are alcohol dehydrogenases (ADH) which have high affinity to alcohol and are present in the cytoplasm. The second step of alcohol degradation is the oxidation of acetaldehyde to acetate catalysed by aldehyde dehydrogenase (ALDH) enzyme system located in the mitochondria. The produced acetate spontaneously breaks down to water and CO₂. Both ADH and ALDH transfer electrons to NAD⁺.

The ethanol can take its effect on the development and progression of liver diseases on at least three ways: (i) via the acetaldehyde and other toxic byproducts of the ethanol degradation pathway, (ii) via the caused biochemical changes and (iii) via oxidative stress.

Toxic byproducts of the ethanol pathway

The oxidation of acetaldehyde to acetate is relatively slow and therefore in excessive drinking allows the accumulation of acetaldehyde in the body. Due to the toxicity of acetaldehyde, several systematic effects of ethanol abuse are mediated, at least in part, by the direct or indirect effects of the elevated acetaldehyde level. Because acetaldehyde is chemically reactive, it can interact with proteins, lipids and DNA.

The main proteins appear to be preferentially modified by acetaldehyde are haemoglobin, albumin, tubulin, lipoproteins, collagen, CYP450 2E1 and ketosteroid reductase. Although the formation of aldehyde adducts during alcohol consumption has been well established, the effects of these adducts and their role in the pathogenesis of liver diseases still needs to be clarified. The calmodulin and tubulin adducts can result in impaired microtubular function and subsequently a disorganization in the hepatocytes that is characterized by structural changes in the liver. The presence of aldehydes increases the collagen production of the liver cells, possibly via an adduct-stimulated way. These disturbances in the extracellular matrix production may lead to formation of scar tissue in the liver (i.e. hepatic fibrosis). These adducts could also elicit an immune response, in which they may trigger harmful immune processes or inflammation that could lead to liver damage.

Biochemical changes

Steatosis, one of the earliest hepatic changes, could play a crucial role both in the initiation and the progression of ALD. As it was mentioned earlier, the oxidation of ethanol to acetaldehyde and subsequently acetate utilizes NAD^+ as an electron acceptor causing a shift in the $\text{NADH}:\text{NAD}^+$ ratio. This reduced state can be involved in the accumulation of lipids during alcohol ingestion with increasing the rate of fatty acid synthesis and esterification and decreasing the β oxidation of free fatty acids.

Oxidative stress

A lot of different factors and processes can cause alcohol induced oxidative stress. Some examples are:

- The shift in the $\text{NADH}:\text{NAD}^+$ ratio
- The effects of the produced acetaldehyde
- Alcohol induced hypoxia
- Alcohol's effect on the immune system (e.g. the cytokine production)
- Alcohol induced increase in the activity of CYP450 2E1

Oxidative stress has also a very important role in the activation of inflammation and the increase in apoptotic hepatocyte death primarily through the enhanced production of $\text{TNF}\alpha$.

Pharmacological and behavioural effects of acetaldehyde

Acetaldehyde accumulation in the periphery produce the symptoms of the so-called “alcohol sensitivity”. These symptoms are often observed in people with deficient ALDH enzyme. These include vasodilatation, increased skin temperature, facial flushing, tachycardia, lowered blood pressure, dry mouth or throat associated with bronchoconstriction, nausea, headache and euphoria. These adverse effects of alcohol consumption can deter from further drinking therefore reduce the susceptibility of developing alcohol dependence (AD).

Although the adverse effects of acetaldehyde are well known, its role in brain is still matter of debates. Some researchers suggest that the level of the acetaldehyde remains low in the brain, mainly its limited diffusion through the blood-brain barrier. By contrary, others claim that in case of high acetaldehyde concentration, it can cross the barrier and take part in the reinforcing mechanism of alcohol consumption.

The alcohol dehydrogenase gene family

There are seven ADH genes, localized on the chromosome region 4q22 in humans. These enzymes are classified in 5 classes based on their enzymatic properties and sequence similarities. Alcohol degradation in the liver primarily involves Class I enzymes. Class I ADHs consist of α , β and γ subunits, encoded by the genes ADH1A, ADH1B, and ADH1C. Since these enzymes have a low K_m , the produced acetaldehyde is normally eliminated shortly after being formed.

Genetic polymorphisms of ADH1B and ADH1C

Although the level of the alcohol consumption is the main determinant of alcohol dependency and chronic liver diseases, their risk, at an individual level, is also affected by genetic factors. Results from twin studies and experimental work on a range of receptors have produced an estimate of 40 to 60% heritability for alcohol dependence, while 49% of the variability of alcohol elimination rate is genetically determined. Coding variations in the corresponding genes influence the kinetic properties of the enzymes and may result in acetaldehyde accumulation. Single nucleotide polymorphisms (SNPs) causing amino acid changes in the NAD^+ coenzyme-binding domain have been described in both ADH1B and ADH1C that modify the enzymatic properties.

The ADH1B*2 allele (rs1229984) results in an arginine to histidine change in the 48th amino acid site that gives rise to the β_2 subunit with a 40-fold higher V_{\max} in homozygous form than the β_1 subunit that is encoded by the wild type ADH1B*1 allele. The ADH1B*3 (rs2066702) encodes the β_3 subunit, containing an arginine to cysteine change at the 370th amino acid site. In its homodimer form, it has 30-fold higher V_{\max} than β_1 . The wild type ADH1C allele, the ADH1C*1 (γ_1 subunit) contains arginine at 272nd and isoleucine at 350th position, while the ADH1C*2 variant, which encodes the γ_2 subunit, contains glutamine

(rs1693482) and valine (rs698), respectively. The V_{\max} of γ_1 is 2-2.5 times higher than that of γ_2 . The two SNPs forming the ADHC*2 allele are in high linkage disequilibrium.

Variants with higher activity (γ_1 , β_2 and β_3) are considered to give rise to acetaldehyde accumulation via faster ethanol degeneration, because the ALDH with unchanged activity is not able to cope with the increased speed of ethanol degradation. The accumulation of acetaldehyde may lead to the previously mentioned adverse effects of this toxic byproduct. These effects may deter from further drinking but, as it is hypothesized, if individuals persist with alcohol consumption, hepatotoxicity and other tissue damage may occur, due to the effects of acetaldehyde.

According to meta-analyses, the ADH1B*1 allele is found to be associated with significantly increased risk of alcoholism (it also means that ADH1B*2 allele is associated with lower risk of alcoholism) both in Caucasians and East Asians but the association is stronger in Asian populations, perhaps because of different haplotypes. For liver disease, the main meta-analysis did not show significant association neither in Asian nor in Caucasian populations. It is important to recognize that since these analyses were conducted using patients with alcoholic liver disease and alcoholic controls, the ADH1B*1 allele was hypothesized to be protective against liver diseases. This also means that the ADH1B*2 allele is thought to be susceptible for liver diseases among alcoholics.

The effect of the ADH1C*2 allele was concluded in the same meta-analyses. It showed significant association with alcohol dependence only among Asians.

Rather less data is available on ADH1B*3 allele. No significant association of ADH1B*3 with alcohol dependence has been found in African Americans. However, this allele proved to be protective against alcohol dependence in Mission Indians in California and in African-American families, according to another study.

The frequency of the polymorphic alleles varies among geographical regions, exhibiting an East-West gradient. The ADH1B*2 allele is relatively rare in Afro-Americans and Caucasians with a frequency of 0-10%. This allele is more frequent among Asian populations, where its frequency varies from 50 to 90 percent, depending on ethnicity.

The ADH1B*3 is mainly found among African Americans, with a prevalence of 10-35% but it is rare or absent in Caucasian and Asian populations.

ADH1C*2 allele frequencies range from about 30 to 50% in Caucasian populations. It is strongly varies in Asian populations, with a higher frequency among East-Asia and lower in Southwest-Asia. In Africa, the frequency of the allele is higher than among Caucasians, but lower than East-Asians. This interesting pattern of allele distribution probably evolved due to the linkage disequilibrium between the different ADH genes and the possible selection mechanisms on ADH1C*2 or other alleles, such as ADH1B*2.

Aldehyde dehydrogenase gene family

There are three classes of ALDHs. The most important enzymes for acetaldehyde oxidation are the cytosolic ALDH1 and the mitochondrial ALDH2. Both enzymes are tetrameric and have low K_m for acetaldehyde. The ALDH2 gene is polymorphic.

The Glu504Lys polymorphism of ALDH2

This polymorphism encoded by a G to A substitution results in replacement of glutamate with lysine at position 504 (the official name of the polymorphism has recently changed from Glu487Lys). The wild allele named ALDH2*1, the mutant allele is the ALDH2*2. Homozygotes for ALDH2*2 have essentially no ALDH2 activity; while heterozygotes have markedly reduced activity. The presence of an ALDH2*2 allele is strongly protective against alcohol dependence due to the severe adverse effects of the accumulated acetaldehyde. But, as in case of ADH1B*2 allele if heterozygotes tolerate heavy drinking may suffer from the harmful effects of the elevated level of acetaldehyde. The inactive allele is relatively common in people of Asian descent, but it is rare or absent in people with Caucasian and African descent.

In our study we have screened our study population for ALDH2 Glu504Lys, although it is very rare in European populations; the mortality of chronic liver diseases is so high in Hungary that we cannot dismiss any of the possible explanations.

Aims

The aim of our study was to determine if the genetic background plays significant role in the high prevalence of chronic liver disease in Hungary. To assess the frequency and the effect of the most frequent ADH polymorphisms, a case-control study was undertaken. By mapping the alcohol consumption patterns of the involved population, the aim of this study was to measure whether these mutations have any effect of drinking habits. Apart from describing the single effects of these polymorphisms, our goal was to analyse their combined effect both on CLDs, AD and alcohol use. The results may help not only to an improved understanding of the high prevalence of chronic liver diseases in Hungary but it may contribute to better understanding of how these polymorphisms modify each other's effects in relation to alcohol consumption and liver disease.

With better understanding of the genetic polymorphisms' role in determining the individual drinking habits and in the development of liver diseases, more personal therapies can be applied. This is very important, since in Hungary, alcoholism is also a very serious issue. On the level of the population, mapping the frequency of allelic variants can help in evolving public health programs more suitable to local specialties to lower the high level of cirrhosis and liver disease.

Materials and methods

Sample collection

In 1998 the School of Public Health in the University of Debrecen and the National Public Health and Medical Officer Service set up a surveillance system in Hungary's four counties (Szabolcs-Szatmár-Bereg, Hajdú-Bihar, Zala and Győr-Moson-Sopron). The General Practitioners' Morbidity Sentinel Station Program (GPMSSP) was established to monitor the morbidity of non-communicable diseases with major public health importance. The involved four counties represent the well-known differences in socio-economic and health status between the eastern and western part of Hungary. The population registered to these practices was representative in terms of age and sex of both the participating counties and the overall Hungarian population. This program also provides a framework for epidemiological researches.

The source population of our study was restricted to men aged 45-64 at the time of data collection. Controls were selected with random systematical sampling. Potential cases were patients with previously diagnosed chronic liver disease. Both cases and controls underwent physical and laboratory tests to verify the previous diagnosis or the absence of the disease. The diagnostic criteria of chronic liver disease were the following: having at least two of the following pre-specified criteria: spider naevi, ascites, palmar and plantar erythema, jaundice, enlarged, firm liver with rounded or nodular edge, and at least one of the following laboratory findings: increased level of serum bilirubin, elevated aspartate transferase activity, elevated alanine transferase activity, elevated gamma-glutamyl-transpeptidase activity, elevated alkaline-phosphatase activity, decreased serum albumin. In case of contradiction between the previous diagnosis and the laboratory results, the affected patients were categorized according the latter.

Blood samples were taken from the involved persons for laboratory and genetic tests and they filled in a questionnaire which contained questions about their financial, marital and educational status, health behaviours and alcohol consumption habits. Patients with hepatitis B or hepatitis C infection were excluded from the analysis.

Altogether, 666 controls and 241 cases were involved in our study; the participation rate was 60%. As for age distribution, controls were representative for the overall Hungarian population ($p=0.424$), while cases were significantly older (55.17 years vs. 53.89 years, respectively, $p=0.0023$). Written informed consent was obtained from each patient. The study was approved by the Regional and Institutional Ethics Committee, Medical and Health Science Centre, University of Debrecen.

Questionnaire-based data collection

Detailed information on alcohol consumption was gathered with a self-completed questionnaire, yielding the following variables.

Education is categorised from 0 to 3, where 0 signifies an individual with 8 years of education or less, 1 signifies having attended secondary school without doing the school leaving exam, 2 signifies completed secondary education and 3 signifies higher education.

Financial status has 3 possible values, from 0 (bad/ very bad) and 1 (adequate) to 2 (good/ very good) based on self-administration.

Detailed information on alcohol consumption was gathered with a self-completed questionnaire, yielding the following variables.

Frequency of drinking, with seven possible outcomes: (i) has never drunk alcohol, (ii) didn't drink in the last twelve months, (iii) drinks less than once a month, (iv) drinks 1 to 3 times in a month, (v) 1 to 2 times in a week, (vi) 3 to 4 times a week and (vii) at least 5 times a week.

Summary drinking data contains 4 categories: (i) non-drinker; (ii) infrequent drinker, consuming alcohol less than 3 times per month; (iii) moderate drinker, if consumption is at least weekly, the weekly total is 14 units or less, and the daily amount is never more than 5 units; (iv) heavy drinker, if more than 14 units are consumed weekly or if the amount more than 5 units is consumed on at least one day of a week (1 unit=15 g pure ethanol).

The number of problem drinkers was measured with the CAGE questionnaire. This questionnaire contains four indirect questions to detect drinking problems. The word CAGE is

an acronym according the names of the questions (**C**ut, **A**nnoyed, **G**uilty, **E**ye-opener). The questions are the following.

1. Have you ever felt that you should cut down on your drinking?
2. Have people annoyed you by criticizing your drinking?
3. Have you ever felt guilty or bad about drinking?
4. Have you ever had a drink first thing in the morning to steady your nerves or to get rid of a hangover (Eye opener)?

According the number of the positive answers we formed two variables.

CAGE score, from 0 to 4, according to the number of positive answers on the CAGE questionnaire.

CAGE status is negative if the number of positive answers is 0 or 1, and positive if it is 2 or more.

DNA preparation

DNA isolations were performed from the leukocytes of blood with EDTA anticoagulation taken by the GPs. We used MagNA Pure LC DNA Isolation Kit –Large Volume (Roche Diagnostics, GmbH, Mannheim, Germany) according to the manufacturer's instructions.

Genotyping

Genotyping was performed on LightCycler 1.5 System (Roche Diagnostics, GmbH, Mannheim, Germany) by real-time polymerase chain reaction (rt-PCR) followed by a melting curve analysis.

ADH1C Arg272Gln (rs1693482) and Ile350Val (rs698) polymorphisms were screened together in a duplex reaction, while ADH1B Arg370Cys (rs2066702) and ADH1B Arg48His (rs1229984) were analyzed in a simplex reaction. The sequencing, where needed, was conducted by Biomi Ltd., Hungary, with one of the adequate PCR primers.

Statistical analysis

Single SNP analysis

Logistic regression was applied to test the association between each genotype and the outcome variables, presence of chronic liver disease, frequency of drinking, summary drinking data, number of positive CAGE answers given and CAGE status. Where variables were non-binary, ordered logistic regression was used.

The logistic regression was either bifactorial (containing only one of the above mentioned explanatory variables) or multifactorial (as well as the genotype, taking into account one of the variables describing alcohol consumption habits as independent risk factors for developing chronic liver disease), including interactions where applicable.

Linkage analysis

To map the linkage between the SNPs, Logarithm of Odds (LOD), D' and r^2 values were calculated using Haploview Software.

Multivariate SNP analysis

To detect the combined effect of the three main polymorphisms (ADH1B*2 and the two polymorphism of ADH1C*2) and the possible epistasis between them, they were entered into a multivariate logistic regression model. Altogether, five groups were formed according to the genotype status of the polymorphisms investigated: the samples in the first group (named wild/wild) were homozygous wild for all three mutations and served as reference group; in the second (wild/heterozygous) they were homozygous for the ADH1B*1 allele and heterozygous for ADH1C*2; the members of the third group (wild/mutant) were also homozygous for ADH1B*1, but they were also homozygous for ADH1C*2; the fourth group (heterozygous/wild) contained carriers for ADH1B*2 and homozygous samples for ADH1C*1, while the fifth group (heterozygous/heterozygous) was formed from samples carrying the ADH1B*2 allele and were heterozygous for ADH1C*2. Logistic regression was used to measure the association between these genotype combinations and CLDs or alcohol consumption habits.

MATERIALS AND METHODS

Odds ratios (ORs) were calculated to estimate the association between risk factors and outcome variables. Odds ratios are indicated in adjusted form, where adjustment included age, education and financial status. Results are expressed with both 95% confidence intervals and p values.

Data were analyzed using STATA 9.0 statistical software (StataCorp LP, Texas, USA).

Results

Single SNP analysis

Identification of a new SNP variant

During the analysis of the ADH1B Arg370Cys (rs2066702) mutation an unexpected polymorphism was detected. Sequencing revealed this to be a recently identified gene variant in which the same amino acid is affected but with substitution of histidine instead of cysteine. This variant is Arg370His (rs75967634). Although this mutation has been submitted recently to PubMed's SNP database, no data are available on its frequency or its effect, so it was included in the further analysis. Its allele frequency was 1% both among cases and controls.

Analysing the included SNPs

Since the ADH1C Arg272Gln and Ile350Val showed the same genotype in all but two samples, they were analyzed together, excluded the different samples, except in the linkage analysis.

The allele frequency of the ADH1B 48His was 4.4% in the cases and 8.6% among controls. The ADH1B 370Cys polymorphism's frequency was below 1%, so it was excluded from the further analysis. The allele frequency of ADH1C 272Gln/350Val was 38.7% both in cases and controls.

The presence of the ADH1B*2 allele was associated with a significantly lower probability of chronic liver disease (OR=0.47; $p=0.003$), also with the frequency of drinking (OR=0.63; $p=0.004$) and with the probability of alcohol dependence, according to CAGE status (OR=0.53; $p=0.009$) e.g. heavy drinkers are less likely to carry ADH1B*2 allele, moreover the carrier status of the mutant allele has a protective effect against being a heavy drinker. Similar strong relationships were found with the number of positive CAGE answers (OR=0.52; $p=0.002$).

To determine whether the genotype acts directly on chronic liver disease or through drinking behaviour, models were created with and without adjustment for alcohol consumption. The clear association between genotypes and disease development disappeared

RESULTS

after controlling the number of positive answers given in the CAGE questionnaire, CAGE status and frequency of drinking (OR=0.63; p=0.123; OR=0.62; p=0.101; OR=0.60; p=0.061, respectively).

To determine whether the association between ADH1B*2 allele and the risk of the development of CLDs varies by alcohol exposure, further models were fitted with interaction between genotype and alcohol exposure indicators. In CAGE negatives, the presence of the ADH1B*2 allele was associated with significantly lower odds of CLDs (OR=0.27; p=0.014), while in CAGE positives, a non-significant effect in the opposite direction was observed (OR=1.21; p=0.500). The effect modification was significant (p=0.026).

The ADH1B 370His allele did not show significant association with any of the examined outcomes.

The ADH1C*2 polymorphism in homozygous form increased the odds of more frequent alcohol drinking (OR=1.513, p=0.028), the higher drinking status according to the summarized drinking data (OR=1.582, p=0.035) and the positive CAGE status (OR=1.780, p=0.016). This allele did not show significant association with the presence of chronic liver diseases.

Linkage analysis

As expected, there is a very high linkage between the two ADH1C mutations. The results showed that in case of ADH1C Arg272Gln and Ile350Val versus ADH1B Arg48His the combination of wild-wild-mutant and mutant-mutant-wild alleles is more frequent in the population than could be expected from the allele frequencies themselves.

Multivariate SNP analysis

As it was described in the Materials and methods, five groups were formed according to the mutational status for ADH1B Arg48His and ADH1C Arg272Gln/Ile350Val. The CAGE status was significantly associated with the heterozygous/wild group, with an odds ratio of 0.550 (p=0.011). Both wild/heterozygous and wild/mutant group resulted in higher odds ratios for positive CAGE status (OR=1.540, p=0.024; OR=1.859, p=0.014).

RESULTS

Interestingly, neither the heterozygous/wild, nor the heterozygous/heterozygous groups showed difference for CAGE status.

Significant association with CLD was found only in case of groups containing the mutant ADH1B*2 allele. Both appeared to be protective, as it was expected on the basis of the single SNP analysis, with odds ratios of 0.398 (heterozygous/wild, $p=0.010$) and 0.461 (heterozygous/heterozygous, $p=0.043$).

To clarify whether these combinations act directly on the risk of chronic liver disease or via drinking habits, these outcome variables were entered together into further logistic regression models. In these models, we used either the presence of chronic liver disease or the CAGE status as an outcome variable and adjusting was made for the other variable. If CAGE status was used as outcome and the presence of chronic liver disease was adjusted for, the association with wild/heterozygous and wild/mutant groups remained significant ($OR=1.756$, $p=0.006$; $OR=2.204$, $p=0.004$). When the presence of CLDs was used as a dependent variable and results were adjusted for CAGE status, only the effect of the heterozygous/wild group on CLD remained significant ($OR=0.368$, $p=0.019$).

To assess if there is any difference in the effects of these polymorphism in drinkers and non-drinkers, additional models were used. When the logistic regression model were restricted to CAGE negative cases and controls, only the heterozygous/wild group showed significant association with CLDs ($OR=0.116$, $p=0.039$). Including only the samples with CAGE positive status, none of the groups showed significant result.

Discussion

Contrary to the alcohol consumption patterns, potential genetic factors as the cause of the increased mortality from the chronic liver diseases in the CEE countries have received less attention. Therefore, in the present study we would like to clarify whether the possible genetic differences between Eastern and Western Europe play a role in the East-West differences in cirrhosis mortality.

Beside this, our goal was to examine the important ADH polymorphisms' joint impacts on chronic liver diseases, alcohol dependence and alcohol drinking habits not only among alcoholic cases and controls but among moderate and rare drinkers as well. The results of this complex analysis may offer insights into the genetic background of alcohol consumption and alcohol related diseases not only among Hungarians but among other Caucasian populations.

An interesting, unexpected finding of our study was the identification of a novel mutation, the ADH1B Arg370His (rs75967634). We have also assessed its frequency. This polymorphic sequence has already been submitted into the PubMed's SNP database, but without any information on the population studied, its frequency, or possible effects. Because of its close proximity to ADH1B Arg370Cys, it cannot be detected with the previously used methods. According to these results it would worth to re-genotype or sequence the samples examined for the presence of ADH1B Arg370Cys, to validate the genotype. Due to its low prevalence, the effect of this SNP needs further investigation, including studies in other populations.

In European populations the average allele frequency of the ADH1B*2 is about 5 percent, varying by ethnicity. This allele was found in 8.31% of our control group, almost twofold higher than reported previously in Western European populations. This may, however, be explained by genetic admixture from neighbouring Slavic populations, given the much higher prevalence observed in the Russian population.

According to our results, carriage of ADH1B 48His allele reduces the risk of developing liver disease (OR=0.47), but this is no longer significant after adjustment for most of the measures of alcohol consumption. It seems that this allele acts directly on alcohol

consumption, and its effect on liver diseases is only indirect. We did not find significant increase in the odds of liver diseases among patients with AD. However, the interaction between the presence of the alcoholism and the allele proved to be significant. This can also mean that although this protective polymorphism has high prevalence among Hungarians, the benefits of carrying it can be neutralized or even inverted by carrying on drinking.

The two main polymorphisms of ADH1C, the Arg272Gln and Ile350Val have an almost complete linkage between each other, as it was expected from previous studies. Although previous meta-analyses and other studies did not find an association between these alleles and alcoholism or liver disease, some researchers have described associations with alcohol drinking habits and/or AD among Europeans. However, while our results confirm the lack of association with CLDs, we did find a significantly increased OR for problem drinking (i.e. positive CAGE status) and heavy drinking in ADH1C*2 homozygous patients. This ambiguity may be due to the differences in categorizing drinking habits in different studies and the relatively minor effect of this genotype. Our results of the linkage disequilibrium completely concur with previous studies. This linkage between these alleles means that polymorphisms with fast ethanol degradation ability are likely to occur together.

To clarify the independent effect of the main polymorphisms of ADH1B and ADH1C, a multivariate analysis was conducted. When CLD or alcohol use habits were used as an outcome variable, the results were what were expected on the basis of the single SNP analysis, except that being heterozygous for the ADH1B 48His allele did not reduce the odds of problem drinking. This can be due to the fact that this allele has an adverse effect on CAGE status in cases and controls, e.g. in cases it did not prove to be protective against CAGE positivism which can result in the lack of significant association.

Interestingly, when CAGE status was used as an outcome variable and an adjustment was made for the presence of CLD, only the association with homozygous ADH1B*1 status remained significant. When CLD was the outcome variable and CAGE status was controlled for, the association with carriage of ADH1B*2 and homozygous for ADH1C*2 remained significant. These results suggest that it is reasonable to suppose that the polymorphisms of ADH1C impact directly on drinking habits only. The outcome of the single SNP analyses also supports this hypothesis. On the contrary, while the ADH1B*2 allele seemed to impact only on drinking habits when analysed individually, the results of the multivariate analysis

DISCUSSION

suggest that in the absence of the controversial ADH1C*2 allele it is significantly protective against chronic liver disease. However, this hypothesis needs to be confirmed by enzymatic tests or other case-control studies.

This study reinforces the observation that the CAGE questionnaire is a more accurate measure of problem drinking than questions on consumption. One well-known issue is that many drinkers under-estimate their consumption (for reasons ranging from social acceptability to forgetfulness).

Nevertheless, our work represents an important step in determining the genetic background of alcoholism and chronic liver diseases. This study not only is the part of the mapping of ADH polymorphisms in Caucasians but this is one of the most complex studies conducted in Europe, involving a remarkably high number of cases and controls. The novel finding of our investigation is that it worth to involve not only alcoholic cases and controls but non-alcoholics as well. With this study set-up we were able to yield novel and interesting findings, including new interactions between ADH SNPs and alcohol dependence or CLDs.

Main achievements and results

1. We have conducted the first case-control study in Hungary to explore the genetic background of chronic liver diseases and alcohol dependence.
2. An unexpected finding was the identification of a novel mutation, the ADH1B Arg370His (rs75967634). We have assessed its frequency, but it has not shown significant association with either the alcohol consumption variables or the chronic liver disease. Due to its low prevalence, the effect of this SNP needs further investigation, including studies in other populations.
3. We have carried out a complex analysis to investigate the important ADH polymorphisms' joint impacts on chronic liver diseases, alcohol dependence and alcohol drinking habits not only among alcoholic cases and controls but among moderate and rare drinkers as well.
4. The ADH1B Arg48His mutation has the strongest affect among the examined SNPs. According to our results, this polymorphism acts mainly on drinking habits, lowering the odds of frequent and problem drinking. If this polymorphism is associated with the wild type ADH1C mutations it seems to be protective against CLDs.
5. We have found a relatively high allele frequency among controls for ADH1B 48His compared with the allele frequency in the Western European countries. However, due to the different effect of this polymorphism in problem drinkers, the benefits of carrying this protective polymorphism can be neutralized or even inverted by carrying on drinking.
6. We have concluded that the two main ADH1C polymorphisms (Arg272Gln and Ile350Val) are almost in complete linkage, and they act directly on drinking habits only, resulted in significantly increased OR for problem drinking (i.e. positive CAGE status) and heavy drinking.
7. According to the results of the linkage analysis, we have concluded that the polymorphisms with fast ethanol degradation ability are likely to occur together.



UNIVERSITY AND NATIONAL LIBRARY UNIVERSITY OF DEBRECEN
KENÉZY LIFE SCIENCES LIBRARY

Register Number: DEENKÉTK /177/2011.

Item Number:

Subject: Ph.D. List of Publications

Candidate: Réka Tóth

Neptun ID: VMUUIIC

Doctoral School: Megelőző Orvostani Intézet

List of publications related to the dissertation

1. **Tóth, R.**, Fiatal, S., Petrovsky, B.É., McKee, M., Ádány, R.: Combined effect of ADH1B rs1229984, rs2066702 and ADH1C rs1693482/ rs698 alleles on alcoholism and chronic liver diseases.
Dis. Markers. "accepted by publisher", 2011.
IF:1.723 (2010)
2. **Tóth, R.**, Pocsai, Z., Fiatal, S., Széles, G., Kardos, L., Petrovsky, B.É., McKee, M., Ádány, R.: ADH1B*2 allele is protective against alcoholism but not chronic liver disease in the Hungarian population.
Addiction. 105 (5), 891-896, 2010.
DOI: <http://dx.doi.org/10.1111/j.1360-0443.2009.02876.x>
IF:4.145

List of other publications

3. Fiatal, S., Szigethy, E., Széles, G., **Tóth, R.**, Ádány, R.: Insertion/deletion polymorphism of angiotensin-1 converting enzyme is associated with metabolic syndrome in Hungarian adults.
J. Renin-Angiotensin-Aldosterone Syst. Epub ahead of print (2011).
DOI: <http://dx.doi.org/10.1177/1470320310394231>
IF:1.6 (2010)
4. Lázár, V., Ecsedi, S., Szöllősi, A., **Tóth, R.**, Vízkeleti, L., Rakosy, Z., Bégány, A., Ádány, R., Balázs, M.: Characterization of candidate gene copy number alterations in the 11q13 region along with BRAF and NRAS mutations in human melanoma.

PUBLICATIONS



UNIVERSITY AND NATIONAL LIBRARY UNIVERSITY OF DEBRECEN
KENÉZY LIFE SCIENCES LIBRARY

Mod. Pathol. 22 (10), 1367-1378, 2009.

DOI: <http://dx.doi.org/10.1038/modpathol.2009.109>

IF:4.406

Total IF: 11,874

Total IF (publications related to the dissertation): 5,868

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

26 August, 2011

