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PNPLA3 Polymorphism: A Key To Understanding And Diagnosing Non-Alcoholic Steatohepatitis.

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ABSTRACT

Background: *Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide and the leading cause of liver-related morbidity and mortality. We aimed to investigate the function of the PNPLA3 gene polymorphism as a non-invasive biomarker for NASH diagnosis. Because invasive diagnostic procedures have limits and non-invasive methods are inaccurate, the primary focus of attention for screening, classifying severity, and tracking the advancement of NASH and liver fibrosis has shifted to the discovery and validation of noninvasive biomarkers.*

Method: *Study populations, in whom NAFLD was diagnosed using an imaging method in the absence of excessive alcohol consumption and viral hepatitis. . In a case-control study, 50 histologically confirmed NAFLD patients and 30 matched healthy controls were genotyped for PNPLA3 rs738409 using real-time PCR. Clinical, biochemical, and histological data were compared across genotypes.*

Results: *The G allele was significantly more frequent in NAFLD patients ($p < 0.001$) and correlated with higher ALT, AST, BMI, and histological severity (steatosis, inflammation, fibrosis). The GG genotype conferred the highest risk The CC genotype lowered the risk of non-alcoholic fatty liver disease by 63%, while the CG genotype increased the risk by 32% and NASH by 30%. GG genotype carriers had a 34% and 40% greater incidence of non-alcoholic fatty liver and NASH, respectively. Individuals with CG + GG genotypes had a 66% higher chance of developing the disease and a 70% higher risk of NASH.*

Conclusion: *PNPLA3 rs738409 is strongly associated with NAFLD and NASH severity. Genotyping may serve as a non-invasive risk stratification tool in clinical practice. Our study confirmed that this genotype can be used as a non-invasive biomarker. But also, Future research may investigate the influence of genetic and environmental factors on tissue damage levels, as well as the impact of this gene on fibrosis and liver cirrhosis. Future genomic analyses through fine mapping or comprehensive sequencing may uncover additional genetic determinants within the PNPLA3 locus.*

Keywords: *PNPLA3; NAFLD; NASH.*

1. INTRODUCTION

As the most prevalent chronic liver disease, non-alcoholic fatty liver disease (NAFLD) affects an estimated 25% of adults worldwide [1]. When there is no major alcohol consumption, pharmaceutical usage, or underlying medical disorders that result in fatty liver, NAFLD is defined by fat infiltration of the liver [2]. It includes a broad spectrum of diseases, such as nonalcoholic steatohepatitis (NASH) and basic hepatic steatosis, which can eventually progress to cirrhosis, end-stage liver disease, hepatocellular carcinoma (HCC), or the requirement for a liver transplant [3].

NAFLD can manifest as a single steatosis, a steatosis combined with portal or lobular inflammation, or a steatosis combined with ballooning (hepatocyte injury) [4]. A high-fructose diet, age, diabetes, and obesity are risk factors [5]. There are also established genetic risk factors for NAFLD. Currently, one of the biggest risk factors for NAFLD is metabolic syndrome [4].

The gold standard for diagnosing, grading, and histologically evaluating nonalcoholic fatty liver disease (NAFLD) is liver biopsy [6]. Early detection of individuals at high risk of developing NAFLD is critical to slow its progression to irreversible and terminal stages, as well as to allow effective management. Currently, NAFLD can be diagnosed using liver histology, imaging techniques, blood biomarkers, or non-invasive prediction scores [7]. Although liver histology is the gold standard for diagnosing and staging its severity, it has been linked to possible consequences and interobserver heterogeneity of particular pathological findings. Pain, transient bacteremia, pneumothorax, and bleeding, which can vary from asymptomatic hematomas to intraperitoneal hemorrhage causing hemodynamic instability, are all

complications of liver biopsy [8].

Because invasive diagnostic procedures have limits and non-invasive methods are inaccurate, the primary focus of attention for screening, classifying severity, and tracking the advancement of NASH and liver fibrosis has shifted to the discovery and validation of noninvasive biomarkers. To diagnose NASH, we will investigate PNPLA3's gene function as a noninvasive biomarker.

PNPLA3, or the human patatin-like phospholipase domain-containing 3 gene, codes for a 481 amino acid protein. The protein's isoleucine to methionine alteration at position 148 (I148M) is encoded by the variation rs738409, which is a cytosine to guanine change. Strong correlations exist between this variant and all types of liver disease. The mechanism underlying PNPLA3 and liver illness remains unclear, despite the fact that this variant is among the best characterized and researched. From a functional standpoint, it is evident that the PNPLA3 protein functions as an enzyme that has the ability to lipase retinyl esters and triglycerides and acyltransferase phospholipids [9]. The mutation I148M in the patatin-like phospholipase domain results in both a gain of functionality, such as increased thioesterase and lysophosphatidic acid acyltransferase activities, and a loss of function that promotes triacylglycerol buildup in hepatocytes [10].

2. METHODOLOGY

We performed a case control study on 50 Egyptian patients, who proved histologically to be NAFLD, who are undergoing bariatric Surgery, will be asked to participate in this study, and 30 healthy control Subjects. This study was conducted for patients attending at Ain-Shams University Hospitals. The patients group included 50 patients with NAFLD proven by histology. Their age ranged between 18th - 60th years. The control group included 30 apparently healthy subjects, age and sex matched, having no acute or chronic illness, and taking no medication.

Patients with Fatty liver due to medication, Chronic liver disease other than nonalcoholic fatty liver disease (NAFLD), alcohol consumption, Decompensated liver cirrhosis and Malignancies and systemic diseases were excluded.

All participants will be subjected to the following: Full history taking, Full Clinical examination including body mass index, Laboratory investigations including: -Complete blood count, -Liver function tests including serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST), total and direct bilirubin, serum albumin, - Lipid profile (LDL, HDL, cholesterol, Triglyceride), -Serum ferritin and Total Iron binding capacity (TIBC), - Random blood glucose, - Thyroid function tests (thyroid stimulating hormone TSH, free thyroxin FT4, free triiodothyronine FT3), - Coagulation profile (INR, PTT, PT). Also, Abdominal Ultrasonography and Liver biopsy for histopathology.

For the PNPLA3 gene Polymorphism, Real-time PCR was used. DNA extraction was done using QIAamp DNA Mini Blood. By Pipeting 20 µL QIAGEN Protease (or proteinase K) into the bottom of a 1.5 mL microcentrifuge tube. Then Add 200 µL sample to the microcentrifuge tube. Add 200 µL Buffer AL to the sample. Mix by pulse-vortexing for 15 s, then, incubate at 56°C for 10 min. after the incubation is complete. Briefly centrifuge the 1.5 mL microcentrifuge tube, add 200 µL ethanol (96–100%) to the sample, and mix again by pulse-vortexing for 15 s. After mixing, briefly centrifuge the 1.5 mL microcentrifuge tube, carefully transfer the sample into the QIAamp Mini spin column and centrifuge at 6000 x g (8000 rpm) for 1 min then add 500 µL Buffer AW1 without wetting the rim. . Carefully open the QIAamp Mini spin column and add 500 µL Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min. carefully open the QIAamp Mini spin column and add 200 µL Buffer AE or distilled water. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

The Real-time PCR procedure was performed using 7500 System Standard 96-well plate. The TaqMan® SNP Genotyping Assays, according to the following reaction mix method: 2X TaqMan® Master Mix a 12.50 µL was used, 20X Assay Working Stock (rs738409) a 1.25 µL was used and Nuclease-free water. With a total volume per well 5.00 µL.

The thermal cycling conditions were performed as the following:

AmpliTaq Gold®, UP, Enzyme Activation: Temp. 95°C. Duration 10 minutes. (HOLD), Denaturation:

Temp. 95°C for 15 seconds. (Cycles 50),
Annealing/ Extension: Temp. 60°C for 90 seconds. (Cycles 50).

3. RESULTS:

The current study was carried out on 80 subjects, comprising 50 Egyptian patients who were histologically proven to have non-alcoholic fatty liver disease (NAFLD) and 30 healthy control subjects. Both study groups were compared according to demographic data, laboratory data and histopathologic liver biopsy results in relation to PNPLA3 gene polymorphism. This study was conducted at Faculty of Medicine Ain Shams Research institute, Ain shams hospitals university.
The collected data was revised, coded, tabulated using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

Genetic statistics

Allele frequency refers to how common an allele is in a population.
The genetic models
If the alleles of the gene of interest are A and B, and A is the risk allele, the three genotype groups would then be AA, AB and BB. This dichotomization of the SNP genotypes can be done as follows:
Dominant: 'AA + AB' versus 'BB',
Recessive: 'AA' versus 'AB + BB'.

Normality of data

Shapiro-Wilk test was done to test the normality of data distribution.

Descriptive statistics:

Mean, Standard deviation (± SD) for numerical data.
Frequency and percentage of non-numerical data.

Analytical statistics:

Student T Test was used to assess the statistical significance of the difference between parametric numerical variable in two study groups.
Mann Whitney Test was used to assess the statistical significance of the difference between non parametric numerical variable in two study groups.
Chi-Square test was used to examine the relationship between two qualitative variables.
A p value is considered significant if <0.05 at confidence interval 95%.
A total of 80 participants met the enrolment criteria and consisted of 30 confirmed health controls and 50 patients with confirmed NAFLD. The clinical data of patients in the NAFLD and control groups are presented in Table 1. Patients with NAFLD had a higher BMI (32.08 ± 4.70 vs. 22.24 ± 1.93, p<0.001) and elevated AST (U/L) (56.48 ± 32.96 vs. 21.83 ± 6.82, p<0.001) also ALT was elevated (61.82 ± 36.60 vs. 19.40 ± 6.05, p<0.001). In addition to there was an increase in INR (1.12 ± 0.13 vs. 0.99 ± 0.15, p<0.001), D.Bil (mg/dL) (0.22 ± 0.10 vs. 0.17 ± 0.09, p=0.026), cholesterol (181.02 ± 25.16 vs. 122.87 ± 9.85, p<0.001) and IR(HOMA) (4.71 ± 0.88 vs. 1.25 ± 0.21, p<0.001). Also, PLT (platelets/µL) counts was observed (235.30 ± 76.88 vs. 300.87 ± 89.99, p<0.001) to be lower and Triglycerides (mg/dL) was decreased (114.96 ± 27.00 vs. 160.67 ± 11.85, p<0.001)

Table 1. Baseline clinical characteristics.

		NAFLD	Control	Test Result
		n=50	n=30	
Age (years)	Mean ± SD	35.64 ± 9.10	34.20 ± 8.63	t: 0.698, p=0.487
Gender	Female	25(50.0%)	17(56.7%)	X2: 0.120, p=0.729
	Male	25(50.0%)	13(43.3%)	
BMI (kg/m2)	Mean ± SD	32.08 ± 4.70	22.24 ± 1.93	Z: 7.195, p<0.001*
Hb (g/dL)	Mean ± SD	12.27 ± 1.30	12.66 ± 0.61	Z: 1.168, p=0.244
TLC (cells/µL)	Mean ± SD	7.02 ± 2.43	7.25 ± 1.97	Z: 0.775, p=0.441

PLT (platelets/µL)	Mean ± SD	235.30 ± 76.88	300.87 ± 89.99	t: 3.462, p<0.001*
AST (U/L)	Mean ± SD	56.48 ± 32.96	21.83 ± 6.82	Z: 6.072, p<0.001*
ALT (U/L)	Mean ± SD	61.82 ± 36.60	19.40 ± 6.05	Z: 6.167, p<0.001*
Albumin (g/dL)	Mean ± SD	4.08 ± 0.72	4.22 ± 0.40	Z: 0.755, p=0.452
INR	Mean ± SD	1.12 ± 0.13	0.99 ± 0.15	Z: 3.608, p<0.001*
T.Bil (mg/dL)	Mean ± SD	0.58 ± 0.21	0.68 ± 0.35	Z: 1.063, p=0.286
D.Bil (mg/dL)	Mean ± SD	0.22 ± 0.10	0.17 ± 0.09	Z: 2.117, p=0.026*
Chole (mg/dL)	Mean ± SD	181.02 ± 25.16	122.87 ± 9.85	Z: 7.255, p<0.001*
Trigly (mg/dL)	Mean ± SD	114.96 ± 27.00	160.67 ± 11.85	Z: 6.524, p<0.001*
IR(HOMA)	Mean ± SD	4.71 ± 0.88	1.25 ± 0.21	t: 21.078, p<0.001*

t: t student test, Z: Mann Whitney test, * for significant p value (<0.05)

Fig 1 shows a comparison between the significance of BMI in NAFLD patient and control group.

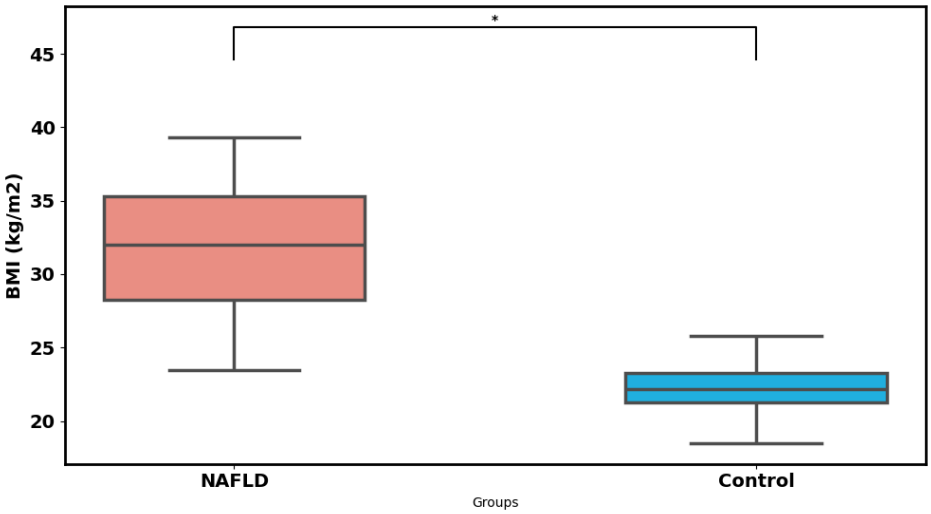

Figure 1.Comparison between studied groups according to BMI.

Fig 2 shows a comparison between the significance Platelets in NAFLD patient and control group.

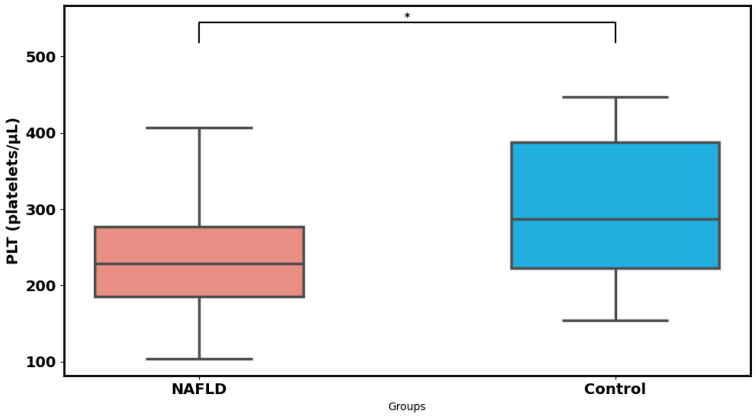

Figure 2.Comparison between studied groups according to platelets.

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Fig 3 shows a comparison between the significance of AST in NAFLD patient and control group.

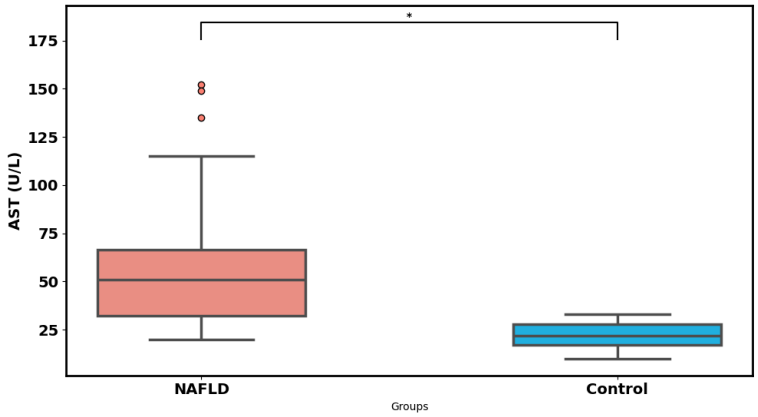

Figure 3.Comparison between studied groups according to AST.

Fig 4 shows a comparison between the significance of ALT in NAFLD patient and control group.

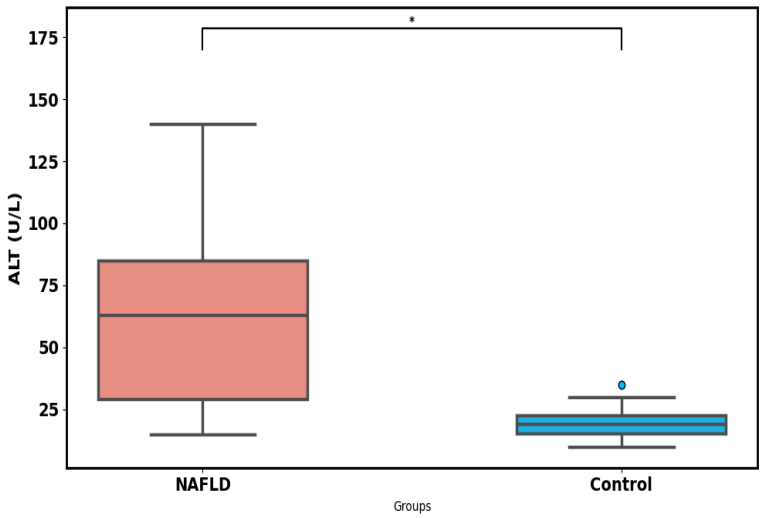

Figure 4.Comparison between studied groups according to ALT.

Fig 5 shows the international normalized ratio (INR) significally difference between NAFLD and control group.

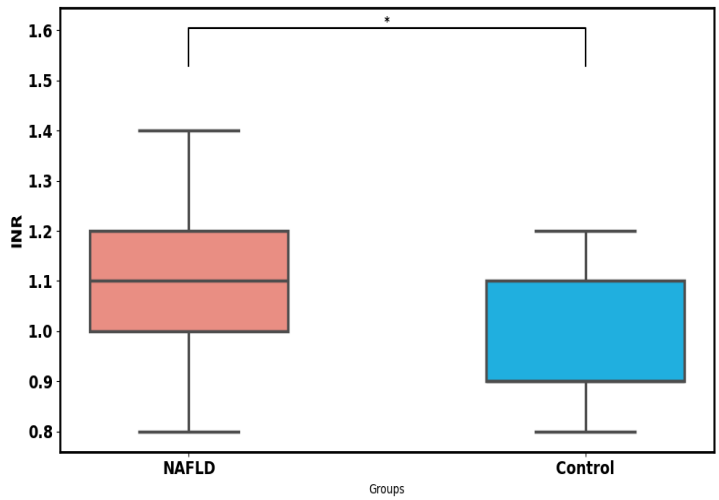

Fig 5 shows a comparison between NAFLD patient and control group in accordance to direct bilirubin which was significaly increased.

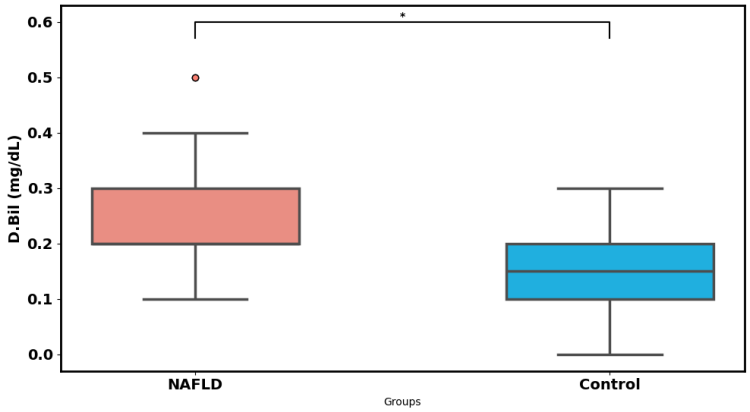

Figure 5.Comparison between studied groups according to direct bilirubin

Fig 6 shows a comparison between NAFLD patients and control group in accordance to cholesterol which was significaly increased.

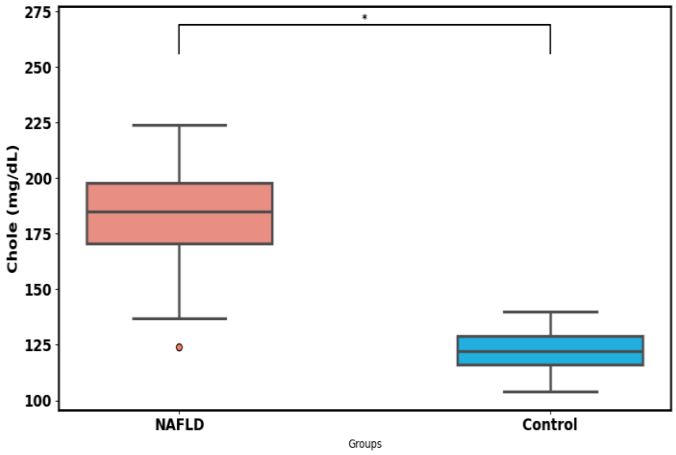

Figure 6.Comparison between studied groups according to total cholesterol.

Fig 7 shows a comparison between NAFLD patient and control group in accordance to triglycerides which was significaly decreased.

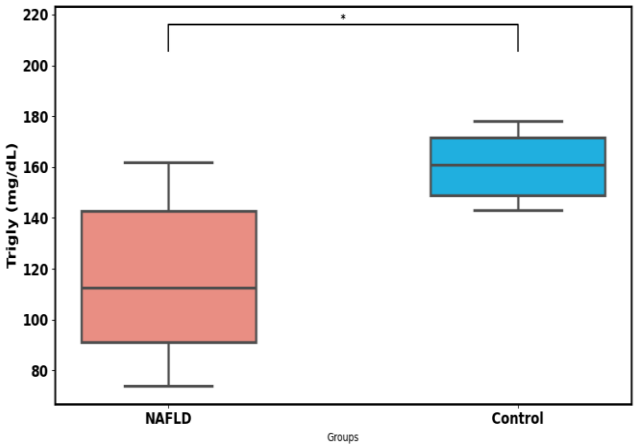

Figure 7. Comparison between studied groups according to triglycerides.

The HOMA-IR (Homeostatic Model Assessment for Insulin Resistance) was compared in fig 8 between NAFLD patient and control group.

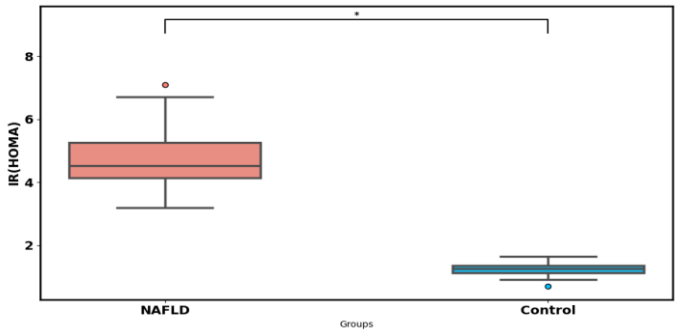

Figure 8. Comparison between studied groups according to HOMA-IR.

According to genotyping: 17 patients (34%) were CC, 16 (32.0%) were GC and 17 (34%) were GG. Control group was 19 healthy (63.3%) were CC, 8 (26.7%) were GC and 3 (10%) were GG. Giving a significant result with X2: 8.083, p=0.018.

By dominant analysis, 17 patients (34%) carried the risk allele (CC), CG+GG dominant model was 33(66.0%). In healthy group 19(63.3%) was CC and 11(36.7%) were CG+GG. (p 0.020, X2: 5.387).

By Recessive model the CC+CG in patients were 33(66.0%) and 17(34.0%) for GG model. Healthy group was 27(90.0%) for CC+CG and 3(10.0%) GG. The analytical test result showed a significant FE: p=0.018*

The overall allele frequency was C/G. The allelic frequency as variant was: 0.5/0.5 for NAFLD, 0.76/0.23 for Control. The observed frequencies were significant X2: 10.028, p=0.002 those result is shown in table 2.

Table 2. Comparison of genotype and allele frequencies between NAFLD and control groups.

		NAFLD	Control	Test Result
		n=50	n=30	
Rs738409 Genotype	CC	17(34.0%)	19(63.3%)	X2: 8.083, p=0.018*
	CG	16(32.0%)	8(26.7%)	
	GG	17(34.0%)	3(10.0%)	
Allele frequency	C/G	0.5/0.5	0.76/0.23	X2: 10.028, p=0.002*
Recessive model	CC+CG	33(66.0%)	27(90.0%)	FE: p=0.018*
	GG	17(34.0%)	3(10.0%)	
Dominant model	CC	17(34.0%)	19(63.3%)	X2: 5.387, p=0.020*
	CG+GG	33(66.0%)	11(36.7%)	

X^2: Chi square test, FE: Fissue exact, * for significant p value (<0.05)

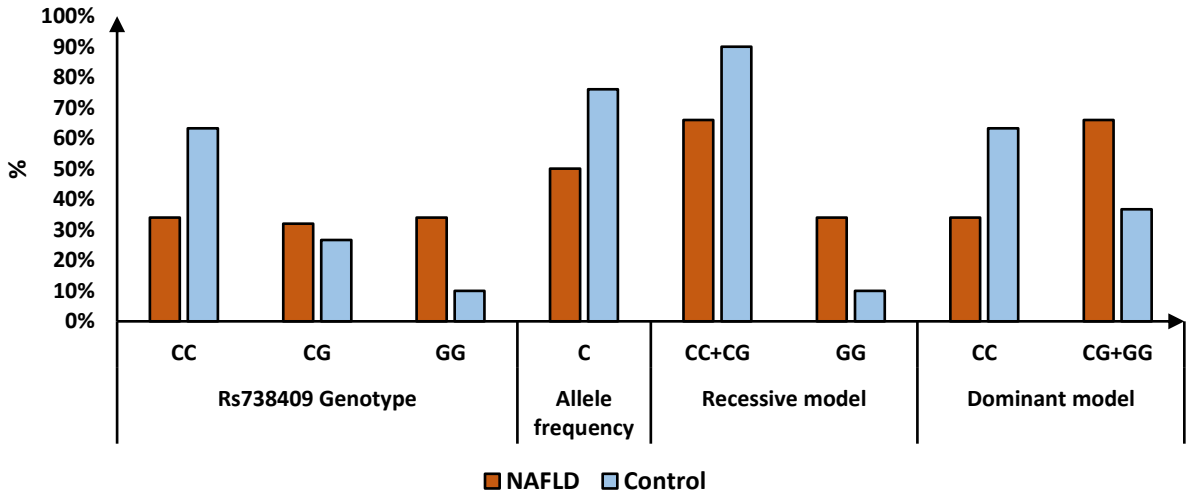

Figure 9. Comparison of genotype and allele frequencies between NAFLD and control groups.

According to genotyping: 6 patients with NASH (30%) were CC, 6 (30.0%) were GC and 8 (40%) were GG. Control group was 19 healthy (63.3%) were CC, 8 (26.7%) were GC and 3 (10%) were GG. Giving a significant result with X2: 7.623, p=0.022

By dominant analysis, 6 NASH patients (30%) carried the risk allele (CC), CG+GG dominant model was 14 (70.0%). In healthy group 19(63.3%) was CC and 11(36.7%) were CG+GG. X2: 4.083, p=0.043

By Recessive model the CC+CG in patients were 12(60.0%) and 8(40.0%) for GG model. Healthy group was 27(90.0%) for CC+CG and 3(10.0%) GG. The analytical test result showed a significant FE: p=0.017

The overall allele frequency was C/G. The allelic frequency as variant was: 0.45/0.55 for NASH, 0.76/0.23 for Control. The observed frequencies were significant X2: 9.116, p=0.003* those results are shown in table 4.

Table 3.Comparison of genotype and allele frequencies between NASH and control groups.

		NASH	Control	Test Result
		n=20	n=30	
Rs738409 Genotype	CC	6(30.0%)	19(63.3%)	X2: 7.623, p=0.022*
	CG	6(30.0%)	8(26.7%)	
	GG	8(40.0%)	3(10.0%)	
Allele frequency	C/G	0.45/0.55	0.76/0.23	X2: 9.116, p=0.003*
Recessive model	CC+CG	12(60.0%)	27(90.0%)	FE: p=0.017*
	GG	8(40.0%)	3(10.0%)	
Dominant model	CC	6(30.0%)	19(63.3%)	X2: 4.083, p=0.043*
	CG+GG	14(70.0%)	11(36.7%)	

X^2: Chi square test, FE: Fissure exact, * for significant p value (<0.05)

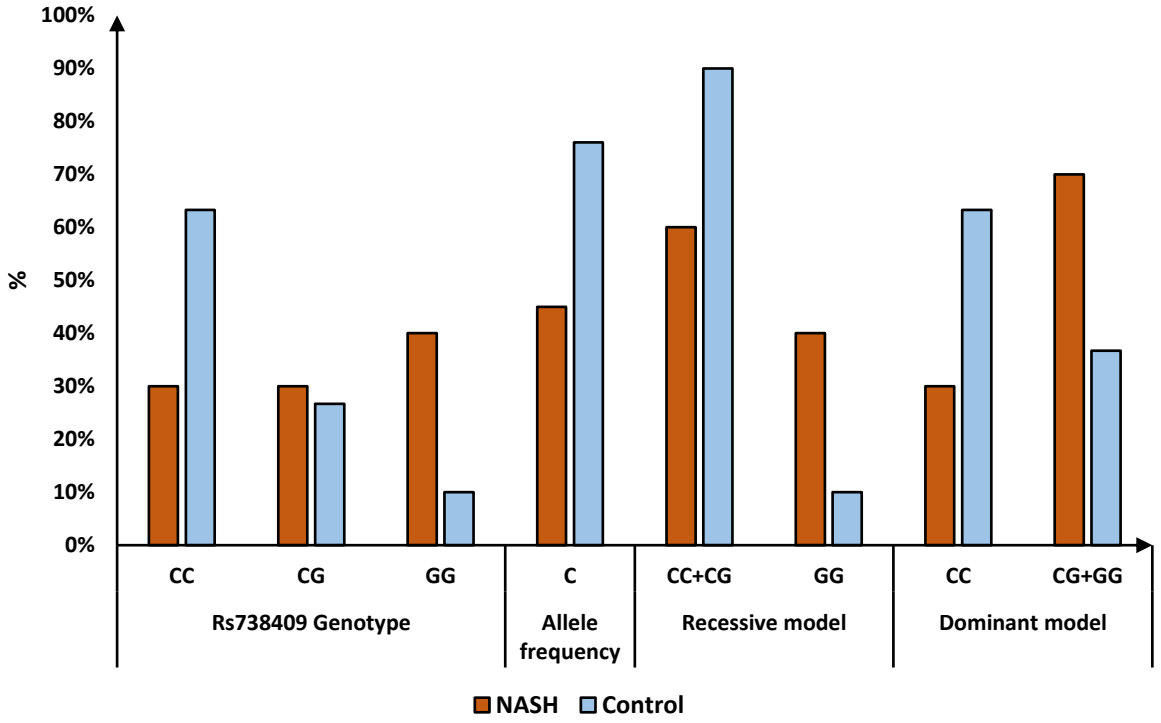

Figure 10. Comparison of genotype and allele frequencies between NASH and control groups.

Table 5. Shows us a Comparison of genotype frequencies in NAFLD group. Differentiating between each grade, with grade I being GG (n=17) 2(11.8%)

CG (n=16) 2(12.5%) and CC (n=17) 2(11.8%). While grade II is GG 4(23.5%), CG 3(18.8%) and CC 3(17.6%). Grade III is GG 5(29.4%), CG 6(37.5%) and CC 6(35.3%). Grade IV GG 5(29.4%), CG 4(25.0%) and CC 5(29.4%). And finally grade V GG 1(5.9%), CG 1(6.2%) and CC 1(5.9%). The test result was X2: 0.420, p=1.000 which was non-significant.

Table 4. Comparison of genotype frequencies in the NAFLD group.

		GG	CG	CC	Test Result
		n=17	n=16	n=17	
Grade	I	2(11.8%)	2(12.5%)	2(11.8%)	X2: 0.420, p=1.000
	II	4(23.5%)	3(18.8%)	3(17.6%)	
	III	5(29.4%)	6(37.5%)	6(35.3%)	
	IV	5(29.4%)	4(25.0%)	5(29.4%)	
	V	1(5.9%)	1(6.2%)	1(5.9%)	

X2: Chi square test

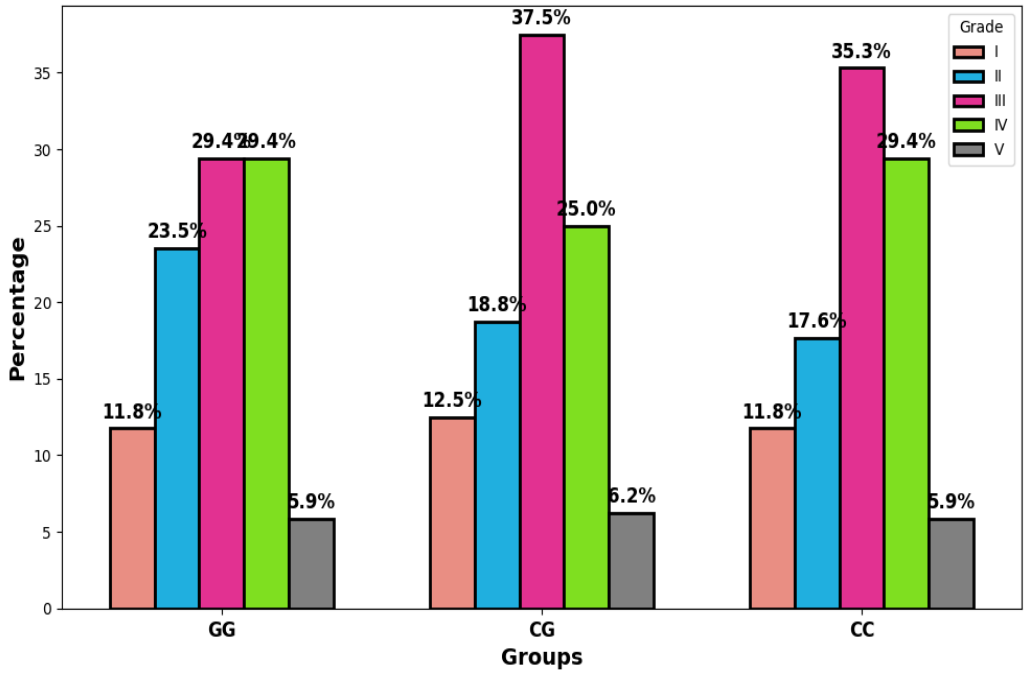

Figure 11. Comparison of genotype frequencies in the NAFLD group.

Table 6. Shows us a Comparison of genotype frequencies in NASH group. Differentiating between each grade, with grade I being GG (n=8) 1(12.5%)

CG (n=6) 1(16.7%) and CC (n=6) 1(16.7%). While grade II is GG 2(25.0%), CG 1(16.7%) and CC 1(16.7%). Grade III is GG 3(37.5%), CG 2(33.3%) and CC 2(33.3%). Grade IV GG 1(12.5%), CG 2(33.3%) and CC 1(16.7%). And finally grade V GG 1(12.5%), CG 0(0.0%) and CC 1(16.7%). The test result was X2: 1.954, p=0.982 which was non-significant.

Table 5. Comparison of genotype frequencies in NASH group.

		GG	CG	CC	Test Result
		n=8	n=6	n=6	
Grade	I	1(12.5%)	1(16.7%)	1(16.7%)	X2: 1.954, p=0.982
	II	2(25.0%)	1(16.7%)	1(16.7%)	
	III	3(37.5%)	2(33.3%)	2(33.3%)	
	IV	1(12.5%)	2(33.3%)	1(16.7%)	
	V	1(12.5%)	0(0.0%)	1(16.7%)	

X2: Chi square test

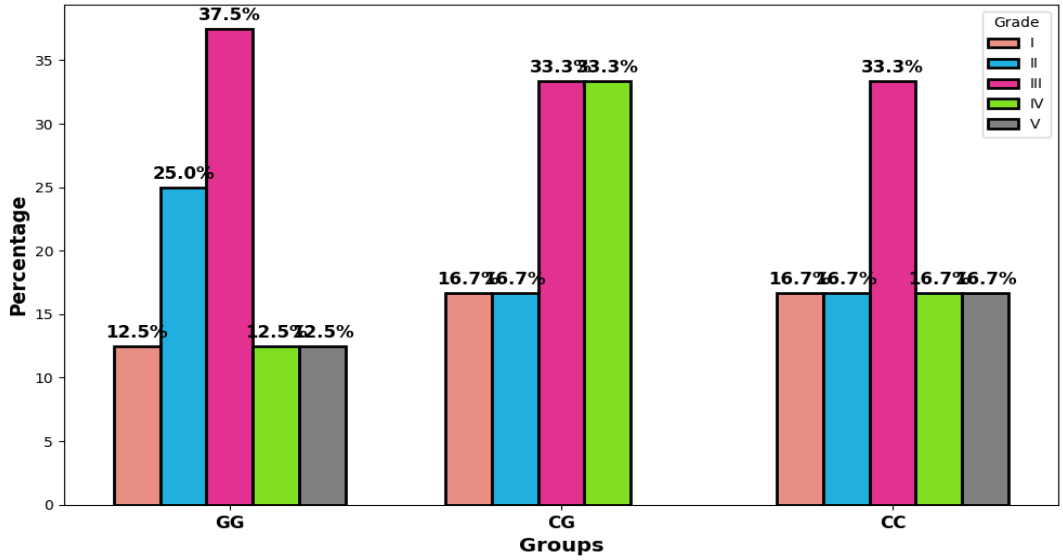

Figure 12. Comparison of genotype frequencies in NASH group

We performed a Comparison of clinical characteristics, dominant model in control group in Table 7. The Mean ± SD measure with n=19 for CC and n=11 for CG+GG. The BMI was CC 22.61 ± 2.03, CG+GG 21.60 ± 1.66 giving us a test result of t: 1.401, p=0.172. The TLC for CC 7.26 ± 1.96, CG+GG 7.24 ± 2.08. the test result was t: 0.028, p=0.978. PLT showed a CC 285.21 ± 88.17, CG+GG 327.91 ± 90.67. test result showed t: 1.265, p=0.216. The AST measure showed CC 22.16 ± 7.03, CG+GG 21.27 ± 6.74 the test showing a result of t: 0.337, p=0.739. Also, ALT CC 20.05 ± 6.60, CG+GG 18.27 ± 5.08, with a t: 0.770, p=0.447. Additionally, Albumin measure was 4.15 ± 0.40 for CC, 4.35 ± 0.39 for CG+GG, the result showed t: 1.329, p=0.195. The INR measure was CC 0.99 ± 0.15, CG+GG 0.97 ± 0.16 results showed Z: 0.430, p=0.673. Cholesterol CC 122.79 ± 10.09, CG+GG 123.00 ± 9.92 resulting in t: 0.055, p=0.956. Triglycerides CC 158.74 ± 12.22 CG+GG 164.00 ± 10.90 test results were t: 1.180, p=0.248. IR(HOMA) CC 158.74 ± 12.22 CG+GG 164.00 ± 10.90 resulting in t: 1.180, p=0.248. All results were insignificant.

Table 6. Comparison of clinical characteristics, dominant model in control group.

		CC	CG+GG	Test Result
		n=19	n=11	
BMI (kg/m2)	Mean ± SD	22.61 ± 2.03	21.60 ± 1.66	t: 1.401, p=0.172
TLC (cells/µL)	Mean ± SD	7.26 ± 1.96	7.24 ± 2.08	t: 0.028, p=0.978
PLT (platelets/µL)	Mean ± SD	285.21 ± 88.17	327.91 ± 90.67	t: 1.265, p=0.216
AST (U/L)	Mean ± SD	22.16 ± 7.03	21.27 ± 6.74	t: 0.337, p=0.739
ALT (U/L)	Mean ± SD	20.05 ± 6.60	18.27 ± 5.08	t: 0.770, p=0.447
Albumin (g/dL)	Mean ± SD	4.15 ± 0.40	4.35 ± 0.39	t: 1.329, p=0.195
INR	Mean ± SD	0.99 ± 0.15	0.97 ± 0.16	Z: 0.430, p=0.673
Chole (mg/dL)	Mean ± SD	122.79 ± 10.09	123.00 ± 9.92	t: 0.055, p=0.956
Trigly (mg/dL)	Mean ± SD	158.74 ± 12.22	164.00 ± 10.90	t: 1.180, p=0.248
IR(HOMA)	Mean ± SD	1.24 ± 0.22	1.27 ± 0.19	t: 0.428, p=0.672

t: t student test, Z: Mann Whitney test

We performed a Comparison of clinical characteristics, dominant model in NAFLD group in Table 8. The Mean ± SD measured n=17 for CC and n=33 for CG+GG. The BMI was CC 32.04 ± 4.31, CG+GG

32.10 ± 4.96 giving us a test result of t: 0.039, p=0.969. The TLC for CC 7.04 ± 2.55, CG+GG 7.01 ± 2.40. the test result was Z: 0.123, p=0.910. PLT showed a CC 218.71 ± 75.61, CG+GG 243.85 ± 77.27. test result showed t: 1.098, p=0.278. AST measure showed CC 53.29 ± 24.28, CG+GG 58.12 ± 36.88 the test showing a result of Z: 0.113, p=0.918. also, ALT CC 59.59 ± 32.92, CG+GG 62.97 ± 38.80, with a t: Z: 0.154, p=0.886. Additionally, Albumin measure was 4.32 ± 0.63 for CC, 3.96 ± 0.74 for CG+GG, the result showed t: 1.731, p=0.090. INR measure was CC 1.09 ± 0.16, CG+GG 1.13 ± 0.12 results showed Z: 1.167, p=0.242. Cholesterol CC 182.12 ± 20.91, CG+GG 180.45 ± 27.38 resulting in t: 0.219, p=0.827. Triglycerides CC 114.53 ± 25.15 CG+GG 115.18 ± 28.28 test results was Z: 0.010, p=1.000 IR(HOMA) CC 4.67 ± 0.67 CG+GG 4.73 ± 0.98 resulting in t: 0.224, p=0.823. Additional characteristics was Hemoglobin CC 12.01 ± 1.44, CG+GG 12.40 ± 1.23.test results t: 0.997, p=0.324. T.Bil CC 0.53 ± 0.19 CG+GG 0.60 ± 0.21 test results was Z: 1.034, p=0.300. D.Bil CC 0.20 ± 0.10 CG+GG 0.23 ± 0.10 test results was Z: 1.352, p=0.152. All results were insignificant.

Table 7. Comparison of clinical characteristics, dominant Model in NAFLD group.

		CG+GG	CC	Test Result
		n=33	n=17	
BMI (kg/m2)	Mean ± SD	32.10 ± 4.96	32.04 ± 4.31	t: 0.039, p=0.969
Hb (g/dL)	Mean ± SD	12.40 ± 1.23	12.01 ± 1.44	t: 0.997, p=0.324
TLC (cells/µL)	Mean ± SD	7.01 ± 2.40	7.04 ± 2.55	Z: 0.123, p=0.910
PLT (platelets/µL)	Mean ± SD	243.85 ± 77.27	218.71 ± 75.61	t: 1.098, p=0.278
AST (U/L)	Mean ± SD	58.12 ± 36.88	53.29 ± 24.28	Z: 0.113, p=0.918
ALT (U/L)	Mean ± SD	62.97 ± 38.80	59.59 ± 32.92	Z: 0.154, p=0.886
Albumin (g/dL)	Mean ± SD	3.96 ± 0.74	4.32 ± 0.63	t: 1.731, p=0.090
INR	Mean ± SD	1.13 ± 0.12	1.09 ± 0.16	Z: 1.167, p=0.242
T.Bil (mg/dL)	Mean ± SD	0.60 ± 0.21	0.53 ± 0.19	Z: 1.034, p=0.300
D.Bil (mg/dL)	Mean ± SD	0.23 ± 0.10	0.20 ± 0.10	Z: 1.352, p=0.152
Chole (mg/dL)	Mean ± SD	180.45 ± 27.38	182.12 ± 20.91	t: 0.219, p=0.827
Trigly (mg/dL)	Mean ± SD	115.18 ± 28.28	114.53 ± 25.15	Z: 0.010, p=1.000
IR(HOMA)	Mean ± SD	4.73 ± 0.98	4.67 ± 0.67	t: 0.224, p=0.823

t: t student test, Z: Mann Whitney test

We performed a Comparison of clinical characteristics, dominant Model in NASH group. in Table 9 The Mean ± SD measure with n=17 for CC and n=33 for CG+GG. The BMI was CC 32.04 ± 4.31, CG+GG 32.10 ± 4.96 giving us a test result of t: 0.039, p=0.969. The TLC for CC 7.04 ± 2.55, CG+GG 7.01 ± 2.40. the test result was Z: 0.123, p=0.910. PLT showed a CC 218.71 ± 75.61, CG+GG 243.85 ± 77.27. test result showed t: 1.098, p=0.278. AST measure showed CC 53.29 ± 24.28, CG+GG 58.12 ± 36.88 the test showing a result of Z: 0.113, p=0.918. also, ALT CC 59.59 ± 32.92, CG+GG 62.97 ± 38.80, with a t: Z: 0.154, p=0.886. Additionally, Albumin measure was 4.32 ± 0.63 for CC, 3.96 ± 0.74

for CG+GG, the result showed t: 1.731, p=0.090. The INR measure was CC 1.09 ± 0.16, CG+GG 1.13 ± 0.12 results showed Z: 1.167, p=0.242. Cholesterol CC 182.12 ± 20.91, CG+GG 180.45 ± 27.38 resulting in t: 0.219, p=0.827. Triglycerides CC 114.53 ± 25.15 CG+GG 115.18 ± 28.28 test results was Z: 0.010, p=1.000 IR(HOMA) CC 4.67 ± 0.67 CG+GG 4.73 ± 0.98 resulting in t: 0.224, p=0.823. Additional characteristics was Hemoglobin CC 12.01 ± 1.44, CG+GG 12.40 ± 1.23.test results t: 0.997, p=0.324. T.Bil CC 0.53 ± 0.19 CG+GG 0.60 ± 0.21 test results was Z: 1.034, p=0.300. D.Bil CC 0.20 ± 0.10 CG+GG 0.23 ± 0.10 test results was Z: 1.352, p=0.152. All results were insignificant.

Table 8. Comparison of clinical characteristics, dominant Model in NASH group.

		CG+GG	**CC**	**Test Result**
		n=14	n=6	
Age (years)	Mean ± SD	35.21 ± 8.68	42.50 ± 9.01	t: 1.702, p=0.106
BMI (kg/m2)	Mean ± SD	36.79 ± 2.43	36.63 ± 1.70	Z: 0.495, p=0.650
Hb (g/dL)	Mean ± SD	12.32 ± 0.94	12.68 ± 0.85	t: 0.810, p=0.429
TLC (cells/µL)	Mean ± SD	6.58 ± 2.45	6.32 ± 0.93	Z: 0.577, p=0.592
PLT (platelets/µL)	Mean ± SD	226.43 ± 60.89	202.50 ± 88.11	Z: 0.577, p=0.602
AST (U/L)	Mean ± SD	89.64 ± 35.58	79.33 ± 17.39	Z: 0.082, p=0.967
ALT (U/L)	Mean ± SD	99.50 ± 26.73	95.17 ± 18.03	Z: 0.165, p=0.901
Albumin (g/dL)	Mean ± SD	4.14 ± 0.60	4.32 ± 0.68	t: 0.598, p=0.557
INR	Mean ± SD	1.08 ± 0.09	1.07 ± 0.17	t: 0.122, p=0.904
T.Bil (mg/dL)	Mean ± SD	0.58 ± 0.20	0.53 ± 0.20	t: 0.465, p=0.648
D.Bil (mg/dL)	Mean ± SD	0.24 ± 0.08	0.25 ± 0.14	t: 0.287, p=0.777
Chole (mg/dL)	Mean ± SD	204.21 ± 12.72	200.17 ± 7.78	t: 0.718, p=0.482
Trigly (mg/dL)	Mean ± SD	142.71 ± 13.94	141.67 ± 10.09	Z: 0.495, p=0.650
IR(HOMA)	Mean ± SD	5.62 ± 0.78	5.37 ± 0.40	t: 0.724, p=0.478

t: t student test, Z: Mann Whitney test

We performed a Comparison of clinical characteristics, recessive Model in control group, in Table 10 The Mean ± SD measure with n=3 for GG and n=27 for CC+CG. The BMI was GG 21.57 ± 2.57, CC+CG 22.31 ± 1.90 giving us a test result of t: 0.629, p=0.535. The TLC for GG 6.90 ± 2.36, CC+CG 7.29 ± 1.97, the test result was t: 0.320, p=0.752. PLT showed a GG 304.67 ± 118.15, CC+CG 300.44 ± 89.20. The test result showed t: 0.076, p=0.940. The AST measure showed GG 27.33 ± 4.04, CC+CG 21.22 ± 6.84 the test showed a result of t: 1.503, p=0.144. Also, ALT GG 22.00 ± 4.58, CC+CG 19.11 ± 6.20, with a t: 0.779, p=0.443. Additionally, Albumin measure was 4.10 ± 0.36 for GG, 4.23 ± 0.41 for CC+CG, the result showed t: 0.543, p=0.591. The INR measure was GG 1.00 ± 0.10, CC+CG 0.99 ± 0.15 results showed Z: 0.277, p=0.803. Cholesterol GG 119.67 ± 9.71, CC+CG 123.22 ± 9.99 resulting in t: 0.586, p=0.562. Triglycerides GG 163.67 ± 14.29 CC+CG 160.33 ± 11.82 test results were Z: 0.380, p=0.729 IR(HOMA) GG 1.26 ± 0.23 CC+CG 1.25 ± 0.21 resulting in t: 0.066, p=0.948. All results were insignificant.

Table 9. Comparison of clinical characteristics, recessive Model in control group.

		CC+CG	GG	Test Result
		n=27	n=3	
BMI (kg/m2)	Mean ± SD	22.31 ± 1.90	21.57 ± 2.57	t: 0.629, p=0.535
TLC (cells/µL)	Mean ± SD	7.29 ± 1.97	6.90 ± 2.36	t: 0.320, p=0.752
PLT (platelets/µL)	Mean ± SD	300.44 ± 89.20	304.67 ± 118.15	t: 0.076, p=0.940

AST (U/L)	Mean ± SD	21.22 ± 6.84	27.33 ± 4.04	t: 1.503, p=0.144
ALT (U/L)	Mean ± SD	19.11 ± 6.20	22.00 ± 4.58	t: 0.779, p=0.443
Albumin (g/dL)	Mean ± SD	4.23 ± 0.41	4.10 ± 0.36	t: 0.543, p=0.591
INR	Mean ± SD	0.99 ± 0.15	1.00 ± 0.10	Z: 0.277, p=0.803
Chole (mg/dL)	Mean ± SD	123.22 ± 9.99	119.67 ± 9.71	t: 0.586, p=0.562
Trigly (mg/dL)	Mean ± SD	160.33 ± 11.82	163.67 ± 14.29	Z: 0.380, p=0.729
IR(HOMA)	Mean ± SD	1.25 ± 0.21	1.26 ± 0.23	t: 0.066, p=0.948

t: t student test, Z: Mann Whitney test

We performed a Comparison of clinical characteristics, recessive Model in NAFLD group, in Table 11. The Mean ± SD measure with n=17 for GG and n=33 for CC+CG. The BMI was GG 33.61 ± 4.22, CC+CG 31.29 ± 4.80 giving us a test result of Z: 1.608, p=0.110. The TLC for GG 6.91 ± 2.48, CC+CG 7.08 ± 2.44. the test result was Z: 0.266, p=0.798. PLT showed a GG 237.53 ± 62.52, CC+CG 234.15 ± 84.21. Test result showed t: 0.146, p=0.885. AST measure showed GG 64.65 ± 36.05, CC+CG 52.27 ± 30.98 the test showing a result of Z: 1.413, p=0.160. also ALT GG 71.59 ± 37.09, CC+CG 56.79 ± 35.87, with a Z: 1.382, p=0.170. Additionally, Albumin measure was 4.06 ± 0.68 for GG, 4.09 ± 0.75 for CC+CG, the result showed t: 0.135, p=0.893. INR measure was GG 1.10 ± 0.11, CC+CG 1.13 ± 0.14 results showed t: 0.670, p=0.506. Cholesterol GG 189.00 ± 21.17, CC+CG 176.91 ± 26.34 resulting in Z: 1.526, p=0.130. Triglycerides GG 123.41 ± 26.25 CC+CG 110.61 ± 26.73 test results was Z: 1.567, p=0.119 IR(HOMA) GG 5.01 ± 0.92 CC+CG 4.56 ± 0.84 resulting in t: 1.716, p=0.093. Additional characteristics was Hemoglobin GG 12.18 ± 1.10, CC+CG 12.32 ± 1.41.test results t: 0.353, p=0.726. T.Bil GG 0.59 ± 0.20 CC+CG 0.57 ± 0.21 test results was Z: 0.389, p=0.701. D.Bil GG 0.25 ± 0.10 CC+CG 0.21 ± 0.09 test results was Z: 1.628, p=0.084. All results were insignificant.

Table 10. Comparison of clinical characteristics, recessive Model in NAFLD group.

		GG	CC+CG	Test Result
		n=17	n=33	
BMI (kg/m2)	Mean ± SD	33.61 ± 4.22	31.29 ± 4.80	Z: 1.608, p=0.110
Hb (g/dL)	Mean ± SD	12.18 ± 1.10	12.32 ± 1.41	t: 0.353, p=0.726
TLC (cells/µL)	Mean ± SD	6.91 ± 2.48	7.08 ± 2.44	Z: 0.266, p=0.798
PLT (platelets/µL)	Mean ± SD	237.53 ± 62.52	234.15 ± 84.21	t: 0.146, p=0.885
AST (U/L)	Mean ± SD	64.65 ± 36.05	52.27 ± 30.98	Z: 1.413, p=0.160
ALT (U/L)	Mean ± SD	71.59 ± 37.09	56.79 ± 35.87	Z: 1.382, p=0.170
Albumin (g/dL)	Mean ± SD	4.06 ± 0.68	4.09 ± 0.75	t: 0.135, p=0.893
INR	Mean ± SD	1.10 ± 0.11	1.13 ± 0.14	t: 0.670, p=0.506
T.Bil (mg/dL)	Mean ± SD	0.59 ± 0.20	0.57 ± 0.21	Z: 0.389,

				p=0.701
D.Bil (mg/dL)	Mean ± SD	0.25 ± 0.10	0.21 ± 0.09	Z: 1.628, p=0.084
Chole (mg/dL)	Mean ± SD	189.00 ± 21.17	176.91 ± 26.34	Z: 1.526, p=0.130
Trigly (mg/dL)	Mean ± SD	123.41 ± 26.25	110.61 ± 26.73	Z: 1.567, p=0.119
IR(HOMA)	Mean ± SD	5.01 ± 0.92	4.56 ± 0.84	t: 1.716, p=0.093

t: t student test, Z: Mann Whitney test

We performed a Comparison of clinical characteristics, recessive Model in NASH group. in Table 12. The Mean ± SD measure with n=8 for GG and n=18 for CC+CG. The BMI was GG 37.24 ± 2.23, CC+CG 36.41 ± 2.19 giving us a test result of Z: 0.964, p=0.354. The TLC for GG 7.04 ± 2.44, CC+CG 6.14 ± 1.83. The test result was Z: 0.964, p=0.354. PLT showed a GG 218.12 ± 38.19, CC+CG 220.00 ± 84.85. The test result showed t: 0.058, p=0.954. AST measure showed GG 91.00 ± 35.86, CC+CG 83.58 ± 28.84 the test showing a result of t: 0.512, p=0.615. also, ALT GG 101.50 ± 27.17, CC+CG 96.00 ± 22.73, with a t: 0.491, p=0.629. Additionally, the Albumin measure was 4.12 ± 0.58 for GG, 4.23 ± 0.65 for CC+CG, the result showed t: 0.381, p=0.708. The INR measure was GG 1.10 ± 0.08, CC+CG 1.07 ± 0.14 results showed t:0.577, p=0.571. Cholesterol GG 205.62 ± 11.84, CC+CG 201.25 ± 11.28 resulting in t: 0.833, p=0.416. Triglycerides GG 146.38 ± 10.99 CC+CG 139.75 ± 13.42 test results were Z: 1.003, p=0.334 IR(HOMA) GG 5.75 ± 0.80 CC+CG 5.41 ± 0.59 resulting in t: 1.084, p=0.293. Additional characteristics were Hemoglobin GG 12.30 ± 0.83, CC+CG 12.52 ± 0.98.test results t: 0.513, p=0.614. T.Bil GG 0.57 ± 0.21 CC+CG 0.56 ± 0.20 test results were t: 0.182, p=0.858. D.Bil GG 0.25 ± 0.09 CC+CG 0.23 ± 0.11 test results were Z: 0.579, p=0.565. All results were insignificant.

Table 11. Comparison of clinical characteristics, recessive Model in NASH group.

		GG	CC+CG	Test Result
		n=8	n=12	
BMI (kg/m2)	Mean ± SD	37.24 ± 2.23	36.41 ± 2.19	Z: 0.964, p=0.354
Hb (g/dL)	Mean ± SD	12.30 ± 0.83	12.52 ± 0.98	t: 0.513, p=0.614
TLC (cells/µL)	Mean ± SD	7.04 ± 2.44	6.14 ± 1.83	Z: 0.964, p=0.354
PLT (platelets/µL)	Mean ± SD	218.12 ± 38.19	220.00 ± 84.85	t: 0.058, p=0.954
AST (U/L)	Mean ± SD	91.00 ± 35.86	83.58 ± 28.84	t: 0.512, p=0.615
ALT (U/L)	Mean ± SD	101.50 ± 27.17	96.00 ± 22.73	t: 0.491, p=0.629
Albumin (g/dL)	Mean ± SD	4.12 ± 0.58	4.23 ± 0.65	t: 0.381, p=0.708
INR	Mean ± SD	1.10 ± 0.08	1.07 ± 0.14	t: 0.577, p=0.571
T.Bil (mg/dL)	Mean ± SD	0.57 ± 0.21	0.56 ± 0.20	t: 0.182, p=0.858
D.Bil (mg/dL)	Mean ± SD	0.25 ± 0.09	0.23 ± 0.11	Z: 0.579, p=0.565
Chole (mg/dL)	Mean ± SD	205.62 ± 11.84	201.25 ± 11.28	t: 0.833, p=0.416

Trigly (mg/dL)	Mean ± SD	146.38 ± 10.99	139.75 ± 13.42	Z: 1.003, p=0.334
IR(HOMA)	Mean ± SD	5.75 ± 0.80	5.41 ± 0.59	t: 1.084, p=0.293

t: t student test, Z: Mann Whitney test

DISCUSSION

As the most prevalent chronic liver disease, non-alcoholic fatty liver disease (NAFLD) affects an estimated 25% of adults worldwide. 1. Concerning the relationship between the PNPLA3 rs738409 gene polymorphism and NAFLD, to further investigate the association between gene polymorphism and disease pathogenesis, and its effects on the genetic susceptibility of NAFLD, investigating the function of the PNPLA3 gene polymorphism may greatly help in the development of a non-invasive biomarker.

In the current study, we examined the relationship between non-alcoholic fatty liver disease and various genotypes of the PNPLA3 rs738409 polymorphism. We found that there is a strong association between this polymorphism and metabolic syndrome with NASH. The current study's findings showed a high correlation between the G-allele of rs738709 and NAFLD, particularly NASH; elevated ALT and AST plasma levels; reduced triglyceride levels in plasma. we highlighted that people with CC genotype, have 63.3% lower risk of developing non-alcoholic fatty liver, while the probability in CG and GG for developing the disease in those with these genotypes were 32% (CG) and 43% (GG) higher. On the other hand, considering the CG + GG groups as a dominant model is 66%, and following a statistical analysis, it was concluded that this group were more likely to develop the disorder than others. Also, the development of NASH had nearly similar percentage being CC at 63.3% at lower risk for developing the disease, CG and GG 30%, 40% respectively, at a higher risk for NASH. While the dominant model being at 70% at higher risk. The effect of the G allele on non-alcoholic fatty liver disease can also be emphasized. A study in India in 2020 also found that the G allele plays a key role in the development of NAFLD 11.

A 2019 meta-analysis indicated that this polymorphism significantly influences the progression of hepatic tissue damage, identifying the G allele as a risk factor for NAFLD. The incidence ratio of the disease in individuals possessing one G allele compared to those lacking it was 1.88, and 4.01 for individuals with homozygous G alleles. This gene is also proposed to elevate serum alanine aminotransferase levels. SNP rs738409 is situated within the PNPLA3 gene, which encodes the protein adiponutrin. Adiponutrin is a protein composed of 481 amino acids, predominantly produced in the internal membrane fraction of human hepatocytes. The common allelic form of rs738409 (148I) is associated with adiponutrin's lipolytic action towards triglycerides, according to one popular explanation of the functional mechanism of SNP rs738409. The minor allelic variant (148M) causes a significant amino acid change adjacent to the catalytic domain, possibly impeding substrate accessibility and diminishing PNPLA3 enzymatic activity towards glycerolipids, thereby contributing to the onset of macrovascular steatosis.

However, some findings indicated a gain of function associated with the SNP rs738409 variant, whereas various murine model studies yielded conflicting results. Consequently, the precise mechanism underlying the correlation between SNP rs738409 and NAFLD remains ambiguous 12. Other meta-analyses on the Asian population indicated that, under the dominant model, carriers of the risk allele "G" exhibited an odds ratio of 2.1 for NAFLD. In the allele model, the odds ratio is 1.92. For both models, the P-value is below 0.001. A meta-analysis by Jiaying et al. (2020) indicates that this gene is implicated in the pathogenesis of non-alcoholic steatohepatitis (NASH) in pediatrics populations and is correlated with biomarkers such as serum alanine transaminase, aspartate transaminase, and gamma-glutamyl transferase, which signify hepatic injury. 13

Obesity has emerged as a significant public health challenge in industrialised nations and subsequently in developing countries. While most obese individuals develop NAFLD, not all experience a severe progression to cirrhosis and hepatocellular carcinoma. Simple steatosis and non-alcoholic

steatohepatitis are two diseases that appear to be distinct from both histological and pathophysiology perspectives. Thus, genetic predispositions, environmental influences, and/or lifestyle choices (dietary habits, physical exercise) may significantly contribute to this situation. A study by Sood et al. (2016) in Japan indicated that the odds ratio of the GG genotype was 36.5% in obese individuals and 47.8% in the non-obese group with fatty liver 14. In our study, the NAFLD population with elevated BMI is more prone to developing NAFLD in the GG population and in heterozygous groups. 15.

The G allele (148Met, rs738409) polymorphism of PNPLA3 is linked to a heightened risk of fatty liver disease. Individuals possessing the rs738409 mutant allele exhibited irregularities in intra-hepatocytic triglyceride metabolism, hence elevating the likelihood of liver steatosis, particularly in instances of overweight, which is uniquely correlated with heightened triglyceride blood levels. 12

Our clinical findings demonstrated an effective significant association between NAFLD and G alleles in individuals afflicted with metabolic syndrome. A separate Chinese study indicated that the G allele in PNPLA3 rs738409 may elevate the risk of NAFLD in the general population, particularly among individuals without MetS, irrespective of dietary patterns and metabolic variables. Our results are contradicted by other research, which indicate that, compared to overweight/obese individuals without MetS, overweight/obese people with MetS had a much higher prevalence of the G allele. Furthermore, several investigations indicate an absence of association between PNPLA3 gene polymorphisms and Metabolic Syndrome (MetS). 16

ALT serves as a marker for liver function, exhibiting excellent sensitivity and specificity; increased ALT levels in patients with fatty liver may indicate mild hepatic injury 17. Individuals who are heterozygous and homozygous for the G allele exhibited markedly elevated ALT levels compared to those who are homozygous for the C allele. Notably, individuals under 20 years of age exhibited markedly elevated ALT levels when homozygous for the G gene. This fact indicates an early development of liver pathology. A recent meta-analysis corroborates our findings, demonstrating that the G allele is a significant risk factor for NAFLD susceptibility and elevated blood ALT levels in adult Asians. 18. The PNPLA3 rs738409 GG genotype is correlated with elevated ALT levels in youth and is more prevalent among obese individuals with Metabolic Syndrome (MetS) 19. This corroborates our findings.

Another meta-analysis examined 7 publications detailing the association between increased ALT levels and the PNPLA3 rs738409 gene polymorphism. The blood ALT levels were examined and compared between GG and CC genotype carriers, as well as between GC and CC genotype carriers. The results indicated that the homozygous GG mutant genotype was linked with significantly higher serum ALT levels. For GC versus CC, the overall effect values revealed $P > .05$, and the consistent results following model modification demonstrated no difference in ALT levels between NAFLD patients with GC and CC genotypes.

These results were consistent with the previous findings that the PNPLA3 rs738409 [G] mutation is linked with the elevated serum ALT level, which might be caused by the liver inflammation or liver cell damages 17. Our analysis indicates that the presence of the G allele, whether in heterozygous or homozygous form, significantly elevates AST and ALT levels while reducing triglycerides, hence contributing to NAFLD. It may serve as a biomarker for vulnerability to both NAFLD and NASH.

The PNPLA3 rs738409 GG genotype is associated already in youths with increased ALT and is more frequent in obese subjects with MetS in all ages. Thus, determination of this polymorphism may help to identify young high-risk candidates for fatty liver disease at an early pre-clinical level. As lifestyle intervention and weight loss have been shown particularly successful in PNPLA3 rs738409 hetero- and homozygote subjects, this fact suggests a possible clinical impact of this genetic test. Most importantly, it underlines the obligatory need for consequent lifestyle intervention as a mainstay of sustained NAFLD treatment in all ages of PNPLA3 rs738409 carriers.

This study confirms a strong association between the PNPLA3 rs738409 polymorphism and NAFLD susceptibility and severity in an Egyptian population. The I148M variant is linked to impaired triglyceride metabolism, hepatic fat accumulation, and progression to NASH and fibrosis. Notably, this risk is independent of BMI and other metabolic traits, underscoring the importance of genetic screening.

Our findings align with global studies demonstrate PNPLA3 as the most significant genetic determinant of NAFLD. The utility of this marker in risk stratification and potential treatment targeting supports its incorporation into clinical algorithms.
Limitations include the relatively small sample size and absence of liver biopsy in controls. Nonetheless, this is the first study to characterize PNPLA3 in a histologically confirmed Egyptian NAFLD cohort, offering valuable insight into regional genetic epidemiology.

CONCLUSION:

This study revealed that individuals with the CC genotype exhibited a 63% reduced likelihood of developing non-alcoholic fatty liver disease, while those with the CG genotype had a 32% increased likelihood of developing non-alcoholic fatty liver and a 30% increased likelihood of developing NASH. Furthermore, individuals with the GG genotype presented a 34% higher risk of developing non-alcoholic fatty liver and a 40% higher risk of NASH. Additionally, the population with CG + GG genotypes demonstrated a 66% increased risk of developing the disease and a 70% increased risk of NASH, indicating the influence of the G allele on non-alcoholic fatty liver disease. We also established that rs738409 is correlated with plasma ALT, AST, cholesterol, BMI, D. Bil., and triglyceride levels.
The PNPLA3 rs738409 polymorphism is significantly associated with NAFLD susceptibility and histologic severity in Egyptian patients. Genotyping may enhance non-invasive risk prediction and enable earlier intervention in high-risk individuals. This variant represents a promising biomarker for precision medicine approaches in NAFLD.
Future research may investigate the influence of genetic and environmental factors on tissue damage levels, as well as the impact of this gene on fibrosis and liver cirrhosis. Future genomic analyses through fine mapping or comprehensive sequencing may uncover additional genetic determinants within the PNPLA3 locus.

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