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Original paper

Association of GCKR and MBOAT7 genetic polymorphisms with non-alcoholic fatty liver disease

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Abstract

Aim of the study: Non-alcoholic fatty liver disease (NAFLD) is one of the most important causes of chronic liver disease (CLD) in both Western and Asian populations. There is wide inter-individual variability in the occurrence of NAFLD and progression to non-alcoholic steatohepatitis (NASH) even after correcting environmental factors, and its true explanation can be provided by heritability. Two such genetic variations, the glucokinase regulator (GCKR) and membrane bound O-acyltransferase domain containing 7 (MBOAT7) genes, in NAFLD patients were studied in the Indian population.

Material and methods: A cross sectional analytical study was conducted in the Department of Gastroenterology at a tertiary care centre. In total 100 subjects in the age range of 18-65 years were included in the study; 50 were patients with NAFLD including fatty liver, NASH and NASH related cirrhosis, and 50 were healthy subjects (No NAFLD). The polymorphisms rs780094 and rs1260326 for GCKR and rs641738 for MBOAT7 were determined using PCR followed by the PCR-RFLP.

Results: GCKR rs780094 minor allele A was more common in NAFLD patients (p = 0.00001). Within the spectrum of NAFLD, the A allele was present frequently among cirrhotics as compared to NASH and fatty liver (p = 0.00001). Morbidly obese individuals showed significant association with the homozygous A allele (p = 0.028). These results were not seen with GCKR rs1260326 across all alleles. In MBOAT7 (rs641738) the frequency of the minor allele T for NAFLD was 84% vs. 80% in healthy subjects (p = 0.79). The association of the T allele among the spectrum of NAFLD was not statistically significant (p = 0.79).

Conclusions: GCKR genetic variant rs780094 was found to be significantly associated with NAFLD. The MBOAT7 (rs641738) genetic variant was not found to be significantly associated with NAFLD.

Key words: SNP, NASH, metabolic syndrome, chronic liver disease.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as the most common cause of chronic liver disease worldwide. The spectrum of NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis, liver cirrhosis and hepatocellular carcinoma [1]. The overall prevalence of NAFLD in Western countries varies in the range 15-40% while in Asian countries it varies in the range 9-40% [2, 3]. Epidemiological studies suggest the prevalence of NAFLD to be around 9-32% in the general Indian population, with a higher incidence amongst overweight/obese and diabetic/prediabetic patients [4, 5]. The occurrence of NAFLD and its progression to non-alcoholic steatohepatitis (NASH) cannot be explained merely based on environmental factors, as familial and twin studies have supported a heritable effect on NAFLD [6, 7]. It has been observed that the concordance of disease severity, including the degree of fibrosis and steatosis, is greater in monozygotic twins than among dizygotic twins. Familial studies demonstrated that first-degree relatives of patients with NAFLD are at much higher risk of the disease than the general population [6, 8].

The role of genetics in NAFLD has been proposed in various studies during the last decade. Among the common genetic variants studied for NAFLD, patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily member 2 (TM6SF2), membrane bound O-acyltransferase domain containing 7 (MBOAT7) and glucokinase regulator (GCKR) are predominantly associated with development and progression of NAFLD [9]. All these genes encode proteins involved in the regulation of hepatic lipid metabolism. GCKR regulates de-novo lipogenesis by controlling the influx of glucose in hepatocytes. The common missense loss-of-function GCKR mutation rs1260326 or rs780094 encoding for the P446L protein variant seems to represent the causal variant underlying the association with hepatic fat accumulation [10]. Specifically, the missense variant rs780094 was associated with a modest risk of having a fatty liver (1.2-fold higher risk of developing NAFLD) [11]. The MBOAT7 gene codes for the enzyme lysophospholipid acyltransferase-1, which causes remodelling of the membranes, through sequential deacylation and reacylation. It catalyses desaturation of the second acyl-chain of phospholipids and specifically transfers a polyunsaturated fatty acid (PUFA), in the form of acyl-CoA, to lysophosphatidylinositol and other lysophospholipids, using as a preferential substrate arachidonoyl-CoA. Thus, it is a fine-tune regulator of the amount of free arachidonic acid, and its loss of function is a potent trigger for hepatic inflammation and fibrosis [12]. Understanding the genetic background may have relevance to surveillance, therapeutic strategies and developing preventive measures for NAFLD. There are limited studies on the genetic background of NAFLD in the Asian population. Hence this study was undertaken to investigate the genetic variants in NAFLD in the Indian population.

Material and methods

Study design and patient selection

The study was carried out after the approval of the Ethical and Scientific Committee of the institute. Informed consent was required to include the patients in the study. This was a cross-sectional analytical study carried out on 100 subjects for 2 years out of whom 50 were NAFLD patients and 50 were healthy subjects.

Patients with non-alcoholic fatty liver disease including fatty liver, NASH and NASH-related cirrhosis were included in the study group. Patients with cirrhosis due to other causes (e.g. viral, autoimmune, Wilson's disease), those with significant alcohol intake (i.e. > 20 grams per day), or those on total parenteral nutrition, pregnant and lactating women, and patients on medications known to induce fatty liver (e.g. amiodarone, oestrogen, tamoxifen, methotrexate) were excluded. A detailed history including demographic profile, comorbidities (diabetes mellitus, hypertension, dyslipidaemia, coronary heart disease), clinical examination, anthropological parameters (e.g. body mass index) and laboratory investigations such as complete blood cell counts, liver function tests, lipid profile, glucose levels, and HbA_{1c} were done for all patients. NAFLD was diagnosed by ultrasound, CT abdomen, MR elastography, FibroScan, ARFI (acoustic radiation force impulse) scan and liver biopsy whenever possible. Healthy controls were those participants who had a normal liver on ultrasound or FibroScan.

Outcome

The primary outcome of the study was to evaluate the association of GCKR (rs780094, rs1260326), MBOAT7 (rs641738) with NAFLD.

Genetic analysis

A 3 ml sample of venous blood was collected from patients in the EDTA bulb. The blood samples were centrifuged at 3000 rpm for 10 minutes. Plasma and buffy coats were separated from the samples and stored at –80°C until further use.

DNA isolation: Genomic DNA was isolated from the buffy coat using the spin-column method of Exgene Cell SV (GeneAll Biotechnology Co. Ltd., Seoul, Korea). The isolated DNA was stored at 4°C until further processing. The allele frequencies of all studied SNP genotypes were in the Hardy-Weinberg equilibrium.

Genotyping

MBOAT7: Primers were manually designed and checked for correct binding sites using primer-BLAST (NCBI) [13]. Amplification-refractory mutation system (ARMS) based sequence-specific primers were designed where the 3' terminal of the reverse primer was changed according to the MBOAT7 mutation (rs641738). The primer sequences used were as follows:

MBOF: 5'-TTTCCTCCTCCAGCAGGA-3' (common forward primer)

MBOR1: 5'-CTGGGGCTCCTCTAGGGA-3' (primer specific for mutant allele (T))

MBOR2: 5'-CTGGGGCTCCTCTAGGGG-3' (primer specific for common allele (C))

Dry desalted primers were obtained from Sigma-Aldrich and reconstituted using an appropriate volume of PCR-grade water as per the manufacturer's instructions before use. PCR was performed using Bioron DFS-Taq polymerase and a complete NH4 Reaction Buffer (Bioron GmbH, Germany). The interpretation was based on a 119 bp amplicon observed with primer combination.

(i) Normal Homozygotes (CC): absence of MBOF and MBOR1 along with detection of MBOF and MBOR2.

(ii) Heterozygotes (CT): detection of MBOF and MBOR1 along with MBOF and MBOR2.

(iii) Mutant Homozygotes (TT): detection of MBOF and MBOR1 along with the absence of MBOF and MBOR2.

GCKR: rs780094 and rs1260326 polymorphisms

Determination of the polymorphism rs780094 and rs1260326 for GCKR was performed using PCR followed by restriction fragment length polymorphism (PCR-RFLP). The primers for both GCKR gene polymorphisms were obtained from the previously described study by Mohás *et al*. [14].

The primer sequences used were as follows for GCKR (rs780094):

Forward primer: 5'- GATTGTCTCAGGCAAA CCTGGTAG-3'

Reverse primer: 5'-CTAGGAGTGGTGGCATA-CACCTG-3'

For GCKR rs1260326, the primer sequences used were as follows:

Forward primer: 5'-TGCAGACTATAGTGGAG-CCG-3'

Reverse primer: 5'-CATCACATGGCCACTGCT-TT-3'

Dry desalted primers were obtained from Sigma-Aldrich and reconstituted using an appropriate volume of PCR-grade water as per the manufacturer's instructions before use. PCR was performed using Bioron DFS-Taq polymerase and a complete KCL Reaction Buffer (Bioron GmbH, Ludwigshafen, Germany). The values of pre-denaturation, primer extension and final extension conditions were the same for both polymorphisms.

Statistical analysis

The association between qualitative variables was assessed by the chi-square (χ^2) test and by Fisher's exact test when the χ^2 test was not valid due to small counts. Comparison of quantitative data measured between binomial qualitative variables was done using the unpaired *t*-test if the data passed the Shapiro-Wilk normality test or the Mann-Whitney *U* test if the data failed the normality test. A p value < 0.05 was considered statistically significant.

Results

Characteristics of study participants

There were 50 patients diagnosed with NAFLD and 50 were healthy subjects (No NAFLD); baseline characteristics of the two groups are shown in Table 1. Out of fifty NAFLD patients (62.0% male, mean age 53.68 years), 20 had fatty liver, 4 patients had biopsy-proven NASH and the remaining patients had NASH-related cirrhosis (n = 26). Genotypes MBOAT7 and GCKR were studied in all subjects. There were no significant differences in age and gender between the two groups (p > 0.05). The mean body mass index (BMI) of NAFLD patients was 28.75 kg/m^2 (morbid obesity – 38.0%, obese – 40.0%, overweight – 12.0%, normal – 10.0%.) Hypertension was present in 54% of NAFLD cases, diabetes was present in 68% of NAFLD cases, and 54% of the NAFLD patients had elevated (more than 150 mg/dl) triglyceride levels. Increased serum levels

Table 1. Baseline characteristics of study participants

Characteristics	NAFLD (mean)	No NAFLD (mean)	*p* value
Age (years) ^	53.68	53.04	0.473
BMI ^	28.75	23.40	8.93E-09
RBS ^	180.62	111.32	3.79E-05
HbA_{1c} ^	7.69	5.01	1.26E-11
Total bilirubin (mg/dl) ^	1.92	0.75	0.001017
Direct bilirubin (mg/dl) ^	1.15	0.20	1.09E-09
AST (mU/ml) ^	73.98	25.78	2.22E-08
ALT (mU/ml) ^	72.98	35.56	0.017332
ALP (mU/ml) ^	103.76	77.62	0.005629
GGTP (mU/ml) ^	55.62	43.76	0.366
Serum albumin (g/dl)	3.05	3.73	4.25E-07
Total proteins (g/dl) ^	6.71	7.08	0.027732
Serum cholesterol ^	143.64	127.30	0.03735
Serum triglycerides ^	174.30	111.02	8.73E-09

Unpaired t-test was applied. ^ Data failed the normality test; hence the Mann-Whitney test was applied. All parameters studied were found to be statistically significant except age and GGTP levels.

NAFLD – patients with nonalcoholic fatty liver disease, No NAFLD – controls, BMI – body mass index, RBS – random blood sugar, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GGTP – γ-glutamyltransferase, ALP – alkaline phosphatase

of ALT, AST, cholesterol, triglycerides, and RBS were found in NAFLD patients as compared to other groups.

MBOAT7 genotype

Presence of the T allele was seen in 84% of NAFLD patients, out of whom the heterozygous trait CT was observed in 80% of patients and the homozygous trait TT was observed in only 4% of patients. Allele T was also observed in 80% of healthy controls. MBOAT7 polymorphisms were similar in NAFLD patients as compared to healthy controls ($p = 0.753$) (Table 2).

Among NAFLD patients ($n = 50$), the T allele was seen in 76.9% of cirrhotic patients, 95% of fatty liver patients and 75% of NASH patients. Also, the T allele was present in 80% of healthy subjects. The association of the T allele among subgroups was not statistically significant among patients with cirrhosis, fatty liver and NASH patients ($p = 0.79$) (Table 3).

Table 2. Distribution of MBOAT7 genotype among study groups

MBOAT7 genotype		Group		Total	*p*-value
		NAFLD	No NAFLD		
CC	*n*	8	10	18	0.753
	%	16.0	20.0	18.0	
CT ^	*n*	40	39	79	
	%	80.0	78.0	79.0	
TT ^	*n*	2	1	3	
	%	4.0	2.0	3.0	
Total	*n*	50	50	100	
	%	100.0	100.0	100.0	

Unpaired t-test was applied. ^ Data failed the normality test; hence the Mann-Whitney test was applied. Presence of the T allele was seen in 84% of NAFLD patients, out of which the hetero-zygous trait CT was observed in 80% of patients and the homozygous trait TT was observed in only 4% of patients. Allele T was also observed in 80% of No NAFLD cases. The difference was not found to be statistically significant (p = 0.79).

Among subgroup analyses based on BMI in the entire cohort ($n = 100$), the T allele of MBOAT7 genotype was seen more commonly in morbidly obese (85%) and obese (80%) subjects. However, this association was not statistically significant ($p = 1.00$) (Table 4).

GCKR genotype rs780094

GCKR rs780094 homozygous allele GG was observed in 24% of the NAFLD patients and 66% of healthy subjects. The frequency of minor allele A was 36% for GA (heterozygous) and 40% for AA (homozygous) in NAFLD patients. The frequency of minor allele A was 28% for GA (heterozygous), and 6% for AA (homozygous) in healthy subjects. GCKR rs780094 polymorphisms had statistically significant association with NAFLD patients as compared to healthy controls ($p < 0.001$) (Table 5) .

Among NAFLD patients ($n = 50$), the A allele was seen in 69.3% of cirrhotic patients (GA = 23.1%, AA = 46.2%) while the homozygous G allele (GG) was seen in 30.7%. The A allele was seen in 80% of fatty liver patients (GA = 50%, AA = 30%) while the homozygous G allele (GG) was seen in 20%. All patients with NASH had the A allele (GA = 50%, AA = 50%). Among healthy controls ($n = 50$), the A allele was present in 34% of subjects. The GCKR rs780094 A allele was statistically significantly associated with subgroups of NAFLD (cirrhosis, fatty liver and NASH) as compared to healthy subjects ($p < 0.001$) (Table 6).

On subgroup analysis based on body mass index, the A allele of GCKR rs780094 genotype was seen more commonly in morbidly obese (70%) with the homozygous A allele seen in 40%. In subjects with normal BMI (69.9%), the homozygous A allele was seen in 33%. The homozygous G allele (GG) was seen in 63%

Table 3. Distribution of MBOAT7 genotype among subgroups

MBOAT7 genotype		Diagnosis				Total	*p*-value
		Cirrhosis#	Fatty liver#	NASH#	No NAFLD		
CC	*n*	6	1	1	10	18	0.795
	%	23.1	5.0	25.0	20.0	18.0	
CT ^	*n*	19	18	3	39	79	
	%	73.1	90.0	75.0	78.0	79.0	
TT ^	*n*	1	1	0	1	3	
	%	3.8	5.0	0.0	2.0	3.0	
Total	*n*	26	20	4	50	100	
	%	100.0	100.0	100.0	100.0	100.0	

^,# Row & column data pooled & chi-square test reapplied with continuity correction. Among NAFLD patients, the T allele was seen in 76.9% of cirrhotic patients, 95% of fatty liver patients and 75% of NASH patients. Also the T allele was present in 80% of No NAFLD subjects. The association of the T allele among subgroups was statistically insignificant (p = 0.79).

Table 4. Distribution of MBOAT7 genotype as per body mass index (BMI) among the study groups

MBOAT7 genotype		BMI				Total	*p*-value
		Morbid obesity#	Obese#	Overweight#	Normal		
CC	*n*	3	6	3	6	18	1.00
	%	15.0	20.0	15.8	19.4	18.0	
CT ^	*n*	17	24	15	23	79	
	%	85.0	80.0	78.9	74.2	79.0	
TT ^	*n*	0	0	1	2	3	
	%	0.0	0.0	5.3	6.5	3.0	
Total	*n*	20	30	19	31	100	
	%	100.0	100.0	100.0	100.0	100.0	

^ # Row & column data pooled & chi-square test reapplied with continuity correction.
The T allele of MBOAT7 genotype was seen more commonly in morbidly obese (85%) and obese (80%) subjects. The association was not statistically significant (p = 1.00).

Table 5. Distribution of GCKR genotype rs780094 among study groups

GCKR genotype rs780094		Group		Total	*p*-value
		NAFLD	No NAFLD		
GG	*n*	12	33	45	0.0000108
	%	24.0	66.0	45.0	
GA	*n*	18	14	32	
	%	36.0	28.0	32.0	
AA	*n*	20	3	23	
	%	40.0	6.0	23.0	
Total	*n*	50	50	100	
	%	100.0	100.0	100.0	

GCKR genotype rs780094 homozygous allele GG was observed in 24% of NAFLD patients and 66% of No NAFLD subjects. Frequency of minor allele A was observed as GA (heterozygous) = 36%, AA (homozygous) = 40% in NAFLD patients and GA = 28%, AA = 6% in No NAFLD subjects. The association of GCKR genotype was statistically significant (p = 0.00001).

Table 6. Distribution of GCKR genotype rs780094 among subgroups

GCKR genotype rs780094		Diagnosis				Total	*p*-value
		Cirrhosis#	Fatty liver#	NASH#	No NAFLD		
GG	*n*	8	4	0	33	45	0.0000108
	%	30.7	20.0	0.0	66.0	45.0	
GA	*n*	6	10	2	14	32	
	%	23.1	50.0	50.0	28.0	32.0	
AA	*n*	12	6	2	3	23	
	%	46.2	30.0	50.0	6.0	23.0	
Total	*n*	26	20	4	50	100	
	%	100.0	100.0	100.0	100.0	100.0	

#Column data pooled & chi-square test reapplied.
Among NAFLD patients, the A allele was seen in 69.3% of cirrhotic patients (GA = 23.1%, AA = 46.2%) (homozygous G allele GG = 30.7%), 80% of fatty liver patients (GA = 50%, AA = 30%) (homozygous G allele GG = 20%) and 100% of NASH patients (GA = 50%, AA = 50%). Also the A allele was present in 34% of No NAFLD subjects. The association of the A allele among subgroups was statistically significant (p = 0.00001).

of obese and 58% of overweight subjects. The association was statistically significant ($p = 0.028$) (Table 7).

GCKR genotype rs1260326

GCKR genotype rs1260326 homozygous allele CC was observed in 40% of the NAFLD patients and 60% of healthy subjects. The frequency of minor allele T in NAFLD patients was 34% for CT (heterozygous), 26% for TT (homozygous). Whereas, the frequency of minor allele T in healthy (No NAFLD) subjects was 20% for CT (heterozygous), 20% for TT (homozygous). The association of the GCKR genotype was statistically insignificant ($p = 0.12$) (Table 8).

Among NAFLD patients ($n = 50$), the T allele was seen in 53.8% of cirrhotic patients (CT = 34.6, TT = 19.2) and homozygous CC was seen in 46%. The T allele was seen in 60% of fatty liver patients (CT = 30%, TT = 30%) while homozygous CC was seen in 40% of fatty liver patients. All patients in the NASH group had the T allele (CT = 50%, TT = 50%). In healthy controls, the T allele was present in 40% of subjects. The association of the T allele with subgroups of NAFLD (cirrhosis, fatty liver and NASH) was not statistically significant as compared to healthy controls ($p = 0.122$) (Table 9).

The T allele of GCKR rs1260326 genotype was seen more commonly in morbidly obese subjects (60%) with the homozygous minor T allele (TT) seen in 20% of morbidly obese subjects. The homozygous C allele (CC) was seen in 68.4% of obese, and 48.4% of overweight subjects and 46.7% of subjects with normal

Table 7. Distribution of GCKR genotype rs780094 as per body mass index (BMI)

GCKR genotype rs780094		BMI				Total	*p*-value
		Morbid obesity	Obese	Overweight	Normal		
GG	*n*	6	12	18	9	45	0.028
	%	30.0	63.2	58.1	30.0	45.0	
GA	*n*	6	4	11	11	32	
	%	30.0	21.1	35.5	36.7	32.0	
AA	*n*	8	3	2	10	23	
	%	40.0	15.8	6.5	33.3	23.0	
Total	*n*	20	19	31	30	100	
	%	100.0	100.0	100.0	100.0	100.0	

The A allele of GCKR rs780094 genotype was seen more commonly in morbidly obese (70%) with homozygous A allele seen in 40% and in subjects with normal BMI (69.9%) the homozygous A allele was seen in 33%. Homozygous G allele (GG) was seen in 63% of obese and 58% of overweight subjects. The association was statistically significant (p = 0.028).

Table 8. Distribution of GCKR genotype rs1260326 among study groups

GCKR genotype rs1260326		Group		Total	*p*-value
		NAFLD	No NAFLD		
CC	*n*	20	30	50	0.122
	%	40.0	60.0	50.0	
CT	*n*	17	10	27	
	%	34.0	20.0	27.0	
TT	*n*	13	10	23	
	%	26.0	20.0	23.0	
Total	*n*	50	50	100	
	%	100.0	100.0	100.0	

GCKR genotype rs1260326 homozygous allele CC was observed in 40% of NAFLD patients and 60% of No NAFLD subjects. Frequency of minor allele T was observed as CT (heterozygous) = 34%, TT (homozygous) = 26% in NAFLD patients and CT = 20%, TT = 20% in No NAFLD subjects. The association of GCKR genotype was statistically insignificant (p = 0.12).

Table 9. Distribution of GCKR genotype rs1260326 among subgroups

GCKR genotype rs1260326		Diagnosis				Total	*p*-value
		Cirrhosis#	Fatty liver#	NASH#	No NAFLD		
CC	*n*	12	8	0	30	50	0.122
	%	46.2	40.0	0.0	60.0	50.0	
CT ^	*n*	9	6	2	10	27	
	%	34.6	30.0	50.0	20.0	27.0	
TT ^	*n*	5	6	2	10	23	
	%	19.2	30.0	50.0	20.0	23.0	
Total	*n*	26	20	4	50	100	
	%	100.0	100.0	100.0	100.0	100.0	

^, # Row & column data pooled & chi-square test reapplied with continuity correction. Among NAFLD patients, the T allele was seen in 53.8% of cirrhotic patients (CT = 34.6, TT = 19.2) (homozygous CC = 46%), 60% of fatty liver patients (CT = 30%, TT = 30%) (homozygous CC = 40%) and 100% (CT = 50%, TT = 50%) of NASH patients. Also the T allele was present in 40% of No NAFLD subjects. The association of the T allele among subgroups was not statistically significant (p = 0.122).

Table 10. Distribution of GCKR genotype rs1260326 as per BMI among study groups

GCKR genotype rs1260326		BMI				Total	*p*-value
		Morbid obesity	Obese	Overweight	Normal		
CC	*n*	8	13	15	14	50	0.512
	%	40.0	68.4	48.4	46.7	50.0	
CT	*n*	8	3	9	7	27	
	%	40.0	15.8	29.0	23.3	27.0	
TT	*n*	4	3	7	9	23	
	%	20.0	15.8	22.6	30.0	23.0	
Total	*n*	20	19	31	30	100	
	%	100.0	100.0	100.0	100.0	100.0	

The T allele of GCKR rs1260326 genotype was seen more commonly in morbidly obese (60%) with the homozygous minor T allele (TT) seen in 20% of morbidly obese subjects. Homozygous C allele (CC) was seen in 68.4% of obese and 48.4% of overweight subjects and 46.7% of subjects with normal BMI. The association was not statistically significant (p = 0.512).

BMI. This association was not statistically significant ($p = 0.512$) (Table 10).

Discussion

Glucokinase regulator and MBOAT7 are among the recently identified genetic variants to be associated with NAFLD. A significant association between GCKR variant rs780094 and NAFLD was found in this study. The association of BMI with MBOAT7 was not found to be statistically significant. NAFLD was observed to be more prevalent in males. MBOAT7 rs641738 was not found to be significantly associated with NAFLD. GCKR rs1260326 was not found to have a statistically significant association with NAFLD.

In this study, MBOAT7 rs641738 was not found to be significantly associated with NAFLD ($p = 0.753$). Our findings were consistent with previous studies performed in other ethnic groups [15-18]. A cross-sectional study conducted by Koo *et al.* in the Asian population found no significant association between the MBOAT7 variant and NAFLD16 [16]. Lin *et al.* also found no association between the MBOAT7 rs641738 variant and hepatic steatosis assessed by ultrasonography in 831 Taiwanese children [15]. The majority of these studies were on the European population. Luukkonen *et al.*

replicated the effects of the MBOAT7 rs641738 variant on NAFLD concerning steatosis, necroinflammation, and fibrosis in 115 European adults [19]. The data suggest that MBOAT7 plays an important role in the development of NAFLD [19].

We identified a significant association between GCKR variant rs780094 and NAFLD (p = 0.00001), which is consistent with findings from previous studies [6, 17, 20, 21]. Corresponding to this study , Yang *et al.* confirmed the association of the GCKR rs780094 variant with NAFLD in Chinese people (p = 0.0072) [22]. The distribution of the A allele among subgroups of NAFLD was also found to be statistically significant. Petta *et al.* also observed an association of GCKR rs780094 with NAFLD severity and progression to NASH [23].

In this study, no association between the GCKR variant rs1260326 and NAFLD was found (p = 0.122). The T allele was most often found in NASH and fatty liver patients. It might suggest its relationship with hepatic steatosis and hepatic fibrosis though it was statistically insignificant. Likewise, Gao *et al.* observed that both GCKR polymorphisms (rs1260326 and rs780094) had no significant association with NAFLD in the Northern Han Chinese population [24]. In contrast, Tan *et al.* observed a significant association of NAFLD with both GCKR rs780094 (p = 0.013) and rs1260326 (p = 0.012) [25]. Santoro *et al.* studied obese children and adolescents of different ethnic backgrounds and found a significant association of the GCKR rs1260326 variant with hepatic steatosis (p = 0.016) [21].

In subgroup analysis based on BMI, GCKR genotype rs780094 was significantly associated with BMI (p = 0.028). In contrast, the rs780094 A allele was shown to be associated with a lower risk of obesity in adult Han Chinese population by Qi *et al.* [26].

There are a few limitations to the study. First, this study had a relatively small sample size.

Second, not all subjects underwent liver biopsy and hence histologic disease severity and its association with genetic polymorphisms was not studied. Lastly, since the study was cross-sectional, it was not possible to study the temporal relation between genetic background and the progression of liver disease over time. Despite such limitations, there are certain strengths of the study. It studied the genetic polymorphism of NAFLD in the Indian population and included a comparison with healthy controls. We performed genetic analysis on the entire study cohort and carried out subgroup analysis based on the spectrum of NAFLD as well as BMI.

To conclude, there is a significant association between the GCKR rs780094 genotype and NAFLD in the Indian population. In this study, the association between GCKR rs1260326 and NAFLD was not significant. Similarly, the MBOAT7 genotype was not significantly associated with NAFLD. GCKR might have an important role to play in Indian patients with NAFLD. Further prospective multi-centre studies spread over demography are needed to estimate the impact of different genetic polymorphisms in NAFLD in India. In the era of precision medicine, this may help in the research of future therapeutics and interventions.

Disclosure

The authors declare no conflict of interest.

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