Open Access
CODEN: IJBNHY
ISSN: 2278-778X
Original Research Article
International Journal of Bioassays

Polymorphisms in alcohol metabolizing genes ADH2 and ADH3 and susceptibility to pancreatitis in alcoholics

Vedangi Aaren*, Godi Sudhakar, L.R.S. Girinadh
Department of Human Genetics, College of Science & Technology, Andhra University, Visakhapatnam-530003, Andhra Pradesh, India.

Received: January 4, 2017; **Revised:** January 23, 2017; **Accepted**: February 14, 2017
Available online: 1st March 2017

Abstract: In both developed and developing countries, overuse of alcohol is a considered as the major cause of acute and chronic pancreatitis. Prolonged overconsumption of alcohol for 5–10 years typically precedes the initial attack of acute alcoholic pancreatitis. It is observed that only a minority (around 5%) of alcoholics develop pancreatitis. It is now established that the pancreas has the capacity to metabolize ethanol. Previous studies have shown that there are two major pathways of ethanol metabolism, oxidative and non-oxidative. Oxidative ethanol metabolism involves the conversion of ethanol to acetaldehyde, a reaction that is catalysed by aldehyde dehydrogenase (ADH) with contributions from cytochrome P450 enzyme (CYP2E1) and possibly also catalase. Genetic factors regulating alcohol metabolism could predispose in developing alcoholic pancreatitis (AP). We investigated the association of polymorphisms in ADH enzymes with the alcoholic pancreatitis in North coastal Andhra Pradesh. Patients with alcoholic pancreatitis (AP; n = 100), alcoholic controls (AC; n = 100), and healthy controls (HC; n = 100) were included in the study. Blood samples were collected from the subjects in EDTA coated vials. DNA was extracted and genotyping for ADH2 and ADH3 was done by PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism). The products were analysed by gel electrophoresis. The frequency distribution of ADH3*1/*1 genotype was significantly higher in AP group (54%) compared with AC (35%), and HC (42%), and was found to be associated with increased risk of alcoholic pancreatitis. There was no statistically significant difference between the frequency distribution of ADH3*1/*1, ADH3*1/*2, and ADH3*2/*2 genotypes between AC and HC. There was no statistically significant difference between the frequency distribution of ADH2*1/*1, ADH2*1/*2, and ADH2*2/*2 genotypes in AP compared with AC and HC. This study shows that carriers of ADH3*1/*1 individuals consuming alcohol are at higher risk for alcoholic pancreatitis than those with other genotypes such as ADH3*1/*2 and ADH3*2/*2.

Key words: Alcoholic pancreatitis; genetic polymorphism; alcohol dehydrogenase.

Introduction

Alcohol consumption is suggested to be a major cause of chronic pancreatitis in 70–80% cases in developed countries [1]. Genetic factors regulating alcohol metabolism could play a role in developing alcoholic pancreatitis [2]. Alcohol tolerance is highly dependent on the presence or absence of alcohol metabolizing enzymes and the subjects having history of alcohol consumption are more susceptible for chronic pancreatitis onset [3]. A high prevalence of chronic pancreatitis in India suggests that it is an endemic zone for chronic pancreatitis and points towards a possible genetic and/or environmental factor as playing an important etiologic role. Although these factors are critical in the etiology of chronic pancreatitis, their role in alcoholic pancreatitis is not clearly elucidated.

Several polymorphisms have been described which are known to influence alcohol metabolism and might have effects on alcohol toxicity. These polymorphisms include genes that are translated into enzymes which metabolize both ethanol to acetaldehyde (alcohol dehydrogenase [ADH], cytochrome P450-2E1 [CYP2E1]) and acetaldehyde to acetate (aldehyde dehydrogenase [ALDH]). Alcohol is mainly oxidized by oxidative pathway.

The major enzyme of oxidative pathway of alcohol metabolism is alcohol dehydrogenase (ADH)- a dimeric Zn-containing protein [4]. ADH is encoded by at least seven gene loci located on the long arm of chromosome 4 (chromosome 4q22). Out of the seven genes ADH2 (ADH1B) and ADH3 (ADH1C) are highly polymorphic [5]. The ADH1B*2 (ADH1B*47His) allele codes for a higher activity enzyme as compared with the ADH1B*1 (ADH1B*47Arg) allele [6]. The most widely studied functional polymorphisms in the ethanol metabolic pathway is the ADH1B Arg47His. Reports on the ADH1B*2 allele (Arg47His polymorphism) frequency in the Indian population are inconsistent [7] Goedde *et al.,* reported a 9.9 per cent ADH1B*2 allele frequency in a heterogeneous sample from the Indian population, whereas another study from India (on the Kachari population) reported a 6.6 per cent frequency.

ADH3 gene has two alleles ADH3*1, ADH3*2 encoding subunits γ^1 (Arginine at position 272 and

***Corresponding Author**:
Dr. Vedangi Aaren,
Department of Human Genetics,
College of Science & Technology,
Andhra University, Visakhapatnam -530003, A.P., India.
E-mail: vedangi.aaren@gmail.com

isoleucine at position 350), and γ^2 (glutamine at position 272 and a valine at position 350), respectively [5]. The subunit γ^1 shows higher ethanol activity than the subunit γ^2 ([8]. In majority of cases the occurrence of these two SNPs have been observed, indicating very high linkage disequilibrium [5].

Materials and Methods

All consecutive patients with alcoholic pancreatitis attending the Gastroenterology and Hepatology department in the King George Hospital, Visakhapatnam were included in the study as per inclusion criteria. Diagnosis was based on clinical and radiological criteria. Inclusion criteria for alcoholic pancreatitis were history of >60 g of alcohol ingestion for at least 6 years, radiological evidence of pancreatitis, and absence of any other cause for pain. Alcohol consumption (history, dosage and type) was taken in a questionnaire. Almost all the patients in the group had pain at presentation. The pain was epigastric in nature usually radiating to back. History of alcohol intake was taken in all the patients (AP and AC). None of the patients in HC group had history of alcohol consumption. In brief, 100 patients with alcoholic pancreatitis, 100 healthy controls (HC), and 100 alcoholic controls (AC) free from any gastrointestinal disorder were recruited in the. Cases and controls were from the same ethnic group and were residents of the northern parts of Andhra Pradesh. The ethics committee of the institution (both the university and the hospital) approved the study. A written pre-informed consent was obtained prior to blood collection from the individuals.

Table 1: The inclusion and exclusion criteria for the recruitment of subjects for the study.

	Inclusion criteria	Exclusion criteria
AP	1. History of >60 g of alcohol intake for at least 6 years. 2. Radiological evidence of pancreatitis 3. Absence of any other cause for pain	1. Occasional alcohol intake 2. All cases of pancreatitis secondary to cholelithiasis, idiopathic or any cause other than alcohol induced pancreatitis. 3. Presence of Pancreatic Cancer
AC	Subjects with no clinical features or previous history of pancreatitis but with a positive history of alcohol consumption.	1. Presence of any other etiological factor like gall stones, hypercalcemia etc. 2. Having any gastrointestinal disorder.
HC	1. Non-alcoholic 2. No family history of Pancreatitis	1. Presence of any other etiological factor like alcoholism, gall stones hypercalcaemia etc. 2. Having any gastrointestinal disorder.

Diagnosis is based on clinical, biochemical and CT, abdominal ultrasound, endoscopic ultrasonography and/ or endoscopic retrograde cholangio-pancreatography (ERCP) by a pancreatologist or an expert gastroenterologist.

DNA isolation

Blood sample (5ml) was collected from each subject in EDTA coated vial. Genomic DNA was extracted by salting out method. The purity and integrity of genomic DNA were checked on agarose gel and by calculating the ratio of absorbance at 260/280nm. The DNA content was quantified by absorbance at 260nm (1 OD= 50mg/ml) before PCR amplification.

Genotyping for ADH2 and ADH3 polymorphism:

Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) approach was used for SNP genotyping. Forward and reverse primer sequences used for the amplification of the ADH1B Arg47His SNP were 5'-AATCTTTTCTGAA TCTGAACAG-3' and 5'-GAAGGGGGGTCACCA GGTTGC-3'. DNA was amplified with specific oligonucleotide primers. The forward and reverse primers used for the amplification of ADH1C Ile349Val SNP were 5' GCTTTAAGAGT AAATATTCTGTCCC 3' and 5' AATCTACCTC TTTCCGAAGC 3', respectively. After amplification, ADH2 amplicons were digested with NmuCI restriction enzyme (RE) and ADH3 amplicons were digested with SspI restriction enzyme at 37°C for overnight. DNA fragments in the RE digested mixture were analysed on 10 per cent polyacrylamide gel electrophoresis (PAGE). For the ADH1B polymorphism, the presence of wild-type Arg and mutant His alleles were indicated by 95 base pair (bp) and 65 bp restriction fragments respectively. For ADH1C polymorphism two bands (67 and 63 bp), three bands (~130, 67, and 63 bp), and one band (130 bp) were present which indicated $ADH3^{1-1,}$ $ADH3^{1-2,}$ $ADH3^{2-2}$ genotypes respectively. (Groppi *et al.,* 1990). [9] [10].

Results

Table 2: Summary of frequency distribution of ADH2 genotypes.

Genotype	AP (n = 100)	AC (n = 100)	HC (n = 100)
ADH2*1/*1	94 (94%)	97 (97%)	95 (95%)
ADH2*1/*2	06 (6%)	03 (3%)	04 (4%)
ADH2*2/*2	00 (0%)	00 (0%)	01 (1%)

Table 3: Summary of frequency distribution of ADH3 genotypes.

Genotype	AP (n = 100)	AC (n = 100)	HC (n = 100)
ADH3*1/*1	54 (54%)	35 (35%)	42 (42%)
ADH3*1/*2	31 (31%)	42 (42%)	50 (50%)
ADH3*2/*2	15 (15%)	23 (23%)	08 (8%)

The prevalence of ADH3*1/ADH3*1 genotype was 54% (54/100) in AP group. Frequency of ADH3*1/ADH3*2 and ADH3*2/ADH3*2 genotype were 31% (31/100) and 15% (15/100), respectively in AP group. ADH3*1/ADH3*1 genotype frequency in AP group was comparable with HC and AC groups. There was no statistically significant difference between the frequency distribution of ADH2*1/*1, ADH2*1/*2, and ADH2*2/*2 genotypes in AP compared with AC and HC.

Table 4: Graphical representation of ADH3 genotypes in all the three groups.

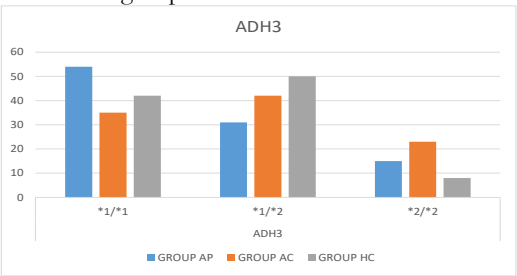

Table 5:

ADH3	*1/*1	*1/*2	*2/*2	Total
Alcoholic controls	35	42	23	100
Alcoholic pancreatitis	54	31	15	100
Healthy controls	42	40	18	100
Total	131	113	56	300

Chi-Square: 16.016, P-value: 0.003, Significant

Table 6:

ADH3		χ^2	P-Value	
Pancreatitis (AP) vs	Alcoholic controls (AC)	7.398	0.025	Significant
	Healthy controls (HC)	2.9136	0.233	Not Significant

Discussion

When each genotype for ADH3 was individually analyzed for patient's vs each control group *1/*1 genotype showed significant association with AP (p=0.006) when compared to alcoholic controls while the others failed to show any association. Studies conducted in past on other populations worldwide have demonstrated varying results some showing strong association of ADH3 polymorphism with ACP (Day *et al.,* 1991; Dumas *et al.,* 1995), some showing either statistically insignificant or no association of ADH3 polymorphism with ACP (Frenzer *et al.,* 2002; Verlaan *et al.,* 2004). Andreas *et al.,* in their study found that the genotype ADH3*2/*2 was more frequent in patients with cirrhosis (40%) than blood donors (12%; OR 4.92, 95% CI 2.36–10.31) and patients with chronic pancreatitis (8%; OR 7.33, 95% CI 2.54–23.78) but was not significantly different from alcoholic controls (23%; OR 2.27, 95% CI 0.95–5.66). In a study in North Indian population study by Singh *et al.,* 2015 the frequency distribution of ADH3*1/*1 genotype was significantly higher in ACP group (59.7%) compared with TCP (38.7%), HC (42%), and AC (37.5%) and was found to be associated with increased risk of alcoholic pancreatitis. Numerous studies have focused on the relationship between (ADH1C) polymorphism (Ile350Val) and pancreatitis risk, but the results have been inconsistent. The most important finding of the present study was the uniquely high frequency of the ADH3*1/*1 genotype being a risk-conferring factor for AP. Therefore, it shows that carriers of ADH3*1/*1 individuals consuming alcohol are at higher risk for alcoholic pancreatitis than those with other genotypes such as ADH3*1/*2 and ADH3*2/*2**.** The results from our present study warrant replication in larger samples and it can also be explained that factors like ethnicity and lifestyle could play a major role in the association of ADH3 polymorphism and AP risk.

References

1. Bhaskar L. V., Thangaraj K., Osier M., Reddy A. G., Rao A. P., Singh L., *et al.,* (2007). Single nucleotide polymorphism of the ALDH2 gene in six Indian populations. Ann. Hum. Biol. 34, 607–619.

2. Vonlaufen A., Wilson J. S., Pirola R. C., Apte M. V. (2007). Role of alcohol metabolism in chronic pancreatitis. Alcohol Res. Health 30, 48–54.

3. Czech E., Hartleb M. (2003). Genetic polymorphism of alcohol dehydrogenase- pathophysiologic implications. Adv. Clin. Exp. Med. 12, 801–809.

4. Quertemont E. (2004). Genetic polymorphism in ethanol metabolism: acetaldehyde contribution to alcohol abuse and alcoholism. Mol. Psychiatry 9, 570–581.

5. Edenberg H. J. (2007). Role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res. Health 30, 5–13.

6. Osier M. V., Pakstis A. J., Goldman D., Edenberg H. J., Kidd J. R., Kidd K. K. (2002). A proline-threonine substitution in codon 351 of ADH1C is common in Native Americans. Alcohol. Clin. Exp. Res. 26, 1759–1763.

7. Harada S., Agarwal D. P., Goedde H. W. (1980). Electrophoretic and biochemical studies of human aldehyde dehydrogenase isozymes in various tissues. Life Sci. 26, 1773–1780.

8. Day C. P., Bashir R., James O. F., Bassendine M. F., Crabb D. W., Thomasson H. R., *et al.,* (1991). Investigation of the role of polymorphism at the alcohol and aldehyde dehydrogenase loci in genetic predisposition to alcohol-related end-organ damage. Hepatology 14, 798–801.

9. Vaswani, M., Prasad, P., & Kapur, S. (2009). Association of ADHIB and ALDH2 gene polymorphisms with alcohol dependence: A pilot study from India. *Human genomics*, *3*(3), 1.

10. Groppi A., Begueret J., Iron A. (1990). Improved methods for genotype determination of human alcohol dehydrogenase (ADH) at ADH2 and ADH3 loci by using polymerase chain reaction directed mutagenesis. Clin. Chem. 36, 1765–1768.

11. Verlaan, Mariette, *et al.,* "Genetic polymorphisms in alcohol-metabolizing enzymes and chronic pancreatitis." *Alcohol and Alcoholism* 39.1 (2004): 20-24.

12. Frenzer, Andreas, *et al.,* "Polymorphism in alcohol-metabolizing enzymes, glutathione S-transferases and apolipoprotein E and susceptibility to alcohol-induced cirrhosis and chronic pancreatitis." *Journal of gastroenterology and hepatology* 17.2 (2002): 177-182.

13. Singh, Divya, *et al.,* "Polymorphism of Alcohol Metabolizing Gene ADH3 Predisposes to Development of Alcoholic Pancreatitis in North Indian Population." *Frontiers in molecular biosciences* 2 (2015).

Cite this article as:
Vedangi Aaren, Godi Sudhakar, L.R.S. Girinadh. Polymorphisms in alcohol metabolizing genes ADH2 and ADH3 and susceptibility to pancreatitis in alcoholics. *International Journal of Bioassays* 6.03 (2017): 5297-5300.
DOI: http://dx.doi.org/10. 21746/ijbio.2017.03.002

Source of support: Nil.
Conflict of interest: None Declared