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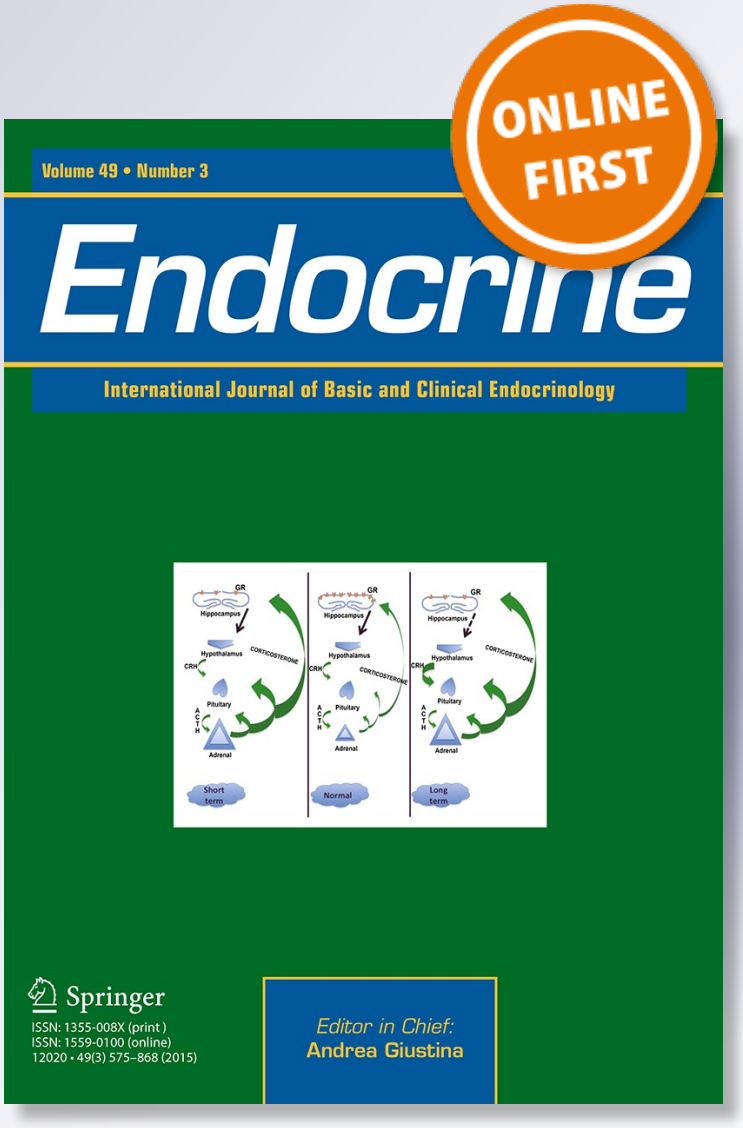

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RESEARCH LETTER

GALNT2 mRNA levels are associated with serum triglycerides in humans

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Introduction

Atherogenic dyslipidemia, characterized by high triglycerides (TG) and low high density lipoprotein (HDL)-cholesterol levels, is a feature of patients with insulin resistance, obesity, and type 2 diabetes (T2D) [1] and plays a major role in shaping the risk of cardiovascular disease. Both TG and HDL-cholesterol serum concentrations are under the control of both environmental factors and up to 95 genetic loci, unraveled by a very large genome-wide association study (GWAs) in approximately 100,000 individuals [2]. Among these loci is *GALNT2,* which encode for ppGal-NAc-T2, involved in O-linked glycosylation. Similarly, studies in rodents have shown that liver *GALNT2* expression modulates HDL-cholesterol concentrations [2]. Based on such studies, it is conceivable that *GALNT2* expression changes play a role on TG and/or HDL-cholesterol levels. To gain further insights about this hypothesis, *GALNT2* expression was measured in peripheral white blood cells (PWBC), from 224 individuals with a wide range of TG and HDL-cholesterol levels, as well as other metabolic parameters and clinical conditions.

Materials and methods

Ethics Statement

Study design and informed consent procedures were approved by the local Institutional Ethic Committee of "Casa Sollievo della Sofferenza" Institute (N°4080/08, 4/8/2009) and performed according to the Helsinki Declaration. All participants gave written informed consent.

Subjects

Fasting blood samples were obtained after overnight rest from 130 non-diabetic individuals (i.e., blood donors) and 94 patients with T2D, recruited at the IRCCS "Casa Sollievodella Sofferenza" (S. Giovanni Rotondo, Gargano, Italy), as a part of an ongoing project on the genetics of T2D and its chronic complications. Anthropometric and clinical features of study subjects were obtained as previously described [3]. Obesity was defined as body mass index (BMI) $\geq$28 kg/m^2. Impaired fasting glucose (IFG) and diabetes were defined as fasting glucose levels of 100–125 and $\geq$126 mg/dl, respectively, after an overnight fast according to ADA 2003 criteria. At variance with patients with T2D who were mostly on anti-hyperglycemic treatments (see "Results" section), IFG individuals ($n = 13$) were not; so, in adjusted and/or stratified analyses, they were grouped together with normoglycemic individuals and then defined as non-diabetic. The insulin resistance index homeostasis model assessment ($HOMA_{IR}$)

Vincenzo Trischitta and Rosa Di Paola have equally supervised the entire study.

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was calculated as fasting serum insulin (mU/l) × fasting plasma glucose (mmol/l)/22.5.

Statistical analyses

Relationships between variables were evaluated by univariate or multivariate analysis, as appropriate, by SPSS 13 software. Since TG levels were not normally distributed, they were log transformed and then used for subsequent analyses. Data are presented as mean ± SD. A p value less than 0.05 was considered as significant. Our sample size had more than 99 % power to detect an association at a p value of 0.001 between *GALNT2* expression and TG levels similar to that reported between TG levels and expression of several other genes in PWBC [4].

RNA extraction, cDNA synthesis, and gene expression analysis from PWBC

Total RNA extraction, cDNA synthesis, and *GALNT2* expression were performed as elsewhere reported [3, 5].

Results

Subjects

Table 1 shows clinical features of study subjects. It is of note that they were characterized by a wide range of both TG and HDL-cholesterol levels, our outcomes of interest. Moreover, study individuals showed a wide range of BMI, excess of adiposity (48.7 % being obese), and glucose homeostasis (5.8 % with IFG and 42 % with T2D). Patients with T2D were mostly treated with anti-hyperglycemic, anti-dyslipidemic (mostly statins), and anti-hypertensive therapies (Table 1). None of non-diabetic individuals was chronically treated with any drug. As expected, TG and HDL-cholesterol levels were strongly and inversely correlated ($r = -0.51$, $p = 2.6 \times 10^{-16}$, Fig. 1a). This association was still significant ($p = 8.4 \times 10^{-15}$) after adjusting for age, gender, and metabolic status as defined by the following conditions: non-obese non-diabetic ($n = 84$), obese non-diabetic ($n = 46$), and diabetic ($n = 94$) individuals.

GALNT2 mRNA expression in PWBC and lipid levels

In all subjects, *GALNT2* mRNA levels were strongly and inversely associated with serum TG concentrations ($r = -0.22$, $p = 0.001$, Fig. 1b). A significant, though less pronounced, direct association was observed between *GALNT2* mRNA and HDL-cholesterol levels ($r = 0.14$, $p = 0.042$). In a model comprising both TG and HDL-cholesterol, *GALNT2* expression was associated with TG ($p = 0.008$), but not with HDL-cholesterol ($p = 0.68$) concentrations. This association was unaffected by age, gender, and metabolic status ($p = 4.3 \times 10^{-4}$).

To address whether insulin resistance mediated the observed association, we took advantage of $HOMA_{IR}$ values available in the 130 non-diabetic individuals. In this subgroup, the association between *GALNT2* expression and serum TG was still significant after adjusting for $HOMA_{IR}$ ($p = 0.028$). Of note, *GALNT2* mRNA levels were not

Table 1 Clinical features of study subjects

Sex (M/F)	156/68
Age (years)	48.6 ± 12.3 (21–80)
BMI (Kg/m^2)	28.8 ± 5.4 (20.4–52.5)
HDL-cholesterol (mg/dl)	48.9 ± 14.8 (21–108)
Triglycerides (mg/dl)	146.7 ± 98.7 (28–767)
Obesity (yes/no)	140/84
IFG (yes/no)	13/211
Diabetes (yes/no)	94/130
Anti-hyperglycemic treatment in diabetic patients	
Diet only	5
OAD	46
Insulin ± OAD	43
Anti-dyslipidemic treatment in diabetic patients (yes/no)	72/22
Anti-hypertensive treatment in diabetic patients (yes/no)	67/27

Continuous variables were reported as mean ± SD and range in parentheses, whereas categorical variables as total number

M males, *F* females, *BMI* body mass index, *Obesity* BMI ≥ 28 kg/m^2, *IFG* Impaired Fasting Glucose (i.e., fasting glucose 100–125 mg/dl), *Diabetes* fasting glucose ≥126 mg/dl, *OAD* oral anti-diabetes drugs

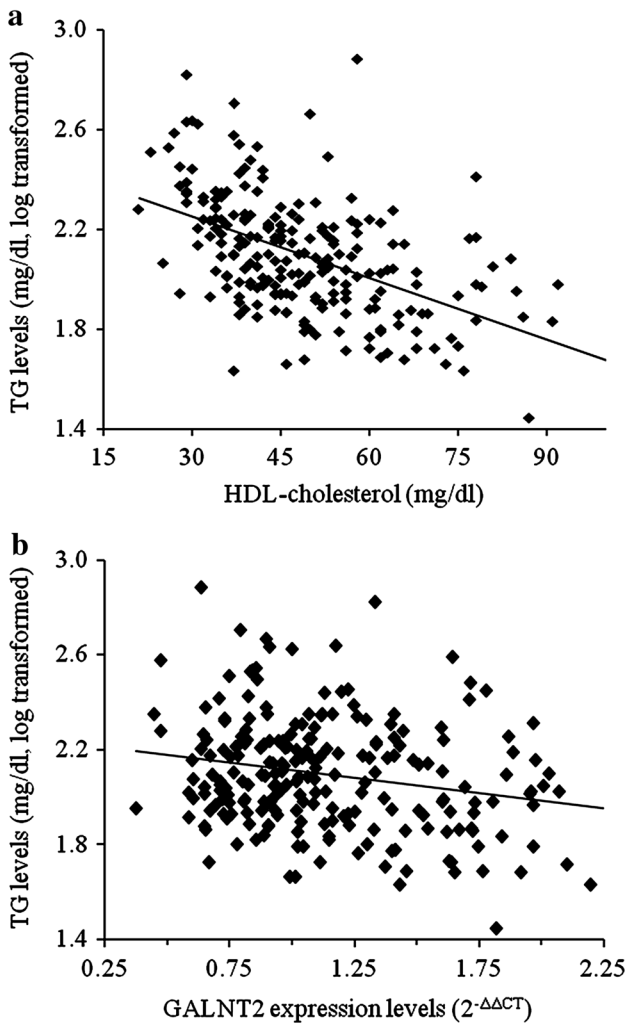

Fig. 1 **a** *Association between plasma TG and HDL-cholesterol levels.* TG and HDL-cholesterol levels were inversely and significantly correlated in 224 individuals ($r = -0.51$, $p = 2.6 \times 10^{-16}$). **b** *Association between plasma TG and GALNT2.* GALNT2 mRNA expression in PWBC was inversely and significantly correlated with plasma TG levels ($r = -0.22$, $p = 0.001$)

correlated with $HOMA_{IR}$ values ($p = 0.53$). In all, these results speak against a role of insulin resistance in the relationship between *GALNT2* and TG.

Among diabetic patients, the association between *GALNT2* and TG levels was still significant when the presence of anti-dyslipidemic treatment was considered as a covariate ($p = 0.007$).

Discussion

This study clearly shows that *GALNT2* expression in human circulating blood cells is strongly associated with serum TG levels in a large sample of individuals with a wide range of metabolic features, such as lipid levels, adiposity measures, and glucose homeostasis. This correlation was independent of several possible confounders, including gender, age, obesity, and diabetes; also insulin resistance in non-diabetic individuals and anti-dyslipidemic treatment in diabetic individuals did not affect the observed association. Although, by their intrinsic nature, association studies cannot inform about cause–effect relationships, our present finding reinforces previous data from GWAs, pointing *GALNT2* as a direct modulator of TG levels in humans [2]. In contrast, no independent association was observed between *GALNT2* mRNA and HDL-cholesterol levels. This latter finding, somehow at variance with studies on liver-specific *GALNT2* changes in rodents [2], speaks against a direct role of GALNT2 expression on HDL-cholesterol in humans.

The correlation between *GALNT2* expression and TG levels was similarly observable among non-diabetic and diabetic individuals, thus suggesting that neither hyperglycemia nor diabetes-related therapies do interfere with it. This indicates that the relationship between *GALNT2* and TG concentrations does not belong to the same pathophysiological pathway linking *GALNT2* to hyperglycemia observed in humans [3] and rodents [6].

As a limitation of our study, we acknowledge that at this present stage it is not known whether GALNT2 protein expression reflects that of *GALNT2* mRNA and do mirror their relationship with serum TG. Unfortunately, since protein lysates were not obtainable from our specimens, no such data were available.

As an additional limitation of our study, we acknowledge that, although PWBC are an easily obtainable cell model widely used for studying gene expression changes in insulin resistance and related abnormalities [5, 7–9], they are not involved in lipid metabolism, thus leaving open the question whether or not our data can be translated into pathophysiological mechanisms controlling serum triglycerides levels. Thus, present data have to be considered as a preliminary, hypothesis generating, finding that needs to be deeper addressed by additional studies in cells responsible of lipid metabolism, including hepatocytes and adipocytes.

In conclusion, our data suggest a role of *GALNT2* expression changes on serum TG and, taken together with previous genetic findings [2], reinforce the hypothesis that GALNT2 plays a role on lipid levels and possibly atherogenic dyslipidemia.

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Compliance with ethical standards

Conflict of Interest The authors declare that they have no conflict of interest.

Statement of Human and Animal Rights All performed procedures were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statement of Informed Consent Informed consent was obtained from all individual participants included in the study.

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