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VIJAYAPURA KARNATAKA

"MOLECULAR CHARACTERIZATION OF PATATIN LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3)GENE IN ALCOHOLIC LIVER DISEASE "

DR SHREYAS

PG IN GENERAL MEDICINE

UNDER THE GUIDANCE OF
Dr. SIDDANAGOUDA.M BIRADAR. MD
PROFESSOR
DEPARTMENT OF GENERAL MEDICINE

CO GUIDE
DR.G.S.KADAKOL
RESEARCH SCIENTIST
HUMAN GENETICS LABORATORY
DEPARTMENT OF ANATOMY

BLDE (DEEMED TO BE UNIVERSITY) Shri B.M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"Molecular Characterization Of Patatin Like Phospholipase Domain Containing 3 (PNPLA3)Gene In Alcoholic Liver Disease "**has been prepared by me under the supervision and guidance **Dr. SIDDANAGOUDA. BIRADAR.MD** PROFESSOR DEPARTMENT OF GENERAL MEDICINE.

This is being submitted to BLDE (Deemed to be University) Shri. B. M. Patil Medical College, Hospital & RC, Vijayapura, Karnataka in partial fulfillment of the requirement for award of master's degree in Pediatrics. This work has not been submitted to any University by me for the award of any degree.

Date: 31-03-2025

Place: Vijayapura

DR SHREYAS

PG IN GENERAL MEDICINE

B.L.D.E. (DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"Molecular Characterization Of Patatin Like Phospholipase Domain Containing 3 (PNPLA3)Gene In Alcoholic Liver Disease "**is a bonafide and genuine research work carried out by **DR SHREYAS** in partial fulfillment of the requirement for the degree of Doctor of Medicine in GENERAL MEDICINE

Date: 31-03-2025

Place: Vijayapura

Dr. SIDDANAGOUDA.M BIRADAR. MD

PROFESSOR

DEPARTMENT OF GENERAL MEDICINE

Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

CERTIFICATE BY THE CO-GUIDE

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Date: 31-03-2025

Place: Vijayapura

Dr.G.S.KADAKOL

Research Scientist

Department of Anatomy

Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY THE HEAD OF DEPARTMENT

This is to certify that the dissertation entitled **"Molecular Characterization Of Patatin Like Phospholipase Domain Containing 3 (PNPLA3)Gene In Alcoholic Liver Disease "**is a bonafide research work done by **DR SHREYAS** under the guidance of **Dr. SIDDANAGOUDA.M BIRADAR. MD** PROFESSOR DEPARTMENT OF GENERAL MEDICINE Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapura.

Date: 31-03-2025

Place: Vijayapura

Dr S N BENTOOR.M.D.

Professor and HOD

Department of GENERAL MEDICINE

Shri B. M. Patil Medical College,

Hospital & Research Centre,

BLDE(Deemed To Be University)

Vijayapura, Karnataka

B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

ENDORSEMENT BY THE PRINCIPAL

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Date: 31-03-2025

Place: Vijayapura

Dr. ARAVIND PATIL.M.S.

Principal, B.L.D.E.

(DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical College

Hospital & Research Centre, Vijayapura.

B.L.D.E. (DEEMED TO BE UNIVERSITY) SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

DECLARATION BY THE CANDIDATE

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Date: 31-03-2025

Place: Vijayapura

DR SHREYAS

Post Graduate Student,

Department of General medicine

B.L.D.E.(DEEMED TO BE UNIVERSITY)

Shri B. M. Patil Medical, College,

Hospital & Research Centre, Vijayapura

BLDE (DEEMED TO BE UNIVERSITY) VIJAYAPURA, KARNATAKA

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ABSTRACT

BACKGROUND

Alcoholic and non-alcoholic fatty liver disease (ALD and NAFLD), or alcoholic and non-alcoholic fatty liver disorders, have grown to be major public health concerns in contemporary nations. Global alcohol consumption was estimated to be 6.18 litres per person on average. Hepatitis, cirrhosis, hepatocellular cancer, and steatosis are the symptoms of alcoholic liver disease (ALD). However, only 6–41% of heavy drinkers who consume more than 60–80 g/day of alcohol (and more than 20 g/day for women) go on to develop cirrhosis. Along with those environmental factors, a number of genetic variants have been linked to ALD and NAFLD. PNPLA3 is a lipoatrophic protein that is a member of the PNPLA family and has 481 amino acids.

The relationship of PNPLA3(148M) with a wide range of liver illnesses, including cirrhosis, fibrosis, non-alcoholic steatohepatitis (NASH), ALD and NAFLD, and hepatocellular carcinoma (HCC), has now been confirmed by numerous genetic investigations.The underlying pathogenic mechanisms, however, are still unknown.[1].

As few studies were done on the Indian population so this study is carried out to find out the Molecular Characterization of patatin like phospholipase domain containing 3(PNPLA3) gene in alcoholic liver disease

AIMS AND OBJECTIVES

Study of Molecular characterization of Patatin like phospholipase domain containing 3 (PNPLA3) gene in alcoholic liver disease.

MATERIALS AND METHODS

It is a cross sectional study done on a Patients admitted with Alcoholic liver disease at BLDE (Deemed to Be University) Shri B M Patil Medical College Hospital and Research Centre, Vijayapura, during the period from May 2023 to Dec 2024.The sample size is 60.

All Alcoholic Liver Disease diagnosed by clinical-history and examination, radiological-ultrasonography and biochemical parameters- like LFT(AST/ALT).

METHODOLOGY

A commercially available DNA kit is used to separate genomic DNA from 300µl of peripheral blood. PCR amplification is performed in a 20µl reaction volume with 0.5µl of each primer (5pmol) and 0.5µl of genomic DNA (75ng/µl to 150 ng/µl). The primer's annealing temperature is determined by annealing it for 10 seconds at 72°C for 154 seconds (primer extension) , followed by the final extension at 72°C which is for 5 minutes. PCR products are verified for their corresponding amplicon size using a standard 100bp ladder in gel electrophoresis.

Statistical Analysis: After the data is gathered and imported into a Microsoft Excel sheet, statistical analyses are performed using SPSS (Statistical Programme for the Four Social Sciences, Version 20). The results are shown as mean, SD, counts, percentages, and graphs. Four continuous variables, each normally distributed, will be compared between the two groups using an independent sample test. When dealing with non-normally distributed variables, the Mann-Whitney U test will be employed. To compare categorical variables between the two groups, the chi-square test or Fisher's exact test is employed.

RESULTS

We found that out of 60 patients , 25 patients (i.e,41.7%) were aged between 41-50 years , followed by 19 patients (31.7%) were between 31-40 years. The mean age and Standard deviation is 42.58 and 8.7 respectively.58 pateitns were Males (96.7%) and 2 were females(3.3%). Majority consumed alcohol for a duration of 11 to 20 years i.e, 35 patietns (58.3%) and followed by for <10 years 19 patients 31.7% .Mean and Standard Deviation of Various parameters like BMI, TB, CB, UB, Albumin, Globulin is 22.0(3.31), 7.23(8.56),5.30(7.75),1.90(1.56),2.6(0.8), 3.4(0.818) respectively. 49 patients (81.7%) had no mutation recorded and 5 patients (8.3%) had rs738409 gene positive and 6 patients(10%) had rs738409, rs738408 genes positive . Out of 60 patients , 11 (18.3%)had the gene mutation, and 49 (81.7%) did not have any mutation. However, this gene is not related to age, gender, years of alcoholic consumption and stage of the disease (p-value>0.05).

CONCLUSION:

This study shows a clear link between certain PNPLA3 gene variations and Alcoholic Liver Disease, especially the rs738409 variant, which seems to be tied to more severe cases. We found that in our study there is no statistically significant between the demographic details, stage of the disease, and years of alcohol consumption.

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ABBREVIATIONS

	Abbreviations
ALD	Alcoholic liver disorders
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
CDC	Centers for Disease Control and Prevention
GKR	glucokinase regulator
HSD17B13	hydroxysteriod 17-beta dehydrogenase 13
HCC	hepatocellular carcinoma
NAFLD	non-alcoholic fatty liver disease
MnSOD	manganese superoxide dismutase
MMP	matrix metalloproteinase
MBOAD	membrane bound O-acyltransferase domain-containing
PDCP3	phospholipase domain-containing protein 3
PNPLA3	patatin-like phospholipase domain-containing protein 3
ROS	reactive oxygen species
TSM2	transmembrane superfamily member 2
TNF	tumor necrosis factor
TB	Total serum bilirubin
BMI	Body mass index
CB	Conjugated bilirubin
UB	Unconjugated bilirubin
AST /ALT	Aspartate aminotransferase /Alanine transaminase
ALP	Alkaline phosphatase

TABLE OF CONTENT

INTRODUCTION:

Alcoholic and non-alcoholic fatty liver disease (ALD and NAFLD), or alcoholic and non-alcoholic fatty liver disorders, have grown to be major public health concerns in contemporary nations.[1]. Many people have traditionally used alcohol as a social activity and to strengthen social ties. The word "moderate" may not apply to everyone, even though moderate alcohol use is linked to cardiovascular advantages. In addition, excessive alcohol use has negative, preventable effects on one's social, professional, interpersonal, and health. Global alcohol consumption was estimated to be 6.18 liters per person on average[2]. Fatty liver, alcoholic hepatitis, and alcoholic cirrhosis—the most severe and permanent form of liver damage linked to alcohol consumption—are all included in the category of alcoholic liver disease.

Alcoholism continues to be a significant global medical and socioeconomic issue. WHO estimates that alcohol consumption in India is 26% among men and 4% among women, which is significantly lower than that of Europeans (90% among men and 81% among women). Even though India's per capita alcohol consumption is about 12.9 L/year, which is similar to the global average, the country's patterns of alcohol consumption differ greatly, with North Indians being far more likely to suffer from alcohol dependence.

In India, alcohol appear to be a contributing factor in 4% of all fatalities, 20% of hospitalizations, 18% of mental health crises, 60% of all injuries reported in emergency rooms and 20% of all brain injuries. [2.] A recent poll in Bangalore found that one-fourth of adult males drank alcohol. Additionally, the investigation showed the unexpected 2% of women regularly consume alcohol, which is a secret.[3, 4.] An estimated 200 million people drink in India, and ALD is thought to be the cause of 1.5 million of these fatalities. Since India's

per capita alcohol intake has increased over the last two decades, drunkenness is probably going to bring additional issues down the road. The liver takes the brunt of alcohol's effects on the body, as it metabolizes over 90% of the alcohol that is consumed.

Hepatitis, cirrhosis, hepatocellular cancer, and steatosis are the symptoms of alcoholic liver disease (ALD). However, only 6–41% of heavy drinkers who consume more than 60–80 g/d of alcohol (and more than 20 g/d for women) go on to develop cirrhosis.Therefore, other coexisting conditions may also have an impact on ALD in addition to alcohol. The pathophysiology and progression of ALD have been linked to a number of agents and environmental factors, such as the kind, quantity, and pattern of drinking, obesity, hyperglycemia, HIV,HCV,HBV, infection, food, toxins, and medicines, among others.[2, 5]

Along with those environmental factors, a number of genetic variants have been linked to ALD and NAFLD.These include cytochrome P4502E1, which helps break down alcohol in the liver; alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which are crucial for alcohol metabolism; and inflammatory markers like Interleukin 1 (IL-1), Interleukin 10 (IL-10), and Tumor Necrosis Factor (TNF) that influence liver inflammation.
Additionally, matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) are involved in tissue remodeling and fibrosis. Genetic factors like PNPLA3, TM6SF2, GCKR, MBOAT7, and HSD17B13 also play significant roles in liver health, affecting fat metabolism, fibrosis risk, and overall liver function.

Triglycerides and retinyl esters have been demonstrated to be hydrolyzed by the lipid droplet-protein such as patatin-like phospholipase domain-containing protein 3 (PNPLA3). [1] Another

name for PNPLA3 is a lipoatrophic protein that is a member of the PNPLA family and has 481 amino acids. Transmembrane protein PNPLA3 is primarily expressed in hepatocytes. The hepatic fat metabolism is significantly impacted by PNPLA3. Its gene mutation may have an impact on fat metabolism, including the hydrolysis and synthesis of fat. The chromosome 22 of humans contains the PNPLA3 gene. The PNPLA3 gene, which encodes i148m, is the subject of the greatest research (rs738,409 [g/C]).

The relationship of PNPLA3(148M) with a wide range of liver illnesses, including cirrhosis, fibrosis, non-alcoholic steatohepatitis (NASH), ALD and NAFLD, and hepatocellular carcinoma (HCC), has now been confirmed by numerous genetic investigations .The underlying pathogenic mechanisms, however, are still unknown.[1].

As few studies where done on Indian population so this study is carried out to find out the Molecular Characterization Of Patatin Like Phospholipase Domain Containing 3(PNPLA3) Gene In Alcoholic Liver Disease

AIMS AND OBJECTIVES

Study of Molecular characterization of Patatin like phospholipase domain containing 3 (PNPLA3) gene in alcoholic liver disease.

REVIEW OF LITERATURE :

Alcoholic hepatitis, fatty liver, and alcoholic cirrhosis—the most severe and permanent form of liver damage associated with alcohol use—are all included in the category of alcoholic liver disease. [7.] About half of cirrhosis in the US is caused by alcohol-associated liver disease (ALD), one of the most common chronic liver diseases worldwide. The phrase "alcohol-associated liver disease" has taken the place of "alcoholic liver disease" in order to avoid the discriminatory label "alcoholic." [8].

Alcoholic liver disease is classified into three histologic phases.

1. Alcoholic Steatosis or Fatty Liver: This disorder results in the accumulation of fat in the liver parenchyma.

2. Alcoholic Hepatitis: This stage is characterized by inflammation of the liver cells, and the outcome depends on how much damage has been done. Although liver failure might result from more severe cases, alcoholic hepatitis can be treated with alcohol abstinence, nutritional assistance, infection treatment, and prednisolone therapy in extreme situations.

3. Alcohol-related Cirrhosis: This type of liver damage is irreversible and causes portal hypertension and cirrhosis complications.[7]. Patients who consume large amounts of alcohol and have underlying ALD may also develop acute alcohol-associated hepatitis ,that is characterized by acute-on chronic liver injury with substantial cholestasis and the characteristic clinical presentation. jaundice [9,10]

Etiology of Alcoholic liver disease

A confluence of immunological, metabolic, genetic, and environmental variables leads to alcoholic liver disease.

The liver can withstand moderate alcohol use, but excessive alcohol consumption impairs the liver's ability to function metabolically. The first stage, referred to as fatty liver or steatosis, that is made of fat in the liver. Alcoholic hepatitis can occasionally result from continued alcohol use after this point. As alcohol use continues, alcoholic liver disease develops into "alcoholic cirrhosis," which is characterized by significant liver cell damage. The stage of alcoholic cirrhosis is characterized by nodules and increasing hepatic fibrosis.

Men are generally less at risk than women when it comes to alcoholic liver issues. A high-fat diet and obesity can really amp up the chances of developing alcoholic liver disease. Plus, if someone has hepatitis C along with it, things can get a lot tougher, with a lower chance of survival and more severe liver damage, and they often start facing these problems earlier in life. The biggest culprits for liver disease? It's all about how much and how long a person drinks, rather than what kind of drink they prefer. Interestingly, we also know that alcoholic liver cirrhosis is linked to something called Protein 3 (PNPLAP3), which has a specific part known as the patatin-like phospholipase domain.

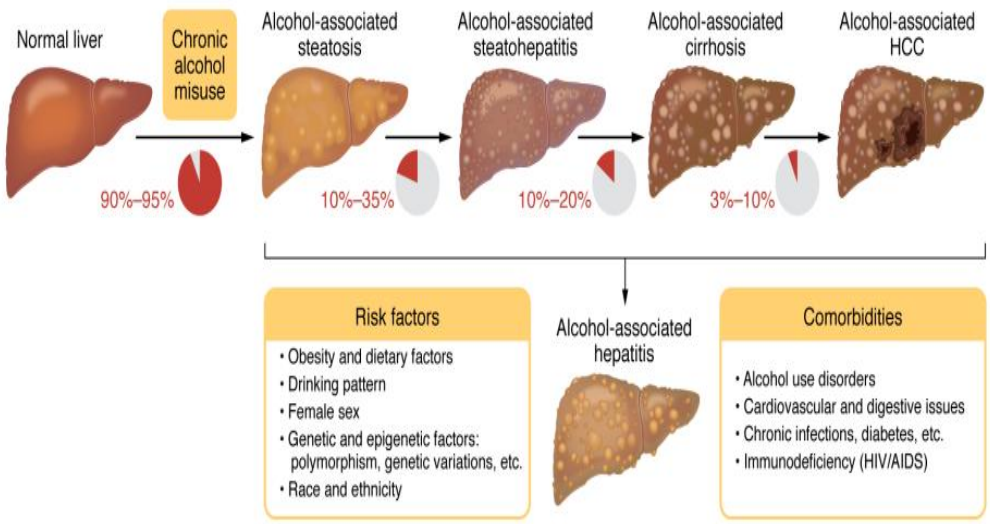


Figure 1: Etilogy of Alcoholic liver disease

Epidemiology of Alcoholic liver disease

In the United States and around the world, alcohol is the most widely abused substance. In the US, it is the most frequent cause of liver disease. Heavy drinkers make up 10–12% of the 61% of Americans who are impacted by it.

Alcoholic liver damage is most common in European countries. Thirty to fifty grams of alcohol every day for more than five years can cause alcoholic liver damage. 30% of patients who have drank more than 40 g of alcohol daily over an extended period of time may develop cirrhosis, and 90percentage of the patients who use > 60 gm of alcohol daily may develop steatosis.

Like many poor countries, India has limits in the quality of its epidemiological data resources on liver illness, including nonexistent electronic databases nationwide, uniformity in reporting, and limitations in diagnostic precision and clinical phenotyping. Although liver illnesses are a leading cause of premature mortality and disability, there is a good amount of evidence that they are also having an increasing influence on the nation's economy and health care resources. India's current cultural-lifestyle shift, includes a of the western diet, sedentary lifestyles, and a liberation from social taboos surrounding alcohol, is contributing to a range of liver diseases that exhibit rapid switchovers.

Among these changes is the growing significance of nonalcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) as causes of liver disease, in addition to viral causative factors. Two significant demographic factors contributing to this shift are the nation's growing population and rising life expectancy. Accordingly, India has implemented supportive health system reaction tactics. Since 2018, India has had a federally sponsored National Viral Hepatitis Control Program that incorporates linkage-to-care (screening at-risk populations, providing medications, and monitoring CLD) and preventive (vaccination, blood safety) techniques. Furthermore, in 2021, a nationwide program to control noncommunicable illnesses was introduced, which includes efforts that incorporate liver disease control in a broader sense into a program to control other noncommunicable diseases.[11,12]

Syndromes of Liver Disease	Etiology	Prevalence (%)	Contribution to Mortality (%)
Acute liver disease	HAV	1.7-33	5-6.3
	HEV	30-50	30-40
	HBV	13.9-27.6	55-60
	Non-A-E virus	14.6-43.9	0.5-2
	Drugs (ATD and others)*	0-15	
CLD including cirrhosis	HBV	17.6-47.9	30-60
	HCV	5.2-44.9	10-22
	Alcohol	10.9-31.9	20-25
	NAFLD/NASH	2.6-43.6	10-15
	Others*	9.7-23.2	5-10
Liver cancer	HBV	46.8	40-60
	HCV	14.8	10-20
	Alcohol	9.6	15-20
	NAFLD/NASH	4.6-19	5-10
Prevalence of population-level risk factors	HBV	2.4-3.7	-
	HCV	0-5.2	-
	NAFLD/NASH	8.7-32	-
	Hazardous drinking habit among alcohol users	44.4	-

Data are taken and summarized from Mukherjee et al.,[3] Sarin et al.,[6] and Anand et al.[7]
*"Others" include autoimmune hepatitis and other autoimmune liver diseases, Wilson's disease, and unknown etiologies.

Table 1: Frequency of epidemiology of different cause of liver disease

Pathophysiology [13,14]

Though they are still not fully understood, the cellular and molecular mechanisms of ALD pathogenesis appear to be connected to a complex of interplay of the genetic, behavioral, and environmental variables. In setting of the ongoing alcohol exposure, steatosis, inflammation, fibrosis, and other histological characteristics of ALD are the outcome of sequential and connected pathophysiological events. Acetaldehyde (AA), the initial metabolite of alcohol breakdown, is directly hazardous, which has played a crucial role in the development of ALD. Through oxidative degradation, alcohol can be converted to AA by two main enzyme systems, with alcohol-dehydrogenase being the mechanism mostly in charge of digesting smaller amounts of alcohol. It is found in the cytosol and is not upregulated when called for. The microsome-based cytochrome P450 2E1 (CYP2E1), on the other hand, is inducible and can be increased 10–20 times in heavy drinkers.[15].

By producing AA, a highly reactive, poisonous, and mutagenic metabolite, both enzyme systems contribute to alcohol-related toxicity in addition to breaking down ethanol (and other organic materials). By producing reactive oxygen species (ROS) including hydrogen peroxide and superoxide anion, CYP2E1 not only produces AA but also leads to oxidative damage. Human hepatic CYP2E1 activity may already rise after consuming 40 g of ethanol each day for a week. CYP2E1 activation in rodents was linked to lipid peroxidation, hydroxyethyl radical generation, NAD phosphate oxidase activity, and the extent of liver damage; these effects may be prevented by employing the CYP2E1 inhibitor clome-thiazole.[16].

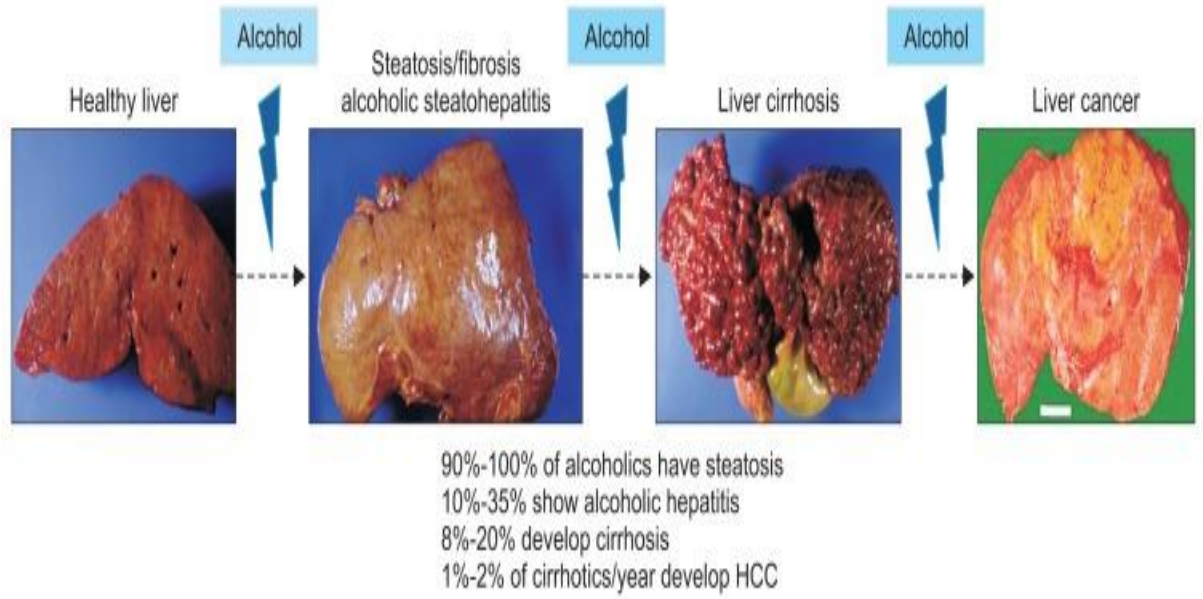


Figure 2: steatosis architectural distortion going to cirrhosis.

Seatosis, which is the first sign of liver problems for people who drink a lot, the main culprits are a bit of a mix-up in how the body handles fats. Basically, there's too much fat coming into the liver from different sources, like fat being released from our body's own reserves and through chylomicrons coming from our diet. On top of that, the liver isn't breaking down fatty acids as it should, and it's also cranking out too much fat and triglycerides. Recently, researchers have discovered that certain protein enzymes involved in processing fats, like PNPLA3 and TM6SF2, may play a role too. Interesting enough, variations in the genes that code for these proteins have been linked to alcoholic liver disease.[17]

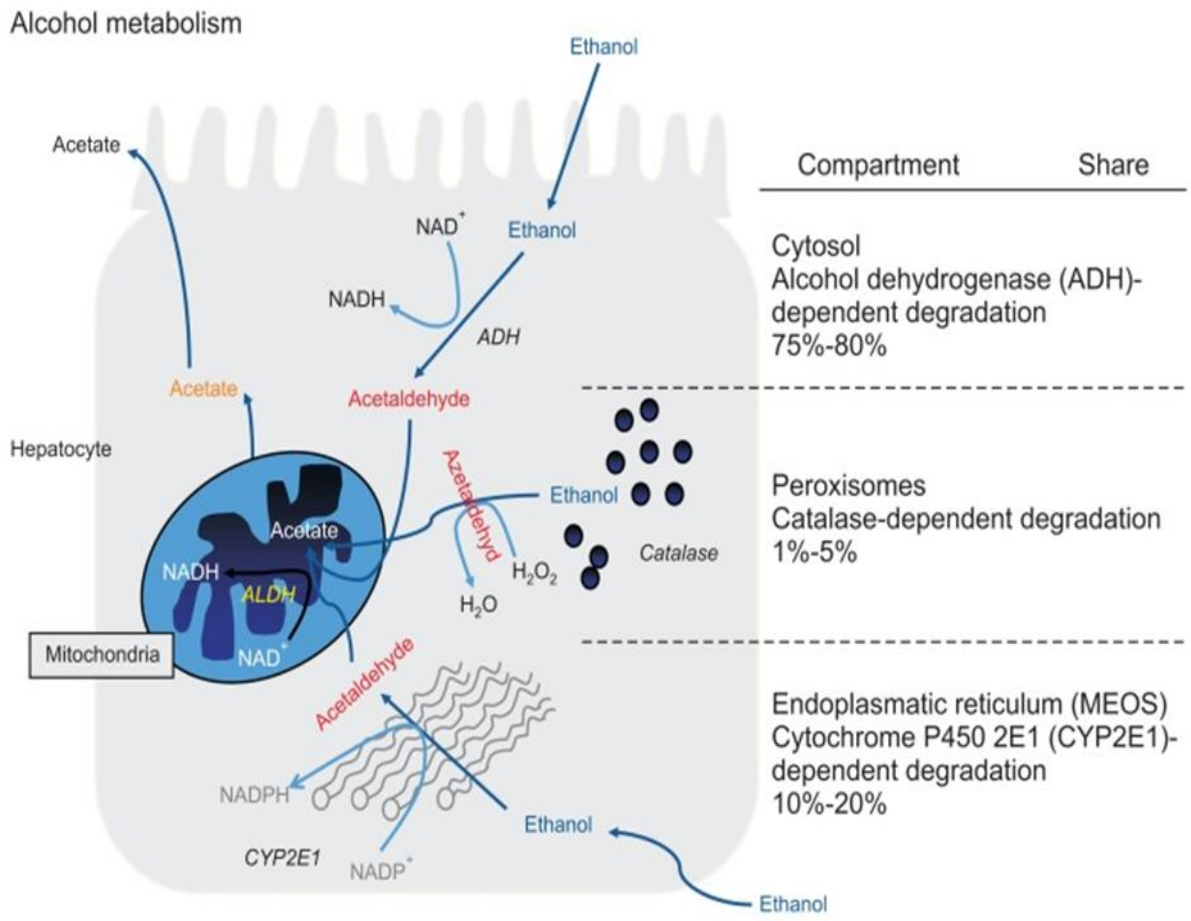


Figure3 : Enzymes ADH, CYP2E1 and catalase For Hepatic metabolism of ethanol

FACTORS MODULATING PROGRESSION OF ALD

Environmental factors [18]

Most people agree that there's a clear link between how much alcohol you drink and your chances of ending up with ALD (alcoholic liver disease). Basically, heavy drinking is pretty much a requirement for developing ALD. According to a study from Italy called the Dionysos Study, people who drink more than 120 grams of pure alcohol every day are at the highest risk of getting alcoholic cirrhosis. ALD was proposed to be influenced by drinking habits; for example, drinking with meals seems to carry a lower risk than drinking alcohol outside on its

own. The scientific consensus is that the impact of various beverages on the risk of ALD is more closely linked to dietary and lifestyle factors, and that the amount of alcohol in some alcoholic beverages matters more than their non-alcoholic contents. The main factor used to make this distinction is the primary histological lesion that a patient with both illnesses has. [18]

According to published evidence, alcohol increases oxidative stress, cytotoxicity, immunological dysfunction, and decreases response to antiviral treatment, all of which speed up the development of liver disease associated with hepatitis C. Similar mechanisms are thought to apply to persons infected with the hepatitis B virus, albeit there is less data on the latter.[19]

Host genetic factors

The disease ALD along with its genetic causes has been substantiated through various clinical findings. A sequence variant in the PNPLA3 (coding for patatin-like phospholipase encoding 3) gene called rs738409C>G, I148M continues to be recognized as the leading risk locus that regulates steatosis and necroinflammation and fibrosis and HCC in alcoholics. Two modern genetic studies analyzed the global disease risk factors in patients with alcoholic cirrhosis and alcoholic hepatitis. Two new genetic spots linked to cirrhosis progression were located. A set of two gene loci named Membrane 6 superfamily member 2 (TM6SF2) and membrane bound O-acyltransferase domain containing 7 (MBOAT7) ($P=9.25\times10-10$) were discovered in the studies of cirrhosis. Investigations proved PNPLA3 rs738409 to represent a genome-wide significant risk locus for developing alcoholic cirrhosis alongside alcoholic hepatitis. The fatty acid trapping process in liver tissue works through PNPLA3 as well as TM6SF2 but MBOAT7 facilitates lipid transfer between phospholipids and lysophospholipids which strongly triggers inflammatory reactions in livers. The patatin domain from a plant protein shows the rs738409

methionine substitution at position 148 to have significant stereotypical effects although animal model studies cannot confirm how mutant PNPLA3 variant influences its functional implications.

The molecular dynamic simulations demonstrate how the change in position 148 from Ile to Met decreases the availability of PNPLA3 substrate triglycerides for interaction with serine residues. The process of lipid trapping occurs simultaneously with a decreased effectiveness of hydrolysis and subsequent fat accumulation.[20].

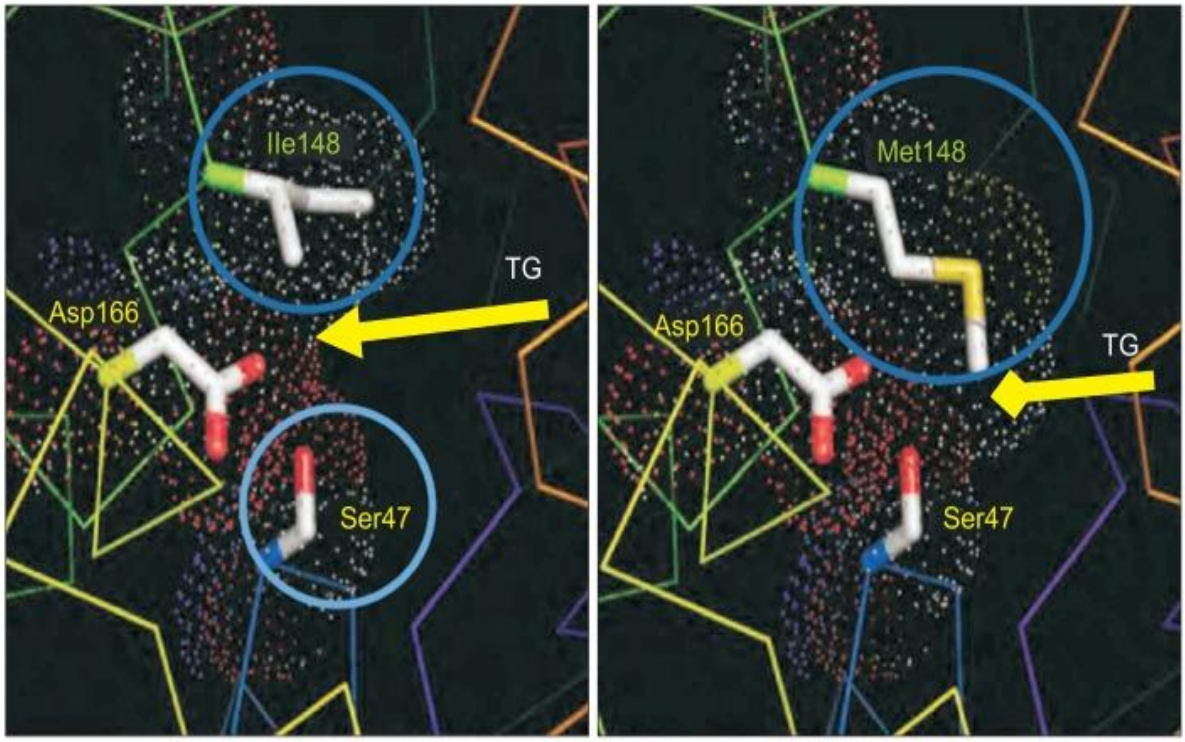


Figure 4: -PNPLA3 rs738409 locus (I148) alters the binding groove instead of the protein's catalytic core.

Patatin-like phospholipase domain-containing protein 3 (PNPLA3) genes [21]

The patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene functions as adiponutrin and attracts strong research interest for its contributions to liver metabolic activities and illnesses. The PNPLA3 gene exists on chromosome 22q13.31 where it produces a protein which initiates triacylglycerol hydrolysis within adipocytes during lipid metabolic processes. The gene variant rs738409 in PNPLA3 produces an I148M mutation through an isoleucine-to-methionine switch at position 148 which helps explain different liver disease manifestation.

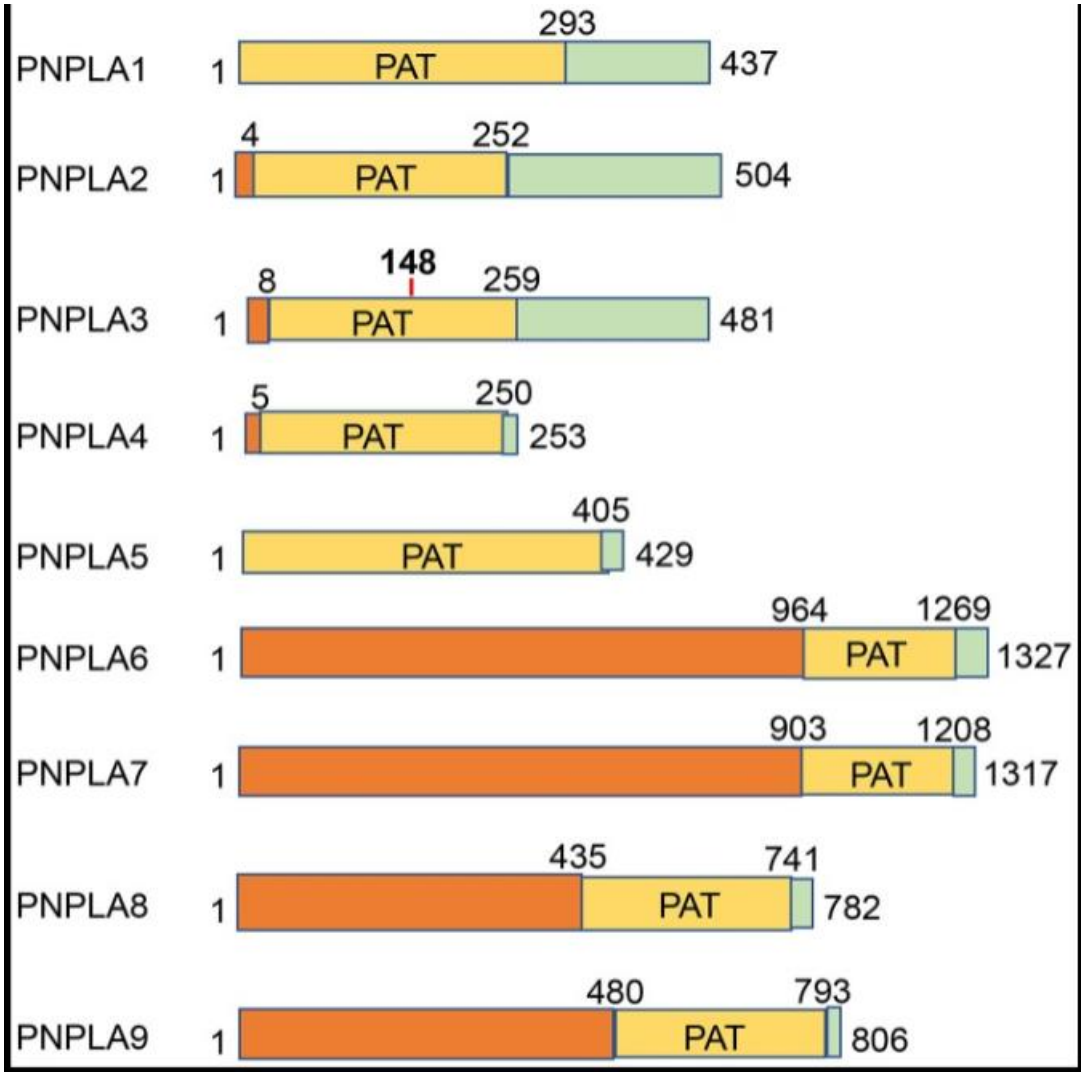


Figure 5: PNPLA family members.

Recurrent alcohol use creates various liver diseases that evolve between simple steatosis and cirrhosis. Research has shown that genetic factors determine why heavy drinkers develop severe liver disease because most individuals who drink heavily do not progress to this advanced stage. Scientists have proven that the PNPLA3 I148M genetic variant functions as a leading factor that decides how ALD advances.[22]

Scientific research shows PNPLA3 I148M polymorphism strongly elevates a person's chance to develop ALD. Research using meta-analysis showed that GG carriers among alcohol consumers face increased risks for alcoholic cirrhosis as well as hepatocellular carcinoma when compared to people with the CC genetic variant. A genetic connection to ALD development proves important for understanding its causes. [23]

It is believed that PNPLA3 I148M polymorphism induces ALD development and this mutated gene causes modifications to liver lipid metabolic processes which create fatty liver damage and then generates liver injuries. The PNPLA3 gene discovery provides new therapeutic directions which focus on treating the pathway responsible for ALD development in individual patients.

Functions Of patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene

The patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene encodes a multifunctional enzyme integral to lipid metabolism, particularly within the liver. This protein has two active properties as triacylglycerol lipase and acylglycerol O-acyltransferase which facilitates triacylglycerol breakdown in adipocytes and hepatocytes. The PNPLA3 enzyme participates in lysophosphatidic acid acylation to produce phosphatidic acid which serves as an essential intermediate for triglycerides and glycerophospholipids creation. [24]

The PNPLA3 gene contains a recognized single-nucleotide polymorphism (SNP) known as rs738409 which alters code 148 from an isoleucine to methionine. Studies have established that the I148M variant strongly elevates the risk for patients to develop non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). The I148M substitution interrupts the enzyme's ability to break down fats creating hepatocyte triglyceride build-up which drives hepatic steatosis development. [25]

The PNPLA3 enzyme assists triglyceride breakdown while helping with polyunsaturated fatty acid movement that leads to the production of big-sized very low-density lipoprotein VLDL particles by the liver. The enzyme's vital function ensures homeostasis of lipids together with energy equilibrium across the liver.[26]

PNPLA influences Alcoholic liver disease

The patatin-like phospholipase domain-containing protein 3 (PNPLA3) gene significantly influences lipid metabolic processes inside the liver. A particular single-nucleotide polymorphism (SNP) rs738409 within this gene produces a genetic alteration which turns isoleucine into methionine at position 148 (I148M). A strong link exists between this variant and heightened susceptibility together with increased severity of alcoholic liver disease (ALD).

Influence on Alcoholic liver disease Susceptibility:[20,26]

The PNPLA3 I148M variant occurs in people demonstrating elevated risk profiles for alcoholic liver disease development according to scientific evidence. This polymorphism shows significant links to the full range of alcoholic liver disease manifestations up to alcoholic fatty liver and alcoholic liver injury through alcoholic cirrhosis and hepatocellular carcinoma stages. The study showed that people with GG genotype of rs738409 SNP develop more severe alcoholic liver disease (ALD) than those who have CC genotype

Impact on Disease Severity:[1]

Studies show that the PNPLA3 I148M variant controls both ALD development risk and the extent of the disease through its effects on liver disease progression. The hepatic fat accumulation among mutation carriers worsens their liver inflammation along with their fibrosis condition. The insufficient lipolysis activity of PNPLA3 mutated protein triggers hepatocellular triglyceride accumulation that produces steatosis and enhances the risk of cirrhosis along with hepatocellular carcinoma development.

Mechanistic Insights:[1]

People with the I148M mutated PNPLA3 enzyme develop ineffective lipase activity which indicates problems with lipid substance metabolism. An improper release of triglycerides by misfunctioning protein accumulates in liver cells that leads to hepatic steatosis and subsequent liver damage. The mutation demonstrates its crucial part in ALD pathogenesis by disrupting lipid processing.

Role of PNPLA3 in Liver Metabolism[26]

Hepatic lipid metabolism depends heavily on the patatin-like phospholipase domain-containing protein 3 (PNPLA3) also referred to as adiponutrin. The liver together with adipose tissues express this enzyme strongly while it serves essential functions in triglyceride breakdown alongside lipid transformation and metabolic balance maintenance. The lipase activity of PNPLA3 converts triglycerides into free fatty acids for maintaining appropriate lipid balance inside hepatocytes.

The PNPLA3 regulation depends on nutritional elements and hormone signals. Fasting conditions lower PNPLA3 expression levels but insulin together with glucose stimulate its expression to enhance lipid storage. The regulatory system plays a vital role to sustain stable lipid conditions inside liver cells.

The rs738409 (I148M) polymorphism in PNPLA3 gene triggered through mutations hinders the gene's enzymatic activity thus disrupting normal lipid metabolic processes. The conversion of isoleucine (I) to methionine (M) at site 148 disrupts the lipolytic activity of PNPLA3 making

triglyceride breakdown slower thus causing hepatocyte lipid droplets to accumulate. The retained lipids cause hepatic steatosis which represents the initial condition of ALD and non-alcoholic fatty liver disease (NAFLD).

The PNPLA3 gene shows a connection to processes of hepatic fibrogenesis. Research shows that the I148M PNPLA3 variant causes an increase of collagen deposition and active hepatic stellate cells that contribute to liver fibrosis development leading to cirrhosis. A deficiency of PNPLA3 enzyme activity disrupts lipid signal molecules thus affecting inflammatory processes and extracellular matrix remodeling while escalating liver damage.

Scientists have established that PNPLA3 mutations create a connection between liver dysfunction from alcohol consumption and metabolic conditions such as NAFLD along with NASH and obesity-based liver complications. The key position of PNPLA3 in lipid control has transformed it into a critical therapeutic target for treating various diseases. Novel therapeutic approaches for ALD and liver-related metabolic diseases exist by either controlling PNPLA3 activity levels or replacing its functional deficiencies.[27]

PNPLA3 GENE POLYMORPHISM AND CHRONIC LIVER DISEASE

Both alcoholic and non-alcoholic liver illnesses frequently start off as simple steatosis and develop into cirrhosis, fibrosis, hepatitis, and even liver cancer. The development of these chronic liver illnesses is influenced by both hereditary and environmental factors. PNPLA3 is one of the genes with the most established effects on ALD and NAFLD. PNPLA3 variant rs738409 (148M) has been implicated in a wide range of chronic liver illness, according to multiple GWAS.

Romeo et al. (2008) [28] discovered a strong association between hepatic fat accumulation in GWAS in European Americans, African Americans, and Hispanics and the PNPLA3(148M) variant. The predominance of NAFLD in these three ancestral populations is supported by the 148Mvariant frequencies, which are 0.49 for Hispanics, 0.23 for European Americans, and 0.17 for African Americans.. PNPLA3(148M) variants have now been strongly linked to both ALD and NAFLD, according to several GWASs. The 148M mutation has been strongly linked to liver cirrhosis in a number of studies. Numerous studies have also demonstrated that the 148M variation is linked to an increased risk of HCC. Additionally, differential gene regulation by microRNAs may result from the PNPLA3 variant rs738409. Hsa-miR-769-3p and hsa-miR 516a-3p have been found to be putative microRNAs that target the 3' UTR of the human PNPLA3 mRNA by an in silico investigation.

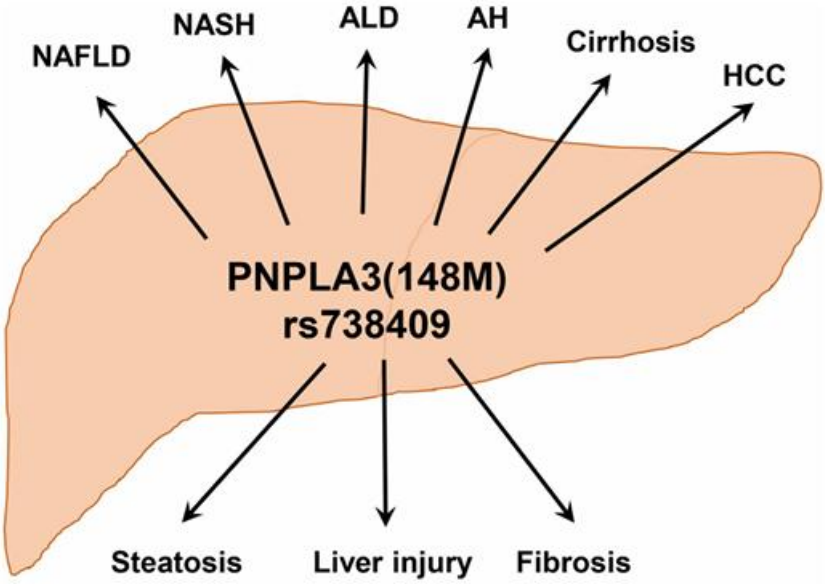


Figure 6: PNPLA3(148M) is associated with a chronic liver diseases.

STRATEGIES - PNPLA3 FOR THE CHRONIC LIVER DISEASE

The PNPLA3(148M) variant is highly prevalent in most ethnic groups, hence developing therapies that precisely target this genetic polymorphism is essential. Depending on the biology of the PNPLA3(148M) mutation, there are several strategies to target it. First, the PNPLA3(148M) mutation can be targeted at RNA level by antisense RNA oligonucleotides, small interfering RNA (siRNA), or small hairpin RNA (shRNA). In a 148M knockin mouse model, triantennary N-acetylgalactosamine (GalNAC3) linked antisense oligonucleotides (ASO) that target Pnpla3 have been shown to significantly reduce hepatic fibrosis, inflammation, and steatosis. This implies that the ASO approach might be beneficial.

In a recent proof-of-concept study, the degradation of Halo-tagged PNPLA3(148M) via proteolysis-targeting chimera (PROTAC) significantly reduced the hepatic triglyceride concentration.

But from a different angle, targeting PNPLA3 might also work since fatty acids or synthetic CGI-58 ligands can regulate the interaction between PNPLA3 and CGI-58. Targeting PNPLA3(148M) in combination has emerged as a promising therapeutic target for several chronic liver diseases, including NASH and ALD.

Genetic Variations in PNPLA3 and Their Implications in Alcoholic Liver Disease

The rs738409 (I148M substitution) polymorphism in PNPLA3 stands out as the most researched genetic variation because it interferes with enzyme function by changing position 148 from isoleucine to methionine. People possessing this variant face higher chances of developing steatosis together with fibrosis and cirrhosis during ALD progression.
Research by Romeo et al. [28](2008) established rs738409 as the main factor governing liver fat content. Their findings showed that people with the 148M allele developed more liver fat which stood as a critical element for worsening ALD effects. The research revealed that PNPLA3 functions as a fundamental biological factor in hepatocellular lipid regulation throughout storage and transport processes.

Tian et al. [29](2010) showed that ALD patients with 148M/M genotype homozygosity suffered from worse ALD severity than non-carrier patients. The study established that the genetic

variant changes lipid metabolic processes while compromising liver function thus making patients more prone to ALD deterioration.

Research by Zhou et al.[30] (2015) revealed how the PNPLA3 I148M variant disrupts triglyceride breakdown which causes hepatic cells and overlaps with hepatocyte damage. The study evidence demonstrates that the mutation alters enzymatic activity which leads to hepatic steatosis and inflammation marks typical of ALD pathology.

The rs738409 (I148M substitution) polymorphism in PNPLA3 stands out as the most researched genetic variation because it interferes with enzyme function by changing position 148 from isoleucine to methionine. People possessing this variant face higher chances of developing steatosis together with fibrosis and cirrhosis during ALD progression.

According to a study by M. Queintin et al., nonalcoholic fatty liver disease is thought to be a complex disease feature that develops when environmental exposures operate upon a sensitive polygenic background composed of multiple distinct modifiers. [31]. PNPLA3 has been identified as a modifier of disease outcome across the whole spectrum of non-alcoholic fatty liver disease (NAFLD), from steatosis to advanced fibrosis and hepatocellular carcinoma. Other recent developments include the identification of TM6SF2 as a putative "master regulator" of metabolic syndrome outcome, which determines not only the risk of advanced liver disease but also the outcomes of cardiovascular disease.

MATERIALS AND METHODS

Method of collection of Data

Study Type: CROSS SECTIONAL STUDY

Study Population: Patients admitted with Alcoholic liver disease at BLDE (Deemed to Be University) Shri B M Patil Medical College Hospital and Research Centre, Vijayapura.

Study Period: May 2023 to December 2024.

Sample Size: 60

Inclusion Criteria:

- All Alcoholic Liver Disease diagnosed by clinical-history and examination, radiological-ultrasonography and biochemical parameters- like LFT(AST/ALT).

Exclusion Criteria:

- HBsAg Positive.
- HCV Positive.
- Hepatocellular carcinoma.
- Drug induced liver injury.

Sample Size:

As per the study done Xiaocheng Charlie Dong[1]on PNPLA3 SNP in Indians can be considered as 3.2%(N%).Considering the confidence limit of these studies to be 95% with 5% level of significance and margin of error 0.05.The sample size computed using the formula

Sample size (n) = $(Z^2 * p*(1-p)) / d^2$

Where,

z is the z score= 1.96

d is the margin of error= 0.05

n is the population size

p is the population proportion =0.032

The estimated sample size of this study is **60.**

Sample Collection And Methodology:

Patients admitted with Alcoholic liver disease admitted and diagnosed with a sample size of 60 cases in Shri B M Patil Medical College Hospital and Research Centre are selected.
A cross sectional study of all patient with Alcoholic liver disease were tested for the gene polymorphism of PNPLA3 by PCR analysis.

Information were collected from each patient through a pre-tested proforma meeting the objectives of the study. The purpose of the study is explained to patients in detail, and written consent were taken. All subjects were Given self-administered questionnaires for the history of alcohol consumption. Medical history and clinical characteristics was collected.

DNA Extraction

1 ml of blood is collected from venipuncture into EDTA coated tube and stored in 4^0C. Genomic DNA is extracted using BIO-RAD THERMOCYCLER 100(PCR MACHINE) from the blood by salting out method using phenol-chloroform and was purified by ethanol precipitation.

Isolation Of Genomic DNA And Quantification

- From 300µl of peripheral blood, genomic DNA is isolated with the help of a commercially available DNA kit.

LIVGEN BLOOD GENOMIC DNA EXTRACTION KIT
Protocol Preparation
Components of Kit

Blood Genomic DNA Extraction Kit	
Cat no	**MP005**
No of Preparation	50
Spin Column	50
Buffer Lysis LC	25ml
Buffer Binding BL	50ml
Proteinase K (Lyophilized)	2 ml stored at -20^{0}C
Reconstitution Buffer(RB)	1ml X2
Wash Buffer (ready t use)	30 ml
Wash Buffer(Concentrated)	10 ml + 90 ml ethanol
Elution Buffer	15 ml

Blood Genomic DNA Purification Protocol

- Add 200µl anticoagulated blood in 1.5 to 2 ml microcentrifuge tube. Add 200 µl Lysis Buffer LC & Add 15 µl Proteinase K Mix thoroughly by vertexing. Incubate at 60^{0}C for 10 mins to yield a homogeneous solution.
- Add 350 µl Buffer Binding BL to the sample, and mix thoroughly by pipetTing 3-4 times.
- Pipet the mixture from step 2 in to the spin column placed in a 2ml collection tube (provided). Centrifuge at 11,000 or 12,000 rpm for 1 min. Discard flow- through.
- Add 400 µl Wash Buffer WA and centrifuge 1 min at 11,000 or 12,000 rpm, Discard flow through.
- Add 600 µl Wash Buffer WB and centrifuge 1 min at 11,000 or 12,000 rpm, Discard flow through..

- Repeat the above step no 5.
- Centrifuge for 3 min at 12,000 rpm to dry the column membrane. Discard flow through and collection tube.
- Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided) and pipet 200 µl Eluent Buffer TE (pre-warm to 60^0C) directly onto the membrane. Incubate at room temperature for 2 minutes, and then centrifuge for 1 min at 11,000 or 12,000 rpm to elute.
- The tube contains the purified DNA. Store the DNA at -20^0C(long term) & 4^0C(Short term).

Quantification of Genomic DNA

- "We used Tecon multimode reader for the quantification of genomic DNA. Tecon multimode reader is a micro-volume UV spectrophotometer specifically designed for the measurement of nucleic acids and purified proteins. Its unique technology holds 0.5-2.5 ul samples between upper and lower measurement surfaces without the use of a cuvette. Tecon multimode reader measures the samples in less than 2 seconds with a high degree of accuracy and reproducibility. The Tecon multimode reader works on the principle, "Nucleic acids absorb light at a wavelength of 260 nm and when 260 nm light source shines on a sample, the amount of light that passes through the sample can be measured, and the amount of light absorbed by the sample can be inferred. For double stranded DNA, an Optical Density (OD) of 1 at 260 nm correlates to a DNA concentration of 50 ng/µl, so that DNA concentration can be easily calculated from OD measurements" as shown in Table no. 2."

Table 2: Quantification of DNA Samples of Alcohol Liver Disease

Sl.No. of DNA samples	OD at 260/280	Concentration DNA in ηg/µl
1	1.89	45.1
2	1.74	64
3	1.43	44
4	1.92	70
5	1.57	136
6	1.98	64
7	1.84	82
8	1.92	73
9	1.65	68
10	1.79	111
11	1.85	64
12	1.81	66
13	1.75	53
14	1.59	65
15	1.66	82
16	1.51	94
17	1.88	49
18	1.92	39
19	1.93	46
20	1.74	100
21	1.65	51.5
22	1.89	85.5
23	1.96	73.9
24	3.05	57

25	2.01	81
26	2.24	125
27	2.09	137
28	1.76	104
29	1.96	92
30	1.58	93
31	1.86	54
32	1.75	65.4
33	1.40	44.5
34	1.90	70.3
35	1.57	95.3
36	1.98	64
37	1.84	82
38	1.92	73
39	1.65	68
40	1.79	111
41	1.85	64
42	1.81	66
43	1.68	54.2
44	1.85	63.5
45	1.74	56.2
46	1.56	48.2
47	1.83	47.2
48	1.53	95.3
49	1.97	64.03

50	1.82	82.4
51	1.90	73.5
52	1.63	68.9
53	1.78	102.1
54	1.84	64.02
55	1.55	66.8
56	1.69	54.8
57	1.84	63.4
58	1.78	56.7
59	1.57	48.4
60	1.88	47.5

Agarose Gel Electrophoresis

- The quality of the isolated DNA was checked under gel electrophoresis. 100 ml of 1% agarose gel was prepared (1gm of Agarose + 100 ml of 1X TAE buffer).The same isolated DNA was quantified under "Nano Drop" (Quawell) and quantity and quality of the DNA was reported.

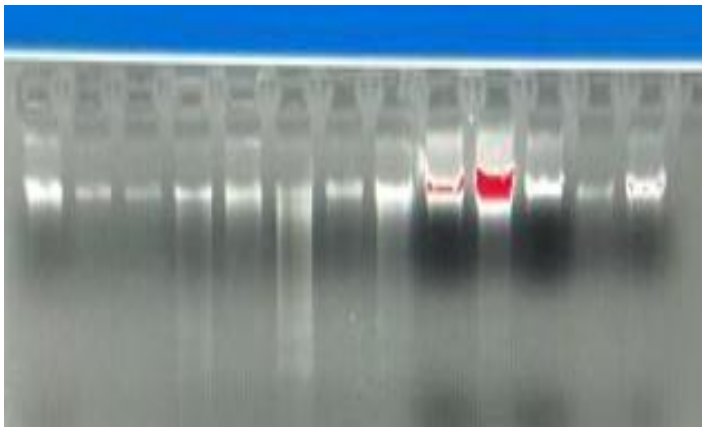


Figure 7 . Agarose gel image of genomic DNA of PNPLA3 in ALD samples.

Exon-Specific Intronic Primer Designing

- Web-based, freely available program PRIMER 3, which is widely accepted, is used for designing PCR primers . Primer 3 is a bioinformatics tool that helps in designing the primers for the target region in the given nucleotide sequence as per the requirement of the user or applications. The designed primers using Primer 3 are reconfirmed for the specificity for its binding site using web-based bioinformatics tool for its In Silco amplification in Insilco PCR (http.//insilico.ehu.es/pcr/). Primers are synthesized by a commercial oligo synthesizer (MWG Biotech, India).As shown in the Table 3 below.

Table 3: Details of the primer sequences and annealing temperatures used for the amplification of exon 3,6 of PNPLA3 Gene.

Sl. No	Primer ID	Sequence	Product Size	Annealing Temperature
1	PNPLA3 3F	5'-TGCTCACTTGGAGAAAGCTTATG-3'	200	56.0^0C
	PNPLA3 3R	5'-CACTTCAGAGGCCCCCAG-3'		
2	PNPLA3 6F	5'-GTTTTCCGTGCCCTTCACAG-3'	252	54.5^0C
	PNPLA3 6R	5'-GAGTGGGTACCTGTAGCGAG-3'		

Polymerase Chain Reaction (PCR)

- "PCR amplification was carried out in a 20µl reaction volume containing 0.5 µl of genomic DNA (75ng/µl to 150 ng/µl), 0.5µl of each primer (5pmol), 0.4µl of dNTP (10pmol), 0.2µl Taq DNA polymerases (3units/ µl), 4 µlTaq Buffer (5X) (BioRad, USA) and the total volume was adjusted to 20µl using molecular biology grade water. Amplification was carried out in Master cycler gradient (Eppendorf, Germany) under the following conditions: an initial denaturation at 94^0C for 4mins, followed by 35 cycles at 94^0C for 30sec (cycle denaturation). The primer annealing temperature was set depending on the annealing temperature of the primer (Table-3) for 10sec ,68^0C for 1min (primer extension) and a final extension at 68^0C for 5 min. PCR products were confirmed for their respective amplicon size by gel electrophoresis with a standard 100bp ladder. The PCR cycling conditions were as follows Initial Denaturation is for 94^0C for 4 mins, Denaturation is 94^0C for 30 sec, Annealing is primer dependent for 10 sec, Elongation 68^0C for 5min & hold at 40^0C."The PCR product of 200bp and 252bp were obtained.

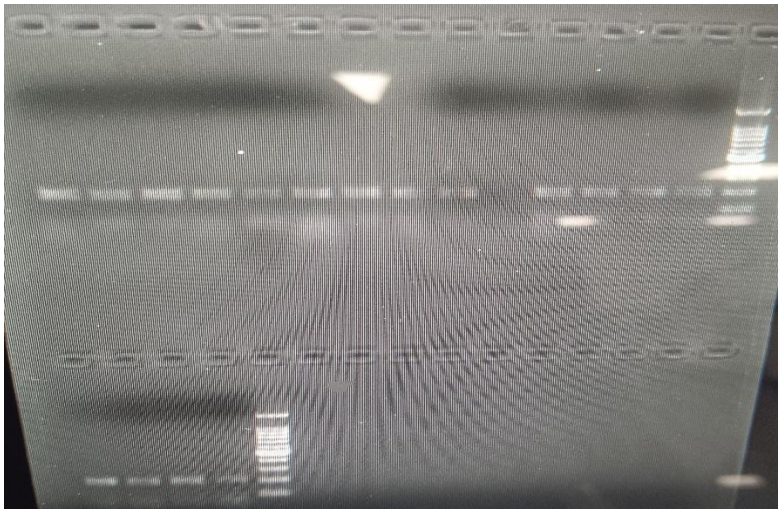
Table 4: PCR Reaction Mixture Components

Component	Volume (µL)	Concentration
Genomic DNA	0.5	25-100 ng/µL
Primers	0.5	5 pmol
dNTPs	0.4	10 pmol
Taq DNA Polymerase (BioRad, USA)	0.2	3 units/µL
5X Taq Buffer(BioRad, USA)	4	Ready-to-use
Molecular Biology-Grade Water	Adjust to 20	-

Table 5: **Thermal Cycling Conditions**

Step	Temperature (Â°C)	Time	Cycles
Initial Denaturation	94	4 minutes	1
Denaturation	94	30 seconds	35
Annealing	Variable	Variable	
Extension	68	1 minute	
Final Extension	68	5 minutes	1

PCR PRODUCT



DNA Sequencing (Capillary-Based)

- PCR products are subjected to capillary-based Big-Dye terminator sequencing. Prior to sequencing, the PCR products are subjected to cycle sequencing and plate processing.

Cycle Sequencing

- As per the Sanger Sequencing protocol, Big-Dye labeling and chain termination were carried out by the cycle sequencing method. To label each base, the PCR amplicon was subjected to a cycle sequencing reaction with a single primer. Big-Dye TM terminator v3.1was used for cycle sequencing (Applied Biosystems, USA) following the manufacturer's guidelines. Cycle sequencing of the PCR products was carried out according to the annealing temperature of the primers.

Sequencing Clean-up (Plate Processing)

- To remove the unbounded florescent DNTPs from the terminator sequencing reaction, 2µl of 3M sodium acetate, and 50µl of 100% ethyl alcohol were added to each sample and incubated at room temperature for 15 minutes to precipitate the DNA. The samples were centrifuged at 4000 rpm for 30 minutes at 4°C. The supernatant was discarded, and the reaction plate was centrifuged in a reverse manner at 300 rpm for 20 seconds. 100µl of 75% alcohol was added to each sample and centrifuged at 4000rpm for15 minutes at 25°C. The supernatant was discarded, and the plate was centrifuged in a reverse manner at 300 rpm for 20 seconds to remove the alcohol completely. The plate was dried at room temperature until the last drop of alcohol dripped off
- 10µl of Hi-Di Formamide was added to each well of the sample plate. The samples were heated to 96°C for 5 minutes and immediately cooled to 4°C to denature and linearise the cycle sequencing products. The processed products were loaded in the sequencer for sequencing.

Sequencing Run

- Sample information sheets which contain analysis protocols along with the sample details were prepared and imported into the data collection software. Prepared samples were analyzed on ABI 3730 genetic analyser (Applied Biosystems, USA) to generate DNA sequences or electropherograms. After completion of the sequencing reaction, the quality of generated sequence was checked by using Sequencing Analysis v5.4software (Applied Biosystems, USA).

Sequence Alignment

- The generated sequences were aligned to their respective reference sequences with the use of Variant reporter software (ABI v1.1). The variant reporter is one of the compatible software of Applied Biosystems designed for automated sequence data analysis. It performs sequence comparisons for novel mutations, known variants, insertions, and deletions. It allows analysis of the sequenced data, comparing the consensus sequences to a known reference sequence. The results of the variant reporter were tabulated in PDF format as the default program of the software.

Statistical Analysis: The gathered data is entered into a Microsoft Excel sheet, and statistical analyses are performed using SPSS (Statistical Package for the Four Social Sciences, Version 20). The results are shown using graphs, counts, percentages, mean, and SD. The four continuous variables that are normally distributed in the two groups will be compared using an independent sample test. The Mann-Whitney U test is used for variables that are not regularly distributed. The two groups' categorical variables are compared using the Chi-square test or Fisher's exact test. If there are more than two groups, an ANOVA will be used; if the data is not normally distributed, the Kruskal-Walli H Test4y will be used.A p-value of less than 0.05 will be deemed statistically significant. Every statistic is run using the two-tailed method.

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RESULTS

Total of 60 patients admitted with Alcoholic liver disease were taken. Out of 60 patients gene sequencing was analysed for PNPLA3 polymorphism. 11 patients (18.3%) had gene mutation and 49 patients (81.7%) does not have any mutation. Majority of the patients were in the stage of Cirrhosis.But this gene is not related to age, gender, years of alcoholic consumption and stage of the disease (p value >0.05).As shown in the tables below.

Table 6: Age wise distribution of study participants

Sl no	Age	Frequency (n)	Percentages %
1	20-30	7	11.7
2	31-40	19	31.7
3	41-50	25	41.7
4	51-60	9	15.0
5	Total	60	100.0

This is the table presents the age of the study participants and found that majority were aged between 41-50 years 25 patients i.e 41.7%(n-25) followed by 31-40 years 19 patients i.e 31.7%(n-19) .The mean age and Standard deviation is 42.58 and 8.7 respectively and it shown in bar diagram

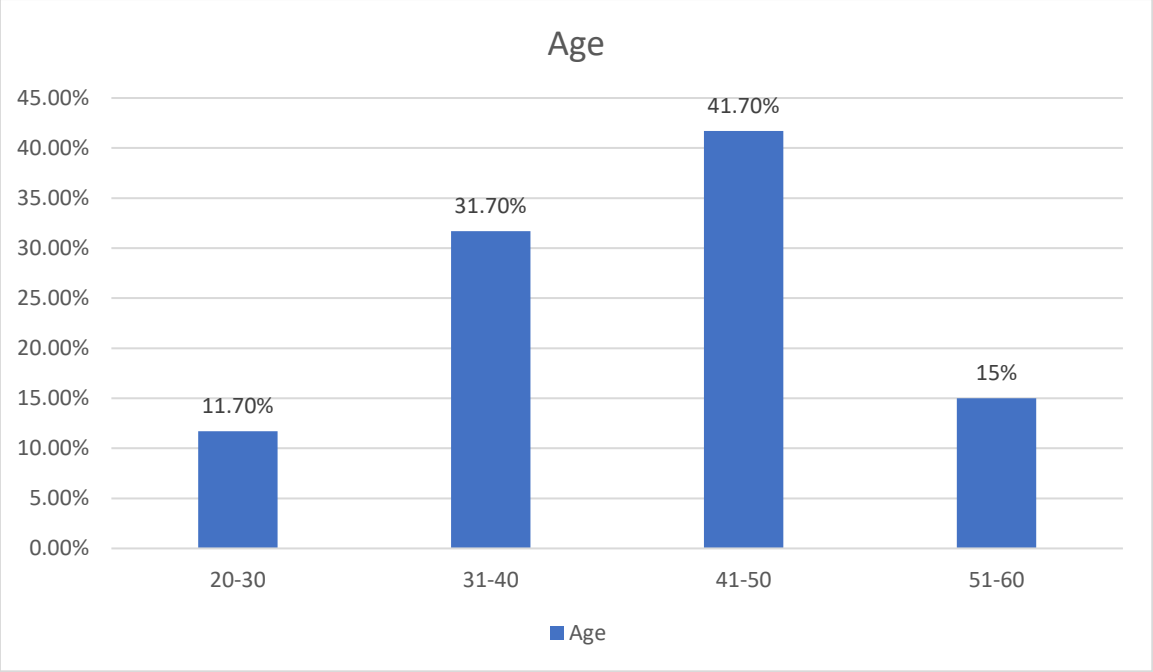

Figure 8: Age wise distribution of study participants

Table 7: Gender wise distribution of study participants

Sl no	Gender	Frequency (n)	Percentages %
1	MALE	58	96.7
2	FEMALE	2	3.3
3	Total	60	100.0

This table presents the gender, among that majority were males 96.7%(n-58) i.e 58 patients and only 3.3% (n-2) were females i.e 2 patients and it is shown in pie diagram

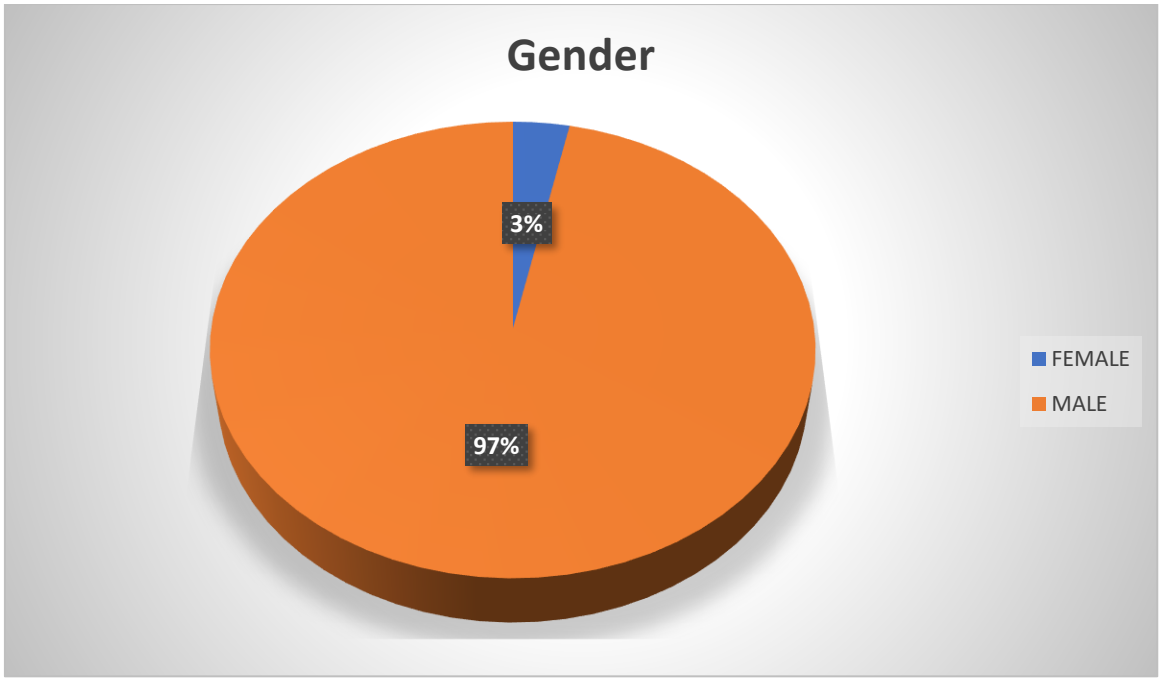

Figure 9: Gender wise distribution of study participants

Table 8: Distribution of Number of years of Alcohol consumption among study participants

Sl no	Number of years of Alcohol consumption	Frequency (n)	Percentages %
1	<10 years	19	31.7
2	11 to 20 years	35	58.3
3	>20 Years	6	10.0
4	Total	60	100.0

This table presents the Number of years of Alcohol consumption and found that majority for a duration of 11 to 20 years of alcohol consumption 58.3 % (n-35) i.e 35 patients and followed by for <10 years 31.7% (n-19) i.e 19 patients and it is shown in pie diagram

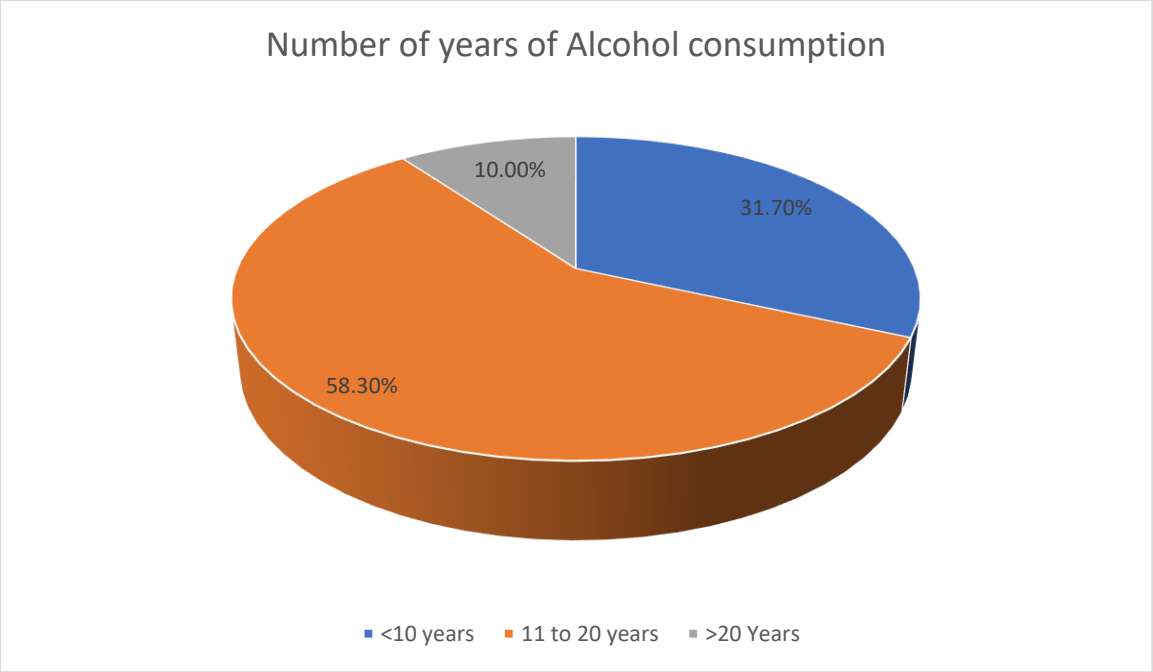

Figure 10: Distribution of Number of years of Alcohol consumption among study participants

Table 9: Mean and Standard deviation of various laboratory parameters (LFT) & BMI

Sl no	Parameters	Mean	Standard Deviation
1	BMI (KG/M ^{2})	22.0	3.31
2	TB (total serum bilirubin)(mg/dl)	7.23	8.56
3	CB (Conjugated bilirubin)	5.30	7.75
4	UB (Unconjugated bilirubin)	1.90	1.56
5	Albumin (Serum)	2.6	0.8
6	Globulin (Serum)	3.4	0.818

This table presents the Mean and Standard Deviation of Various laboratory parameters & BMI like (Serum analysis) TB, CB, UB, Albumin, Globulin is 22.0(3.31), 7.23(8.56),5.30(7.75),1.90(1.56),2.6(0.8), 3.4(0.818) respectively and it is shown in bar diagram

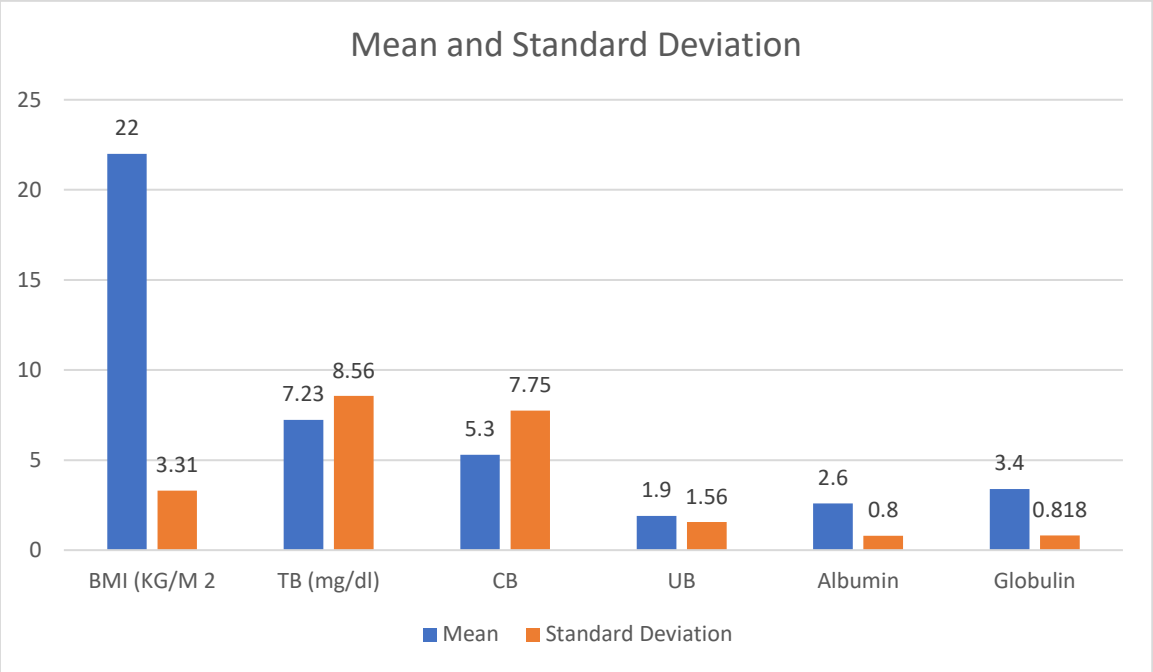

Figure 11: Mean and Standard deviation of various parameters

Continued with other laboratory parameters below .

Table 10: Mean and Standard deviation of Laboratory parameters

Sl no	Laboratory parameters	Mean	Standard Deviation
1	AST	131.15	157.2
2	ALT	77.5	223.3
3	ALP	149.53	67.6
4	Hb	9.2	2.8
5	PLATELET COUNT	134000.00	83203.651
6	TC	10968.92	6736.823

This table presents the mean and standard deviation of laboratory parameters like AST, ALT, ALP, Hb level, Platelet count and Total count and it is shown in bar digarm

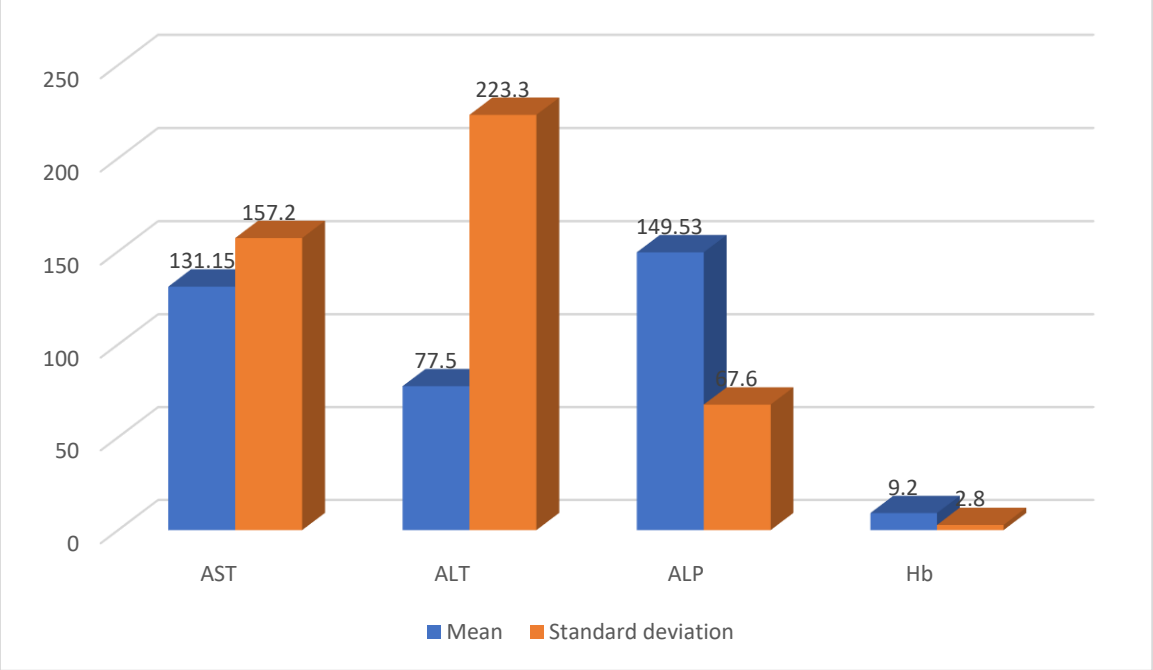

Figure 12: Mean and Standard deviation of Laboratory parameters

Table 11: Distribution of study participants according to Stage of the disease

Sl no	Stage of the disease	Frequency (n)	Percentages %
1	ALCOHOL GASTRITIS	1	1.7
2	STEATOSIS	1	1.7
3	STEATOHEPATITIS	16	26.60
4	CIRRHOSIS	42	70.0
5	Total	60	100.0

This table presents the Stage of the disease and found that 70% (n-42) had Cirrhosis and followed by 26.6% (n-16) STEATOHEPATITIS ,that include steatosis, hepatitis and it is shown in bar diagram

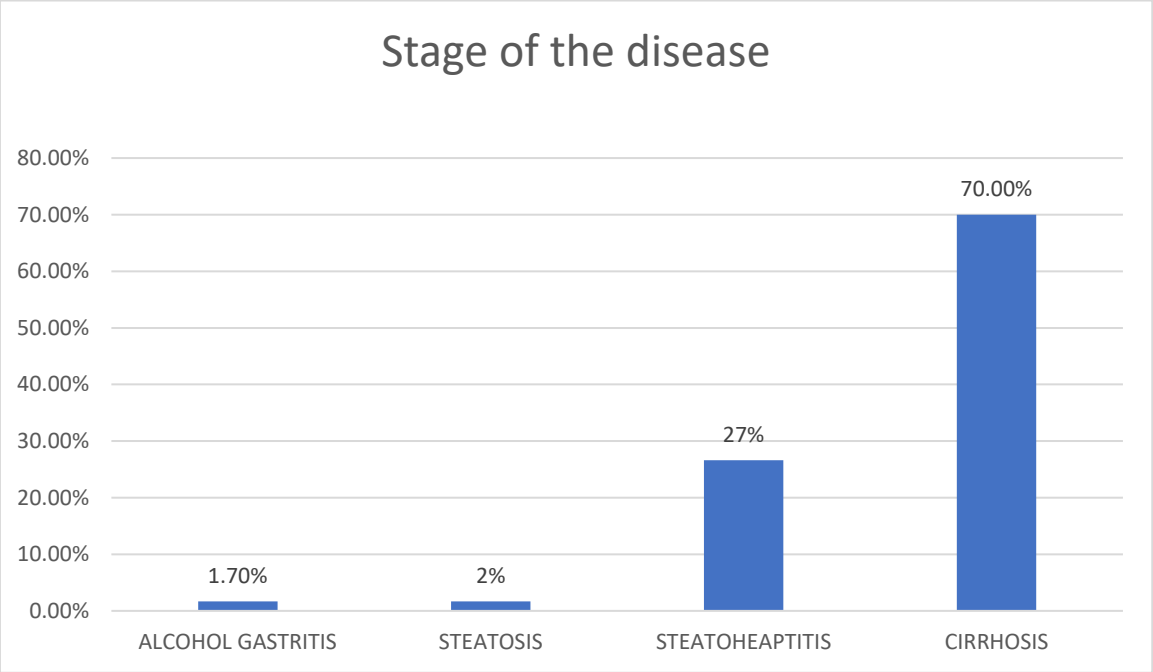

Figure 12: Distribution of study participants according to Stage of the disease

Table 12: Distribution of study participants according to genetic study

Sl no	genetic study	Frequency	Percentages
1	No mutations recorded	49	81.7
2	rs738409	5	8.3
3	rs738409, rs738408	6	10.0
4	Total	60	100.0

This table presents the Genetic study of study participants and found that 81.7%(n-49) had no mutation recorded and 8.3% (n-5) had rs738409 gene and then 10% (n-6) had rs738409, rs738408 genes

Table 13: Mutations observed in exon 3 of PNPLA3 Gene among 60 patients with alcohol-associated liver disease (ALD)

gDNA position	cDNA position	Amino acid position	Status	Variant type	Phenotype/ Disease	Condition	Sample ID PNPLA3 no.
g.10109 C>G	c.444 C>G	p. I148M	rs738409	Missense variant	Benign	Homozygous & Heterozygous	6,20,32,49, 57
g.10112 C>T	c.447 C>T	p. P149P	rs738408	Synonymous variant	Benign	Homozygous	4,15,18,26, 28,56.
g.10109 C>G	c.444 C>G	p. I148M	rs738409	Missense variant	Benign	Homozygous & Heterozygous	4,15,18,26, 28,56.

This table demonstrate the PNPLA3 gene mutations in exon 3 among sixty alcohol-associated liver disease (ALD) patients through this tabular representation.

Out of sixty samples, 5 patients 8.3% (n-5) had rs738409 gene mutation and 6 patients 10% (n-6) had both rs738409 and rs738408 genes mutation. So a total of 11 patients 18.3% had gene mutation in our study.

1. Missense Variant (rs738409, p.I148M)

The Isoleucine to Methionine (I148M) substitution occurs because of the g.10109C>G (c.444C>G) mutation. The exon 3 PNPLA3 gene variation belongs to the benign missense classification as Homozygous mutation frequency 8.3% (05 patients) and Heterozygous mutation frequency 10% (06 patients).

2. Synonymous Variant (rs738408, p.P149P):

The genetic change at g.10112C>T (c.447C>T) maintains Proline amino acid as its original Proline form. A benign synonymous variant with 10% (06 patients) homozygous frequency. The analysis reveals rs738409 (I148M) and rs738408 (P149P) occur in 18.3% of patients with ALD thus having an equivalent total occurrence rate. The missense variant I148M poses risks for liver disease susceptibility because it was already observed in previous research so scientists should study its role in ALD progression.

Sl. no	gDNA position	cDNA position	Amino acid position	Status	Variant type	Phenotype/ Disease	Condition	Frequency (%)
1	g.10109C>G	c. 444 C>G	p. I148M	rs738409	Missense variant	Benign	Homozygous Heterozygous	10% (06) 8.3% (05)
2	g.10112C>T	c. 447C>T	p. P149P	rs738408	Synonymous variant	Benign	Homozygous	10% (06)

In patients with Alcohol-Associated Liver Disease (ALD) the PNPLA3 gene's exon 3 contained two essential genetic variants rs738409 (I148M) and rs738408 (P149P), which were studied during analysis. Analysis of the rs738409 variant demonstrates its nature as a missense mutation because it changes the chemical composition of position 148 from Isoleucine (I) to Methionine (M). This research study considers the rs738409 variant benign because it links to non-alcoholic fatty liver disease (NAFLD) while being known for its association with liver diseases. This allele manifests in patients with ALD but indicates it may function as a key factor in developing susceptibility to the disease. The rs738408 variant (g.10112C>T, c.447C>T, p.P149P) represents a synonymous mutation since it preserves protein sequence although it might exist with additional risk-associated mutations.

The two variants appeared in 11 patients out of sixty (18.3%) showing a low to moderate infection frequency in this patient group. The rs738409 variant manifested both as homozygous and heterozygous state thus indicating its possible critical importance for ALD progression. The rs738408 variant shows lack of impact on protein functionality since it principally proves to be neutral. The examination implies that rs738409 may play a role in making patients more susceptible to ALD while acknowledging the necessity for additional functional studies. A larger group of study participants would be essential to validate these discovered relationships as well as define PNPLA3 gene variant impact on ALD progression.Comparison Between gene mutation and no mutation group are described below .

Table 14: Association between Age and Genetic mutation

Sl no	Age	Genetic study mutation		P value
1		Negative	Present	0.719
2	20- 30 years	5(10.2%)	2 (18.2%)	
3	31- 40 years	15 (30.6%)	4 (36.4%)	
4	41-50 years	22 (44.9%)	3 (27.3%)	
5	51-60 years	7 (14.3%)	2 (18.2%)	
6	Total	49 (100%)	11 (100%)	

X^2=1.34,df- 3

This table shows the association between Age and Genetic mutation and its is not significant

Table 15: association between Sex and Genetic mutation

Sl no	Sex	Genetic study mutation		P value
		Negative	Present	
	Male	47 (95.9%)	11(100.0%)	0.496
	Female	2(4.1%)	0.0%	
L;.	Total	49 (100%)	11 (100%)	60 (patients)

X^2=4.64,df- 1

This table shows the association between sex and Genetic mutation and its is not significant

Table 16: Asscociation between years of alcohol consumption and Genetic mutation

Sl no	years of alcohol consumption	Genetic study mutation		P value
		Negative	Present	
1	<10 years	16 (32.7%)	3 (27.3%)	0.924
2	11- 20 years	28 (57.1%)	7 (63.6%)	
3	>20 years	5 (10.2%)	1 (9.1%)	
4	Total	49 (100%)	11 (100%)	60 (patients)

X^2=1.58,df- 1

This table shows the association between years of alcohol consumption and Genetic mutation and its is not significant

Table 17: Association between stage of disease and Genetic mutation

Sl no	stage of disease	Genetic study mutation		P value
		Negative	Present	
1	Alcoholic gastritis	1 (2.0%)	0.0%	0.702
2	Cirrohosis	32 (65.3%)	10 (90.9%)	
3	Steatosis	1 (2.0%)	0.0%	
	Steatohepatitis	15 (30.61%)	1 (9.09%)	
4	Total	49 (100%)	11 (100%)	60 (patients)

X^2=2.990,df- 1

This table shows the association between stage of disease and Genetic mutation and its is not significant

Table 20: Association between BMI , Lab parameters and Genetic mutation

Lab parameter's	Genetic study mutation		P value
	Negative	Present	
TB(Serum total bilirubin)	7.07 (8.6)	7.93 (8.51)	0.616
CB(Conjugate bilirubin)	5.19 (7.8)	5.85 (7.77)	0.672
UB(Unconjugate bilirubin)	1.89 (1.65)	1.97 (1.12)	0.777
ALBUMIN (Serum)	2.68 (0.798)	2.76 (0.86)	0.630
GLOBULIN(Serum)	3.42 (0.778)	3.65 (1.00)	0.202

AST	112.76 (106.28)	213.09 (287.67)	0.02
ALT	80.67 (246.19)	63.64 (56.96)	0.634
ALP	151.96 (71.03)	138.73 (51.58)	0.28
HB g/dl (Hemoglobin)	9.23 (2.8)	9.50 (3.02)	0.644
TLC (total leukocyte count)	11463(7257)	8767.27 (2899.11)	0.016
PLATELET COUNT	142.82(85.53)	94.73(60.44)	0.0016
BMI	22.06 (3.3)	21.74 (3.37)	0.63

AST, TLC (total leukocyte count), and Platelet Count show **statistically significant** differences between the groups ($p < 0.05$).

Other parameters do not show significant differences.

DISCUSSION

In our study, we noticed that most participants were between age group of 41 and 50 years,i.e 25 patients making up about 41.7% (n=25) of the group. The next was 31 to 40 years, which accounted for 31.7% (n=19)i.e 19 patients. On an average, the participants were of age 42.58 years (mean age), with a standard deviation of 8.7. We also found that majority were males 96.7% (n=58), while just Females were 3.3% (n=2).

Majority consumed alcohol for a duration of 11 to 20 years 58.3% (n-35) i.e 35 patients and followed by 19 patients for <10 years 31.7% (n-19) . Mean and Standard Deviation of Various parameters like BMI, TB, CB, UB, Albumin, Globulin is 22.0(3.31), 7.23(8.56),5.30(7.75),1.90(1.56),2.6(0.8), 3.4(0.818) respectively.

Out of 60 samples,11 patients 18.3%(n-11) had gene mutation and 49 (81.7%) does not have mutation. But this gene is not related to age, gender, years of alcoholic consumption and stage of the disease (p value >0.05).

A study by **Suthat et al** published in *Clinical Gastroenterology and Hepatology* pointed out that younger people are more at risk for severe alcoholic hepatitis (AH), which can lead to alcohol-related liver disease (ALD). It turns out that the younger folks often experience worse forms of this condition.[32]

Similarly, **Suthat et al** emphasized a male dominance in AH cases, which aligns with what we've seen in other research—men tend to drink more heavily, resulting in higher ALD rates. However, it's critical to remember that women can develop liver issues even with lower alcohol intake, likely because of differences in how their bodies process alcohol.[32]

In our study, we looked at how long people had been drinking, and found that 58.3% (n=35)had been consuming alcohol for 11 to 20 years. About 31.7% (n=19) had been drinking for less than 10 years. A related study by **Suthat et al** also found that once they reach certain limit of heavy drinking, the amount or duration of alcohol doesn't directly correlate with developing AH or ALD. So, it seems like other factors, like genetics, may play a important role in how these diseases develop.

Our study found that cirrhosis is super common, affecting about 70% of patients with alcohol-related liver disease (ALD), with steatohepatitis tagging along for around 26.6%. This is pretty much in line with what **Sagar's team** found in a big hospital in India, showing that 70% of ALD patients had cirrhosis, and 28% of those cases were pretty serious decompensated cirrhosis. Another study on drinking habits in India pointed out that 38.31% of heavy drinkers ended up developing liver cirrhosis. All these findings really emphasize just how tough it is for folks dealing with heavy alcohol consumption in India.[33]

It's been all over research when it comes to liver diseases because it leads to an I148M change in the PNPLA3 gene. In our group, we saw that 8.3% of the participants carried the rs738409 mutation on its own, and another 10% had both the rs738409 and rs738408 mutations. So, that adds up to 18.3% of individuals having that rs738409 variant.

A study done by **Zhang** and the team, which included 507 ALD patients and 645 healthy folks from Han Chinese backgrounds, found a solid link between the rs738409 G allele and being more susceptible to ALD. They found that G allele was higher among ALD patients compared to healthy ones, an odds ratio (OR) of 1.93, almost doubling the risk for ALD for those carrying the G allele.[34]

On a similar note, another study in Europe looked at 330 ALD patients and 328 healthy controls and reported that the rs738409 G allele was considerably more common among the ALD patients (OR = 1.54). Plus, this G allele was connected to higher risks of steatosis, fibrosis, and cirrhosis. Multivariate analysis emphasized rs738409 as the top independent factor linked to cirrhosis risk (OR = 2.08).

A U.S. study led by **Bhanu Prakash** found that among heavy drinkers, a specific gene variant called rs738409 is strongly linked to Alcoholic Liver Disease (ALD). This connection still holds up even when you take diabetes into account, which really reinforces how important this variant is when it comes to being at risk for ALD.[35]

So, what we found in our study really lines up with what other researchers have already shown—namely, that the PNPLA3 rs738409 variant is a big player in the risk for ALD. Plus, we've got some folks in our group who have both the rs738409 and rs738408 mutations, which makes it clear we need to dig deeper into how these gene variations work together to influence the risk and progression of ALD. Understanding this could help personalize prevention and management strategies for ALD specifically for individuals.

A study by **Hotta et al.** also found that in Japan, patients with the G-allele of rs738409 are likely to develop Non-Alcoholic Fatty Liver Disease (NAFLD). They discovered that this gene variant is linked to higher levels of certain liver enzymes and ferritin, and even the extent of liver scarring. Our findings suggest that PNPLA3 might play a role in pushing NAFLD towards more severe liver fibrosis.[36]

Strengths

- This study dives into the genetic details of the PNPLA3 gene and its link to Alcoholic Liver Disease (ALD), adding to what we already know about genetic risk factors.
- We made sure DNA extraction and quality checks were done using proper protocols, so we could trust the genotyping results.
- We had a solid group of ALD patients in our study, which helps keep things clear by minimizing mixed causes of liver disease.
- By comparing our results with past studies, we strengthen our findings and also point out some genetic differences that might be unique to specific populations.

Recommendations

- For future research, it'd be great to use a bigger sample size and involve multiple centers to really nail down the connection between PNPLA3 genetic variations and ALD.
- We should also look into how PNPLA3 works at a functional level to understand the molecular paths that lead to disease progression.
- Long-term studies that follow patients with certain PNPLA3 variants could help spot markers that predict how severe ALD might get.

Limitations

- One downside is that our sample size was on the smaller side, which might make it hard to apply these findings to a wider group.
- We only focused on two PNPLA3 variants (rs738409 and rs738408); there are likely other genetic variations that could impact ALD risk that we didn't consider.
- We didn't take a deep look at environmental factors like diet, smoking, and other health conditions, which could also play a role in how the disease progresses aside from genetics.

CONCLUSION

This study shows a clear link between certain PNPLA3 gene variations and Alcoholic Liver Disease, especially the rs738409 variant, which seems to be tied to more severe cases. Our results back up what's already out there in the literature regarding PNPLA3's role in liver issues like fibrosis, steatosis, and cirrhosis. We found that in our study there is no statistically significant between the demographic details, stage of the disease, and years of alcohol consumption.

While we've gained important insights into how genetics can influence ALD risk, there's still a need for more research with larger groups and in-depth functional studies to really confirm these findings and look into possible treatments.

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ANNEXURES

INFORMED CONSENT FORM

TITLE OF RESEARCH	**:**	MOLECULAR CHARACTERIZATION OF PATATIN LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3) GENE IN ALCOHOLIC LIVER DISEASE
GUIDE	**:**	**DR SIDDANAGOUDA.M BIRADAR**
P.G. STUDENT	**:**	**DR SHREYAS**

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to find the molecular characterization of PNPLA3 polymorphism in alcohol liver disease.

PROCEDURE:

I understand that I will undergo detailed history and clinical examination and investigations.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and I may experience some pain during the above mentioned procedures.

BENEFITS:

I understand that my participation in this study will help to study the association of PNPLA3 polymorphism in alcohol liver disease.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulation of hospital. If the data is used for publication the identity will not be revealed.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or withdraw from study at any time.

INJURY STATEMENT:

I understand in the unlikely event of injury to me during the study I will get medical treatment but no further medical compensation.

_____

(Signature of patient)

ಮಾಹಿತಿ ನೀಡಿದ ಒಪ್ಪಿಗೆ ನಮೂನೆ

ಸಂಶೋಧನೆಯ ಶೀರ್ಷಿಕೆ : ಆಲ್ಕೋಹಾಲಿಕ್ ಲಿವರ್ ಡಿಸೀಸ್‌ನಲ್ಲಿ 3 (PNPLA3) ಜೀನ್ ಒಳಗೊಂಡಿರುವ ಫಾಸ್ಫೋಲಿಪೇಸ್ ಡೊಮೇನ್‌ನಂತಹ ಪಾಪಾಟಿನ್‌ನ ಆಣ್ವಿಕ ಗುಣಲಕ್ಷಣ

ಮಾರ್ಗದರ್ಶಿ: ಡಾ.ಸಿದ್ದನಗೌಡ.ಎಂ ಬಿರಾದಾರ

ಪಿ.ಜಿ. ವಿದ್ಯಾರ್ಥಿ: ಡಿ. ಶ್ರೇಯಸ್

ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ:

ಆಲ್ಕೋಹಾಲ್ ಪಿತ್ತಜನಕಾಂಗದ ಕಾಯಿಲೆಯಲ್ಲಿ PNPLA3 ಪಾಲಿಮಾರ್ಫಿಸಂನ ಆಣ್ವಿಕ ಗುಣಲಕ್ಷಣಗಳನ್ನು ಕಂಡುಹಿಡಿಯುವುದು ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವಾಗಿದೆ ಎಂದು ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ.

ವಿಧಾನ:

ನಾನು ವಿವರವಾದ ಇತಿಹಾಸ ಮತ್ತು ಕ್ಲಿನಿಕಲ್ ಪರೀಕ್ಷೆ ಮತ್ತು ತನಿಖೆಗಳಿಗೆ ಒಳಗಾಗುತ್ತೇನೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಅಪಾಯಗಳು ಮತ್ತು ಅನಾನುಕೂಲಗಳು:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಯಾವುದೇ ಅಪಾಯವಿಲ್ಲ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ ಮತ್ತು ಮೇಲೆ ತಿಳಿಸಿದ ಕಾರ್ಯವಿಧಾನಗಳ ಸಮಯದಲ್ಲಿ ನಾನು ಸ್ವಲ್ಪ ನೋವನ್ನು ಅನುಭವಿಸಬಹುದು.

ಪ್ರಯೋಜನಗಳು:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯು ಆಲ್ಕೋಹಾಲ್ ಯಕೃತ್ತಿನ ಕಾಯಿಲೆಯಲ್ಲಿ PNPLA3 ಪಾಲಿಮಾರ್ಫಿಸಂನ ಸಂಬಂಧವನ್ನು ಅಧ್ಯಯನ ಮಾಡಲು ಸಹಾಯ ಮಾಡುತ್ತದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಗೌಪ್ಯತೆ:

ಅಧ್ಯಯನದಿಂದ ಉತ್ಪತ್ತಿಯಾಗುವ ವೈದ್ಯಕೀಯ ಮಾಹಿತಿಯು ಆಸ್ಪತ್ರೆಯ ದಾಖಲೆಯ ಭಾಗವಾಗುತ್ತದೆ ಮತ್ತು ಆಸ್ಪತ್ರೆಯ ಗೌಪ್ಯತೆ ಮತ್ತು ಗೌಪ್ಯತೆ ನಿಯಂತ್ರಣಕ್ಕೆ ಒಳಪಟ್ಟಿರುತ್ತದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ. ಡೇಟಾವನ್ನು ಪ್ರಕಟಣೆಗೆ ಬಳಸಿದರೆ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ಹೆಚ್ಚಿನ ಮಾಹಿತಿಗಾಗಿ ವಿನಂತಿ:

ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಅಧ್ಯಯನದ ಕುರಿತು ಹೆಚ್ಚಿನ ಮಾಹಿತಿಯನ್ನು ಕೇಳಬಹುದು ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

ಭಾಗವಹಿಸುವಿಕೆಯ ನಿರಾಕರಣೆ ಅಥವಾ ಹಿಂತೆಗೆದುಕೊಳ್ಳುವಿಕೆ:

ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆಯು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ ಮತ್ತು ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಅಥವಾ ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಲು ನಿರಾಕರಿಸಬಹುದು.

ಗಾಯದ ಹೇಳಿಕೆ:

ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ನನಗೆ ಗಾಯದ ಅಸಂಭವ ಸಂದರ್ಭದಲ್ಲಿ ನಾನು ವೈದ್ಯಕೀಯ ಚಿಕಿತ್ಸೆಯನ್ನು ಪಡೆಯುತ್ತೇನೆ ಆದರೆ ಹೆಚ್ಚಿನ ವೈದ್ಯಕೀಯ ಪರಿಹಾರವನ್ನು ಪಡೆಯುವುದಿಲ್ಲ ಎಂದು ನಾನು ಅರ್ಥಮಾಡಿಕೊಂಡಿದ್ದೇನೆ.

_____ (ರೋಗಿಯ ಸಹಿ)

STUDY SUBJECT CONSENT FORM:

I confirm that Dr. SHREYAS explained to me the purpose of this research, the study procedure that I will undergo and the possible discomforts and benefits that I may experience, in my own language.

I have been explained all above in detail in my own language and I understand the same. I agree to give my consent to participate as a subject in this research project.

SIGNATURE OF PARTICIPANT DATE

ANNEXURE IX

PROFORMA

Name of the patient : IP NO:

Age and Sex : Religion:

Address: Occupation:

Annual income:

Presenting Complaints :

Past history:

Personal history:

1 . Diet- Veg/Mixed

2. Sleep

3. Appetite

4. Bowel and Bladder Habits

5. Habits: Alcoholic history- amount of alcohol/day:

Type of alcohol :

Duration of consumption:

Family history:

GENERAL PHYSICAL EXAMINATION :

Built :

Nourishment :

Ht(Cm) : Wt(kg): BMI:

Pallor: Lymphadenopathy:

Cyanosis: Edema:

Clubbing:

GENERAL EXAMINATION (HEAD TO TOE) :

1. scalp and hair:
2. eyes:
3. ears:
4. nose:
5. oral cavity:
6. neck:
7. chest:

8. upper limbs

9. abdomen:

10. lower limbs:

Vital parameters a) Pulse : b) BP:

c) temperature: d) Respiratory rate:

SYSTEMIC EXAMINATION:

ABDOMEN EXAMINATION:

INSPECTION:

PALPATION:
PERCUSSION:
AUSCULTATION:

CARDIOVASCULAR SYSTEM

RESPIRATORY SYSTEM
CENTRAL NERVOUS SYSTEM

INVESTIGATIONS:	
1) liver function test	
Bilirubin, total	
Bilirubin conjugated (direct)	
Bilirubin (indirect)	
Alanine aminotransferase (ALT/SGPT)	
Aspartate aminotransferase (AST/SGOT)	
Alkaline phosphatase	
Protein total	
Albumin	
Globulin	
A/G ratio	
2) URINE ROUTINE:	
3) HBsAG	
4) HCV	
5) PT/INR	
6) complete blood count	
Hb	gm/dl
Total count	Cells/cumm

Differential count Neutrophils	% %
Lymphocytes	%
Eosinophils	%
Basophils	%
Monocytes	%
ESR	
Platelets	

STOOL (IF REQUIRED) 1) ROUTINE:

2) FOR OCCULT BLOOD:

ULTRASOUND DIAGNOSIS:

UPPER GI ENDOSCOPY (IF REQUIRED):

SAMPLE COLLECTION FOR GENE ANALYSIS

CONCLUSION :

Diagnosis -

Stage of disease

FOLLOW UP OF PATIENT DURING HOSPITAL STAY:

SIGNATURE:

DATE:

BLDE

(DEEMED TO BE UNIVERSITY)

Declared as Deemed to be University u/s 3 of UGC Act, 1956

Accredited with 'A' Grade by NAAC (Cycle-2)

The Constituent College

SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 928/2023-24

10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinized the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "MOLECULAR CHARACTERIZATION OF PAPATIN LIKE PHOSPHOLIPASE DOMAIN CONTAINING 3 (PNPLA3) GENE IN ALCOHOLIC LIVER DISEASE".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR.SHREYAS

NAME OF THE GUIDE: DR.SIDDANANGOUDA M.BIRADAR, PROFESSOR, DEPT. OF GENERAL MEDICINE.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
College: Phone: +918352-262770, Fax: +918352-263019, E-mail: bmpmc.principal@bldedu.ac.in

Master chart

CHIEF COMPI	AMOUI	BMI	TB (n	CB	UB	ALB	GLOE	AST	ALT (	ALP	Hb (g	TC (ce	PLATEI	USG	STOC	UPPEF	STAGE OF	GENETIC STUDY
PAIN ABDOM	115	28	6.9	4.6	2	2.4	3.4	79	63	283	8.3	6800	77000	HEPATOSPI	ND(N	GRAD	CIRRHOS	recorded
ABDOMINAL	115--15	24	0.9	0.3	1	4.5	2.8	47	32	109	15	####	22000	GRADE 1 FA	ND	GRAD	STEATOH	recorded
ABDOMINAL	115	22	1	0.4	1	1.8	3.4	30	12	91	13	####	1E+05	LIVER CAPS	ND	NIL	CIRRHOS	recorded
ABDOMINAL	51	19	1.9	0.7	1	2.2	5	65	22	175	5.8	9320	71000	COARSE EC	ND	NIL	CIRRHOS	rs738408
BLOOD IN VC	52	26	1.5	0.2	1	3.8	3.2	67	23	80	16	7970	2E+05	GRADE 1 FA	ND	EROSI	ALCOHOI	recorded
ABDOMEN D	115	28	0.3	0.1	0	2.5	3.3	44	18	134	9.5	####	2E+05	HEPATOME	ND	ND	CIRRHOS	rs738409
BLOOD IN ST	154	23	5.8	4	2	3.4	5	##	51	200	10	6930	80000	LIVER CAPS	ND	SMALI	CIRRHOS	recorded
BLOOD IN VC	115	20	5.3	2.8	3	2.9	3.2	##	33	109	8.4	####	94000	GRADE 1 FA	ND	SMALI	CIRRHOS	recorded
B/L LL SWELL	57	33	42	39	3	2.5	4.2	82	23	126	9.7	6610	2E+05	HEPATOSPI	ND	ND	CIRRHOS	recorded
INVOLUNTAF	115	21	0.7	0.4	0	1.9	2.3	65	60	304	13	####	1E+05	HEPATOME	ND	ND	STEATOH	recorded
ABDOMEN D	230	26	8.9	2.4	7	3.6	1.4	##	87	233	11	####	98000	HEPATOME	ND	ND	CIRRHOS	recorded
ABDOMEN D	57	21	0.7	0.2	1	2.2	3	53	31	120	9.7	8630	4E+05	ADVACNED	ND	ND	CIRRHOS	recorded
ABDOMEN D	57	18	6	3.1	3	2.1	3.9	40	33	188	8.7	####	71000	NORMAL S	ND	ND	STEATOH	recorded
PAIN ABDOM	57	21	1.2	0.6	1	2	4.4	81	28	126	8.3	####	48000	LIVER CAPS	ND	LARGE	STEATOH	recorded
ABDOMEN D	115	18	25	22	3	2.3	3.5	##	64	108	11	####	2E+05	HEPATOME	ND	ND	CIRRHOS	rs738408
BLOOD IN VC	115	24	2	0.7	1	2	2	70	32	133	4.2	####	2E+05	ALTERED E	POSI	GRAD	CIRRHOS	recorded
BLOOD IN VC	57	24	2.3	0.8	2	4.2	1.6	##	81	173	7.4	####	2E+05	HEPATOSPI	ND	LARGE	CIRRHOS	rs738408
ABDOMEN PA	230-28	20	29	19	10	2.9	3.7	##	92	307	10	####	3E+05	HEPATOSPI	ND	ND	STEATOH	recorded
ABDOMEN D	57-115	21	18	15	2	2.4	3.4	##	45	233	10	####	2E+05	NORMAL S	ND	ND	STEATOH	recorded
EPIGASTRIC F	115	17	1.8	0.7	0	4.5	3.2	##	85	174	16	9160	1E+05	GRADE 2 FA	ND	ND	STEATOH	rs738409
B/L LLSWELL	115	15	2.7	1	2	3.6	2.8	80	38	167	7.1	####	2E+05	NORMAL S	NEGA	ND	HEPATITI	recorded
ABDOMEN D	57	24	13	10	3	1.9	3.8	##	90	42	8.8	5130	45000	NORMAL S	ND	NORM	CIRRHOS	recorded

PAIN ADBDO	115-17(	26	3.5	2.1	1	4.4	3	##	180	113	11	5170	45000	NORMAL S	ND	ND	CIRRHOS	recorded
ABDOMEN D	115	21	3.8	1.6	2	2.3	3	43	36	112	10	2690	43000	NORMAL S	ND	ND	CIRRHOS	rs738409, rs738408
PAIN ABDOM	28-57	25	15	13	1	2.6	2.8	##	###	214	11	4950	88000	NORMAL S	ND	ND	HEPATITI	No mutations recorded
ALTERED SEN	115-24(	25	6.6	4.3	2	2.5	3.5	##	220	147	14	8040	66000	NORMAL S	ND	ND	CIRRHOS	rs738409, rs738408
ABDOMEN D	115	30	5.5	4.5	1	1.8	3.2	72	37	205	8.5	8650	1E+05	HEPATOME	ND	ND	CIRRHOS	recorded
ABDOMEN D	57-115	20	26	24	2	2.1	3.8	##	30	162	11	####	2E+05	NORMAL S	ND	ND	CIRRHOS	recorded
LOSS OF APPI	115	21	22	19	3	2.6	3.8	##	75	135	11	####	1E+05	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ABDOMEN D	57-115	20	9.4	7.2	2	1.8	5	##	28	88	6.8	5560	24000	LIVER SHO	ND	ND	CIRRHOS	rs738409
FEVER WITH	115	20	4.8	1.6	3	3.7	2.6	64	78	98	8.1	####	1E+05	LIVER SHO	ND	LOW (	CIRRHOS	recorded
GENERALISEI	32	21	1	0.4	1	2.2	3.5	46	18	108	6.3	6430	2E+05	NORMAL S	ND	ND	CIRRHOS	recorded
ABDOMEN	57-60	19	0.8	0.3	1	1.8	3.2	32	25	182	4.6	####	2E+05	LIVER SOW	POSIT	ND	CIRRHOS	No mutations recorded
ABDOMEN D	115	20	7.2	5.2	2	2.1	3.9	81	18	126	5.6	####	2E+05	NORMAL S	POSIT	ND	CIRRHOS	No mutations recorded
BREATHLESSI	115	20	1.5	1.4	0	1.2	3.1	93	42	129	8.3	####	1E+05	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ABDOMEN D	60-84	25	18	16	2	2.9	3.4	##	26	140	8.7	####	2E+05	HEPATOME	POSIT	ND	CIRRHOS	No mutations recorded
YELLOWISH [	115	20	2.5	0.5	2	3.5	2.4	36	23	47	2.7	1110	19000	GRADE 1 F/	NEGA	ND	STEATOSI	No mutations recorded
BREATHLESSI	57	19	1.2	0.7	1	2.6	3	30	31	120	10	####	3E+05	NORMAL S	ND	ND	CIRRHOS	recorded
ABDOMEN D	57	20	3.7	2.9	1	2.1	3.6	42	33	152	11	7070	55000	NORMAL S	ND	ND	STEATOH	recorded
BLOOD IN VC	57-115	23	3.6	0.8	3	2.5	4.2	47	22	107	8	####	78000	CHRONIC L	ND	HIGH (	CIRRHOS	recorded
YELLOWISH [	57	23	17	14	3	2.7	4.1	##	43	202	8.5	####	1E+05	CIRRHOSIS	ND	ND	CIRRHOS	recorded
BLOOD IN VC	115	18	2.4	0.7	2	3.3	4.1	23	26	384	9.6	4010	94000	NORMAL S	POSIT	LOW (	CIRRHOS	recorded
BLOOD IN VC	57	19	0.5	0.3	0	4.1	3.5	##	105	134	15	6950	2E+05	HEPATOME	ND	LOW (	HEPATITI	recorded
ABDOMEN P/	115-17(	24	1.2	0.6	1	2.7	2.6	33	12	41	14	5810	3E+05	COMPLEX /	ND	ND	CIRRHOS	recorded
ABDOMEN D	115	26	1.6	1	1	3	4.5	##	57	157	9	####	4E+05	HEPATOME	ND	ND	CIRRHOS	recorded
ABDOMEN D	115	20	17	14	3	2.9	4.1	##	28	177	6.4	####	1E+05	HEPATOME	ND	HIGH (	CIRRHOS	No mutations recorded

ABDOMEN D	115	22	7.5	5	3	3.1	4.8	##	42	241	10	8630	61000	NORMAL S	ND	ND	CIRRHOS	rs738409
PAIN ABDOM	115	20	2.7	0.6	2	3.3	2.5	76	100	78	2.4	2200	66000	HEPATOSPI	ND	ND	STEATOH	No mutations recorded
ABDOMEN D	57-115	19	9.8	5.5	4	2.3	5.3	80	30	122	9.4	7590	1E+05	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ABDOMEN D	115-17(	23	2.7	1.2	2	4	2.7	52	60	245	8.8	9200	81000	HEPATOME	ND	LOW (	STEATOH	No mutations recorded
ABDOMEN D	115	20	1.4	0.8	1	2.3	4.5	##	47	112	10	7210	1E+05	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ABDOMEN D	115	23	5.9	2.7	3	2	4.4	74	37	106	8.2	6540	82000	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ABDOMEN D	115	19	5	2.9	2	2.7	3.6	##	44	187	12	9900	2E+05	MILD HEAI	ND	ESOPH	CIRRHOS	No mutations recorded
ABDOMEN P/	115	24	5.2	2.2	3	2.1	3.7	##	70	50	6.8	8610	1E+05	CIRRHOSIS	ND	ND	CIRRHOS	rs738409, rs738408
ABDOMEN D	115	20	23	20	3	2.9	3.6	89	34	124	8.1	8600	20000	NORMAL S	ND	ND	CIRRHOS	rs738409
ALTERED SEN	57-115	19	0.9	0.1	1	1.8	2.6	##	58	181	11	4750	58000	HEPATOME	ND	ND	STEATOH	No mutations recorded
ABDOMEN D	115	23	0.7	0.1	1	1.2	2.8	23	25	82	5.2	8650	1E+05	NORMAL S	ND	ND	CIRRHOS	No mutations recorded
ALTERED SEN	115	20	1.8	0.0	1	4	3	##	51	75	11	4500	2E+05	HEPATOME	ND	ND	STEATOH	No mutations recorded

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