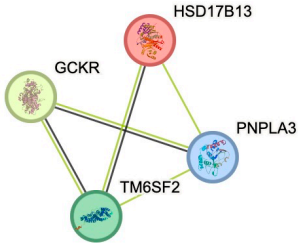
A.

GWAS Study	Mapped Gene	Variant	Location	p-value
GCST90275041	PNPLA3	rs738409	22:43928847	1×10^{-66}
GCST90275041	TM6SF2	rs58542926	19:19268740	7×10^{-53}
GCST90104598	GCKR	rs1260326	2:27508073	7×10^{-14}
GCST010861	HSD17B13	rs9992651	4:87311358	3×10^{-7}

B.



C.

Protein	SNP	Variant Substitution	Substitution Score	Predicted Effect
PNPLA3	rs738409	I148M	0.02	Deliterious
TM6SF2	rs58542926	E167K E167Ter	0.24	Tolerated
GCKR	rs1260326	L446P L446R	0.14 0.31	Tolerated Tolerated
HSD17B13	rs9992651	Intron variant	-	-

Figure S1 A) Screening of Genome-wide association studies catalog for MASLD-related genetic variants identifying 4 genes with variants with p-value < 0.001. B) Protein-protein interaction network using STRING online tool showing the interaction of the four mapped genes. The required score was set to 0.7 (high confidence). C) Prediction of the impact of the selected genetic variants on protein function using the SIFT predictive tool. A score < 0.05 predicts the variation to be “deleterious”; whereas a score ≥ 0.05 would be “tolerated”.

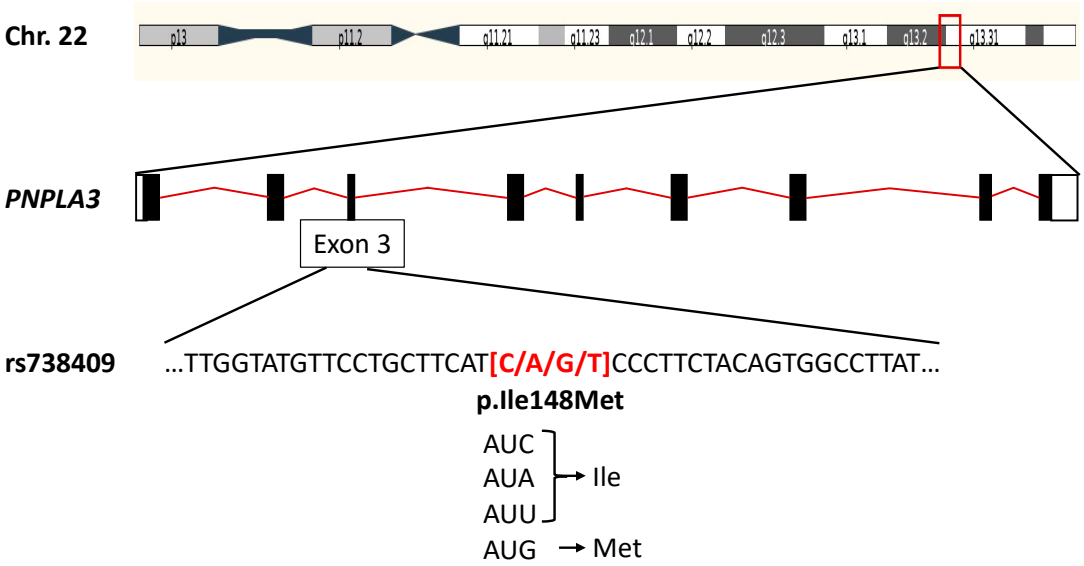


Figure S2 Location of *PNPLA3* gene on chromosome 22 and location of rs738409 within exon 3 with all possible alleles.

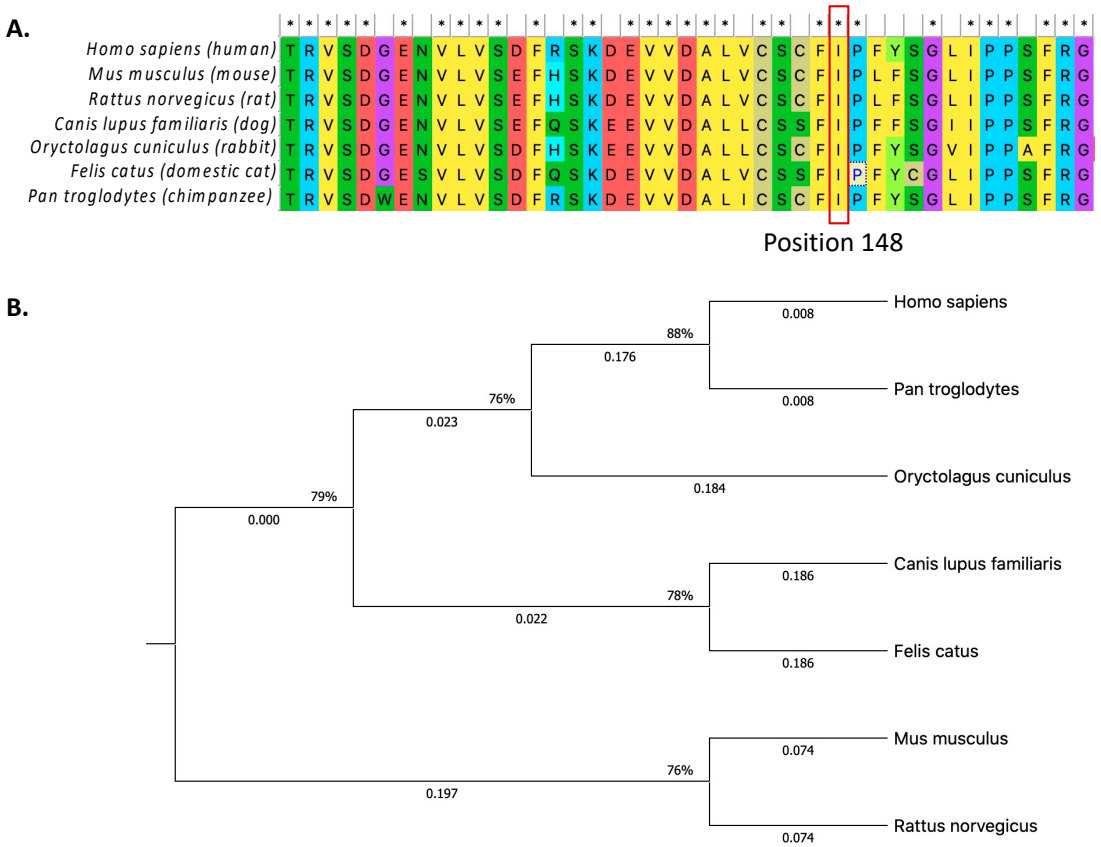


Figure S3 A) Multiple sequence alignment of PNPLA3 protein in common model organisms and domestic animals. The location of the variant (148 in human protein) is marked with a box. B) Phylogenetic tree conducted in MEGA X using UPGMA method with Poisson correction.

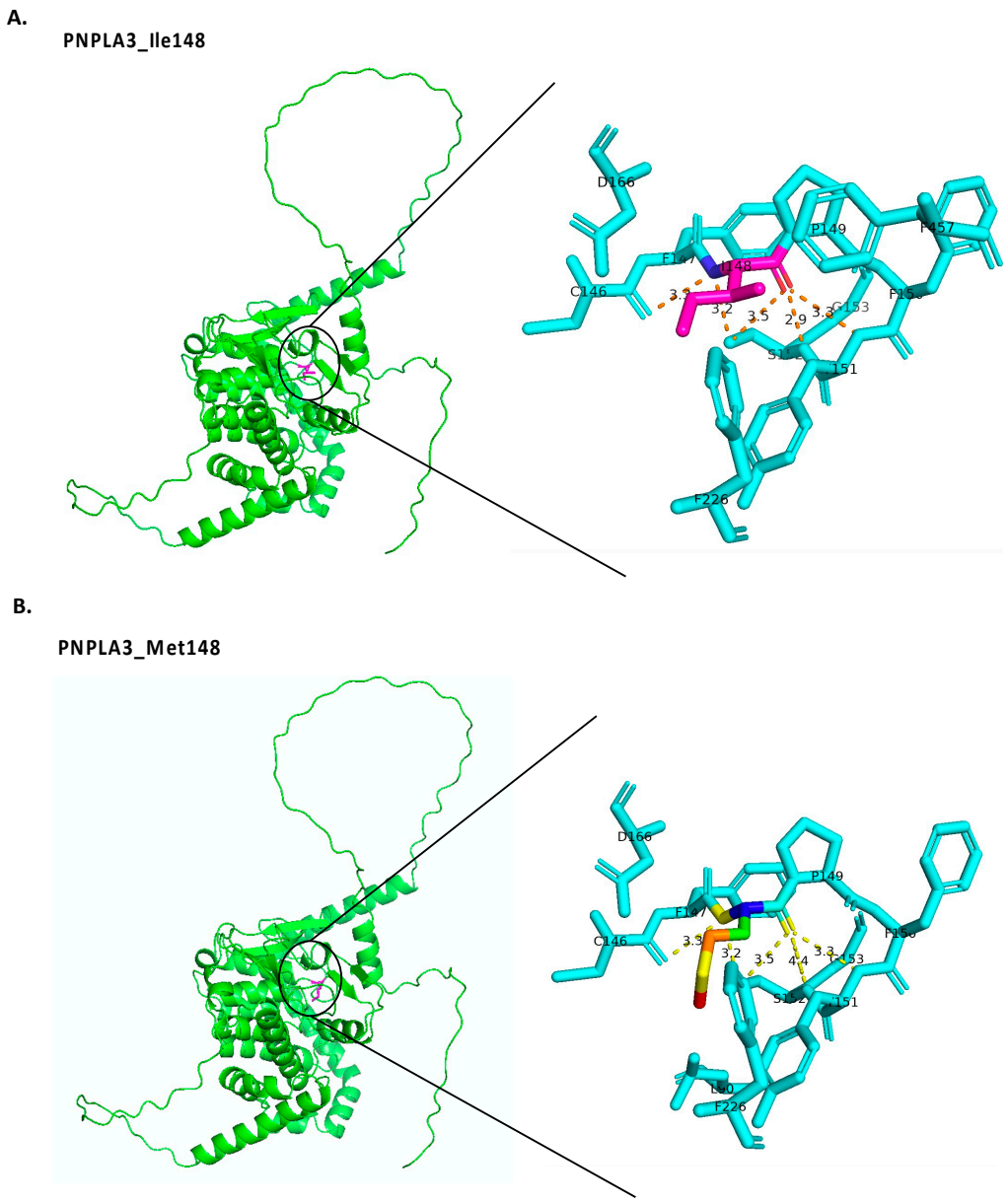


Figure S4 Comparison of the 3D structure of PNPLA3_Ile148 (A) and PNPLA3_Met148 (B) showing the main polar distances where Met148 had a larger distance compared to Ile148.

Table S1: The proportions of participants with genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C in MASLD cases and controls.

	MASLD cases (n = 205)		controls (n = 187)		P-value	FDR corrected p-value
	No.	%	No.	%		
PNPLA3 rs738409 C>G						
Genotype						
CC	100	48.8	119	63.6		
CG	83	40.5	60	32.1	0.004**	0.016*
GG	22	10.7	8	4.3		
$^{HW}\chi^2$(p-value)			0.900			
Allele	(n = 410)		(n = 374)			
C	283	69	298	79.7	0.001**	-
G	127	31	76	20.3		
TM6SF2 rs58542926 C>T						
Genotype						
CC	170	82.9	157	84.0	^{FE}P=0.940	0.940
CT	34	16.6	30	16.0		
TT	1	0.5	0	0.0		
^{HW}FE (p-value)			0.614			
Allele	(n = 410)		(n = 374)			
C	374	91.2	344	92.0		-
T	36	8.8	30	8.0	0.702	
HSD17B13 rs9992651 G>A						
Genotype						
GG	150	73.5	130	71.4	0.279	0.558
GA	46	22.5	49	26.9		
AA	8	3.9	3	1.6		
$^{HW}\chi^2$(p-value)			0.504			
Allele	(n = 408)		(n = 364)			
G	346	84.8	309	84.9	0.973	-
A	62	15.2	55	15.1		
GCKR rs1260326 T>C						
Genotype						
TT	70	34.5	62	34.1	0.824	0.940
TC	102	50.2	88	48.4		
CC	31	15.3	32	17.6		
$^{HW}\chi^2$(p-value)			0.936			
Allele	(n = 406)		(n = 364)			
T	242	59.6	212	58.2	0.701	-
C	164	40.4	152	41.8		

p-value estimated by Chi Square test for categorical datasets, *<0.05; ** < 0.01.
^{FE}P Fisher's Exact corrected p-value.
$^{HW}\chi^2$: Chi square for goodness of fit for Hardy-Weinberg equilibrium.

Table S2: The proportions of participants with genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C in MASLD cases with liver fibrosis, MASLD cases without liver fibrosis and controls.

	MASLD cases with liver fibrosis (n = 131)		MASLD cases without liver fibrosis (n=74)		Controls (n = 187)		P-value	FDR corrected p-value
	No.	%	No.	%	No.	%		
PNPLA3 rs738409 C>G								
Genotype								
CC	59	45	41	55.4	119	63.6	0.003**	0.012*
CG	54	41.2	29	39.2	60	32.1		
GG	18	13.7	4	5.4	8	4.3		
Allele	(n = 262)		(n=148)		(n=374)			
C	172	65.6	111	75	298	79.7	< 0.001 ***	-
G	90	34.4	37	25	76	20.3		
TM6SF2 rs58542926 C>T								
Genotype								
CC	110	84	60	81.1	157	84		
CT	21	16	13	17.6	30	16	^{FE}P=0.526	0.526
TT	0	0	1	1.4	0	0		
Allele	(n = 262)		(n=148)		(n=374)			
C	241	92.0	133	89.9	344	92	0.706	-
T	21	8.0	15	10.1	30	8		
HSD17B13 rs9992651 G>A								
Genotype								
GG	100	76.3	50	68..5	130	71.4	^{FE}P=0.350	0.526
GA	26	19.8	20	27.4	49	26.9		
AA	5	3.8	3	4.1	3	1.6		
Allele	(n = 262)		(n=146)		(n=364)			
G	226	86.3	120	82.2	309	84.9	0.547	-
A	36	13.7	26	17.8	55	15.1		
GCKR rs1260326 T>C								
Genotype								
TT	49	34.5	21	28.4	62	34.1	0.504	0.526
TC	59	50.2	43	58.1	88	48.4		
CC	21	15.3	10	13.5	32	17.6		
Allele	(n = 258)		(n=148)		(n=364)			
T	157	60.9	85	57.4	212	58.2	0.740	-
C	101	39.1	63	42.6	152	41.8		

p-value estimated by Chi Square test for categorical datasets, ** < 0.01; *** < 0.001.
^{FE}P Fisher's Exact corrected p-value.

Table S3. Association of genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C in MASLD cases with liver fibrosis, MASLD cases without liver fibrosis, and controls (After adjusting for the risk factors (age, sex, BMI, WC, HDL, TG, diabetes mellitus, and hypertension)).

	Control (Ref.) (n = 187) versus MASLD cases with liver fibrosis (n = 131)		Control (Ref.) (n = 187) versus MASLD cases without liver fibrosis (n = 74)	
	p-value [1]	OR (95%C.I)	p-value [2]	OR (95%C.I)
PNPLA3 rs738409 C>G				
Genotype				
CC(Ref.) (n=219)		1.000		1.000
CG (n=143)	0.783	1.234(0.277 -5.486)	0.632	0.715 (0.181-2.828)
GG (n=30)	0. 477	9.348 (0.020 -4454.064)	0.043*	9.085(1.073-76.885)
TM6SF2 rs58542926 C>T				
Genotype				
CC(Ref.) (n=327)		1.000		1.000
CT (n=64)	0.242	3.434 (0.435-27.107)	0.594	0.647 (0.131 -3.201)
TT (n=1)	0.995	0.994(0.182-5.429)	-	-
HSD17B13 rs9992651 G>A				
Genotype				
GG(Ref.) (n=280)		1.000		1.000
GA (n=95)	0.219	3.439 (0.480-24.641)	0.940	0.941 (0.188-4.705)
AA (n=11)	1.000	-	0.455	1.759 (0.400-7.739)
GCKR rs1260326 T>C				
Genotype				
TT(Ref.) (n=132)		1.000		1.000
TC (n=190)	0.436	0.489(0.081-2.962)	0.922	0.925 (0.191-4.474)
CC(n=63)	0.414	0.275(0.012-6.100)	0.353	2.728 (0.328-22.689)

Ref., reference group; OR, odds ratio; C.I., confidence interval; p-value[1] and p-value[2] estimated by logistic regression analysis, *<0.05; ** < 0.01.

Table S4: The proportions of participants with genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C in subjects with different severities of liver fibrosis.

PNPLA3 rs738409 C>G					
	CC n(%)	CG n(%)	GG n(%)	P value	FDR corrected p-value
F0-1 F2 F3 F4	160(73) 43(19.6) 14(6.4) 2(1)	89(62.2) 36(25.2) 12(8.4) 6(4.2)	12(40) 11(36.7) 5(16.7) 2(6.7)	^{FE}P=0.003**	0.012*
TM6SF2 rs58542926 C>T					
	CC n(%)	CT n(%)	TT n(%)	P value	FDR corrected p-value
F0-1 F2 F3 F4	217(66.4) 75(22.9) 27(8.3) 8(2.4)	43(34.1) 15(12.9) 4(6.4) 2(3.1)	1(100) 0(0) 0(0) 0(0)	^{FE}P=0.943	0.943
HSD17B13 rs9992651 G>A					
	GG n(%)	AG n(%)	AA n(%)	P value	FDR corrected p-value
F0-1 F2 F3 F4	180(64.3) 70(25) 24(8.6) 6(2.1)	69(72.6) 16(16.8) 7(7.4) 3(3.2)	6(54.5) 4(36.4) 0(0) 1(9.1)	^{FE}P=0.260	0.365
GCKR rs1260326 T>C					
	TT n(%)	TC n(%)	CC n(%)	P value	FDR corrected p-value
F0-1 F2 F3 F4	83(62.9) 35(26.5) 9(6.8) 5(3.8)	131(68.9) 44(23.2) 13(6.8) 2(1.1)	42(66.7) 11(17.5) 7(11.1) 3(4.8)	^{FE}P=0.274	0.365

^{FE}P Fisher's Exact corrected p-value, *<0.05; ** < 0.01.

Table S5. Association of genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C with different severity of liver fibrosis in MASLD by logistic regression analysis (After adjusting for the risk factors (age, sex, BMI, WC, HDL, TG, diabetes mellitus, and hypertension).

	F0-1 (Ref.) (n = 261) VS F2 (n = 90)		F0-1 (Ref.) (n = 261) VS F3 (n = 31)		F0-1 (Ref.) (n = 261) VS F4 (n = 10)	
	p-value [1]	OR (95%C.I)	p-value [2]	OR (95%C.I)	p-value [3]	OR (95%C.I)
PNPLA3 rs738409 C>G						
Genotype CC(Ref.) (n=219) CG (n=143) GG (n=30)	 0.585 0.008**	 1.000 1.098(0.785-1.537) 5.482 (1.572-19.112)	 0.606 0.017 *	 1.000 1.135(0.701-1.840) 9.641 (1.493-62.233)	 0.299 0.034*	 1.000 1.666 (0.635 -4.372) 107.296 (1.411-8157.152)
TM6SF2 rs58542926 C>T						
Genotype CC(Ref.) (n=327) CT (n=64) TT (n=1)	 0.734 1.000	 1.000 1.152(0.510-2.604) -	 0.245 1.000	 1.000 0.455(0.121-1.717) -	 0.982 -	 1.000 0.976 (0.121-7.891) -
HSD17B13 rs9992651 G>A						
Genotype GG(Ref.) (n=280) GA (n=95) AA (n=11)	 0.012* 0.949	 1.000 0.341(0.148-0.787) 1.030(0.414-2.561)	 0.190 0.999	 1.000 0.473(0.155-1.448) -	 0.762 0.502	 1.000 0.733 (0.098-5.471) 1.933 (0.282-13.222)
GCKR rs1260326 T>C						
Genotype TT(Ref.) (n=132) TC (n=190) CC(n=63)	 0.206 0.922	 1.000 0.633(0.312-1.286) 0.946(0.311-2.876)	 0.530 0.483	 1.000 0.709(0.242-2.075) 1.660(0.404-6.825)	 0.168 0.839	 1.000 0.262(0.039-1.757) 0.766 (0.059-9.968)

Ref., reference group; OR, odds ratio; C.I., confidence interval; p-value[1], p-value[2], and p-value[3] estimated by logistic regression analysis, *<0.05; ** < 0.01.

Table S6: The proportions of participants with genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C in subjects with different severities of hepatic steatosis.

PNPLA3 rs738409 C>G					
	CC n(%)	CG n(%)	GG n(%)	P value	FDR corrected p-value
S0-1	141(64.4)	70(49)	9(30)	0.001**	0.004**
S2	38(17.4)	32(22.4)	12(40)		
S3	40(18.3)	41(28.7)	9(30)		
TM6SF2 rs58542926 C>T					
	CC n(%)	CT n(%)	TT n(%)	P value	FDR corrected p-value
S0-1	183(56)	37(57.8)	0		
S2	67(20.5)	15(23.4)	0	^{FE}P=0.465	0.465
S3	77(23.5)	12(18.8)	1(100)		
HSD17B13 rs9992651 G>A					
	GG n(%)	AG n(%)	AA n(%)	P value	FDR corrected p-value
S0-1	152(54.3)	57(60)	5(45.5)		
S2	65(23.2)	14(14.7)	3(27.3)		0.465
S3	63(22.5)	24(25.3)	3(27.3)	^{FE}P=0.419	
GCKR rs1260326 T>C					
	TT n(%)	TC n(%)	CC n(%)	P value	FDR corrected p-value
S0-1	68(51.5)	115(60.5)	32(50.8)	0.422	0.465
S2	33(25)	34(17.9)	15(23.8)		
S3	31(23.5)	41(21.6)	16(25.4)		

p-value estimated by Chi Square test for categorical datasets, ** < 0.01.

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^{FE}P Fisher's Exact corrected p-value.

Table S7. Association of genotypes of *PNPLA3* rs738409 C>G, *TM6SF2* rs58542926 C>T, *HSD17B13* rs9992651 G>A, and *GCKR* rs1260326 T>C with different severity of hepatic steatosis in MASLD by logistic regression analysis.

	S0-1 (Ref.) (n = 220) VS S2 (n = 82)		S0-1 (Ref.) (n = 220) VS S3 (n = 90)	
	p-value [1]	OR (95%C.I)	p-value [2]	OR (95%C.I)
PNPLA3 rs738409 C>G				
Genotype CC(Ref.) (n=219) CG (n=143) GG (n=30)	 0.060 0.001**	 1.000 1.696(0.978-2.942) 4.947(1.941-12.609)	 0.006** 0.012*	 1.000 2.065(1.226-3.478) 3.525(1.312-9.471)
TM6SF2 rs58542926 C>T				
Genotype CC(Ref.) (n=327) CT (n=64) TT (n=1)	 0.763 -	 1.000 1.107(0.571-2.147) -	 0.468 -	 1.000 0.771(0.381-1.558) -
HSD17B13 rs9992651 G>A				
Genotype GG(Ref.) (n=280) GA (n=95) AA (n=11)	 0.096 0.649	 1.000 0.574(0.299-1.103) 1.403(0.326-6.045)	 0.956 0.620	 1.000 1.016(0.580-1.779) 1.448(0.336-6.241)
GCKR rs1260326 T>C				
Genotype TT(Ref.) (n=132) TC (n=190) CC(n=63)	 0.086 0.927	 1.000 0.609(0.346-1.072) 0.966(0.460-2.026)	 0.385 0.805	 1.000 0.782(0.449-1.362) 1.097(0.526-2.288)

Ref., reference group; OR, odds ratio; C.I., confidence interval; p-value[1], and p-value[2] estimated by logistic regression analysis, *<0.05; ** < 0.01.

Table S8. A) Clinical characteristics of *PNPLA3* rs738409 G and *TM6SF2* rs58542926 T carriers and non-carriers in the study population.

Phenotype	*PNPLA3* rs738409 C>G		P-value	FDR corrected p-value	*TM6SF2* rs58542926 C>T		P-value	FDR corrected p-value
	Carriers	Non-carriers			Carriers	Non-carriers		
Diabetes (n) (Diabetic/ Prediabetic/ Normal)	(40/6/127)	(38/8/173)	0.364	0.54	(18-2-45)	(60/12/255)	0.226	0.565
Hypertension (n) (Hypertensive/ Normal)	(64/109)	(61/158)	0.054	0.162	(18/47)	(107/220)	0.427	0.864
FBG(mg/dl)	97.23±26.901	96.56±28.982	0.935	0.939	105.68±36.151	95.10±25.854	0.017*	0.128
PPBS(mg/dl)	115.72±33.881	112.73±26.457	0.689	0.939	115.98±25.591	113.66±30.772	0.157	0.565
SBP(mmHg)	119.96±17.263	116.75±12.854	0.029*	0.145	118.15±14.481	118.17±15.152	0.888	0.982
DBP(mmHg)	81.79±10.159	79.95±11.717	0.015*	0.113	80.97±12.111	80.72±10.885	0.922	0.982
WC(cm)	106.29±13.680	106.28±13.268	0.939	0.939	103.0±11.622	106.94±13.688	0.013*	0.128
BMI (kg/m^2)	30.13±5.709	30.27±6.388	0.821	0.939	29.52±5.217	30.34±6.248	0.461	0.864
ALT(U/L)	30.782±27.718	22.333±17.377	0.002**	0.030*	27.631±24.177	25.750±22.652	0.607	0.911
AST (U/L)	28.961±20.710	24.197±11.292	0.052	0.162	26.508±11.899	26.258±17.044	0.200	0.565
GGT (U/L)	31.473±24.184	29.959±32.840	0.066	0.165	27.323±15.735	31.284±31.292	0.774	0.968
TC(mg/dl)	180.069±42.223	187.068±44.515	0.114	0.244	183.40±38.546	184.095±44.593	0.982	0.982
LDL-C(mg/dl)	119.15±36.787	123.805±36.619	0.323	0.538	123.646±32.870	121.374±37.472	0.594	0.911
HDL-C(mg/dl)	38.416±11.055	38.206±11.605	0.907	0.939	38.138±10.504	38.33±11.528	0.749	0.968
TG(mg/dl)	125.457±62.10	124.032±76.150	0.256	0.480	112.338±61.119	127.110±71.718	0.062	0.310

B) Clinical characteristics of *HSD17B13* rs9992651 A and *GCKR* rs1260326 C carriers and non-carriers in the study population.

Phenotype	*HSD17B13* rs9992651 G>A		P-value	FDR corrected p-value	*GCKR* rs1260326 T>C		P-value	FDR corrected p-value
	Carriers	Non-carriers			Carriers	Non-carriers		
Diabetes (n) (Diabetic/ Prediabetic/ Normal)	(21/5/80)	(56/9/215)	0.780	0.947	(54-6-193)	(23-8-101)	0.141	0.423
Hypertension (n) (Hypertensive/ Normal)	(35/71)	(90/190)	0.870	0.947	(78-175)	(45-87)	0.515	0.858
FBG(mg/dl)	98.43±32.636	95.71±24.285	0.771	0.947	97.57±30.778	95.67±22.759	0.659	0.913
PPBS(mg/dl)	115.16±29.238	113.74±30.439	0.350	0.947	114.62±30.598	113.40±29.170	0.817	0.913
SBP(mmHg)	118.18±14.035	118.11±15.556	0.884	0.947	117.32±13.523	119.58±17.720	0.063	0.236
DBP(mmHg)	81.96±13.653	80.32±10.059	0.584	0.947	80.12±11.444	81.88±10.472	0.043*	0.236
WC(cm)	107.01±13.264	106.21±13.538	0.749	0.947	106.38±13.820	106.08±12.498	0.902	0.913
BMI (kg/m^2)	30.51±5.956	30.18±6.149	0.524	0.947	30.21±6.308	30.17±5.559	0.913	0.913
ALT(U/L)	25.160±20.459	26.676±23.919	0.850	0.947	24.561±21.314	29.229±25.494	0.056	0.236
AST (U/L)	25.322±12.571	26.672±17.601	0.947	0.947	24.625±13.320	29.434±20.499	0.014*	0.210
GGT (U/L)	27.858±17.036	31.799±32.985	0.879	0.947	29.735±30.705	32.286±27.024	0.269	0.504
TC(mg/dl)	187.679±41.059	182.461±44.862	0.246	0.947	182.237±43.759	187.735±43.238	0.243	0.504
LDL-C(mg/dl)	124.465±33.936	120.621±38.038	0.323	0.947	121.249±37.531	123.222±34.920	0.750	0.913
HDL-C(mg/dl)	38.226±11.583	38.190±11.255	0.852	0.947	38.379±11.097	38.198±11.499	0.875	0.913
TG(mg/dl)	133.104±85.935	121.921±63.63	0.495	0.947	119.664±60.259	133.303±84.558	0.264	0.504

Values are presented as numbers (n) or mean ± standard deviation.
BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase; FBG, fasting blood glucose; PPBS, post-prandial blood sugar; TC, total cholesterol; TG, triacylglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol. P-value estimated by the Chi-Square test or Mann-Whitney test for categorical or non-normally distributed continuous, respectively, *<0.05; ** < 0.01.

Table S9. A power calculation of sample size to evaluate the effect of genetic variations on MASLD.

MASLD cases	controls	Test family	Total sample size	Effect size	α error probability	Achieved power
PNPLA3 rs738409 C>G						
n=205	n=187	X^2	392	0.3	0.05	0. 9988964
TM6SF2 rs58542926 C>T						
n=205	*n=187*	X^2	392	0.3	0.05	0.9988964
HSD17B13 rs9992651 G>A						
n=204	*n=182*	X^2	386	0.3	0.05	0.9987241
GCKR rs1260326 T>C						
n=203	*n=182*	X^2	385	0.3	0.05	0.9986930

X^2 Chi Square test