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Association of polymorphisms in *GCKR* and *TRIB1* with nonalcoholic fatty liver disease and metabolic syndrome traits

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Abstract. In several genome-wide association studies, nonalcoholic fatty liver disease and alanine aminotransferase susceptibility variants have been identified in several genes, including *LYPLAL1*, *ZP4*, *GCKR*, *HSD17B13*, *PALLD*, *PPP1R3B*, *FDFT1*, *TRIB1*, *COL13A1*, *CPN1*, *ERLIN1*, *CWF19L1*, *EFCAB4B*, *PZP*, and *NCAN*. To investigate the relationship between these genes and nonalcoholic fatty liver disease in the Japanese population, we genotyped 540 patients and 1012 control subjects for 18 variations. We performed logistic regression analyses to characterize the association between the tested variations and nonalcoholic fatty liver disease. Metabolic syndrome and histological traits were also analyzed by linear regression. We also examined *GCKR* rs780094, *TRIB1* rs2954021, and *PNPLA3* rs738409 for epistatic effects. The A-allele of rs780094 in *GCKR* ($P = 0.0024$) and the A-allele of rs2954021 *TRIB1* ($P = 4.5 \times 10^{-5}$) were significantly associated with nonalcoholic fatty liver disease. *GCKR* rs780094 was also associated with decreased plasma glucose, and increased triglycerides in the patient and control groups. *GCKR* rs780094 was also associated with an increased ratio of visceral to subcutaneous fat area in the patients with nonalcoholic fatty liver disease. Variations in *GCKR*, *TRIB1*, and *PNPLA3* independently influenced nonalcoholic fatty liver disease and had no epistatic effects. Our data suggest variations in *GCKR* and *TRIB1* are involved in the development of nonalcoholic fatty liver disease.

Key words: *GCKR*, *TRIB1*, Nonalcoholic fatty liver disease, Metabolic syndrome, Japanese

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) has been recognized as an important health concern [1, 2]. NAFLD is a spectrum of liver diseases ranging from simple steatosis, through steatohepatitis (NASH), to fibrosis and ultimately cirrhosis. The frequency of patients presenting with NAFLD has increased in Japan in proportion to the increase in the population with metabolic syndrome [3]. NAFLD is observed in 20–30% of the population in Japan and

approximately 1–3% of them are considered to have NASH, similar to American and European populations [3, 4].

In addition to environmental factors, genetic factors are important in the development of NAFLD [5]. In a previous search for these genetic factors, we found that variations in peroxisome proliferator-activated receptor γ coactivator 1 α (*PPARGC1A*), angiotensin II type 1 receptor (*AGTRI*), and nitric oxide synthase 2 (inducible) (*NOS2*) are associated with NAFLD in Japanese individuals [6–8]. Genome-wide association studies (GWAS) have shown that SNPs in the patatin-like phospholipase domain containing 3 (*PNPLA3*) influence NAFLD and plasma liver enzymes [9–12]. We reported that the risk allele (G) of rs738409 in *PNPLA3* is strongly associated with NAFLD, as well as with

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increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and fibrosis stage in Japanese patients with NAFLD [13]. In a recent GWAS, we found that polymorphisms in the SAMM50 sorting and assembly machinery component (*SAMM50*), parvin, β (*PARVB*), and *PNPLA3* were associated with the development and progression of NAFLD [14].

Several other susceptibility loci for NAFLD [9-12, 14] and ALT [15-17] have been reported in GWAS, but these loci have not been confirmed in the Asian population. We investigated the association between SNPs identified by GWAS and NAFLD in the Japanese population.

Materials and Methods

Study subjects

The entire study was conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from each subject, and the protocol was approved by the ethics committee of Kyoto University, Yokohama City University, Hiroshima University, and Kurume University.

We enrolled 1012 control subjects (general population) from among Japanese volunteers undergoing medical examinations for common disease screening. Control subjects were retained from our previous study (control-2) [14]. Japanese patients with NAFLD who underwent liver biopsy (488 with NASH and 52 with simple steatosis) were enrolled; 392 (NAFLD-1) and 98 (NAFLD-2) of these subjects were retained from the previous study [14]. Control and NAFLD subjects were collected at Yokohama City University Hospital, Hiroshima University Hospital, and Kurume University Hospital. Patients with the following diseases were excluded from the study: viral hepatitis (hepatitis B and C, Epstein-Barr virus infection), autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, hemochromatosis, α 1-antitrypsin deficiency, Wilson's disease, drug-induced hepatitis, and alcoholic hepatitis (present or past daily consumption of more than 20 g alcohol per day). None of the patients showed clinical evidence of hepatic decompensation, such as hepatic encephalopathy, ascites, variceal bleeding, or a serum bilirubin level greater than two-fold the normal upper limit.

Liver biopsy tissues were stained with hematoxylin and eosin, reticulin, and Masson's trichrome stain. The histological criterion for NAFLD diagnosis is macrovesicular fatty change in hepatocytes with dis-

placement of the nucleus toward the cell edge [18]. When more than 5% of hepatocytes are affected by macrovesicular steatosis, patients are diagnosed as having either steatosis or NASH; minimal criteria for the diagnosis of NASH included the presence of >5% macrovesicular steatosis, inflammation, and liver cell ballooning, typically with predominantly centrilobular (acinar zone 3) distribution [19, 20]. The degree of steatosis was graded as follows, based on the percentage of hepatocytes containing macrovesicular fat droplets: grade 0, no steatosis; grade 1, <33% hepatocytes containing macrovesicular fat droplets; grade 2, 33-66% of hepatocytes containing macrovesicular fat droplets; and grade 3, >66% of hepatocytes containing macrovesicular fat droplets [21]. Hepatitis activity (necroinflammatory grade) was also determined on the basis of the composite NAS, as described by Kleiner *et al.* [22]. NAS is the unweighted sum of the scores for steatosis, lobular inflammation, and hepatocellular ballooning, and ranges from 0 to 8. Fibrosis severity was scored according to the method of Brunt [18] and was expressed on a 4-point scale as follows: 0, none; 1, perivenular and/or perisinusoidal fibrosis in zone 3; 2, combined pericellular portal fibrosis; 3, septal/bridging fibrosis; and 4, cirrhosis.

Clinical and laboratory evaluation

Patient weight and height were measured using a calibrated scale after removing shoes and heavy clothing, if present. Venous blood samples were obtained after overnight fasting (12 h) to measure plasma glucose, hemoglobin A1c (HbA1c), total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, serum AST, and ALT. All blood chemistry was measured using conventional methods.

The patients underwent CT imaging (in the supine position) to measure visceral fat area (VFA) and subcutaneous fat area (SFA) at the umbilical level (L4-L5); these values were calculated using the FatScan software program (N2system, Osaka, Japan) [23]. Clinical characteristics are shown in Table 1.

DNA extraction and SNP genotyping

Genomic DNA was extracted using Genomix (Talent Srl, Trieste, Italy) for blood samples collected from each subject. Invader probes (Third Wave Technologies, Madison, WI, USA) were designed for 18 SNPs previously identified as susceptibility loci for NAFLD [10, 11] or ALT [15-17]. The SNPs were

Table 1 Clinical characteristics

	NAFLD	Control	P
n	540	1012	—
Men/Women	285/255	500/512	0.21 ^{a)}
Type 2 diabetes (%)	250 (46.3%)	66 (6.5%)	1.1×10 ^{-76 a)}
Age (year)	50.5 ± 14.3	53.1 ± 15.3	0.0013
BMI (kg/m ²)	28.0 ± 5.0	22.7 ± 3.2	1.9×10 ⁻¹⁰⁷
Plasma glucose (mg/dL)	118.5 ± 36.1	98.2 ± 19.0	1.2×10 ⁻⁶¹
HbA1c (%)	6.4 ± 1.3	5.5 ± 0.7	6.2×10 ⁻⁶³
Total cholesterol (mg/dL)	212.4 ± 39.3	208.5 ± 36.2	0.21
Triglycerides (mg/dL)	167.0 ± 105.2	110.0 ± 88.5	1.4×10 ⁻⁵⁷
HDL-cholesterol (mg/dL)	52.9 ± 14.8	62.7 ± 15.5	3.3×10 ⁻³⁷
SBP (mmHg)	128.1 ± 14.7	124.5 ± 19.1	1.0×10 ⁻⁴
DBP (mmHg)	78.5 ± 11.6	76.3 ± 11.6	2.2×10 ⁻⁴
AST (IU/L)	50.1 ± 29.9	23.0 ± 10.2	1.2×10 ⁻¹¹⁹
ALT (IU/L)	81.0 ± 56.3	20.3 ± 11.8	4.1×10 ⁻¹⁷¹
VFA (cm ²) ^{b)}	128.6 ± 58.3	—	—
SFA (cm ²) ^{b)}	209.9 ± 102.6	—	—
V/S ratio ^{b)}	0.71 ± 0.39	—	—
Steatosis grade (1-3)	1.6 ± 0.7	—	—
Lobular inflammation (0-3)	1.3 ± 0.7	—	—
Hepatocyte ballooning (0-2)	1.1 ± 0.7	—	—
NAS (0-8)	4.0 ± 1.6	—	—
Fibrosis stage (0-4)	1.7 ± 1.0	—	—

P-values for comparison of the clinical data between the simple steatosis and NASH groups were obtained by the Mann–Whitney U-test. ^{a)}, ratios were analyzed by χ^2 -test. ^{b)}, n = 439

as follows: rs12137855 near lysophospholipase-like 1 (*LYPLALI*); rs2499604 near zona pellucida glycoprotein 4 (*ZP4*); rs780094 in glucokinase (hexokinase 4) regulator (*GCKR*); rs6834314 near hydroxysteroid (17- β) dehydrogenase 13 (*HSD17B13*); rs2710833 near palladin, cytoskeletal associated protein (*PALLD*); rs343062 in chromosome 7, position 35,549,066; rs4240624 and rs2126259 near protein phosphatase 1, regulatory subunit 3B (*PPP1R3B*); rs2645424 in farnesyl-diphosphate farnesyltransferase 1 (*FDFT1*); rs2954021 near tribbles homolog 1 (*TRIB1*); rs1227756 in collagen, type XIII, α 1 (*COL13A1*); rs10883437 and rs11597390 near carboxypeptidase N, polypeptide 1 (*CPNI*); rs2862954 in ER lipid raft associated 1 (*ERLIN1*); rs17668255 in CWF19-like 1, cell cycle control (*CWF19L1*); rs887304 in EF-hand calcium binding domain 4B (*EFCAB4B*); rs6487679 near pregnancy-zone protein (*PZP*); and rs2228603 in neurocan (*NCAN*). SNPs were genotyped by Invader assay as described [24], with a success rate of >98.0%.

Statistical analysis

We categorized the genotypes as 0, 1, or 2, depending on the number of risk alleles present. Odds ratios

(OR) and P-values, adjusted for age, sex, logarithmically transformed body mass index (BMI), and the presence of type 2 diabetes mellitus (DM), were calculated by multiple logistic regression analysis. Multiple linear regression analyses were performed to test the independent effect of each allele on biochemical traits, and histological and anthropometric parameters, accounting for the effects of other variables (i.e., age, sex, and logarithmically transformed BMI). BMI, fasting plasma glucose, triglycerides, AST, ALT, VFA, SFA, and VFA to SFA (V/S) ratio values were logarithmically transformed before performing multiple linear and logistic regression analyses. Simple comparisons of the clinical data between NAFLD and control groups were carried out using the Mann–Whitney U-test. Male: female and the presence of DM ratios were analyzed by χ^2 -test. To test SNP×SNP epistasis for case–control population-based samples, we used the logistic regression model for each SNP1 and SNP2, and fit the model in the form of $Y = \beta_0 + \beta_1 \times \text{SNP1} + \beta_2 \times \text{SNP2} + \beta_3 \times \text{SNP1} \times \text{SNP2} + \beta_4 \times \text{age} + \beta_5 \times \text{sex} + \beta_6 \times \log_{10}(\text{BMI}) + \beta_7 \times \text{DM}$. Statistical analyses were performed using PLINK 1.07 (<http://pngu.mgh.harvard.edu/purcell/plink>) [25] and R software (<http://www.r-project>).

org/). P -values less than 2.8×10^{-3} (0.05/18) was considered significant.

Results

First, we examined the association of 18 SNPs with NAFLD and control subjects. We performed multiple logistic regression analysis using genotypes, age, sex, BMI, and the presence of DM as independent variables. Two SNPs, rs780094 in *GCKR* ($P = 0.0024$) and rs2954021 in *TRIB1* ($P = 4.5 \times 10^{-5}$), were significantly associated with NAFLD (Table 2). No other SNPs showed significant associations with NAFLD.

Minor allele frequencies (MAFs) of eight SNPs were no more than 0.06. The lack of a significant association, especially in those SNPs with small MAF, is most likely due to the relatively low power of this study. All SNPs were in Hardy–Weinberg equilibrium ($P > 0.05$), with the exception of rs12137855 ($P = 0.025$) in the NAFLD patients.

Next, we examined the association of rs780094 and rs2954021 with metabolic syndrome traits in NAFLD. As reported previously [11], rs780094 in *GCKR* is associated with lower plasma glucose ($P = 0.0047$), higher triglycerides ($P = 0.0029$), and higher diastolic blood pressure ($P = 0.018$) (Table 3). An association

Table 2 Association tests of SNPs in patients with NAFLD and control subjects

Chr	SNP ID	position (build 132)	Nearby gene	Allele1/ allele2	Risk allele	Risk allele frequency		P -value	OR (95%CI)
						NAFLD	Control		
1	rs12137855	219,448,378	<i>LYPLAL1</i>	T/C	C	0.94	0.95	0.72	0.93 (0.61 - 1.40)
1	rs2499604	238,103,501	<i>ZP4</i>	A/G	A	0.46	0.48	0.59	0.95 (0.77 - 1.16)
2	rs780094	27,741,237	<i>GCKR</i>	G/A	A	0.62	0.55	0.0024	1.37 (1.12 - 1.68)
4	rs6834314	88,213,808	<i>HSD17B13</i>	G/A	A	0.69	0.66	0.30	1.12 (0.91 - 1.38)
4	rs2710833	169,409,958	<i>PALLD</i>	T/C	T	0.13	0.12	0.53	0.91 (0.67 - 1.23)
7	rs343062	35,549,066	no gene	T/C	T	0.46	0.47	0.92	0.99 (0.81 - 1.22)
8	rs4240624	9,184,231	<i>PPP1R3B</i>	G/A	A	0.99	0.99	0.40	0.64 (0.23 - 1.79)
8	rs2126259	9,185,146	<i>PPP1R3B</i>	A/G	G	0.99	0.99	0.37	0.62 (0.22 - 1.74)
8	rs2645424	11,684,463	<i>FDFT1</i>	C/T	C	0.23	0.21	0.30	1.13 (0.90 - 1.43)
8	rs2954021	126,482,077	<i>TRIB1</i>	A/G	A	0.52	0.45	4.5×10^{-5}	1.53 (1.25 - 1.88)
10	rs1227756	71,588,504	<i>COL13A1</i>	A/G	G	0.72	0.72	0.82	1.03 (0.82 - 1.29)
10	rs10883437	101,795,361	<i>CPN1</i>	A/T	T	0.83	0.82	0.64	0.94 (0.73 - 1.21)
10	rs11597390	101,861,435	<i>CPN1</i>	A/G	G	0.96	0.95	0.80	0.94 (0.59 - 1.51)
10	rs2862954	101,912,064	<i>ERLIN1</i>	C/T	T	0.97	0.95	0.58	1.16 (0.69 - 1.94)
10	rs17668255	102,000,701	<i>CWF19L1</i>	T/C	C	0.97	0.95	0.54	1.17 (0.70 - 1.97)
12	rs887304	3,757,548	<i>EFCAB4B</i>	A/G	A	0.005	0.001	0.70	1.46 (0.22 - 9.64)
12	rs6487679	9,371,332	<i>PZP</i>	C/T	C	0.11	0.11	0.75	1.05 (0.77 - 1.43)
19	rs2228603	19,329,924	<i>NCAN</i>	T/C	T	0.05	0.06	0.41	0.82 (0.51 - 1.32)

The OR for each SNP was adjusted simultaneously for age, sex, logarithmically transformed BMI, and the presence of DM. Bold entries indicate P -value < 0.05 .

Table 3 Tests of association between significant SNPs and metabolic traits in NAFLD

SNP ID	rs780094				rs2954021			
	NAFLD		Control		NAFLD		Control	
	β	P -value	β	P -value	β	P -value	β	P -value
Plasma glucose	-0.018	0.0047	-0.007	0.014	-0.003	0.67	-0.003	0.29
Total cholesterol	0.182	0.94	1.464	0.34	2.663	0.28	0.187	0.90
Triglycerides	0.037	0.0029	0.039	2.5×10^{-5}	0.013	0.30	0.005	0.58
HDL-cholesterol	-0.588	0.49	-0.946	0.12	0.750	0.39	0.014	0.98
SBP	0.000	1.00	-0.280	0.69	-0.180	0.88	-1.102	0.13
DBP	2.222	0.018	-0.082	0.86	-1.115	0.24	-0.311	0.50
AST	0.005	0.72	0.009	0.21	-0.009	0.51	0.007	0.33
ALT	-0.008	0.58	0.019	0.036	-0.017	0.27	0.012	0.18

Data were derived from linear regression analysis. Values of FPG, triglycerides, AST, and ALT were logarithmically transformed. Each metabolic phenotype was adjusted simultaneously for age, sex, and logarithmically transformed BMI. Bold entries indicate P -value < 0.05 .

Table 4 Association between significant SNPs and histological traits in NAFLD subjects

SNP ID	Steatosis grade		Lobular inflammation		Hepatocyte ballooning		NAS		Fibrosis stage	
	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value
rs780094	0.013	0.76	0.006	0.89	0.004	0.92	0.002	0.99	0.049	0.42
rs2954021	0.005	0.92	-0.140	0.0016	-0.072	0.09	-0.214	0.029	-0.152	0.014

Data were derived from linear regression analysis. Each phenotype was adjusted for age, sex, logarithmically transformed BMI, and the presence of DM. Bold entries indicate *P*-value < 0.05.

Table 5 Association between significant SNPs and anthropometric parameters in NAFLD subjects

SNP ID	BMI		VFA*		SFA*		V/S ratio*	
	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value	β	<i>P</i> -value
rs780094	-0.004	0.37	0.023	0.051	-0.011	0.26	0.036	0.0067
rs2954021	-0.007	0.11	-0.024	0.048	-0.023	0.021	0.002	0.87

Data were derived from linear regression analysis. Values of BMI, VFA, SFA, and V/S ratio were logarithmically transformed. BMI and V/S ratio were adjusted for age and sex. VFA and SFA were adjusted for age, sex, and logarithmically transformed BMI. Bold entries indicate *P*-value < 0.05. *, n = 439.

Table 6 Logistic analysis of 3 SNPs on the association between NAFLD and control subjects

Explanatory variables	<i>P</i> -value	OR (95% CI)
rs738409	4.1×10^{-13}	2.20 (1.78 - 2.72)
rs2954021	9.7×10^{-5}	1.52 (1.23 - 1.88)
rs780094	0.0011	1.42 (1.15 - 1.76)

The *P*-value and OR were derived from the following logistic regression model:

$$Y = \beta_0 + \beta_1 \times \text{rs738409} + \beta_2 \times \text{rs2954021} + \beta_3 \times \text{rs780094} + \beta_4 \times \text{age} + \beta_5 \times \text{sex} + \beta_6 \times \log_{10}(\text{BMI}) + \beta_7 \times \text{DM}.$$

between rs780094 with lower plasma glucose ($P = 0.014$) and higher triglycerides ($P = 2.5 \times 10^{-5}$) was also observed in the control subjects. Originally, rs2954021 in *TRIB1* was reported to be associated with ALT [16]; however, we observed no association with ALT or AST in the NAFLD and control subjects. The A-allele of rs2954021 in *TRIB1*, which is a risk allele of NAFLD, was associated with lower lobular inflammation, NAS, and fibrosis grade, and rs780094 in *GCKR* was not associated with histological phenotype (Table 4). We also examined anthropometric parameters in the NAFLD patients and found that rs780094 in *GCKR* was associated with increased V/S ratio ($P = 0.0067$) (Table 5).

Next, we tested SNP×SNP epistasis, including rs738409 in *PNPLA3*, which was an SNP for NAFLD susceptibility. No SNP pairs showed significant epistatic effects on NAFLD (data not shown). We performed multiple logistic regression analysis of three genotypes (rs780094, rs2954021, and rs738409), age, sex, logarithmically transformed BMI, and the presence of DM as independent variables and found that the effects of these SNPs were independent (Table 6).

Discussion

After the first report of an association of *PNPLA3* rs738409 with NAFLD [9], many replication studies and meta-analysis were performed in various populations, verifying the importance of rs738409 in the development of NAFLD [11-14, 26, 27]. GWAS for NAFLD and ALT yielded SNPs in genes other than *PNPLA3* [15-17]; however, a few replication studies produced conflicting results. Among the 18 SNPs in this study, only six SNPs (rs2499604, rs780094, rs2645424, rs1227756, rs2862954, and rs6487679) were included in our previous GWAS [14]. The *P*-values for six SNPs exceeded the cut-off levels (5.0×10^{-5}) and proceeded to the second stage of analysis. The JSNP database (<http://snp.ims.u-tokyo.ac.jp/>) was used as a control; we made no adjustment for age, sex, BMI, or the presence of DM because clinical information was not available. The NAFLD sample size in our GWAS was relatively small (n = 392). Therefore, we investigated 18 SNPs associated with NAFLD and ALT susceptibility, including the six SNPs described

above, in a larger set of NAFLD patients and control subjects for whom clinical information was available.

In this study, we confirmed the association of rs780094 in *GCKR* and rs2954021 in *TRIB1*. The A-allele of rs780094 in *GCKR* was associated with NAFLD in subjects of European descent [11, 28, 29]. No association between the A-allele of rs780094 and NAFLD was observed in African American and Hispanic Americans [28, 29]. A study in Asian populations (Indian, Malay, and Chinese) was not conclusive, due to small sample size [30]. GWAS in the Japanese population reported by Kawaguchi *et al.* showed a weak association ($P=0.011$) [12]. Therefore, rs780094 in *GCKR* is associated with NAFLD in the Japanese population. Other NAFLD susceptibility SNPs in *LYPLAL1*, *PPP1R3B*, and *NCAN* were not associated with NAFLD in our study. *LYPLAL1* rs12137855 and *NCAN* rs2228603 were not associated with NAFLD in another Japanese study [12]. This may be due to the relatively lower power of this study, since the MAFs were no more than 0.05. These results may be also due to ethnic differences in linkage disequilibrium (LD) patterns, ethnic-specific association, and gene/environment interactions.

The A-allele of rs780094 in *GCKR* was associated with decreased fasting plasma glucose and increased triglycerides, as reported by Speliotes *et al.* [11]. An association between increased triglycerides and rs780094 has been reported in previous GWAS [31]. The A-allele of rs780094 in *GCKR* was associated with an increased V/S ratio in our study. These data suggest the A-allele of rs780094 in *GCKR* is related to the development of NAFLD through the increased serum triglycerides caused by visceral fat accumulation.

TRIB1 rs2954021 is associated with increased ALT

[16]. *TRIB1* rs17321515, which is in LD ($r^2 = 1.00$), is associated with increased triglycerides [32]. Although rs2954021 was associated with NAFLD, this SNP was not associated with ALT or triglycerides in our study. Although further study is necessary, these and previous results suggest *TRIB1* rs2954021 is related to the development of NAFLD through increased triglycerides.

We previously showed that *PNPLA3* rs738409 is associated with NAFLD severity [14]. *GCKR* rs780094 and *TRIB1* rs2954021 were not associated with histological traits and ALT levels, suggesting these SNPs are not related to NAFLD severity. *PNPLA3* rs738409 was not associated with metabolic syndrome traits [14]. These results were confirmed in this study (490 subjects overlapped) and *PNPLA3* rs738409 was not associated with VFA ($P = 0.32$) or V/S ratio ($P = 0.14$). The effects of *GCKR*, *TRIB1*, and *PNPLA3* on NAFLD were different. Indeed, *GCKR* rs780094, *TRIB1* rs2954021, and *PNPLA3* rs738409 were independently associated with NAFLD.

In conclusion, *GCKR* rs780094 may be involved in the development of NAFLD but does not affect disease severity. Our study suggests *GCKR* rs780094, *TRIB1* rs2954021, and *PNPLA3* rs738409 affect NAFLD through different mechanisms.

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Disclosure Statement

The authors have nothing to disclose.

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