



TM6SF2 rs58542926 variant affects postprandial lipoprotein metabolism and glucose homeostasis in NAFLD^S

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Abstract Mechanisms underlying the opposite effects of transmembrane 6 superfamily member 2 (TM6SF2) rs58542926 C>T polymorphism on liver injury and cardiometabolic risk in nonalcoholic fatty liver disease (NAFLD) are unclear. We assessed the impact of this polymorphism on postprandial lipoprotein metabolism, glucose homeostasis, and nutrient oxidation in NAFLD. Sixty nonobese nondiabetic normolipidemic biopsy-proven NAFLD patients and 60 matched controls genotyped for TM6SF2 C>T polymorphism underwent: indirect calorimetry; an oral fat tolerance test with measurement of plasma lipoprotein subfractions, adipokines, and incretin glucose-dependent insulinotropic polypeptide (GIP); and an oral glucose tolerance test with minimal model analysis of glucose homeostasis. The TM6SF2 T-allele was associated with higher hepatic and adipose insulin resistance, impaired pancreatic β -cell function and incretin effect, and higher muscle insulin sensitivity and whole-body fat oxidation rate. Compared with the TM6SF2 C-allele, the T-allele entailed lower postprandial lipemia and nefaemia, a less atherogenic lipoprotein profile, and a postprandial cholesterol (Chol) redistribution from smaller atherogenic lipoprotein subfractions to larger intestinal and hepatic VLDL1 subfractions. Postprandial plasma VLDL1-Chol response independently predicted the severity of liver histology. In conclusion, the TM6SF2 C>T polymorphism affects nutrient oxidation, glucose homeostasis, and postprandial lipoprotein, adipokine, and GIP responses to fat ingestion independently of fasting values. These differences may contribute to the dual and opposite effect of this polymorphism on liver injury and cardiometabolic risk in NAFLD.—Musso, G., U. Cipolla, M. Cassader, S. Pinach, F. Saba, F. De Michieli, E. Paschetta, D. Bongiovanni, L. Framarin, N. Leone, M. Berrutti, F. Rosina, S. Corvisieri, F. Molinaro, A. Sircana, and R. Gambino. TM6SF2 rs58542926 variant affects postprandial lipoprotein metabolism and glucose homeostasis in NAFLD. *J. Lipid Res.* 2017. 58: 1221–1229.

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Nonalcoholic fatty liver disease (NAFLD) confers an increased risk of liver-related complications [largely limited to its progressive form, nonalcoholic steatohepatitis (NASH)], type 2 diabetes (T2DM), and CVD (1, 2). The wide inter-individual variability in the risk of hepatic and extra-hepatic complications in NAFLD may reflect the interplay between genetic and environmental factors. While in the general population an association between the type and amount of dietary fat and the development of obesity, CVD, and T2DM has been demonstrated (3), data linking dietary fat to the presence and severity of NAFLD are controversial (4, 5). We hypothesized that a genetically determined susceptibility to dietary fat lipotoxicity modulates liver injury and cardiometabolic risk in NAFLD.

The SNP, rs58542926 C>T, in the transmembrane 6 superfamily member 2 (TM6SF2) gene has recently been

Abbreviations: AI, adaptation index; AUC, area under the curve; CGI, CP-genic index; CHO, carbohydrate; Chol, cholesterol; CHO_{ox}, carbohydrate oxidation; CRP, C-reactive protein; DI, disposition index; Fat_{ox}, fat oxidation; FFM, fat-free mass; GIP, glucose-dependent insulinotropic polypeptide; HDL-C, HDL-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; IAUC, incremental area under the curve; ICAM, intercellular adhesion molecule; IGI, insulinogenic index; IR, insulin resistance; LDL-C, LDL-cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; npRQ, nonproteic respiratory quotient; OFTT, oral fat tolerance test; OGIS, oral glucose insulin sensitivity index; OGTT, oral glucose tolerance test; OR, odds ratio; oxLDL, oxidized LDL; REE, resting energy expenditure; RQ, respiratory quotient; T2DM, type 2 diabetes; Tg, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; TRLP, triglyceride-rich lipoprotein; VCO₂, carbon dioxide production; VO₂, oxygen consumption.

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linked to the severity of NAFLD in genome-wide association studies (6, 7): the TM6SF2 T-allele, encoding the E167K amino acidic substitution, results in reduced transcript levels of its product protein, which is expressed in humans in the liver, intestine, adipose tissue, and pancreatic β -cells and has unclear biological function (8, 9).

The TM6SF2 C>T variant has been linked to a reduced LDL-cholesterol (LDL-C) level and cardiovascular risk and to an increased risk of T2DM (10, 11). Mechanisms connecting the TM6SF2 C>T polymorphism to liver injury and cardiometabolic risk are unclear. The impaired hepatic VLDL secretion associated with the TM6SF2 T-allele (8, 9) may not be the main mechanism mediating NASH, as enhanced lipid storage into neutral triglycerides (Tgs) protects against liver injury (12). Furthermore, the reduced CVD risk associated with the TM6SF2 T-allele is not fully explained by lower fasting cholesterol (Chol) levels (13). Postprandial lipemia is an emerging cardiometabolic risk factor, independently of fasting lipid levels (14), and dietary fat lipotoxicity has been implicated in liver injury in NASH (3–5): Hypothesizing that dietary fat lipotoxicity may mediate the impact of TM6SF2 on liver disease and cardiometabolic risk in NAFLD, we assessed the effect of the TM6SF2 C>T variant on postprandial lipoprotein metabolism and on glucose homeostasis in biopsy-proven NAFLD patients and healthy controls.

METHODS

Participants

There are no data on the impact of the TM6SF2 C>T variant on postprandial lipoprotein metabolism and glucose homeostasis. Based on available data on the impact of the TM6SF2 C>T variant on fasting lipid levels (6–8, 10) and on the impact of NAFLD on lipoprotein and glucose metabolism (12, 15), considering a type I error of 0.05 and a type II error of 0.20, at least 18 T-allele carriers per arm were needed to detect a significant difference in parameters related to lipoprotein metabolism [incremental area under the curve (IAUC) Tg and LDL-C] and glucose homeostasis (whole-body and tissue insulin sensitivity, β -cell function) within different TM6SF2 genotypes in NAFLD patients.

As obesity, dyslipidemia, and diabetes may modify the effect of the TM6SF2 C>T variant on glucose/lipid metabolism, adipokines, and liver disease, subjects with obesity (BMI ≥ 30 kg/m²), diabetes [fasting plasma glucose ≥ 126 mg/dl or plasma glucose ≥ 200 mg/dl at +2 h on oral glucose tolerance test (OGTT) or antidiabetic drugs], overt dyslipidemia (fasting serum Chol ≥ 200 mg/dl or plasma Tg ≥ 200 mg/dl), or clinical signs/symptoms of CVD were excluded.

Sixty nonobese nondiabetic normolipidemic biopsy-proven NAFLD patients referred to two hepato-metabolic clinics were included (criteria for diagnosis of NAFLD are detailed in the supplemental Appendix). Each pathological feature of liver biopsy was read by a single pathologist (Renato Parente, HUMANITAS Gradenigo) blinded to the patients' clinical-biochemical characteristics and scored according to the NASH Clinical Research Network criteria; NASH was defined according to current recommendations (1).

Sixty randomly identified healthy controls, i.e., nondiabetic nonobese normolipidemic individuals without evidence of CVD,

randomly selected from a population-based cohort study, matched for TM6SF2 C>T genotype, age, gender, BMI, and waist circumference were included (12). Criteria to rule out NAFLD in controls are detailed in the supplemental Appendix.

Patients and controls were characterized for lifestyle habits, routine biochemistry, adipokine profile, markers of inflammation, and endothelial dysfunction, as detailed below. The homeostatic model assessment of insulin resistance (HOMA-IR) index was calculated as the product of the fasting glucose and insulin concentration divided by 22.5 (16). Participants gave their consent to the study, which was conducted according to the Helsinki Declaration and was approved by the Institutional Review Board of San Giovanni Battista Hospital, Turin, Italy.

Genetic analyses. Genotyping for the TM6SF2 rs58542926 C/T SNP utilized the real-time allele discrimination method, using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA). The TaqMan genotyping reaction was run on a 7300HT fast real-time PCR (Applied Biosystems). We also genotyped our population for the PNPLA3 SNP, rs738409 C/G, and for the apoE genotype, which have been previously linked to both NAFLD and lipid metabolism (17), to assess their interference with outcome variables (detailed in the supplemental Appendix).

Dietary and physical activity record. Participants filled in the validated European Prospective Investigation into Cancer and Nutrition (EPIC) 7 day alimentary questionnaire and the Minnesota-Leisure-Time-Physical-Activity questionnaire, and data were analyzed as described in the supplemental Appendix.

Anthropometry. Percent body fat was estimated by the bioelectrical impedance analysis method (TBF-202; Tanita, Tokyo, Japan), closely correlating with dual X-ray absorption (18). Abdominal visceral fat area (square centimeters) was estimated using Stanforth equations validated against computed tomography in blacks and Caucasians (19).

Indirect calorimetry and substrate oxidation rates. After an overnight (12 h) fast, participants underwent indirect calorimetry measurement of oxygen consumption (VO₂) and carbon dioxide production (VCO₂) using an open circuit indirect calorimeter with a ventilated-hood system (Deltatrac™ II; Datex Instrumentarium Corp., Helsinki, Finland) (see supplemental Appendix). Whole-body respiratory quotient (RQ) and nonprotein RQ (npRQ) were calculated as VCO₂/VO₂. Resting energy expenditure (REE) and whole-body carbohydrate (CHO) oxidation (CHO_{ox}) and fat oxidation (Fat_{ox}) rates were calculated from VO₂ and VCO₂ by using stoichiometric equations and appropriate energy equivalents (20). REE and substrate oxidation rates were corrected for fat-free mass (FFM).

Markers of cardiovascular risk/endothelial dysfunction and adipokines. Serum C-reactive protein (CRP) and soluble adhesion molecules, E-selectin and intercellular adhesion molecule (ICAM)-1, were measured as validated markers of CVD risk, endothelial dysfunction, and subclinical atherosclerosis (21, 22) (detailed in the supplemental Appendix). Circulating adipokines, adiponectin, TNF- α , resistin, and leptin, were measured by immunoenzymatic methods (see the supplemental Appendix).

OGTT-derived indexes of glucose homeostasis. Participants underwent a standard 75 g OGTT and indexes of glucose homeostasis were calculated (detailed in the supplemental Appendix). Whole-body oral glucose insulin sensitivity index (OGIS) and

hepatic and muscle insulin resistance (IR) indexes were calculated as previously proposed and validated against clamp in non-diabetic subjects (23, 24). The adipose tissue IR index was calculated as fasting NEFAs \times fasting insulin (15). The minimal model technique was used to calculate the following indexes of β -cell function: the insulinogenic index (IGI), the CP-genic index (CGI), and the two integrated indexes of β -cell function, the disposition index (DI) and adaptation index (AI), which relate β -cell insulin secretion to IR. The DI and AI were previously validated against the frequently sampled intravenous glucose tolerance test in NAFLD and nondiabetic subjects (21, 25), and reliably predict T2DM development (26).

Incretin effect

To assess whether differences in β -cell function were related to a reduced incretin stimulatory effect on β -cells, a frequently sampled intravenous glucose tolerance test was performed and the incretin effect, i.e., the effectiveness of ingested glucose in stimulating β -cell insulin secretion compared with intravenous glucose, was assessed (see the supplemental Appendix).

Oral fat tolerance test. Participants underwent a 10 h oral fat tolerance test (OFTT) (14) with measurement of the following parameters (methods detailed in the supplemental Appendix): 1) Plasma total Chol, Tg, NEFA, and HDL-cholesterol (HDL-C). 2) Tg-rich lipoprotein (TRLP) subfractions and LDL. TRLPs were isolated through preparative ultracentrifugation and their total Tg and Chol content was subsequently measured as described in the supplemental Appendix. Two VLDL subfractions with decreasing Sf values (VLDL1: Sf >100; VLDL2: Sf = 20–100) were separated and their Chol and Tg content was determined (see supplemental Appendix). VLDL apoB48 and apoB100 were separated by SDS-polyacrylamide gel electrophoresis using 3.9% gel (detailed in supplemental Appendix). LDL-C content was measured with a standardized homogeneous enzymatic colorimetric method in order to avoid Tg effects on LDL determination (Sentinel) (see supplemental Appendix). 3) Lipid-induced oxidative stress: oxidized LDLs (oxLDLs). LDL conjugated dienes, validated markers of oxLDLs, were determined by capillary electrophoresis (detailed in supplemental Appendix). 4) Glucose-dependent insulinotropic polypeptide (GIP), adiponectin, and resistin. GIP is an emerging modulator of lipid metabolism independently of its incretin effect on pancreatic β -cell function. Dietary fat is the most potent stimulator of GIP secretion (27) and TM6SF2 protein is expressed by human intestinal cells (12); furthermore, acute and chronic administration of GIP, but not of glucagon-like peptide-1, reduces Fat_{ox} and energy expenditure (28), induces adipocyte dysfunction and proinflammatory adipokine secretion (29), and promotes development of obesity-associated metabolic disorders (30), including NAFLD, which were all reversed by GIP antagonists (28). Plasma GIP, as well as resistin and adiponectin, which have been linked to both liver disease severity and lipoprotein metabolism in NAFLD, were measured as detailed in the supplemental Appendix.

Statistical analysis

Differences across groups were analyzed by ANOVA followed by Bonferroni correction when variables were normally distributed; otherwise, the Kruskal-Wallis test, followed by the post hoc Dunn test, was used. Normality was evaluated by the Shapiro-Wilk test. The Fisher or chi-square test was used to compare categorical variables, as appropriate. Hardy-Weinberg equilibrium was assessed using the χ^2 test. To adjust for multiple comparison testing, the Benjamini-Hochberg false discovery rate correction was applied to raw *P* values in all comparisons; significance was set at an adjusted *P* value threshold of 0.05 (31). The area under the curve (AUC) and IAUC of

parameters measured during the OFTT and the OGTT were computed by the trapezoid method. Due to the low prevalence of TM6SF2 TT homozygotes and to the overlapping clinical characteristics with heterozygous CT carriers, TM6SF2 TT carriers were combined with CT heterozygotes for group comparisons. Differences were considered statistically significant at *P* < 0.05.

Analysis of dietary, anthropometric, and metabolic parameters and of genetic polymorphisms was made using the Spearman correlation test to assess correlation among different variables. Based on available evidence (6–8, 10), the TM6SF2 C>T variant was modeled as a dominant model of inheritance, that is, quantitative predictor variables reflecting the number of risk alleles (0, 1, or 2).

When a relation was found on univariate analysis, multivariate logistic regression was used to identify independent predictors of selected outcome variables of interest, namely: 1) for liver histology, the presence of NASH and of advanced (stage 3) fibrosis; 2) for CVD risk, serum CRP and endothelial adhesion molecules, E-selectin and ICAM-1; 3) for whole-body nutrient oxidation rates, CHO_{ox} and Fat_{ox}; 4) for glucose homeostasis, OGTT-derived parameters of whole-body/tissue IR and of β -cell function; and 5) for postprandial lipid metabolism, the IAUC of Tg, LDL-C, oxLDL, and of the main TRLP subfractions. For this analysis, continuous variables were divided into quartiles and independent predictors of the highest quartile of outcome variables were assessed after log transformation of skewed data. The independent predictors were those variables found to be related to the outcome variables on univariate analysis. Data are expressed as mean \pm SEM, unless otherwise specified (STATISTICA software, 5.1; Statsoft Italia, Padua, Italy).

RESULTS

Subjects' characteristics

The main features of patients and controls grouped according to the TM6SF2 C>T genotype are reported in **Table 1**. In study participants, the prevalence of TM6SF2 CC homozygotes was 64%, of CT heterozygotes was 34%, and of TT carriers was 2%. The distribution of the TM6SF2 CT genotype was in Hardy-Weinberg equilibrium (6, 7, 8). NAFLD, as a group, had higher HOMA, serum CRP, and endothelial adhesion molecules, E-selectin and ICAM-1, and lower HDL-C and adiponectin than controls. Within NAFLD patients and controls, TM6SF2 CT/TT carriers showed lower serum CRP and endothelial adhesion molecules than TM6SF2 CC genotype carriers (Table 1). Among NAFLD patients, 42% had NASH and 16% had advanced fibrosis. The TM6SF2 T-allele carriers had more severe liver histology than their counterpart genotype (Table 1).

Alimentary record

There was no difference in daily total energy, macro- and micro-nutrients, types of fat, and antioxidant vitamin intake between patients with NAFLD and controls and among different TM6SF2 genotypes (not shown).

Indirect calorimetry

While the TM6SF2 C>T variant did not affect REE, the proportion of energy derived from Fat_{ox} and CHO_{ox} differed between TM6SF2 genotypes: TM6SF2 T-allele carriers had lower RQ and nprQ, indicating that they oxidized more fat and less CHO than CC homozygotes (Table 1).

TABLE 1. Main clinical, biochemical, and histological parameters of biopsy-proven NAFLD patients and controls grouped according to TM6SF2 C/T polymorphism (n = 120)

	Controls			NAFLD		
	TCM6F2 CC (n = 40)	TCM6F2 CT/TT (n = 20)	<i>P</i>	TM6SF2 CC (n = 40)	TM6SF2 CT/TT (n = 20)	<i>P</i>
Age (years)	42 ± 2	42 ± 2	0.851	42 ± 2	40 ± 2	0.851
Sex (% males)	68	65	0.693	68	65	0.693
BMI (kg/m ²)	25.6 ± 0.5	25.9 ± 0.6	0.731	25.6 ± 0.5	25.8 ± 0.6	0.690
Fat mass (%)	22 ± 2	22 ± 2	0.872	23 ± 2	22 ± 2	0.232
Waist (cm)	89 ± 3	90 ± 4	0.482	89 ± 2	90 ± 2	0.426
WHR	0.91 ± 0.02	0.91 ± 0.03	0.756	0.92 ± 0.03	0.92 ± 0.03	0.731
AVF (cm ²)	99 ± 5	103 ± 6	0.731	101 ± 5	97 ± 6	0.832
Smokers (%)	31	30	0.410	33	31	0.390
Systolic BP (mm Hg)	118 ± 3	123 ± 2	0.291	121 ± 2	127 ± 2	0.280
Diastolic BP (mm Hg)	80 ± 2	84 ± 2	0.130	83 ± 2	87 ± 5	0.122
AST (U/l)	15 ± 1	16 ± 2	0.591	32 ± 2	41 ± 4 ^c	0.131
ALT (U/l)	19 ± 2	2312	0.678	70 ± 5	88 ± 6 ^c	0.111
GGT (U/l)	35 ± 5	43 ± 4	0.702	89 ± 16	108 ± 18	0.089
Tg (mg/dl)	98 ± 211	86 ± 10	0.879	94 ± 17	85 ± 13	0.561
Total Chol (mg/dl)	179 ± 9	168 ± 7	0.311	187 ± 11	173 ± 12	0.132
HDL-C (mg/dl)	54 ± 2	55 ± 2	0.210	52 ± 2 ^b	54 ± 2 ^c	0.118
LDL-C (mg/dl)	103 ± 6	94 ± 6	0.131	107 ± 5	95 ± 10	0.210
Glucose (mg/dl)	99 ± 3	90 ± 3	0.394	100 ± 10	90 ± 7	0.273
Insulin (μU/ml)	7.2 ± 1.8	6.3 ± 1.2	0.569	13.7 ± 3.8	15.9 ± 6.4	0.543
HOMA-IR	1.9 ± 0.9	1.3 ± 0.8	0.298	3.55 ± 1.1	2.9 ± 0.90	0.220
METS (h/week)	21.2 ± 1.0	22.2 ± 1.7	0.413	22.7 ± 1.5	21.9 ± 1.4	0.639
RQ	0.81 ± 0.01	0.77 ± 0.01	0.001	0.81 ± 0.01	0.78 ± 0.01	0.003
npRQ	0.81 ± 0.02	0.76 ± 0.01	0.001	0.82 ± 0.01	0.77 ± 0.01	0.0009
REE (kcal/24 h/kg/FFM)	29.5 ± 1.8	29.9 ± 2.0	0.711	29.7 ± 1.5	28.4 ± 1.7	0.302
Fat _{ox} (mg/kg/FFM/min)	1.23 ± 0.05	1.54 ± 0.05	0.0009	1.22 ± 0.06	1.50 ± 0.08	0.002
CHO _{ox} (mg/kg/FFM/min)	2.00 ± 0.10	1.42 ± 0.11	0.001	1.99 ± 0.11	1.45 ± 0.10	0.002
Hs-CRP (mg/l)	1.9 ± 0.2	1.1 ± 0.4	0.009	3.1 ± 0.2 ^a	2.0 ± 0.2 ^b	0.001
E-selectin (ng/ml)	31.1 ± 3.1	20.1 ± 4.6	0.010	51.3 ± 4.8 ^a	28.9 ± 3.1 ^b	0.002
ICAM-1 (ng/ml)	239.1 ± 4.6	191.8 ± 5.3	0.028	285.1 ± 5.2 ^a	228.6 ± 6.0 ^b	0.009
TNF-α (pg/ml)	1.20 ± 0.18	1.08 ± 0.21	0.512	1.18 ± 0.17	0.99 ± 0.25	0.471
Leptin (pg/ml)	1,830 ± 399	1,793 ± 224	0.430	1,746 ± 275	1,914 ± 201	0.711
ApoE genotype (%)						
2-3	16	14	0.573	14	16	0.689
3-3	66	67	0.312	67	67	0.911
3-4	18	19	0.690	19	17	0.892
PNPLA3 (%)						
CC	41	55	0.671	41	55	0.671
CG	41	33	0.312	41	33	0.312
GG	8	12	0.218	8	12	0.218
Abdominal obesity (%)	17	20	0.691	17	20	0.691
IGR (%)	19	8	0.231	21	10	0.289
Hypertension (%)	30	27	0.379	51	49	0.592
Low HDL-C (%)	13	9	0.398	16	9	0.401
High Tg (%)	13	9	0.412	14	8	0.379
Met sy (%)	37	29	0.311	40§	31 ^c	0.297
Steatosis (% hep.)	—	—	—	25 ± 3	32 ± 4	0.168
NAFLD activity score	—	—	—	2.0 ± 0.2	4.0 ± 0.3	0.0001
Fibrosis stage	—	—	—	0.2 ± 0.1	1.0 ± 0.2	0.0001
NASH (%)	—	—	—	31	61	0.045

Data are presented as mean ± SEM, unless otherwise specified. Statistically significant *P* values are in bold. AVF, abdominal visceral fat area; BP, blood pressure; hs-CRP, highly sensitive CRP; WHR, waist-on-hip ratio; IGR, impaired glucose regulation; METS, metabolic equivalent of activity; Met sy, metabolic syndrome (according to the joint statement of the American Diabetes Association, the International Diabetes Federation, and the National Heart, Lung, and Blood Institute); MTP, microsomal Tg transfer protein; SREBF, sterol regulatory element-binding factor. Met sy requires the presence of three or more of the following criteria: 1) abdominal obesity, waist circumference ≥102 cm (males) and ≥88 cm (females); 2) high Tgs, ≥150 mg/dl (1.7 mmol/l) or on drug treatment for elevated Tgs; 3) low HDL-C, <40 mg/dl (1.0 mmol/l) (males) or <50 mg/dl (1.3 mmol/l) (females) or on drug treatment for reduced HDL-C; 4) hypertension, systolic BP ≥130 mm Hg and/or diastolic BP ≥85 mm Hg or on drug treatment; 5) high fasting plasma glucose (FPG): FPG ≥100 mg/dl (5.6 mmol/l) or on drug treatment for elevated glucose.

^a*P* < 0.01 versus controls.

^b*P* < 0.05 versus controls bearing the same genotype.

^c*P* < 0.01 versus controls bearing the same genotype.

OGTT-derived indexes of glucose homeostasis

The time course of plasma glucose and serum insulin during the OGTT is reported in supplemental Fig. S1. In patients and controls, TM6SF2 T-allele carriers showed higher hepatic and adipose IR and enhanced muscle insulin sensitivity compared to CC homozygotes. The TM6SF2 CT/TT

genotype also displayed impaired pancreatic β-cell function and incretin effect compared to CC homozygotes (**Table 2**).

OFTT

Within patients and controls, the TM6SF2 CT/TT genotype showed lower postprandial Tg, VLDL1-Tg,

TABLE 2. OGTT-derived indexes of glucose homeostasis in patients with biopsy-proven NAFLD and controls, grouped according to TM6SF2 rs58542926 C/T genotype (n = 120)

	TM6SF2 C/T Genotype					
	Controls			NAFLD		
	CC (n = 40)	CT/TT (n = 20)	<i>P</i>	CC (n = 40)	CT/TT (n = 20)	<i>P</i>
OGIS (ml min ⁻¹ m ⁻²)	427.9 ± 13.5	442.6 ± 15.1	0.318	385.5 ± 7.4 ^a	392.2 ± 11.0 ^a	0.810
Hepatic IR (g/dl glucose·μU/ml _{ins} ·min ⁻¹)	2,615.7 ± 126.4	3,298.4 ± 173.5	0.001	4,180.1 ± 107.4 ^a	4,779.7 ± 182.1 ^b	0.002
Muscle IS	0.014 ± 0.002	0.021 ± 0.001	0.028	0.012 ± 0.001	0.018 ± 0.002	0.002
Adipose IR (mmol/l/pmol/l)	21.2 ± 2.0	30.1 ± 1.4	0.0004	48.6 ± 4.2 ^b	88.4 ± 6.8 ^b	0.0001
Hepatic extraction (%)	74 ± 3	72 ± 4	0.414	73 ± 5	69 ± 7	0.582
IGI (μU _{insulin} g ⁻¹ glucose)	187 ± 11	112 ± 14	0.009	171 ± 19 ^c	106 ± 13 ^b	0.001
CGI (ng _{C-pep} g ⁻¹ glucose)	511 ± 12	401 ± 11	0.0009	502 ± 13 ^c	394 ± 16 ^b	0.001
DI (μU _{insulin} g ⁻¹ glucose ml ⁻¹ m ⁻²)	80,124 ± 4,318	48,615 ± 4,379	0.001	52,136 ± 3,615 ^c	37,639 ± 1,713 ^b	0.0001
AI (ng _{C-pep} g ⁻¹ glucose ml ⁻¹ m ⁻²)	220,709 ± 12,138	175,241 ± 8,136	0.009	189,420 ± 8,372 ^c	142,671 ± 9,139 ^b	0.001
Incretin effect (%)	72.6 ± 3.2	47.3 ± 2.9	0.0002	70.9 ± 3.1	45.2 ± 4.1	0.0001

Data are presented as mean ± SEM, unless otherwise specified. Statistically significant *P* values are in bold. IS, insulin sensitivity.

^a*P* < 0.05 versus controls.

^b*P* < 0.01 versus controls.

^c*P* < 0.05 versus controls bearing the same genotype.

NEFA, and oxLDL responses, a higher increase in postprandial Chol content in the VLDL1 and VLDL2 subfractions of intestinal and hepatic origin, and a slight, but statistically significant, postprandial LDL-C decrease as compared with the TM6SF2 CC genotype (Table 3, Fig. 1A–D, supplemental Fig. S2). The TM6SF2 CT/TT genotype also showed lower postprandial GIP and higher resistin responses than homozygous CC carriers (Table 3, Fig. 1F, G).

Independent predictors of outcome variables on multiple logistic regression analysis

Liver histology. NASH was independently predicted by IAUC VLDL1-Chol [odds ratio (OR) = 1.60; 95% CI, 1.1–2.2; *P* = 0.009], while advanced (stage 3) fibrosis was predicted by IAUC adiponectin (OR = 1.41; 95% CI, 1.1–2.0; *P* = 0.021) and IAUC VLDL1-Chol (OR = 1.53; 95% CI, 1.1–2.2; *P* = 0.010).

Circulating markers of CVD risk. IAUC Tg and IAUC oxLDLs independently predicted CRP (OR = 1.51; 95% CI, 1.05–2.65; *P* = 0.006 and β = 1.48; 95% CI, 1.08–2.54; *P* = 0.005, respectively), E-selectin (OR = 1.56; 95% CI, 1.11–2.61; *P* = 0.002 and OR = 1.54; 95% CI, 1.19–2.63; *P* = 0.0009, respectively), and ICAM-1 (OR = 1.54; 95% CI, 1.18–2.78; *P* = 0.009 and OR = 1.52; 95% CI, 1.07–2.77; *P* = 0.010, respectively). Whole-body Fat_{ox} was independently predicted by IAUC adiponectin (OR = 1.49; 95% CI, 1.14–2.59; *P* = 0.002) and IAUC GIP (β = 0.49; 95% CI, 0.18–0.88; *P* = 0.012). The independent determinants of OGTT-related glucose homeostasis parameters and of postprandial lipoprotein and adipokine responses during the OFTT are reported in Table 4.

DISCUSSION

The main findings of our study are the following: 1) The TM6SF2 C>T variant modulated postprandial lipid metabolism: despite similar fasting lipid levels, TM6SF2 CT/TT

carriers showed lower postprandial Tg, NEFA, and oxLDL responses, higher HDL-C levels, and a Chol redistribution from LDL to larger intestinal and hepatic TRLP subfractions. TM6SF2 T-allele carriers also had higher incretin GIP and resistin elevations after fat ingestion. 2) Postprandial plasma VLDL1-Chol elevation independently predicted the severity of liver histology in NAFLD, while Tg and oxLDL responses were independently associated with markers of CVD risk. 3) The TM6SF2 C>T variant affected tissue IR, pancreatic β-cell function, and whole-body substrate oxidation rate, the latter possibly through modulation of the GIP response to dietary fat.

Postprandial lipemia is an independent cardiometabolic risk factor in the Western world and, consistently, individuals spend most of the day in the postprandial phase rather than in fasting conditions (14). The effect of the TM6SF2 variant on dietary fat metabolism may contribute to the dual and opposite effect of this SNP on liver disease severity and on CVD risk in NAFLD (32): following fat ingestion, TM6SF2 T-allele carriers showed a shift in Chol content from LDL to larger intestinal and hepatic VLDL subfractions, which are preferentially taken-up by liver cells and adipocytes through the LDL receptor-related protein (33, 34) and the VLDL receptor (35), thereby triggering hepatocyte apoptosis and adipocyte dysfunction (33–35). The independent association of postprandial VLDL-Chol response with liver histology is consistent with recent data, demonstrating an important role for TRLP uptake in promoting high fat-induced liver injury (36) and linking Chol concentration in VLDL subclasses to hepatic Chol content, inflammation, and fibrosis (37). These findings suggest that the TM6SF2 T-allele-associated postprandial lipoprotein pattern may divert toxic Chol away from the vessel walls into the liver and adipose tissue, enhancing liver injury and adipose dysfunction and protecting from CVD.

The independent association of CVD risk markers with postprandial Tg and oxLDL responses, which were lower in TM6SF2 T-allele carriers, is also consistent with

TABLE 3. OFTT parameters in patients with NAFLD and controls grouped according to TCM6F2 rs58542926 C/T genotype (n = 120)

Parameter	Controls			NAFLD		
	TCM6F2 CC (n = 40)	TCM6F2 CT/TT (n = 20)	<i>P</i>	TCM6F2 CC (n = 40)	TCM6F2 CT/TT (n = 20)	<i>P</i>
Fasting Tg (mg/dl)	98 ± 11	86 ± 10	0.812	94 ± 15	85 ± 18	0.513
IAUC Tg (mg/dl × h)	141 ± 12	79 ± 10	0.001	525 ± 21 ^b	297 ± 20 ^b	0.00001
Fasting NEFA (mmol/l)	0.35 ± 0.23	0.47 ± 0.28	0.712	0.50 ± 0.29	0.63 ± 0.31	0.711
IAUC NEFA (mmol/l × h)	1.93 ± 0.27	0.82 ± 0.15	0.00009	5.24 ± 0.22 ^b	2.31 ± 0.28 ^c	0.0001
Fasting VLDL1-Tg (mg/dl)	42 ± 9	40 ± 10	0.812	52 ± 12	36 ± 10	0.201
IAUC VLDL1-Tg (mg/dl × h)	408 ± 29	123 ± 14	0.0001	922 ± 37 ^b	497 ± 31 ^c	0.00002
Fasting VLDL2-Tg (mg/dl)	30 ± 7	31 ± 7	0.813	36 ± 8	42 ± 9	0.312
IAUC VLDL2-Tg (mg/dl × h)	56 ± 10	89 ± 13	0.301	137 ± 14	131 ± 19	0.611
Fasting VLDL1-Chol (mg/dl)	10 ± 2	12 ± 2	0.812	14 ± 4	16 ± 4	0.713
IAUC VLDL1-Chol (mg/dl × h)	41 ± 4	92 ± 7	0.00009	97 ± 9 ^c	199 ± 11 ^b	0.000001
Fasting VLDL2-Chol (mg/dl)	15 ± 3	13 ± 3	0.712	18 ± 3	20 ± 4	0.611
IAUC VLDL2-Chol (mg/dl × h)	11 ± 1	32 ± 2	0.00009	37 ± 2 ^c	108 ± 4 ^c	0.000001
Fasting LDL-C (mg/dl)	103 ± 6	94 ± 6	0.131	107 ± 5	95 ± 10	0.210
IAUC LDL-C (mg/dl × h)	-10 ± 2	-24 ± 2	0.003	-20 ± 3 ^{c,d}	-51 ± 3 ^b	0.0001
Fasting VLDL1 apoB48 (mg/dl)	2.1 ± 0.4	2.0 ± 0.5	0.812	2.7 ± 0.9	2.4 ± 0.9	0.511
IAUC VLDL1 apoB48 (mg/dl × h)	4.5 ± 0.9	1.9 ± 0.5	0.0002	8.7 ± 1.4 ^b	4.3 ± 1.0 ^c	0.00001
Fasting VLDL2 apoB48 (mg/dl)	1.8 ± 0.4	1.5 ± 0.4	0.509	2.3 ± 0.6	2.1 ± 0.7	0.421
IAUC VLDL2 apoB48 (mg/dl × h)	1.5 ± 0.3	2.9 ± 0.5	0.008	1.6 ± 0.3	5.8 ± 0.6 ^b	0.0001
Fasting VLDL1 apoB100 (mg/dl)	3.7 ± 1.0	3.5 ± 1.1	0.712	4.5 ± 1.6	4.2 ± 1.7	0.913
IAUC VLDL1 apoB100 (mg/dl × h)	10.0 ± 1.5	3.9 ± 0.9	0.00009	22.4 ± 3.5 ^b	11.7 ± 2.9 ^c	0.00001
Fasting VLDL2 apoB100 (mg/dl)	3.7 ± 0.7	3.2 ± 0.9	0.802	5.2 ± 0.9	4.8 ± 1.1	0.611
IAUC VLDL2 apoB100 (mg/dl × h)	4.6 ± 0.9	8.3 ± 1.0	0.015	13.8 ± 1.9 ^b	24.5 ± 2.6 ^b	0.00001
Fasting LDL C.D. (uA 234 nm/uA 200 nm × 100)	7.3 ± 1.6	7.9 ± 1.8	0.902	7.5 ± 1.8	7.1 ± 1.6	0.616
IAUC LDL C.D. (uA 234 nm/uA 200 nm × 100 × h)	2.1 ± 0.1	0.8 ± 0.2	0.0009	15.1 ± 1.0 ^b	5.2 ± 1.2 ^a	0.00001
Fasting HDL-C (mg/dl)	54 ± 2	55 ± 2	0.210	52 ± 2	54 ± 2	0.212
IAUC HDL-C (mg/dl × h)	-14 ± 2	2 ± 1	0.0001	-56 ± 4 ^b	-18 ± 2 ^c	0.00009
Fasting GIP (pg/ml)	18.8 ± 6.4	16.5 ± 6.1	0.712	22.1 ± 9.5	11.9 ± 5.2	0.211
IAUC GIP (pg/ml × h)	571.9 ± 18.5	266.4 ± 20.1	0.000008	703.9 ± 20.1 ^b	379.6 ± 24.4	0.000002
Fasting adiponectin (ng/ml)	8,631 ± 782	9,515 ± 812	0.412	6,161 ± 572	5,575 ± 650	0.713
IAUC adiponectin (ng/ml × h)	11,071 ± 912	12,916 ± 926	0.513	1,768 ± 246	1,536 ± 494	0.423
Fasting resistin (ng/ml)	3.4 ± 0.9	3.1 ± 1.0	0.912	3.8 ± 0.9	3.3 ± 0.9	0.301
IAUC resistin (ng/ml × h)	0.1 ± 0.1	1.5 ± 0.3	0.008	2.8 ± 1.1 ^a	6.4 ± 11.9 ^b	0.0000001

Oral fat load parameters of patients with NAFLD and controls according to TM6SF2 genotype. Data are presented as mean ± SEM. Statistically significant *P* values are in bold. C.D., conjugated dienes.

^a*P* < 0.05 versus controls.

^b*P* < 0.01 versus controls.

^c*P* < 0.05 versus controls bearing the same genotype.

^d*P* < 0.01 versus controls bearing the counterpart genotype.

an important role for postprandial lipoprotein metabolism in mediating the cardioprotective role of the T-allele observed in large epidemiological studies (7, 10) The lower postprandial Tg response in TM6SF2 T-allele carriers may be due to lower fat absorption or greater chylomicron clearance. The lower increase in NEFA is not consistent with greater chylomicron clearance, which would have increased plasma NEFA through spillover. Additionally, a recent report showed that the TM6SF2 T-allele impairs Tg processing and secretion in enterocytes (38), confirming that reduced Tg absorption may underlie the lower postprandial lipemia observed in TM6SF2 T-allele carriers. If confirmed by larger studies, these findings may have therapeutic implications, as Chol-lowering interventions may reduce Chol hepatotoxicity in TM6SF2 T-allele carriers, irrespective of fasting Chol levels.

We also evaluated the impact of the TM6SF2 SNP on glucose homeostasis, as both NAFLD and the TM6SF2 C>T variant have been associated with an increased risk of T2DM (2, 11). The TM6SF2 gene variant affected tissue insulin sensitivity and pancreatic β-cell function: the TM6SF2 T-allele was associated with an impaired incretin effect and

β-cell function, possibly via reduced incretin secretion or action on β-cells, which express TM6SF2 protein (13). These findings may help to select NAFLD carriers of the TM6SF2 at-risk genotype, who are also at higher risk of T2DM, for targeted preventive interventions improving β-cell dysfunction, including incretin mimetics. An intriguing finding was the impact of the TM6SF2 SNP on muscle insulin sensitivity and whole-body Fat_{ox} rates, both effects related to postprandial adiponectin and GIP responses to fat (Table 4).

Consistent with our data, adiponectin stimulates muscle Fat_{ox} and insulin sensitivity, while GIP potently reduces energy expenditure and Fat_{ox} (39). The link between TM6SF2 and incretins and the role of GIP antagonism to enhance Fat_{ox} and insulin sensitivity warrant future investigation. In the meantime, it should be noted that the GIP increase induced by dipeptidyl peptidase-IV inhibitors, currently evaluated in NAFLD, may attenuate the benefits of glucagon-like peptide-1 elevation (40).

In conclusion, a maladaptive response to a chronic daily repetitive metabolic challenge, like fat ingestion, may link the TM6SF2 C>T variant to liver injury and cardiometabolic

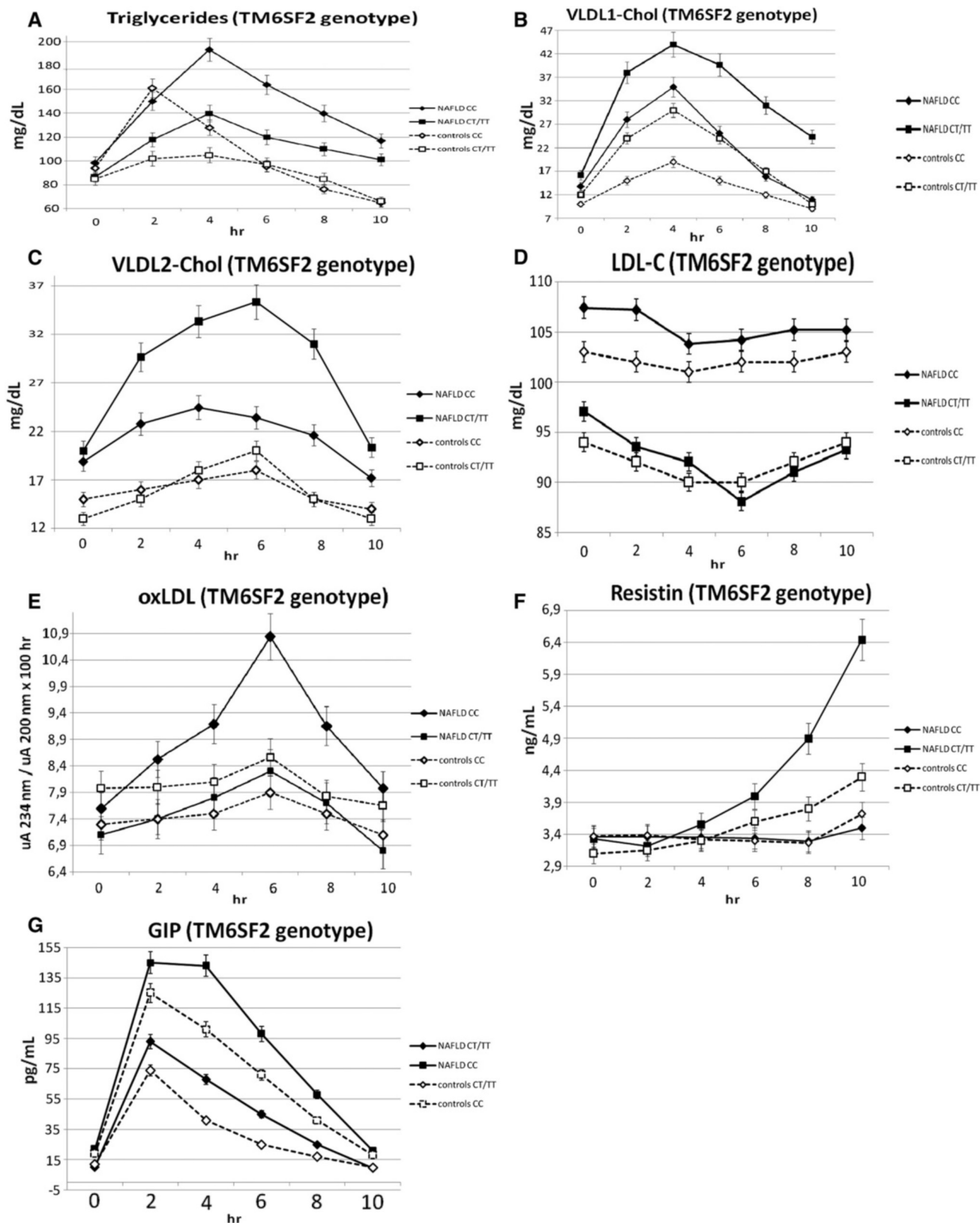


Fig. 1. OFFT: postprandial responses in plasma Tgs (A), VLDL1-Chol (B), VLDL2-Chol (C), LDL-C (D), oxLDL (E), resistin (F), and GIP (G). Patients and controls were grouped according to TM6SF2 genotype. Data are presented as mean \pm SEM (n = 120).

disease in NAFLD. Future research should unravel the underlying molecular pathways in different tissues and organs, allowing therapeutic interventions tailored to

individual risk profile and mechanism of injury (41–43). The strength of our study is the careful selection and thorough characterization of participants. The limitations

TABLE 4. Independent predictors of parameters related to glucose and lipid metabolism in biopsy-proven NAFLD subjects and matched controls on multivariate logistic regression analysis (n = 120)

Outcome Variable	Independent Predictor	OR (95% CI)	P
OGTT-related parameters			
of glucose homeostasis			
OGIS	IAUC adiponectin	1.50 (1.15–2.51)	0.001
Hepatic IR	IAUC adiponectin	0.54 (0.16–0.86)	0.001
	IAUC resistin	1.58 (1.12–2.63)	0.006
Adipose tissue IR	PNPLA3	1.52 (1.06–2.76)	0.008
	IAUC VLDL1-Chol	1.45 (1.05–2.59)	0.002
Muscle IS	IAUC adiponectin	1.47 (1.07–2.46)	0.011
	IAUC GIP	0.49 (0.18–0.91)	0.012
IGI	TM6SF2	0.49 (0.04–0.83)	0.009
	IAUC adiponectin	1.49 (1.04–2.50)	0.004
DI	TM6SF2	0.51 (0.16–0.86)	0.001
	IAUC adiponectin	1.49 (1.12–2.55)	0.009
CGI	TM6SF2	0.46 (0.11–0.81)	0.001
	IAUC adiponectin	1.68 (1.04–2.50)	0.003
AI	TM6SF2	0.43 (0.10–0.70)	0.001
	IAUC adiponectin	1.79 (1.23–2.84)	0.002
Incretin effect	TM6SF2	0.45 (0.11–0.80)	0.009
	IAUC GIP	0.51 (0.16–0.86)	0.007
OFTT parameters			
IAUC Tgs	IAUC adiponectin	0.50 (0.14–0.87)	0.003
	TM6SF2	0.47 (0.02–0.82)	0.001
IAUC VLDL1-Tg	IAUC adiponectin	0.49 (0.13–0.84)	0.001
	TM6SF2	0.43 (0.08–0.78)	0.0009
IAUC VLDL1-Chol	TM6SF2	1.69 (1.11–2.81)	0.00002
IAUC VLDL2-Chol	TM6SF2	1.55 (1.15–2.60)	0.0009
IAUC VLDL1-apoB100	TM6SF2	0.49 (0.13–0.83)	0.002
IAUC VLDL2-apoB100	TM6SF2	0.45 (0.10–0.81)	0.004
IAUC VLDL1-apoB48	TM6SF2	0.44 (0.02–0.80)	0.0001
IAUC VLDL2-apoB48	TM6SF2	0.51 (0.06–0.91)	0.023
IAUC LDL-C	TM6SF2	0.50 (0.15–0.85)	0.003
	Fasting LDL-C	1.91 (0.36–3.11)	0.0008
IAUC LDL conjugated dienes	IAUC VLDL1-Tg	1.89 (1.23–2.95)	0.0001
IAUC HDL-C	IAUC VLDL1-Tg	0.52 (0.17–0.87)	0.009
IAUC GIP	TM6SF2	1.88 (1.21–3.01)	0.001
IAUC resistin	TM6SF2	1.58 (1.13–2.92)	0.012

IS, insulin sensitivity.

are the small number of subjects and the cross-sectional design, which prevents any causal inference between the TM6SF2 variant and the abnormalities in lipid and glucose metabolism, and requires confirmation by larger follow-up studies.

A further caveat is that we did not directly measure hepatic and muscle insulin sensitivity, but rather estimated them from the time course of glucose and insulin during the OGTT. This method assumes a similar intestinal glucose absorption rate across TM6SF2 genotypes, as a faster glucose absorption rate in TM6SF2 T-allele carriers would cause a steeper increase and an earlier peak and fall in plasma glucose regardless of any actual differences in tissue insulin sensitivity. However, the visual inspection of the plasma glucose curve during the OGTT (supplemental Fig. S1) shows a similar slope in the 0–30 min ascending limb of the curve across the TM6SF2 genotypes and the same peak time (+60 min), making differences in glucose absorption very unlikely to occur.

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