Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 04 July 2026
Revised 10 Aug 2026
Accepted 21 Aug 2026

Natural Resources for Human Health 2026, Vol. 6 (18s): 515–527

KEYWORDS:
NAFLD; Vitamin D; Vitamin D protein gene; rs7975232; rs2228570; Hepatic steatosis

Association Between Vitamin D Protein Gene Polymorphisms and Non-Alcoholic Fatty Liver Disease Risk

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ABSTRACT: Background

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease worldwide, affecting approximately one-quarter of the global population, and is strongly influenced by genetic predisposition. Vitamin D contributes to hepatic steatosis, fibrosis, and insulin resistance, and low vitamin D levels have been linked to an increased risk of NAFLD. Vitamin D receptor protein (VDR) is a key nuclear receptor that binds to vitamin D, so polymorphisms in its gene may influence vitamin D availability and, in turn, NAFLD risk and severity. Data on the relationship between VDR single-nucleotide polymorphisms (SNPs) and NAFLD remain limited. In this study, we aim to investigate the association between the VDR gene polymorphisms rs7975232 and rs2228570 and the risk and severity of NAFLD.

Methods

This cross-sectional study enrolled 100 participants (50 with NAFLD, diagnosed by abdominal ultrasound and transient elastography, and 50 controls) over 12 months, all attending the Department of Hepatology, Gastroenterology and Infectious Diseases, Faculty of Medicine, Benha University. Serum 25(OH)D was measured by enzyme-linked immunosorbent assay (ELIZA) method according to manufacture instructions and regarding to rs7975232 and rs2228570 were genotyped by TaqMan allelic-discrimination real-time PCR.

Results

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Serum vitamin D was significantly lower in the NAFLD group than in controls (17.07±5.64 vs 28.38±3.04 ng/mL, p<0.001). Among NAFLD patients, the rs7975232 AA genotype was associated with lower vitamin D (12.57±1.71 vs 19.86±7.89 ng/mL, p=0.002) and a higher steatosis grade (269.92±11.77 vs 252.00±11.19, p<0.001) than the CC genotype. Similarly, the rs2228570 TT genotype was associated with lower vitamin D (14.25±4.50 vs 19.25±5.88 ng/mL, p=0.013) and a higher steatosis grade (266.95±11.42 vs 247.33±3.12, p<0.001) than the CC genotype. Within the NAFLD group, both rs7975232 and rs2228570 dosage correlated positively with cholesterol (r=0.369 and r=0.309, respectively; p<0.001 and p=0.002, respectively) and triglycerides level (r=0.440 and r=0.409, respectively; both p<0.001) In addition, both rs7975232 and rs2228570 dosage correlated positively with steatosis grade (r=0.539 and r=0.654, respectively; both p<0.001) and steatosis grade (r=0.412 and r=0.490, respectively; both p<0.001), and rs2228570 dosage also correlated with the FIB-4 index (r=0.437, p<0.001). A combined model of vitamin D and both SNPs achieved the best discrimination for NAFLD (AUC 0.961, 95% CI 0.910–0.996).

Conclusion

The rs2228570 and rs7975232 VDR variants are associated with lower vitamin D levels and greater hepatic steatosis, and genotype information modestly improves the diagnostic performance of vitamin D for NAFLD. Because the study is cross-sectional, these findings cannot establish causality or disease progression; longitudinal and mechanistic studies are needed.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting approximately one-quarter of the global population, and its clinical relevance has grown further as the prevalence of viral hepatitis B and C has declined [1]. NAFLD encompasses a spectrum of disorders ranging from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH) [2], and is diagnosed when hepatic steatosis exceeds 5% after exclusion of secondary causes of fatty liver [2]. More recently, the term metabolic dysfunction-associated steatotic liver disease (MASLD) has been introduced, defined as hepatic triglyceride accumulation in the presence of at least one cardiometabolic risk factor (obesity, diabetes, hypertension, hypertriglyceridemia and hypercholesteremia), in contrast to NAFLD which is a pathological diagnosis and diagnosis of exclusion [3].

Vitamin D is a fat-soluble secosteroid classically recognized for its role in calcium and bone homeostasis, but increasingly implicated in a wide range of non-skeletal outcomes [4]. In the liver, vitamin D helps limit adipose-tissue inflammation [5], hepatic fibrosis [6], triglyceride accumulation in hepatocytes, and insulin resistance [7]. Several cross-sectional and prospective studies have linked vitamin D deficiency to an increased risk and severity of NAFLD, while vitamin D supplementation has been reported to improve hepatic steatosis [1, 8, 9].

There is growing evidence that vitamin d levels help to improve adipose tissue inflammation liver fibrosis and triglycerides accumulation in hepatocytes and insulin resistance. [5,6,7,10]. Some previous cross-sectional studies showed that vitamin D deficiency was associated with an increased risk of NAFLD, and vitamin D levels were negatively associated with the severity of NAFLD [1]. Several prospective studies also pointed out that serum vitaminD deficiency was accompanied by an increased risk of NAFLDwhile Vitamin D supplementation could also improve hepatic steatosis [8.9]

Vitamin D receptor, (VDR) is a key nuclear receptor that binds to vitamin D. According to current evidence, vitamin D receptor is one of the most important key mediators for the biological activities of vitamin D. The interaction of vitamin D and this receptor plays an important role in adipocyte differentiation both in vitro and in vivo[11, 12]. Because VDR governs the response of circulating vitamin D, polymorphisms in its encoding gene (GC) could plausibly alter the gene expressions and, in turn, the risk and severity of NAFLD [13]. Variants such as rs4588 and rs7041 in the GC gene have already been associated

with reduced serum 25-hydroxyvitamin D [25(OH)D] [14], but data specifically relating the GC variants rs7975232 and rs2228570 to NAFLD risk remain scarce.

We therefore aimed to investigate the association between the VDR gene polymorphisms rs7975232 and rs2228570 and the risk and severity of NAFLD in a cross-sectional cohort of Egyptian adults.

Patients and Methods

Study design and setting

This cross-sectional study was conducted among 100 participants attending the Department of Hepatology, Gastroenterology, and Infectious Diseases, Faculty of Medicine, Benha University, over a 12-month period, following informed consent from all participants. Participants were allocated to an NAFLD group (n=50), diagnosed by abdominal ultrasound and transient elastography, and a control group without NAFLD (n=50). The study was approved by the Research Ethics Committee of Benha University (MS 1/2/2024, approval no. 9554710776) and was conducted in accordance with the Declaration of Helsinki.

Inclusion and exclusion criteria

Patients older than 18 years with non-alcoholic fatty liver disease, defined as alcohol consumption <20 g/day, negative viral hepatitis markers, no medication known to cause hepatic steatosis, and steatosis confirmed by transient elastography, were eligible for the NAFLD group [15-17]. Participants with a history of drug-induced liver injury, liver transplantation, psychiatric disorders, hepatocellular carcinoma, or positive hepatitis B or C serology were excluded from the study.

Clinical and anthropometric assessment

All participants underwent measurement of height, weight, body mass index (BMI), and waist circumference (measured midway between the lower costal margin and the anterior superior iliac spine; considered abnormal if >90 cm in men or >80 cm in women). Body weight was measured to the nearest 0.5 kg. Blood pressure was measured in the sitting position after 5 minutes of rest using a periodically calibrated mercury sphygmomanometer, to the nearest 2 mmHg; hypertension was defined as current antihypertensive treatment or a blood pressure ≥140/90 mmHg.

Laboratory assessment

Venous blood was collected after an overnight fast for a complete blood count, liver function tests, a lipid profile (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol), a coagulation profile, C-reactive protein (CRP), and alpha-fetoprotein (AFP).

Imaging assessment

Abdominal ultrasonography was performed in all participants. Liver stiffness and the controlled attenuation parameter (CAP) were measured by transient elastography (FibroScan® 502, Echosens, Paris, France) after at least 3 hours of fasting, with a minimum of 10 valid measurements per patient; a CAP value ≥238 dB/m defined hepatic steatosis [18].

Vitamin D quantification

Serum 25(OH)D was measured by a validated colorimetric method on serum that was separated promptly and stored at −20°C for up to 6 months. Vitamin D deficiency was defined as a serum 25(OH)D level <20 ng/mL

Genotyping of VDR gene polymorphisms

Genomic DNA was extracted from whole blood using the QIAamp® DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. DNA concentration and purity (A260/280 ratio of 1.7–1.9) were verified using a NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). Genotyping of the VDR (GC) gene polymorphisms rs7975232 and rs2228570 was performed by TaqMan® allelic-discrimination real-time PCR on the Applied Biosystems StepOne™ Real-Time PCR System (Thermo Fisher Scientific, Singapore), in accordance with the manufacturer's guidelines.

Statistical analysis

Data were analyzed using IBM SPSS Statistics, version 27 (IBM Corp., Armonk, NY, USA). Normality of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Quantitative variables are presented as mean ± SD and were compared between two groups using the independent-samples t-test, and among the three genotype groups using one-way analysis of variance (ANOVA) with post-hoc pairwise comparisons; genotype groups sharing the same superscript letter (^{a},^{b},^{c}) did not differ significantly. Categorical variables are presented as number (%) and were compared using the chi-square test or Fisher's exact test, as appropriate. Spearman's rank correlation was used to relate risk-allele dosage (coded 0, 1, or 2 according to the number of minor alleles carried) to anthropometric, laboratory, and hepatic-severity parameters. Multivariable logistic regression (L2-penalized, with 1,000 bootstrap resamples) was used to estimate genotype-carrier associations with NAFLD presence, moderate-to-severe steatosis, and elevated FIB-4 risk, adjusting for vitamin D, BMI, age, and sex (and additionally diabetes for the FIB-4 outcome); robust linear regression was used for the CAP-score outcome, with B representing the adjusted mean difference in CAP score. FIB-4 analyses represent derived fibrosis risk rather than direct histological fibrosis staging. Receiver operating characteristic (ROC) curve analysis, with 95% confidence intervals derived from 2,000 bootstrap resamples, was used to evaluate the discriminatory performance of vitamin D alone and in combination with genotype for NAFLD/steatosis presence. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

A total of 100 participants were enrolled: 50 with NAFLD and 50 controls without NAFLD. The NAFLD and control groups were comparable in age, sex distribution, and smoking status (Table 1). However, participants with NAFLD had a significantly greater waist circumference and BMI, and a higher prevalence of diabetes and hypertension, than controls (all p<0.001; Table 1). On clinical examination, hepatomegaly was identified in 25.0% of the NAFLD group compared with 0.0% of controls and splenomegaly was identified in 6.0% of the NAFLD compared to none in the control. (p<0.001; Table 1).

Nearly all laboratory parameters differed significantly between groups, with the exception of platelet count, albumin, and creatinine. The NAFLD group showed higher hemoglobulin levels and WBC count compared to the controls (Table 2). The NAFLD group had significantly higher ALT, AST, GGT, ALP, total bilirubin, INR, total cholesterol, triglycerides, LDL-cholesterol, AFP, and CRP, and significantly lower HDL-cholesterol and serum vitamin D (17.07±5.64 vs 28.38±3.04 ng/mL, p<0.001) than controls (Table 2).

Our study showed significant differences in both genotype distributions between NAFLD patients and controls (P<0.001 for each SNP). For ApaI gene (rs7975232), CA and AA genotypes were associated with higher odds of NAFLD than CC [OR=14.50 (95% CI: 4.97–42.30) and OR=11.70 (95% CI: 3.24–42.28), respectively]. The A allele was more frequent in NAFLD patients than controls (55.0% versus 19.0%) and was associated with a 5.21-fold increase in NAFLD odds. For FokI gene (rs2228570), CT and TT genotypes were associated with higher odds than CC [OR=6.10 (95% CI: 2.22–16.74) and OR=25.19 (95% CI: 6.10–104.05), respectively], while the T allele was associated with a 6.67-fold increase in odds. The rs7975232 distribution deviated from Hardy–Weinberg equilibrium in controls (P=0.008), whereas rs2228570 did not (P=0.347) (Table 3).

Restricting the analysis to the 50 NAFLD patients, the rs7975232 AA genotype was associated with lower serum vitamin D than the CC genotype (12.57±1.71 vs 19.86±7.89 ng/mL, p=0.002) and a higher steatosis grade, together with more advanced fibrosis stage. 69% of the patients with AA genotype were S2/S3, compared 87.5% of the patients with CC genotype were S1(Table 4). Also, almost two thirds of the patients with AA genotype were F2/3 compared to 87.5% of the patients with the CC genotype were F1 (Table 4). A similar pattern was observed for rs2228570: TT carriers had lower vitamin D than CC carriers (14.25±4.50 vs 19.25±5.88 ng/mL, p=0.013) and a higher steatosis grade and fibrosis stage (Table 4). 65% of the patients with TT genotype were S2/S3, compared all of the patients with CC genotype were S1(Table 4). Also, three quatres of the patients with TT genotype were F3 compared to all of the patients with the CC genotype were F1 (Table 4).

Risk-allele dosage for both SNPs correlated positively with waist circumference and BMI, and with several markers of hepatic injury and metabolic risk, including ALT, AST, total bilirubin, INR, total cholesterol, triglycerides, CRP, and AFP, and correlated inversely with serum vitamin D (all p<0.05; Table 5). Within the NAFLD group, both rs7975232 and rs2228570 dosage correlated positively with cholesterol (r=0.369 and r=0.309, respectively; p<0.001 and p=0.002, respectively) and triglycerides level (r=0.440 and r=0.409, respectively; both p<0.001) (Table 5). Within the NAFLD group, both rs7975232 and rs2228570 dosage correlated positively with CAP score(r=0.539 and r=0.654, respectively; both p<0.001) and steatosis grade

(r=0.412 and r=0.490, respectively; both p<0.001), and rs2228570 dosage also correlated with the FIB-4 index (r=0.437, p<0.001) (Table 5).

ApaI gene (rs7975232) A-carrier status remained independently associated with NAFLD [adjusted OR=11.86 (95% CI: 3.30–54.20)], moderate/severe steatosis [adjusted OR=9.00 (95% CI: 1.77–138.49)] and elevated FIB-4 risk [adjusted OR=5.88 (95% CI: 1.05–65.56)], but its adjusted association with steatosis grade was not statistically significant (Table 6). In contrast, FokI (rs2228570) T-carrier status was independently associated with a 12.77-unit higher CAP score and with moderate/severe steatosis [adjusted OR=28.94 (95% CI: 7.68–125.68)], but was not independently associated with NAFLD or elevated FIB-4 after adjustment (Table 6).

Serum vitamin D alone showed excellent discrimination for NAFLD (AUC 0.930, 95% CI 0.866–0.982), with a Youden-optimal cutoff of <22.0 ng/mL (sensitivity 88.0%, specificity 98.0%). Discrimination improved further when vitamin D was combined with genotype information, and the model incorporating vitamin D and both SNPs achieved the highest AUC (0.961, 95% CI 0.910–0.996; sensitivity 90.0%, specificity 98.0%) (Figure 1).

DISCUSSION

NAFLD is a common, multifactorial chronic liver disease driven by diet, lifestyle, and genetic predisposition, and is closely associated with diabetes, metabolic syndrome, obesity, and insulin resistance [19]. It represents the hepatic manifestation of metabolic syndrome, with steatosis arising from the inability of adipocytes to store excess fat; accelerated hepatocyte senescence in this setting increases the long-term risk of hepatocellular carcinoma [1]. The disease has recently been reconceptualized as MASLD, defined by hepatic triglyceride accumulation with at least one cardiometabolic risk factor, rather than the two risk factors required for MAFLD [3].

Consistent with this metabolic underpinning, our NAFLD and control groups were similar in age, sex, and smoking status, but the NAFLD group had a significantly greater waist circumference and BMI and a higher prevalence of diabetes and hypertension. These findings aligned with Wang et al. (2022) [11], who found no significant difference in age or sex between NAFLD and control groups in a Chinese cohort of 1,137 NAFLD patients and 1,886 controls, but reported significantly higher BMI and blood pressure in the NAFLD group.

Vitamin D is a pleiotropic, fat-soluble hormone involved in immune modulation and cell proliferation and differentiation [21]; reduced vitamin D availability could therefore plausibly increase hepatic inflammation and the severity of steatosis and fibrosis. Because VDR is both the principal receptor of vitamin D and an independent anti-inflammatory and immunoregulatory mediator in chronic liver disease [22], variants that impair VDR function could affect development of NAFLD.

Our study showed significant differences in both genotype distributions between NAFLD patients and controls (P<0.001 for each SNP). For rs7975232, CA and AA genotypes were associated with higher odds of NAFLD than CC [OR=14.50 (95% CI: 4.97–42.30) and OR=11.70 (95% CI: 3.24–42.28), respectively]. The A allele was more frequent in NAFLD patients than controls (55.0% versus 19.0%) and was associated with a 5.21-fold increase in NAFLD odds. For rs2228570, CT and TT genotypes were associated with higher odds than CC [OR=6.10 (95% CI: 2.22–16.74) and OR=25.19 (95% CI: 6.10–104.05), respectively], while the T allele was associated with a 6.67-fold increase in odds. The rs7975232 distribution deviated from Hardy–Weinberg equilibrium in controls (P=0.008), whereas rs2228570 did not (P=0.347). This was supported partially by Zhang et al. (2021) who found out that rs2228570-C might contribute to low risk of NAFLD in the Chinese Han Population [20].

This was reflected in our genotype-stratified findings: among NAFLD patients, the rs7975232 AA genotype was associated with lower vitamin D than the CC genotype and a higher steatosis grade. A similar pattern was seen for rs2228570, where the TT genotype was associated with lower vitamin D and a higher steatosis grade than the CC genotype, indicating that both mutant genotypes were linked to lower vitamin D and greater hepatic steatosis. This is broadly consistent with Zhang et al. (2021) [20], who reported a significant association between rs2228570 and NAFLD, although unlike our findings they did not find a significant association for rs7975232; this discrepancy may partly reflect their much larger sample of 1,114 NAFLD cases compared with our 50.

Elevated total cholesterol, LDL-cholesterol, and particularly triglycerides are central to NAFLD pathogenesis, since hepatic triglycerides accumulate from the esterification of free fatty acids (FFAs) and glycerol; FFAs are derived from adipose-tissue

lipolysis, dietary intake, and de novo lipogenesis, while their disposal occurs via β-oxidation, re-esterification and storage, or export as very-low-density lipoprotein (VLDL). Hepatic fat accumulation therefore reflects a net imbalance favoring fat synthesis and delivery over fat export and oxidation [23]. In our study, the rs7975232 AA genotype was associated with higher total cholesterol and triglycerides than the CA and CC genotypes and higher LDL-cholesterol, consistent with the correlations between rs7975232 dosage and lipid parameters.

Both risk alleles correlated positively with waist circumference, BMI, and several components of the metabolic and hepatic-injury profile, including ALT, AST, triglycerides, and CRP an association pattern that plausibly explains their link to greater NAFLD risk and steatosis severity, since these are recognized components of the metabolic syndrome. Rashidi et al. (2021) found that there was a positive correlation between A carrier status in ApaI gene and obesity [24]. However, Fiaz et al. (2021), found no correlation between ApaI gene variants and hypertension [25]. In addition, another study found no association between the gene variants of ApaI and Diabetes [26]. Lastly, regarding ApaI gene polymorphism, Andreeva et al. (2019) concluded that there was positive correlation between A- allele and cholesterol levels [27]. Regarding FokI gene variants, Fiaz et al.(2021) and Alharbi et al.(2021) agreed with us that there is positive correlation between T- allele and hypertension and diabetes [25,26]. Also, in line with our findings, Sharifi et al. (2025) found a positive correlation between T-allele and cholesterol levels [28]. Consistent with this, rs7975232 and rs2228570 dosage correlated positively with CAP score and steatosis grade among NAFLD patients, with the strongest correlations observed for CAP score ($r=0.539$ and $r=0.654$, respectively; both $p<0.001$); rs2228570 dosage also correlated with the FIB-4 index ($r=0.437$, $p<0.001$). This was reinforced regarding FokI gene variants, TC/TT as seen in Gibson et al. (2018) [29]. However, no study was found regarding comparing the CAP score and the gene polymorphisms of ApaI gene.

The current study showed that rs7975232 A-carrier status remained independently associated with NAFLD, moderate/severe steatosis and elevated FIB-4 risk, but its adjusted association with CAP score was not statistically significant. Currently, there are no studies comparing ApaI genotypes on risk of NAFLD and degree of steatosis. However, Baur et al(2011), a study conducted on 251 patients with HCV, found out that ApaI rs7975232 AA genotypes was significantly associated with fibrosis progression [23]. Yet, Arai et al(2019) found no association [30].

In contrast, rs2228570 T-carrier status was independently associated with a 12.77-unit higher CAP score and with moderate/severe steatosis, but was not independently associated with NAFLD or elevated FIB-4 after adjustment. In partial agreement with our study, Gibson et al(2018), concluded that TT/CT genotype was associated with higher degree of steatosis, and inflammation and fibrosis in the liver biopsy compared to the CC genotype [29].

Finally, serum vitamin D alone showed excellent discrimination for NAFLD (AUC 0.930), with an optimal cutoff of <22.0 ng/ml. Adding genotype information further improved discrimination, and the combined model incorporating vitamin D and both SNPs achieved the highest AUC, with 90.0% sensitivity and 98.0% specificity, suggesting that VDR genotyping may offer incremental value alongside vitamin D measurement for NAFLD risk stratification.

Limitations

This study has several limitations. The cross-sectional design precludes conclusions about causality or disease progression. The overall sample size, and particularly the size of individual genotype subgroups, was modest, which may have limited statistical power and widened the confidence intervals of some adjusted estimates. The study was conducted at a single center, which may limit generalizability to other populations. Finally, the genotype-based multivariable and ROC models require external validation before they can be used for individual risk prediction.

CONCLUSION

Both the rs2228570 and rs7975232 VDR gene polymorphisms were associated with lower serum vitamin D levels and greater hepatic steatosis severity, and rs7975232 A-carrier status and rs2228570 T-carrier status were each independently associated with distinct aspects of NAFLD risk and severity after adjustment for metabolic confounders. Combining vitamin D measurement with VDR genotyping improved discrimination for NAFLD beyond vitamin D alone. However, the cross-sectional design does not allow conclusions about disease progression, and further mechanistic and longitudinal studies are required to determine whether these variants, or the VDR pathway more broadly, represent viable therapeutic or risk-stratification targets.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Committee of Benha University (MS 1/2/2024, approval no. 9554710776). Written informed consent was obtained from all participants.

Conflict of interest

All authors declare that they have no conflict of interest.

Financial support and sponsorship

The authors declare that this research did not receive any specific grant from any public, commercial, or not-for-profit funding agency.

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Table 1. Demographic, anthropometric, and clinical characteristics of the NAFLD and control groups

Variable	NAFLD (n=50)	Control (n=50)	Test	P value
Age, years (mean±SD)	45.96±10.95	42.98±14.14	t=1.178	0.242
Male sex, n (%)	21 (42.0%)	20 (40.0%)	χ^2=0.041	1.000

Variable	NAFLD (n=50)	Control (n=50)	Test	P value
Female sex, n (%)	29 (58.0%)	30 (60.0%)	–	–
Waist circumference, cm (mean±SD)	90.72±6.61	79.33±8.28	t=7.598	<0.001
BMI, kg/m² (mean±SD)	32.80±3.87	25.10±1.93	t=12.589	<0.001
History of diabetes mellitus, n (%)	25 (50.0%)	5 (10.0%)	χ^2=19.048	<0.001
History of hypertension, n (%)	29 (58.0%)	5 (10.0%)	χ^2=25.668	<0.001
Current smoking, n (%)	7 (14.0%)	8 (16.0%)	χ^2=0.078	1.000
Hepatomegaly(%)	25(50.0%)	0(0%)	χ^2=120.666	<0.001
Splenomegaly(%)	3(6.0%)	0(0%)	χ^2=120.666	<0.001

Table 2. Laboratory and biochemical parameters of the NAFLD and control groups

Parameter	NAFLD (mean±SD)	Control (mean±SD)	Test	P value
Hemoglobin, g/dL	12.56±1.19	11.60±1.81	t=3.123	0.002
WBCs, ×10³/μL	8.39±1.94	6.63±1.92	t=4.550	<0.001
Platelets, ×10³/μL	257.56±54.40	272.07±82.73	t=-1.036	0.303
ALT, U/L	62.64±23.76	29.94±5.27	t=9.500	<0.001
AST, U/L	58.70±18.26	32.46±6.16	t=9.626	<0.001
GGT, U/L	43.66±18.21	33.30±7.31	t=3.733	<0.001
Albumin, g/dL	3.73±0.36	3.77±0.40	t=-0.639	0.524
ALP, U/L	93.04±20.20	69.68±18.86	t=5.977	<0.001
Total bilirubin, mg/dL	1.34±0.53	0.76±0.19	t=7.246	<0.001
INR	1.47±1.68	0.98±0.07	t=2.039	0.047
Creatinine, mg/dL	0.93±0.16	0.95±0.16	t=-0.680	0.498
Total cholesterol, mg/dL	188.22±29.79	160.78±17.70	t=5.599	<0.001
Triglycerides, mg/dL	192.02±38.08	148.72±18.10	t=7.262	<0.001
HDL, mg/dL	36.16±7.50	41.58±7.54	t=-3.604	<0.001

Parameter	NAFLD (mean±SD)	Control (mean±SD)	Test	P value
LDL, mg/dL	121.12±25.33	111.42±14.82	t=2.338	0.021
AFP, ng/mL	12.82±3.89	6.41±3.54	t=8.616	<0.001
CRP, mg/dL	8.90±2.35	4.63±1.29	t=11.267	<0.001
Serum 25(OH)D, ng/mL	17.07±5.64	28.38±3.04	–	<0.001

WBC, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; INR, international normalized ratio; HDL/LDL, high-/low-density lipoprotein; AFP, alpha-fetoprotein; CRP, C-reactive protein. 25(OH)D, 25-hydroxyvitamin D;

Table 3. Distribution of ApaI (rs7975232) and FokI (rs2228570) genotypes and alleles in NAFLD patients and controls

Variant/model	NAFLD n (%)	Control n (%)	OR (95% CI)	P value
rs7975232 genotype (global)				<0.001
CC	8 (16.0%)	36 (72.0%)	Reference	—
CA	29 (58.0%)	9 (18.0%)	14.50 (4.97–42.30)	<0.001
AA	13 (26.0%)	5 (10.0%)	11.70 (3.24–42.28)	<0.001
A allele	55 (55.0%)	19 (19.0%)	5.21 (2.76–9.84)	<0.001
Hardy–Weinberg equilibrium in controls				0.008
rs2228570 genotype (global)				<0.001
CC	9 (18.0%)	34 (68.0%)	Reference	—
CT	21 (42.0%)	13 (26.0%)	6.10 (2.22–16.74)	<0.001
TT	20 (40.0%)	3 (6.0%)	25.19 (6.10–104.05)	<0.001
T allele	61 (61.0%)	19 (19.0%)	6.67 (3.51–12.66)	<0.001
Hardy–Weinberg equilibrium in controls				0.347

Table 4. Relation between the studied parameters and ApaI (rs7975232) and FokI (rs2228570) genotype among NAFLD patients

Panel A. rs7975232 genotype (NAFLD patients only)						
Parameter	**Category**	**AA (n=13)**	**CA (n=29)**	**CC (n=8)**	**Test**	**P value**

Serum 25(OH)D, ng/mL		12.57±1.71[a]	18.31±5.07[b]	19.86±7.89[b]	F=7.294	0.002
Steatosis grade	S1	4 (30.8%)	19 (65.5%)	7 (87.5%)	χ^2=9.318	0.029
	S2	7 (53.8%)	10 (34.5%)	1 (12.5%)		
	S3	2 (15.4%)	0 (0.0%)	0 (0.0%)		
Fibrosis stage†	F1	5 (38.5%)	19 (65.5%)	7 (87.5%)	χ^2=10.386	0.017
	F2	6 (46.2%)	10 (34.5%)	0 (0.0%)		
	F3	2 (15.4%)	0 (0.0%)	1 (12.5%)		
Panel B. rs2228570 genotype (NAFLD patients only)						
Parameter	**Category**	**CC (n=9)**	**CT (n=21)**	**TT (n=20)**	**Test**	**P value**
Serum 25(OH)D, ng/mL		19.25±5.88[ab]	18.81±5.62[a]	14.25±4.50[b]	F=4.817	0.013
Steatosis grade	S1	9 (100.0%)	14 (66.7%)	7 (35.0%)	χ^2=12.405	0.005
	S2	0 (0.0%)	7 (33.3%)	11 (55.0%)		
	S3	0 (0.0%)	0 (0.0%)	2 (10.0%)		
Fibrosis stage	–	–	–	–	χ^2=9.617	0.023
	F1	9(100.0%)	5(23.8%)	1(5.0%)		
	F2	0(0.0%)	10(47.6%)	4(20.0%)		
	F3	0(0.0%)	6(28.5%)	15(75.0%)		

25(OH)D, 25-hydroxyvitamin D

Table 5. Spearman correlation of VDR risk-allele dosage with studied parameters

Parameter	**rs7975232 A-dosage: r**	**P1**	**rs2228570 T-dosage: r**	**P2**
Age, years	0.020	0.843	0.180	0.073
Waist circumference, cm	0.432	<0.001	0.239	0.017
BMI, kg/m²	0.319	0.001	0.490	<0.001
Hemoglobin, g/dL	0.233	0.020	0.119	0.239
WBCs, ×10³/μL	0.204	0.042	0.228	0.023
Platelets, ×10³/μL	-0.162	0.108	-0.258	0.010

Parameter	rs7975232 A-dosage: r	P1	rs2228570 T-dosage: r	P2
ALT, U/L	0.276	0.005	0.392	<0.001
AST, U/L	0.348	<0.001	0.467	<0.001
GGT, U/L	0.117	0.245	0.244	0.014
Albumin, g/dL	-0.166	0.098	-0.094	0.354
ALP, U/L	0.126	0.211	0.370	<0.001
Total bilirubin, mg/dL	0.379	<0.001	0.346	<0.001
INR	0.353	<0.001	0.410	<0.001
Creatinine, mg/dL	-0.115	0.256	-0.085	0.398
Total cholesterol, mg/dL	0.369	<0.001	0.309	0.002
Triglycerides, mg/dL	0.440	<0.001	0.409	<0.001
HDL, mg/dL	-0.297	0.003	-0.185	0.065
LDL, mg/dL	0.181	0.072	0.124	0.218
AFP, ng/mL	0.286	0.004	0.293	0.003
CRP, mg/dL	0.483	<0.001	0.523	<0.001
FIB-4 index	0.244	0.014	0.437	<0.001
Serum 25(OH)D, ng/mL	-0.499	<0.001	-0.578	<0.001
CAP score	0.539	<0.001	0.654	<0.001
Steatosis grade	0.412	0.003	0.490	<0.001

WBC, white blood cells; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; HDL/LDL, high-/low-density lipoprotein; AFP, alpha-fetoprotein; CRP, C-reactive protein; FIB-4, fibrosis-4 index; 25(OH)D, 25-hydroxyvitamin D; CAP, controlled attenuation parameter.

Table (6): Multivariable regression analysis of genotype carrier models for steatosis and derived fibrosis risk

Outcome	Predictor	B	Adjusted OR (Exp B)	95% CI	P value
NAFLD/steatosis presence	rs7975232 A-carrier (CA/AA vs CC)	2.47	11.86	3.30–54.20	<0.001
NAFLD/steatosis presence	rs2228570 T-carrier (CT/TT vs CC)	0.66	1.94	0.45–10.30	0.364
CAP score	rs7975232 A-carrier (CA/AA vs CC)	8.62	—	−1.83–19.08	0.106

CAP score	rs2228570 T-carrier (CT/TT vs CC)	12.77	—	7.21–18.34	<0.001
Moderate/severe steatosis (S2–S3)	rs7975232 A-carrier (CA/AA vs CC)	2.20	9.00	1.77–138.49	0.014
Moderate/severe steatosis (S2–S3)	rs2228570 T-carrier (CT/TT vs CC)	3.37	28.94	7.68–125.68	<0.001
Elevated FIB-4 risk (≥1.3)	rs7975232 A-carrier (CA/AA vs CC)	1.77	5.88	1.05–65.56	0.044
Elevated FIB-4 risk (≥1.3)	rs2228570 T-carrier (CT/TT vs CC)	0.32	1.38	0.12–25.19	0.772

NAFLD, Non-Alcoholic Fatty Liver Disease , FIB-4, Fibrosis-4 index, CAP, Controlled attenuation parameter

Figure F1. ROC curves for prediction of NAFLD/steatosis presence using vitamin D and genotype models.

