TM6SF2 may drive postprandial cholesterol toxicity away from the vessel walls to the liver in NAFLD

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RUNNING TITLE: *TM6SF2* and cholesterol metabolism in NAFLD

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Word count: 598

Tables: 1

ABBREVIATIONS: TM6SF2: Transmembrane 6 superfamily member 2;MRS:magnetic resonance spectroscopy; VLDL: very low density lipoprotein; oxLDL: oxidized low density lipoprotein; IAUC: incremental area under the curve; ICAM: intercellular adhesion molecule; VLDL-Ch: VLDL cholesterol.

KEY WORDS: NAFLD, NASH, TM6SF2, lipemia, postprandial

Conflict of interest: no author has any present or past conflict of interest to disclose

Financial support: this work received no financial support

Author's contributions:

Giovanni Musso: designed research, conducted research, analyzed data, wrote paper, has primary responsibility for final content;

Maurizio Cassader: conducted research, analyzed and discussed data, approved final version of the paper;

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Roberto Gambino: conducted research analyzed and discussed data, approved final version of the paper

To the Editor:

we read with interest the article by Zhou et al.[1] reporting on the impact of the *Transmembrane 6 Superfamily Member 2* gene (*TM6SF2*) rs58542926 C>T variant on circulating triacylgycerol signatures, liver fat content and insulin sensitivity in NAFLD: they found that the TM6SF2 T-allele was associated with higher 1H-MRS-assessed liver fat content, lower fasting triacylgycerol levels and a distinct lipidomic signature. To provide further mechanistic insight into the dual and opposite effect of TM6SF2 C>T variant on liver injury and on cardiovascular disease in NAFLD [2, 3], we examined the impact of this polymorphism on postprandial lipoprotein subfractions and on postprandial plasma cytokeratin-18 fragments, markers of hepatocyte apoptosis [4] in 55 normolipidemic non-diabetic biopsy-proven NAFLD patients and 55 healthy controls, randomly selected from a population-based study [5] and genotyped for TM6SF2 C>T variant by the real-time allele discrimination method, using TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster city, CA).

Participants underwent an oral fat tolerance test as previously described [6].

Due to the low prevalence of homozygous TM6SF2 T-allele carriers (1%), these subjects were analyzed together with heterozygous TM6SF2 C/T carriers. Despite comparable fasting lipid values, TM6SF2 T-allele carriers showed a lower postprandial triglyceridaemia and a striking redistribution of cholesterol from smaller, atherogenic LDL and oxLDL particles to larger VLDL1 and VLDL2 subfractions, as compared with C-allele carriers (Table 1). This phenomenon was reflected by a marked postprandial reduction in triacylgycerol/cholesterol ratio in VLDL particles (Table 1).

On multiple regression analysis, IAUC VLDL1-Cholesterol (VLDL1-Ch) during the oral fat load independently predicted NAFLD activity score (β=0.394, p=0.022), IAUC CK-18 (β=0.412, p=0.018) and fibrosis score (β=0.402, p=0.019), while IAUC oxLDL

predicted circulating E-selectin (β=0.418, p=0.011) and ICAM-1 (β=0.451, p=0.012), validated markers of early atherosclerosis and endothelial dysfunction.

While mechanisms underlying postprandial cholesterol distribution among lipoproteins require kinetic studies to be elucidated, these findings indicate TM6SF2 C>T variant may affect dietary cholesterol lipotoxicity, thereby contributing to the dual and opposite impact of this polymorphism on liver injury and CVD risk in NAFLD. In fact, VLDL1 particles are predominantly taken up by liver cells through the low-density lipoprotein receptor-related protein (LRP) and the VLDL-receptor(VLDLR) and following their uptake, they may trigger high fat-induced hepatocyte apoptosis and Kupffer cell and hepatic stellate cell activation in experimental NASH models [7, 8]. Consistent with these findings, circulating VLDL-Ch closely correlated with hepatic cholesterol content, inflammation, fibrosis, and cell injury in patients with NASH [9] and a growing body of experimental and human evidence supports a role for hepatic cholesterol accumulation in the pathogenesis of liver injury in NASH [10].

TM6SF2 C>T variant may modulate dietary cholesterol distribution among different lipoproteins in the postprandial phase, which accounts for a substantial part of the day in the Western countries nowadays: as different lipoproteins are taken up by different tissues, T-allele diverts toxic cholesterol away from the vessel walls into the liver, thereby promoting liver injury and protecting from atherosclerosis.

While these findings require kinetic studies, they may provide the rationale for evaluating cholesterol-lowering medications, like statins or ezetimibe, to unload the liver of cholesterol accumulation in TM6SF2 T-allele carriers, even in the absence of fasting hypercholerserolemia.

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Author names in bold designate shared co-first authorship

Table 1.Clinical, biochemical, histological and oral fat tolerance test parameters in patients with NAFLD and controls grouped according to TCM6F2 rs58542926 C>T genotype (n=110).

Clinical-histological parameters						
	Controls			**NAFLD**		
Parameter	**TCM6F2 CC (n=35)**	**TCM6F2 CT/TT (n=20)**	**P**	**TCM6F2 CC (n=35)**	**TCM6F2 CT/TT (n=20)**	**P**
Age (yr)	43±3	44±3	0.987	43±2	44±3	0.914
Gender (% males)	60	62	0.718	65	61	0.713
BMI(kg/m2)	25.7±0.5	25.8±0.5	0.815	25.7±0.5	25.8±0.6	0.798
Hs-CRP (mg/L)	1.9±0.3	1.1±0.3	**0.019**	3.0±0.2†	1.9±0.2§	**0.022**
E-selectin (ng/mL)	31.0±3.3	20.2±4.2	**0.019**	51.0±4.2†	28.2±3.0§	**0.009**
ICAM-1 (ng/mL)	243.1±4.3	190.1±5.0	**0.043**	286.1±4,8†	226.2±5.3§	**0.01**
NAFLD Activity Score	-	-	-	1.9±0.2	4.0±0.2	**0.005**
Fibrosis stage	-	-	-	0.2±0.1	1.0±0.2	**0.009**
Oral Fat Tolerance Test parameters						
	Controls			**NAFLD**		
Parameter	**TCM6F2 CC (n=35)**	**TCM6F2 CT/TT (n=20)**	**P**	**TCM6F2 CC (n=35)**	**TCM6F2 CT/TT (n=20)**	**P**
Fasting Tg(mg/dL)	100±11	95±15	0.991	94±11	91±18	0.713
IAUC Tg (mg/dL x hr)	139±11	89±14	**0.01**	482±18†	299±17†	**0.001**
Fasting VLDL1-Tg (mg/dL)	45±11	46±12	0.898	51±11	42±12	0.413
IAUC VLDL1-Tg (mg/dL x hr)	416±31	119±19	**0.004**	951±25†	503±30§	**0.006**
Fasting VLDL1-Ch (mg/dL)	12±4	14±3	0.913	16±5	18±5	0.846
IAUC VLDL1-Ch (mg/dL x hr)	43±5	91±6	**0.002**	102±11§	202±9†	**0.0001**
Fasting VLDL1-Tg/Ch ratio	3.8±0.3	3.3±0.5	0.387	3.2±0.4	2.6±0.6	0.672

IAUC VLDL1-Tg/Ch ratio	9.7±1.1	1.3±0.3	**0.0002**	9.3±1.9§	2.5±0.9†	**0.00003**
Fasting VLDL2-Tg (mg/dL)	31±8	33±9	0.901	38±10	43±10	0.412
IAUC VLDL2-Tg (mg/dL x hr)	64±13	83±18	0.413	139±17	143±21	0.713
Fasting VLDL2-Ch (mg/dL)	16±4	15±5	0.812	19±5	22±6	0.732
IAUC VLDL2-Ch (mg/dL x hr)	13±2	33±4	**0.002**	42±3§	112±5§	**0.0001**
Fasting VLDL2-Tg/Ch ratio	1.9±0.4	2.2±0.6	0.396	2.1±0.5	1.8±0.6	0.578
IAUC VLDL2-Tg/Ch ratio	4.9±0.9	2.3±0.5	**0.008**	3.3±0.8	1.2±0.3	**0.018**
Fasting LDL-C(mg/dL)	28±3	27±6	0.982	25±5	26±4	0.819
IAUC LDL-C (mg/dL x hr)	15±3	-11±3	**0.001**	24±4 # §	-21±5†	**0.002**
Fasting oxLDL (uA 234 nm/uA 200 nm x 100)	7.3±1.6	7.9±1.8	0.912	7.5±1.8	7.1±1.6	0.692
IAUC oxLDL (uA 234 nm/uA 200 nm x 100 x hr)	2.1±0.1	0.8±0.2	**0.001**	15.1±1.0†	5.2±1.2*	**0.00007**
Fasting HDL-C(mg/dL)	56±2	54±2	0.312	50±3	52±4	0,358
IAUC HDL-C (mg/dL x hr)	-11±2	1±2	**0.002**	-51±4†	-23±3§	**0.008**
Fasting CK-18 (I.U./L)	99±10	102±13	0.592	172±18	259±17	**0.030**
IAUC CK-18 (I.U./L x hr)	62±10	159±12	**0.031**	232±19†	389±22†	**0.002**

Data are presented as mean ± SEM, unless otherwise specified. Statistically significant P values are written in bold characters.

Abbreviations: IAUC: incremental area under the curve; Tg: triglyceride; C.D. : conjugated dienes; Ch: cholesterol; CK-18: cytokeratin-18 fragments; ICAM: intercellular adhesion molecule; CRP: C-reactive protein; oxLDL: oxidized LDLs, assessed by capillary electrophoresis as conjugated dienes

* $p<0.05$ vs. controls

† $p<0.01$ vs. controls

§ p<0.05 vs. controls bearing the same genotype

‡ p<0.01 vs. controls bearing the same genotype

¶ p<0.05 vs. controls bearing the counterpart genotype

# p<0.01 vs. controls bearing the counterpart genotype

Differences between groups were analyzed by ANOVA for normal variables; otherwise the Mann-Whitney test was used for nonparametric variables. Normality was evaluated by Shapiro-Wilk test. Fisher or chi square test were used to compare categorical variables, as appropriate. Differences were considered statistically significant at $p<0.05$. 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. Analysis of different parameters and of genetic polymorphisms was made using Spearman correlation test. Genetic polymorphisms were modeled as an additive effect, that is, quantitative predictor variables reflecting the number of risk alleles (0, 1, or 2).When a relation was found on univariate analysis, multiple linear regression and logistic regression analyses were used to estimate relationship between different variables, after log transformation of skewed data.