# Association of PNPLA3 Gene Polymorphisms with the Development and Progression of Hepatocellular Carcinoma in Egyptian patients

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## ABSTRACT:

This study aimed to examine the relationship between hepatocellular carcinoma (HCC) susceptibility and two single nucleotide polymorphisms (SNPs) of PNPLA3: rs738409 C > G and rs3747207 G > A. An investigation was carried out on a case-control group of 47 patients with hepatocellular carcinoma (HCC)—20 females and 27 males—with a mean age of 60.62 ± 8.06 years. The control group was made up of 47 volunteers who were in good health and had unrelated, healthy blood donors. They do not suffer from any long-term illnesses and are often recruited. based on the Hardy-Weinberg equilibrium standards. In patients with HCC, the frequency of PNPLA3 rs738409C/G polymorphism was determined to be 23 (48.9%) CC, 22 (46.8%) CG, and 2 (4.3%) GC; in contrast, the control group had 22 (46.8%) CC, 21 (44.7%) CG, and 4 (8.5%) GG [P<0.05]. No statistically significant association was discovered between the PNPLA3 rs738409C/G allele or genotype frequency with the risk of HCC. In contrast, the genotype frequencies of PNPLA3 rs738407A/G I148M in the HCC group were 8 (17.0%) GG, 30 (63.81%) GA, and 9 (19.1%) AA, while control group consisted of 20 (42.6%) GG, 25 (53.2%) GA, and 2 (4.3%) AA. In the total samples, there was a statistically significant positive connection between the risk of HCC and the AA genotype and the A allele. In conclusion, our study suggested that the PNPLA3 rs738407 (GG) genotype and A allele were risk factors for HCC patients.

**Keywords:** *PNPLA3*, Polymorphism, HCC, Susceptibility, Egyptian population

## 1. INTRODUCTION

Primary liver cancer is the second greatest cause of cancer-related death Worldwide and the fourth most frequent cancer (Qin *et al*., 2022). It poses a severe threat to people's lives and health. The most prevalent primary liver cancer is hepatocellular carcinoma (HCC). It was now the third most common cause of cancer-related mortality and the fifth most common cancer globally. Owing to the ongoing hepatitis C virus epidemic and non-alcoholic fatty liver disease, the prevalence of HCC is rising, resulting in around 690,000 more deaths annually (Gomaa *et al*., 2013). A substantial fraction of HCC may be caused by chronic hepatitis C virus infection, according to compelling epidemiologic data (El-Serag, 2012). The search for additional biological markers or risk factors is necessary since, although standard methods like tumor markers and ultrasonography are essential for HCC diagnosis, they are insufficient for early detection of HCC. Evaluation of the

genetic risk factors for hepatocellular carcinoma (HCC) may be helpful in detecting cases of the disease and enhancing effective early intervention protocols (Vauthey, 2005).

The transmembrane protein encoded by the patatin-like phospholipase domain-containing 3 gene (PNPLA3), also referred to as adiponectin, is expressed on the hepatocyte membrane and can regulate inflammatory mediators, lipid metabolism, and other processes (Nomair *et al*., 2021, Gavril OI, *et al*., 2021; Xiang H, *et al*., 2021, Kanda *et al*., 2021). PNPLA3 gene polymorphisms and non-alcoholic fatty liver disease (NAFLD) (Mansoor *et al*., 2021), alcoholic liver disease (ALD) (Stasinou *et al*., 2021), liver fibrosis (LF) (Manchiero *et al*., 2017, Oliveira *et al*., 2021), and liver cirrhosis (LC) (Shao X, *et al*., 2021) are currently the subject of numerous studies. Conversely, there isn't much focus on the link between PNPLA3 gene polymorphisms and HCC. Thus, more investigation into the connection between PNPLA3 gene polymorphisms and HCC risk is highly valuable. When the Dallas Heart Study, a cohort study including a diverse population, was conducted in 2008, the scientific community's focus on PNPLA3 increased dramatically. This study reported a strong association between hepatic steatosis and inflammatory risk and the PNPLA3 genetic variant rs738409 C>G. Separate confirmations in additional cohorts and liver disorders were also reported (Fuchs *et al*., 2014). The study by Valenti et al. (Valenti *et al*., 2011) revealed the first documented link between the PNPLA3 genetic variant and hepatic malignancy. This association was subsequently verified by other research, supporting the close relationship between hepatic carcinogenesis and HCV-related cirrhosis. When examined in Non-alcoholic fatty liver disease NAFLD and Alcoholic liver disease (ALD) backgrounds, the association between the PNPLA3 genotype and the prevalence of HCC in the presence of cirrhosis is strong, and a recent meta-analysis supported this finding (Chen *et al*., 2015).

This work aimed to evaluate the Association of polymorphisms in the PNPLA3 genes with hepatocellular carcinoma in Egyptian population.

## 2.MATERIALS AND METHODS

### 2.1. Study subjects

Ninety-four Egyptian patients receiving treatment at Menoufia University's National Liver Institute Hospital served as the subjects of this case-control research. The Institutional Ethics Committee approved the study code of the ph.D proposal :SREC290124B10051 (29-01-2024), and all participants provided informed consent. The entire medical history, clinical examination, and several laboratory tests, such as CBC, liver enzymes, renal function markers, alpha-fetoprotein level, and viral hepatitis indicators, were performed for each enrolled individual. The serum sample's leftover aliquots were stored at -80ºC.

### 2.2. PNPLA3 genotyping

The ABIOpureTM total DNA extraction kit (Bothell, WA 98021 USA) was used to extract DNA from peripheral blood following the kit methodology. PNPLA3 rs738409C/G I148M genotyping was performed through polymerase chain reaction followed by Restriction Fragment Length Polymorphism (PCR-RFLP). The PCR was done using a 2X PCR master mix, along with the forward primer 5′-CCCTGCTCACTTGGAGAAAG-3′ and the reverse primer 5′-TGTCACCGGAATAGGGAGGA-3′ and 2 ul of the extracted DNA. The rs738409 variant's PCR cycling conditions were as follows: in short, the genomic DNA was denatured for five minutes at 95 °C. Thereafter, it underwent

thirty-five cycles of 95 °C for one minute, 58 °C for thirty seconds, 72 °C for one minute, and a final extension step at 72 °C for ten minutes. The 139 bp amplified fragment was digested using *NlaIII* restriction enzyme from Thermo Fisher Scientific Inc. (USA). The GG genotype displayed two fragments of 112 and 27 bp, while the CC genotype produced a 139 bp output using the PCR technique. Samples with heterozygous genotype (G/C) revealed three bp fragments: 139, 112, and 27. PNPLA3 rs738407's forward primer pair was 5-TCCTTCAACACTTGGCTCAT-3, while the reverse primer was 5-AAGTATTCGCGAGGAACCTC-3. After PCR product *MluC1* digestion, the digested product was electrophored in a 3% agarose gel with ethidium bromide stain (Sigma-Aldrich, Germany), and the digested fragments were visualized under a UV transilluminator**.**

## 3. RESULTS

Table (1) represents a summary of the demographic data-based comparison between the two groups under study. A total of 47 participants, ranging in age from 43.0 to 80.0 years, with a mean age of 59.43 ± 9.99 years for the control group, and 47 patients, ranging in age from 44.0 to 77.0 years, with a mean age of 60.62 ± 8.06 years, made up the patient group. Regarding the age mean, there was a statistically insignificant difference between the two groups. matching sex when the p-value is greater than 0.05 and there is no discernible difference between the study groups**.**

In patients and controls, PNPLA3 rs738409C/G genotyping was found to be independent according to the application of Hardy Weinberg Equilibrium (p>0.05). The frequency of PNPLA3 rs738409C/G genotypes in the HCC group was 48.9%, 46.8%, and 4.3% for CC, CG, and GG genotypes respectively, while the frequency in the control group was 46.8%, 44.7%, 8.5% for CC, CG, and GG genotypes respectively (P=0.808). No statistically significant association was discovered between the PNPLA3 rs738409C/G allele or genotype with the risk of HCC (Table 2, figure 1). Conversely, the genotype frequencies of PNPLA3 rs738407A/G I148M in the HCC group met the equilibrium criterion, with 8 (17.0%) representing GG, 30 (63.81%) representing GA, and 9. In contrast to 31.2%, 49.8%, and 21.8%, the control group consisted of 20 (42.6%) GG, 25 (53.2%) GA, and 2 (4.3%) AA (observed). In the total samples, there was a statistically significant positive connection between the risk of HCC and the AA genotype and the A allele (Table 2, figure 2)

It was thought that HCC had a very diverse genetic signature. had a comparatively greater risk of HCC (p=0.006) in comparison to AA genotypes. Patients carrying the A allele with the PNPLA3 rs738407 AA genotype may have been at increased risk of HCC than control subjects (p=0.005). PNPLA3 rs738407 genotype and allele distributions are displayed in (Table 2). Therefore, we looked at how the PNPLA3 rs738407 affected the various clinicopathological risk factors that HCC patients had. The distributions of PNPLA3 rs738407 genotypes in HCC patients were categorized based on factors such as liver enzyme, AFP, lesion size and number in instances of HCC, and HCV infection (Table 3& 4). There was no statistically significant difference in the association between the PNPLA3 rs738407 various genotypes and risk variables (liver enzyme, AFP, size and number of lesions in HCC cases, and other clinicopathological risk factors among the HCC patients). All of these findings point to the A allele and PNPLA3 rs738407 (GG) genotype as risk factors for HCC patients.

## 4. DISCUSSION

The annual incidence of HCC is 6.5% in women and 7.9% in men, ranking fifth and seventh in the globe, respectively (Jemal *et al*., 2017). The incidence and prevalence of HCC have steadily increased in areas with high rates of hepatitis B and hepatitis C virus infections (El-Serag, 2012). Because HBV and HCV infections are so common in Egypt, the incidence rate of HCC in the last ten years has doubled (El-Garem, *et al*., 2013). It suggested combining serum alpha-fetoprotein (AFP) with hepatic ultrasonography for the diagnosis of HCC. According to Singal *et al*. (2009), liver ultrasonography is advised as the main surveillance method for HCC; it has a moderate sensitivity of roughly 60% and a higher specificity of roughly 85–90%. Although they have not been well evaluated for HCC surveillance, magnetic resonance imaging (MRI) and computed tomography (CT) scans are nevertheless employed in clinical practice. Compared to ultrasound, CT and MRI are linked to a higher detection rate of HCC (Kobayashi *et al*., 1985). Because AFP measurement is widely accessible, easy to use, and reasonably priced, it is also frequently utilized for HCC surveillance. Because of its poor sensitivity and specificity in identifying HCC, AFP by alone is not advised as an HCC surveillance test (Paul *et al*., 2007). In order to determine if polymorphisms in the PNPLA3 genes were linked to the risk of HCC in the Egyptian population, we chose two SNPs for this investigation. 47 HCC patients and 47 healthy controls participated in our study to examine the relationship between PNPLA3 SNPs and HCC susceptibility. The findings indicated that rs738407 was strongly linked to higher HCC susceptibility. Our study adds to our knowledge of the pathophysiology of HCC and offers a fresh approach to its management. Two groups were used in the current study: With a mean age of 59.43 years, Group A consisted of 47 healthy control subjects, divided into 21 (44.7%) males and 26 (55.3%) females. Group B consisted of twenty female and twenty male HCC patients that were taken from the National Liver Institute Hospital in Menoufia, Egypt. They are 60.62 years old on average. PCR-RFLP test was used to genotype PNPLA3 rs738409C/G I148M, and PNPLA3 rs738407. Regarding the biochemical parameters, we discovered that, in comparison to the healthy group, there was a substantial decrease in albumin levels with HCC and a highly statistically significant increase in AST, ALT, and direct bilirubin levels. According to the findings of Carr *et al*. (2014), patients with HCC who had aberrant bilirubin levels were not as likely to recover as those who had normal bilirubin levels. Additionally, in individuals with both small and large tumors, their AFP levels were higher. Bilirubin levels and measures of the aggressiveness of HCC were related. Up till now, the majority of studies on PNPLA3 gene polymorphisms have focused on the relationship between rs738409 and the risk of NAFLD. The study by Hikmet Akkiz et al. demonstrated that rs738409 significantly increases the risk of NAFLD in the Turkish population in an unadjusted regression model (Akkiz and Taskin, 2021). Furthermore, Brazilian persons with the rs738409-GG genotype have a 3.29-fold greater risk of non-alcoholic fatty liver disease (NAFLD), according to a study by Daniel F. Mazo *et al*, (2019). Additionally, a study (Falleti *et al*., 2011) found a correlation between PNPLA3 rs738409 and the risk of HCC and showed that rs738409 is a reliable indicator of the development of HCC. But since the aforementioned study involved white Italian patients, racial disparities might be the cause of the discrepancy between our findings and the study's. Notably, our investigation showed that the rs28

rs738409 genotype was not clearly connected with HCC susceptibility, despite the fact that no association was found between the alleles and genotypes of rs738409 and HCC susceptibility.

Our analysis between PNPLA3 SNPs and HCC susceptibility in 47 HCC patients and 47 healthy controls revealed a strong correlation between rs2896019 and enhanced HCC susceptibility. Our study adds to our knowledge of the pathophysiology of HCC and offers a fresh approach to its management. Our work investigated the link between PNPLA3 SNPs and HCC susceptibility in terms of age, gender, and smoking status given the various factors that can lead to HCC occurrence. First, individuals over 55 years of age had an elevated risk of HCC; the mean age of HCC cases in our study was 55 years. This could be the result of the body's immune deteriorating with age, which raises the risk of HCC. Regarding gender, research from all across the world has demonstrated that HCC is a cancer that primarily affects men. The most effective multilocus prediction model was rs738409, rs3747207, rs4823173, and rs2896019 combined. The notion that SNP-SNP interactions affect HCC risk may be strengthened by this combination. To completely comprehend the complex interactions between PNPLA3 SNPs in the development of HCC, more study is still necessary, according to Gong *et al*. (2022). The goal of the current study was to examine the SNPs of a single gene, PNPLA3. We will choose several genes that are on the same molecular pathway as PNPLA3 and the accompanying SNPs for the follow-up research in or\der to better understand the connection between genetic polymorphisms and HCC.

## 5. CONCLUSION

Our study suggested that the PNPLA3 rs738407 (GG) genotype and A allele were risk factors for HCC patients, while no correlation was found between the PNPLA3_rs738409 genotype and HCC risk. To completely comprehend the complex interactions between PNPLA3 SNPs in the development of HCC, more study is still necessary as selecting multiple genes that share the same biochemical pathway as PNPLA3 and the associated SNPs to gain a deeper understanding of the relationship between genetic variants and HCC.

## 6. AUTHOR CONTRIBUTIONS:

The conception and design of this study were collaboratively developed by all authors. H.A. was responsible for the collection of samples, while material preparation and data analysis were conducted by H.A., and M.S. The manuscript drafting and revisions were equally shared among F.A., M.E., M.S., and G.B. All authors provided valuable input and feedback during the manuscript's evolution, and they collectively approved the final version of the manuscript.

## 7. COMPLIANCE WITH ETHICAL STANDARDS

**Funding:** Not applicable

**Conflict of interest:** The authors declare that there are no conflicts of interest**.**

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**Table 1:** The demographic data between the studied groups

| | Patients (n = 47) | | Control (n = 47) | | Test of sig. | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **Gender** | | | | | | |
| Male | 27 | 78.7 | 21 | 44.7 | $\chi^2$= 11.525* | 0.345 |
| Female | 20 | 21-3 | 26 | 55.3 | | |
| **Age (years)** | | | | | | |
| Min. – Max. | 44.0 – 77.0 | | 43.0 – 80.0 | | t= 0.637 | 0.526 |
| Mean ± SD. | 60.62 ± 8.06 | | 59.43 ± 9.99 | | | |

$\chi^2$: Chi square test t: Student t-test p: p value for comparing between the studied groups
*: Statistically significant at p ≤ 0.05

**Table 2:** Comparison between the two studied groups according to PNPLA3 gene

| PNPLA3 gene | HCC (n = 47) | | Control (n = 47) | | $\chi^2$ | p |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **rs738409** | | | | | | |
| CC | 23 | 48.9 | 22 | 46.8 | 0.720 | $^{MC}p$= 0.808 |
| CG | 22 | 46.8 | 21 | 44.7 | | |
| GG | 2 | 4.3 | 4 | 8.5 | | |
| $^{HW}p_1$ | **0.245** | | **0.746** | | | |
| **Allele** | | | | | | |
| C | 68 | 72.3 | 65 | 69.1 | 0.231 | 0.631 |
| G | 26 | 27.7 | 29 | 30.9 | | |
| **rs738407** | | | | | | |
| GG | 8 | 17.0 | 20 | 42.6 | 10.052* | 0.007* |
| GA | 30 | 63.8 | 25 | 53.2 | | |
| AA | 9 | 19.1 | 2 | 4.3 | | |
| $^{HW}p_1$ | 0.057 | | 0.091 | | | |
| **Allele** | | | | | | |
| G | 46 | 48.9 | 65 | 69.1 | 7.941* | 0.005* |
| A | 48 | 51.1 | 29 | 30.9 | | |

**Table (3):** Relation between rs738407 and different parameters in HCC group (n = 47)

| | rs738407 | | | | | | Test of sig. | p |
|---|---|---|---|---|---|---|---|---|
| | GG (n= 8) | | GA (n= 30) | | AA (n= 9) | | | |
| | No. | % | No. | % | No. | % | | |
| **Age (years)** | | | | | | | | |
| <60 | 4 | 50.0 | 12 | 40.0 | 3 | 33.3 | $\chi^2$= 0.580 | $^{MC}$p= 0.836 |
| ≥60 | 4 | 500 | 18 | 60.0 | 6 | 66.7 | | |
| Min. – Max. | 51.0 – 73.0 | | 43.0 – 71.0 | | 41.0 – 77.0 | | F= 0.049 | 0.952 |
| Mean ± SD. | 60.75 ± 8.89 | | 60.37 ± 6.96 | | 61.33 ± 11.30 | | | |
| Median | 60.50 | | 61.0 | | 64.0 | | | |
| **Gender** | | | | | | | | |
| Male | 7 | 87.5 | 22 | 73.3 | 8 | 88.9 | $\chi^2$= 1.087 | $^{MC}$p= 0.675 |
| Female | 1 | 12.5 | 8 | 26.7 | 1 | 11.1 | | |
| **HCV** | | | | | | | | |
| Negative | 4 | 50.0 | 12 | 40.0 | 3 | 33.3 | $\chi^2$= 0.580 | $^{MC}$p= 0.836 |
| Positive | 4 | 50.0 | 18 | 60.0 | 6 | 66.7 | | |
| **Child Pugh Score** | | | | | | | | |
| A | 4 | 50.0 | 18 | 60.0 | 3 | 33.3 | $\chi^2$= 3.526 | $^{MC}$p= 0.576 |
| B | 4 | 50.0 | 11 | 36.7 | 6 | 66.7 | | |
| C | 0 | 0.0 | 1 | 3.3 | 0 | 0.0 | | |
| **Size of focal lesion** | | | | | | | | |
| <5 | 6 | 75.0 | 15 | 50.0 | 8 | 88.9 | $\chi^2$= 4.904 | $^{MC}$p= 0.080 |
| ≥5 | 2 | 25.0 | 15 | 50.0 | 1 | 11.1 | | |
| Min. – Max. | 3.0 – 14.0 | | 1.50 – 14.0 | | 2.0 – 5.0 | | H= 5.674 | 0.059 |
| Mean ± SD. | 5.25 ± 3.67 | | 5.47 ± 3.19 | | 3.24 ± 1.05 | | | |
| Median | 4.0 | | 4.75 | | 3.0 | | | |
| **Number of focal lesion** | | | | | | | | |
| 1 | 7 | 87.5 | 23 | 76.7 | 5 | 55.6 | $\chi^2$= 7.675 | $^{MC}$p= 0.173 |
| 2 | 0 | 0.0 | 4 | 13.3 | 3 | 33.3 | | |
| 3 | 1 | 12.5 | 0 | 0.0 | 0 | 0.0 | | |
| >3 | 0 | 0.0 | 3 | 10.0 | 1 | 11.1 | | |
| **Pro Conc (A1AT concentration)** | | | | | | | | |
| Min. – Max. | 58.0 – 100.0 | | 51.70 – 100.0 | | 34.20 – 75.0 | | F=3.192 | 0.051 |
| Mean ± SD. | 75.95 ± 13.82 | | 72.91 ± 13.45 | | 60.97 ± 14.83 | | | |
| Median | 75.30 | | 71.50 | | 66.0 | | | |
| **AFP** | | | | | | | | |
| Min. – Max. | 2.80 – 633.0 | | 2.40 – 9902.0 | | 6.90 – 733.0 | | H= 4.901 | 0.086 |
| Mean ± SD. | 163.3 ± 248.5 | | 1626.8 ± 3088.1 | | 206.8 ± 302.2 | | | |
| Median | 31.90 | | 142.5 | | 46.50 | | | |

**Table (4):** Relation between rs738407 and different parameters in HCC group (n = 47)

| | rs738407 | | | Test of sig. | p |
|---|---|---|---|---|---|
| | GG (n= 8) | GA (n= 30) | AA (n= 9) | | |
| **Hb** | | | | | |
| Min. – Max. | 9.80 – 13.50 | 9.20 – 14.90 | 9.70 – 16.50 | F=0.049 | 0.952 |
| Mean ± SD. | 12.45 ± 1.26 | 12.59 ± 1.54 | 12.53 ± 2.16 | | |
| Median | 12.90 | 12.85 | 12.80 | | |
| **RBCs** | | | | | |
| Min. – Max. | 3.0 – 5.20 | 3.02 – 5.20 | 3.10 – 6.0 | F=0.023 | 0.977 |
| Mean ± SD. | 4.36 ± 0.68 | 4.38 ± 0.56 | 4.28 ± 0.94 | | |
| Median | 4.45 | 4.35 | 4.33 | | |
| **TLC** | | | | | |
| Min. – Max. | 4.50 – 11.0 | 2.0 – 8.40 | 2.50 – 7.30 | F=2.374 | 0.105 |
| Mean ± SD. | 6.19 ± 2.20 | 4.83 ± 1.73 | 5.80 ± 1.53 | | |
| Median | 5.40 | 4.90 | 6.10 | | |
| **PLT** | | | | | |
| Min. – Max. | 107.0 – 255.0 | 32.0 – 209.0 | 41.0 – 123.0 | H=12.278* | 0.002* |
| Mean ± SD. | 184.5 ± 59.84 | 105.5 ± 40.34 | 91.0 ± 26.81 | | |
| Median | 183.5 | 99.0 | 94.0 | | |
| **INR** | | | | | |
| Min. – Max. | 1.0 – 1.40 | 1.0 – 1.50 | 1.26 – 2.10 | H=4.365 | 0.113 |
| Mean ± SD. | 1.22 ± 0.14 | 1.25 ± 0.15 | 1.45 ± 0.28 | | |
| Median | 1.21 | 1.25 | 1.30 | | |
| **Creatinine** | | | | | |
| Min. – Max. | 0.70 – 1.0 | 0.50 – 1.70 | 0.44 – 1.40 | H=1.663 | 0.435 |
| Mean ± SD. | 0.86 ± 0.09 | 0.97 ± 0.26 | 0.94 ± 0.25 | | |
| Median | 0.90 | 0.95 | 0.90 | | |
| **AST** | | | | | |
| Min. – Max. | 26.0 – 200.0 | 22.0 – 210.0 | 13.0 – 85.0 | H=0.404 | 0.817 |
| Mean ± SD. | 65.63 ± 57.29 | 56.93 ± 33.76 | 56.22 ± 22.87 | | |
| Median | 46.50 | 50.0 | 64.0 | | |
| **ALT** | | | | | |
| Min. – Max. | 20.0 – 145.0 | 11.0 – 239.0 | 14.0 – 110.0 | H=0.396 | 0.821 |
| Mean ± SD. | 51.63 ± 46.09 | 47.83 ± 40.07 | 45.89 ± 28.60 | | |
| Median | 34.0 | 40.0 | 40.0 | | |
| **Bil T** | | | | | |
| Min. – Max. | 0.60 – 2.70 | 0.52 – 3.50 | 0.90 – 2.20 | H=3.209 | 0.201 |
| Mean ± SD. | 1.18 ± 0.67 | 1.37 ± 0.73 | 1.60 ± 0.50 | | |
| Median | 0.95 | 1.14 | 1.70 | | |
| **Bil D** | | | | | |
| Min. – Max. | 0.11 – 1.0 | 0.12 – 2.10 | 0.22 – 1.15 | H=4.315 | 0.116 |
| Mean ± SD. | 0.39 ± 0.33 | 0.58 ± 0.49 | 0.70 ± 0.36 | | |
| Median | 0.25 | 0.40 | 0.70 | | |
| **Albumin** | | | | | |
| Min. – Max. | 2.40 – 4.20 | 2.10 – 4.40 | 2.10 – 4.20 | F=0.027 | 0.973 |
| Mean ± SD. | 3.28 ± 0.62 | 3.23 ± 0.64 | 3.27 ± 0.60 | | |
| Median | 3.25 | 3.10 | 3.20 | | |

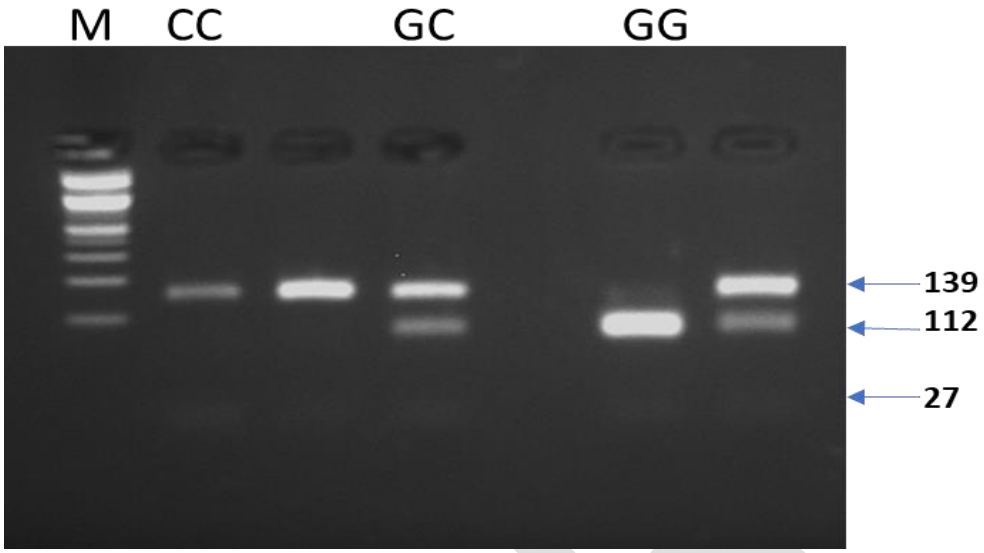


**Figure 1.** PCR-RFLP study of the mutation rs738409C/G I148M in PNPLA3. The 139 bp fragment length of the CC genotype cannot be broken up into individual bands by the NlaIII restriction enzyme. NlaIII, however, cleaves the G allele, producing two fragments (112 bp and 27 bp). Three bands are produced by the heterozygote (139 bp, 112 bp, and 27 bp). 100 bp ladder DNA marker is Lane M.

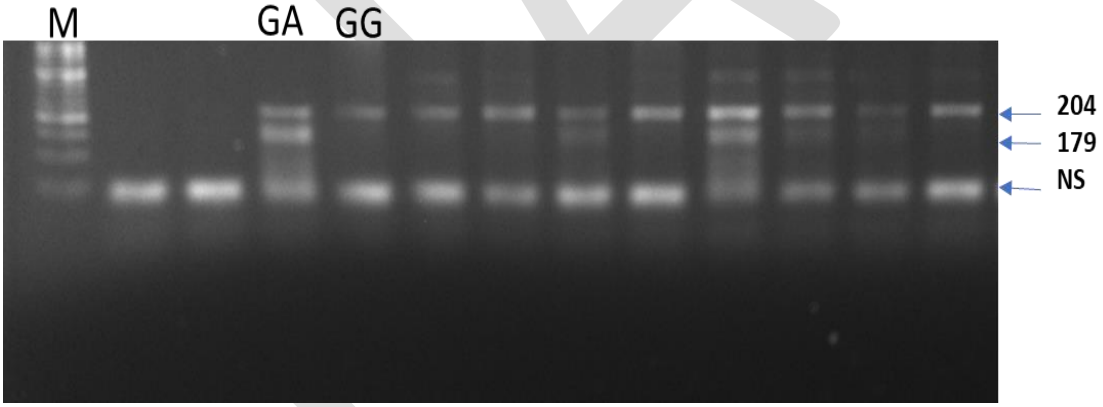


**Figure 2.** PCR-RFLP analysis of the PNPLA3 rs738407_polymorphism. *MluC1* restriction enzyme cannot cleave GG genotype with a single band with a fragment length of 204 bp. While the G allele is cleaved by *MluC1* and yields two fragments (179 bp and 31,25 bp). The heterozygote generates three bands (204bp, 179 bp, 31, 25 bp). Lane M = 150 bp ladder DNA marker.

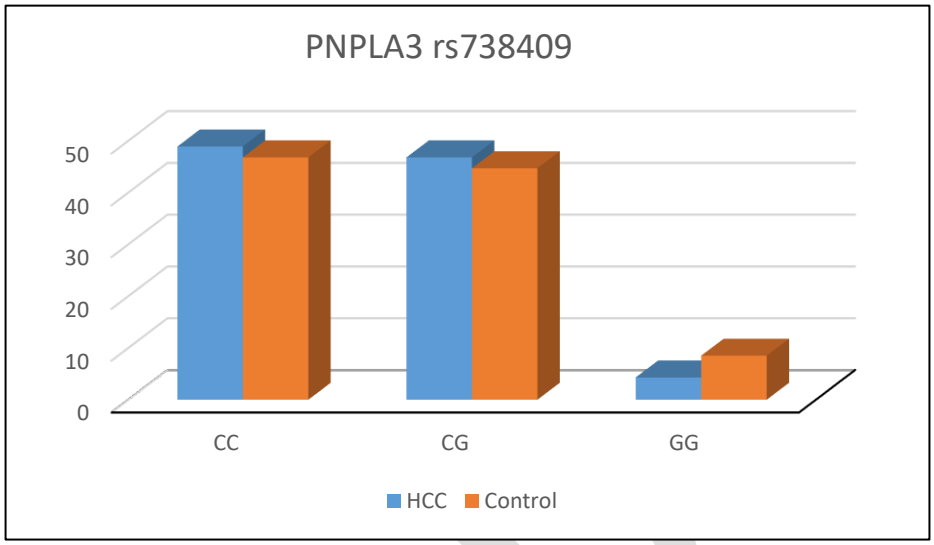


**Figure (3):** Comparison between the two studied groups according to PNPLA3 rs738409C/G

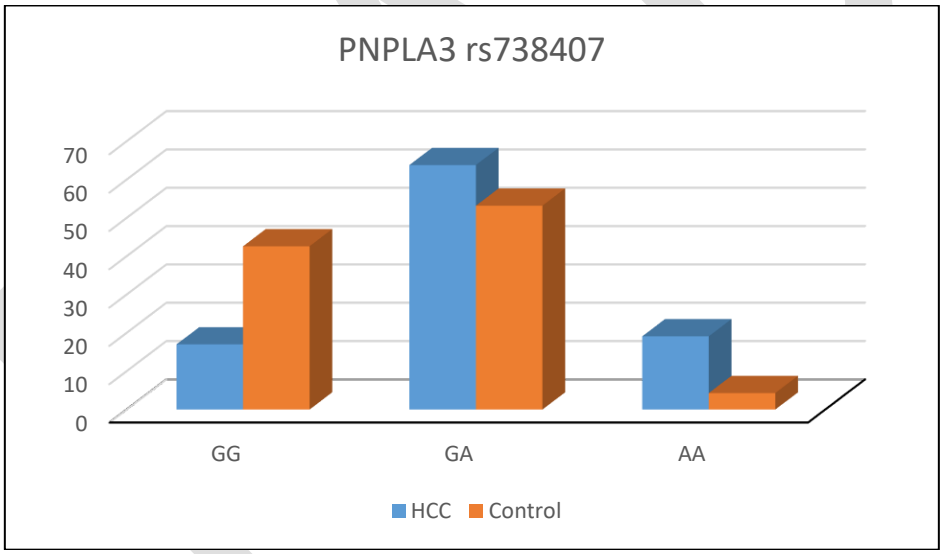


**Figure (4):** Comparison between the two studied groups according to PNPLA3 rs738407A/G I148M