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RUNNING TITLE: PNPLA3, *TM6SF2* and renal function in NAFLD

Giovanni Musso[1]M.D., Maurizio Cassader[2] Ph.D. Roberto Gambino[2] Ph.D.

[1]*Gradenigo Hospital, Italy*

[2]*Department of Medical Sciences, University of Turin, Italy*

Corresponding author:

Giovanni Musso

Gradenigo Hospital

Corso Regina Margherita 8,

10132 Torino, Italy.

Phone: +39-11-3475944237

Fax: +39118151320

E-mail: giovanni_musso@yahoo.it

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To the Editor:

we read with interest the article by Dongiovanni et al. (1) reporting on the impact of the rs58542926 C>T variant of the *Transmembrane 6 superfamily member 2* gene (*TM6SF2*) on liver histology and cardiovascular disease (CVD) risk in NAFLD. Elucidating mechanisms connecting NAFLD to CVD would have important preventive and therapeutic implications in these patients.

Chronic kidney disease (CKD) is a frequent , underappreciated condition and an established risk factor for CVD (2). In a recent meta-analysis of observational studies, the presence and severity of NAFLD at baseline were associated with an increased incidence and stage of CKD, independently of traditional risk factors (3).

CKD might be a mediator or a marker of an increased CVD risk in NAFLD. We thus explored the impact of the two polymorphisms in *PNPLA3* and *TM6SF2* genes, both modulating liver disease severity and CVD risk in NAFLD, on renal function in a cohort of 202 non-obese non-diabetic subjects (61 with biopsy proven non-cirrhotic NAFLD)(Table 1).CKD, eGFR and microalbuminuria were defined according to current Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (see legend to Table 1)(2).

We found that the presence of PNPLA3 G allele was associated with lower eGFR and with a higher prevalence of microalbuminuria and CKD(Table 1). Conversely, the TM6SF2 T allele was associated with higher eGFR and with a lower prevalence of albuminuria and CKD ($p<0.05$). On multiple regression analysis, PNPLA3 polymorphism remained independently associated with eGFR ($\beta=-0.368$, $p=0.029$) and with a higher risk of microalbuminuria (OR 3.52, 95%CI 1.19-7.81, $p=0.028$) and of CKD (OR 3.87, 95%CI 1.18-7.30, $p=0.0021$).

Whether CKD, a correctable risk factor, mediates the effects of these polymorphisms on CVD risk in NAFLD may have relevant screening and therapeutic implications for these patients and warrants confirmation in large follow-up studies.

Legend to Table 1

∗ $p<0.05$ vs. controls

† $p<0.05$ vs. controls with PNPLA3 CC genotype

Data are presented as mean ± SEM. 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 $p<0.05$. 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.

Abbreviations:

BP: blood pressure; C: cholesterol; CKD: chronic kidney disease, defined by persistent(>3 months) albuminuria and/or eGFR<60 ml/min/1.73 m^2

eGFR: estimated glomerular filtration rate, calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation;

HOMA-IR: homeostasis model assessment of insulin resistance; Met Sy: metabolic syndrome as defined according to the joint statement of American Heart Association, International Diabetes Federation and National Heart Lung and Blood Institute; PNPLA3: patatin-like phospholipase domain-containing 3; TM6SF2: Transmembrane 6

superfamily member 2; Tg: triglycerides.

Microalbuminuria was defined by an albumin-creatinine ratio of 30-300 mG/ on fresh morning urine sample;

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