ORIGINAL ARTICLE

GALNT2 expression is associated with glucose control and serum metabolites in patients with type 2 diabetes

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Abstract

Aims Aim of this study was to investigate in type 2 diabetes whether expression level of *GALNT2*, a positive modulator of insulin sensitivity, is associated with a metabolic signature.

Methods Five different metabolite families, including acylcarnitines, aminoacids, biogenic amines, phospholipids and sphingolipids were investigated in fasting serum of 70 patients with type 2 diabetes, by targeted metabolomics. *GALNT2* expression levels were measured in peripheral white blood cells by RT-PCR. The association between *GALNT2* expression and serum metabolites was assessed using false discovery rate followed by stepwise selection and, finally, multivariate model including several clinical parameters as confounders. The association between *GALNT2* expression and the same clinical parameters was also investigated.

Results *GALNT2* expression was independently correlated with HbA1c levels (P value = 0.0052), a finding that is the likely consequence of the role of *GALNT2* on insulin sensitivity. *GALNT2* expression was also independently associated with serum levels of the aminoacid glycine (P value = 0.014) and two biogenic amines phenylethylamine (P value = 0.0065) and taurine (P value = 0.0011). The association of *GALNT2* expression with HbA1c was not mediated by these three metabolites.

Conclusions Our data indicate that in type 2 diabetes the expression of *GALNT2* is associated with several serum metabolites. This association needs to be further investigated to understand in depth its role in mediating the effect of GALNT2 on insulin sensitivity, glucose control and other clinical features in people with diabetes.

Introduction

Insulin resistance is a common pathogenic ground for many highly prevalent diseases. These include atherogenic dyslipidemia [1], type 2 diabetes, obesity, hypertension [1],

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and closely related cardiovascular disease and renal dysfunction [1, 2], all major causes of morbidity and mortality worldwide [3]. Unraveling the intimate molecular signature of insulin signaling would contribute to understanding the pathogenesis of all the above-mentioned diseases and is therefore urgently needed.

In the last few years several evidences suggested that *GALNT2* (coding for ppGalNAc-T2, involved in the initiation step of O-linked glycosylation [4, 5]), modulates insulin sensitivity [6]. In fact, in cultured human liver cells (HepG2) *GALNT2* down-regulation reduces insulin-induced insulin receptor, IRS-1 and protein kinase beta Akt2 phosphorylation, as well as expression of gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) [7]. Also, GALNT2 over-expression in mouse pre-adipocytes fibroblasts (3T3L1) stimulates adipocyte maturation and enlargement, through increasing insulin signaling [8]. Although, the biological mechanism through which GALNT2 affects insulin signaling is still unknown, the inverse correlation between *GALNT2* and *ENPP1* (a negative modulator of insulin signaling) expression, suggests that *ENPP1* down-regulation mediates, at least in part, the effect of *GALNT2* on insulin sensitivity [9–14].

In addition, several data from both humans and animal models consistently demonstrated the contribution of GALNT2 to several highly prevalent metabolic abnormalities related to insulin resistance, namely atherogenic dyslipidemia [6, 15–23], type 2 diabetes [24, 25], obesity [6, 17, 26] and polycystic ovary syndrome [27]. Unfortunately, the exact biological mechanisms through which *GALNT2* affect insulin signaling is not completely understood [6].

Thanks to the recent advances in bioinformatics and technology, measuring hundreds or thousands of metabolites in biological samples has unraveled specific signatures related to altered metabolic states, including insulin resistance, type 2 diabetes and obesity [28].

We investigated whether *GALNT2* expression is characterized by a specific metabolic signature. In details, five different metabolite families were investigated, including acylcarnitines, aminoacids, biogenic amines, phospholipids and sphingolipids.

Research design and methods

Participants

The study cohort consisted of 70 patients with type 2 diabetes (according to the American Diabetes Association 2003 criteria), belonging to the Gargano Mortality Study 2 (GMS, [29]) including individuals recruited from 2008 to 2010 at the Endocrine Unit of Fondazione Istituto di Ricovero e Cura a Carattere Scientifico "Casa Sollievo della Sofferenza" in San Giovanni Rotondo. Our 70 study patients were randomly selected among those whose RNA sample at recruitment was available. The study protocols and the informed consent procedures were approved by the local Institutional Ethic Committee.

Metabolite quantification and normalization

Metabolites were measured in baseline fasting serum at the Genome Analysis Center, Helmholtz Zentrum München with a targeted metabolomics approach by Absolute*IDQ* p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria), as previously described [29]. The assay includes free carnitine, 40 acylcarnitines (Cx:y), 21 amino acids (19 proteinogenic + citrulline + ornithine), 21 biogenic amines, hexoses (sum of hexoses: ~ 90 to 95% glucose), 90 glycerophospholipids (14 lysophosphatidylcholines [lysoPC] and 76 phosphatidylcholines [PC], and 15 sphingolipids [SMx:y]). Three quality control samples (sex-mixed human plasma provided by the manufacturer) and one zero sample (PBS) were included in each randomized plate.

GALNT2 expression levels

Gene expression levels were measured in peripheral white blood cells by using Gene Expression Assay on Demand Kit Reagents (Applera Life Technologies, Carlsbad, CA), by means of RT-PCR as previously described [24]. Expression levels of *GALNT2* were calculated by using the comparative DCT method normalizing, the amount of *GALNT2* was normalized to GAPDH, B actin and 18S considered together (geometric mean) [30] and related to a control RNA as calibrator ($2^{-\Delta\Delta CT}$).

Statistical analysis

Patients' baseline characteristics are reported as mean ± SD, or median and interquartile range for continuous skewed variables (|skewness| > 1) and frequency and percentage for categorical variables. Values of serum metabolites below the limit of detection have been replaced by the limit of detection itself.

For pre-processing of data, normal distribution and skewness were tested in all metabolites and covariates. Since the metabolites' distributions were skewed, all concentrations were $\log_2$ transformed and standardized. The $2^{-\Delta\Delta CT}$ data of *GALNT2* expression levels were also standardized. Clinical parameters with percentage of missing value less than 5% (i.e., BMI 1.4% and HbA1c 2.8%) were imputed with random forest method [31].

The association between *GALNT2* expression and serum metabolites within each metabolite family (i.e., acylcarnitine, amino acids, biogenic amines, glycerophospholipids

and sphingolipids), was firstly assessed in a univariate model by using false discovery rate (FDR) to take into account multiple comparisons and then in a multivariate model including age, sex, smoking habits, BMI, HbA1c, diabetes duration, eGFR, and anti-hypertensive, anti-hyperglycemia and lipid-lowering therapies. Finally, in order to minimize potential multicollinearity issues, metabolites that were independently associated with *GALNT2* expression, entered jointly a stepwise selection (SSE criterion: *p* value of the F-statistic to enter and to remove term to the model less than 0.05 and greater than 0.10 respectively). A *P* value < 0.05 was considered statistically significant.

All analyses were performed using SAS 9.4 (SAS Institute, Cary, NC) and Matlab R2022—Statistics and Machine Learning Toolbox (The MathWorks, Inc., Natick, MA).

Results

Study patients

Clinical features as well as diabetes duration and ongoing treatments of the 70 study participants with type 2 diabetes are reported in Table 1.

In univariate analyses, *GALNT2* expression levels were correlated with HbA1c ($\beta \pm SE = -0.198 \pm 0.069$;

Table 1 Clinical characteristics of study patients (n = 70)

Women (%)	28.6
Age (years)	55.3 ± 10.1
Current smokers (%)	24.3
Diabetes duration (years)	11 ± 7.8
BMI (kg/m²)	30.5 ± 5.4
HbA1c (%)	7.7 ± 2.1
eGFR (mL/min/1.73 m²)	101.2 (74.5–109.6)
HDL-cholesterol (mg/dL)	43.2 ± 11.4
Triglycerides (mg/dL)	125.5 (88–173)
Anti-hypertensive therapy (%)	72.9
Anti-hyperglycemia therapy	
Diet (%)	4.3
OA (%)	42.8
Insulin ± OA (%)	52.9
Lipid-lowering therapy	
None (%)	21.4
Statins (%)	75.7
Others (%)	2.9

Continuous variables were reported as mean ± SD or as median (interquartile range) for skewed variables (skewness > |±1|), whereas categorical variables as total frequencies

BMI, Body Mass Index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate as CKD-EPI formula [51]; HDL, high density lipoprotein; OA, Oral agents

Table 2 Association between GALNT2 expression levels and patients' clinical features

	β ± SE	P value	P value*
Age (years)	− 0.001 ± 0.012	0.95	0.89
Gender	0.36 ± 0.26	0.18	0.29
Current smokers (%)	− 0.41 ± 0.28	0.15	0.17
BMI (kg/m²)	− 0.045 ± 0.02	*0.044*	0.15
Diabetes duration (years)	− 0.011 ± 0.016	0.47	0.79
HbA1c (%)	− 0.198 ± 0.069	*0.0052*	*0.026*
eGFR (mL/min/1.73 m²)	0.009 ± 0.005	0.061	0.36
Anti-hypertensive therapy (%)	− 0.329 ± 0.268	0.22	0.24
Anti-hyperglycemia therapy	− 0.287 ± 0.205	0.17	0.89
Lipid-lowering therapy	− 0.319 ± 0.261	0.23	0.38

BMI, Body Mass Index; HbA1c, glycated hemoglobin; eGFR, estimated glomerular filtration rate as CKD-EPI formula [51]

*P value in a multivariate model including all variables listed in the table

Italic font indicate a nominal statistical significance

P value = 0.0052) and BMI ($\beta \pm SE = -0.045 \pm 0.22$; *P* value = 0.044) but not with other features (Table 2). Only the association with HbA1c remained significant in a fully adjusted model comprising all available clinical information (Table 2).

GALNT2 and metabolites

Five out of 188 metabolites measured (i.e., carnosine, DOPA, dopamine, nitrotyrosine, *cis*-4-Hydroxyproline) were excluded from the analyses because their value was below the detection limit in > 80% samples.

Correlation between *GALNT2* expression and metabolites was investigated separately in the five metabolite families. After adjusting for multiple comparisons with false discovery rate (FDR) procedure [32], *GALNT2* was associated with two aminoacids (asparagine and glycine: $\beta \pm SE = -0.37 \pm 0.11$ and -0.38 ± 0.11, *P* values = 0.014 for both) and three biogenic amines (ADMA, phenylethylamine and taurine: $\beta \pm SE = -0.34 \pm 0.11$, 0.33 ± 0.11 and -0.39 ± 0.11, *P* values being 0.023, 0.023 and 0.011, respectively) (Fig. 1A). Conversely, no associations with acylcarnitines, phospholipids and sphingolipids were observed (Supplementary Table 1).

All five metabolites remained significantly associated with *GALNT2* mRNA levels in a multivariate model that included age, gender, smoking habits, BMI, HbA1c, diabetes duration, eGFR, and current treatments (*P* values = 0.0027, 0.000049, 0.013253, 0.035 and 0.00067, for glycine, asparagine, ADMA, phenylethylamine and taurine, respectively), thus indicating that their correlation with *GALNT2* expression is independent of the most important clinical variables (Fig. 1B).
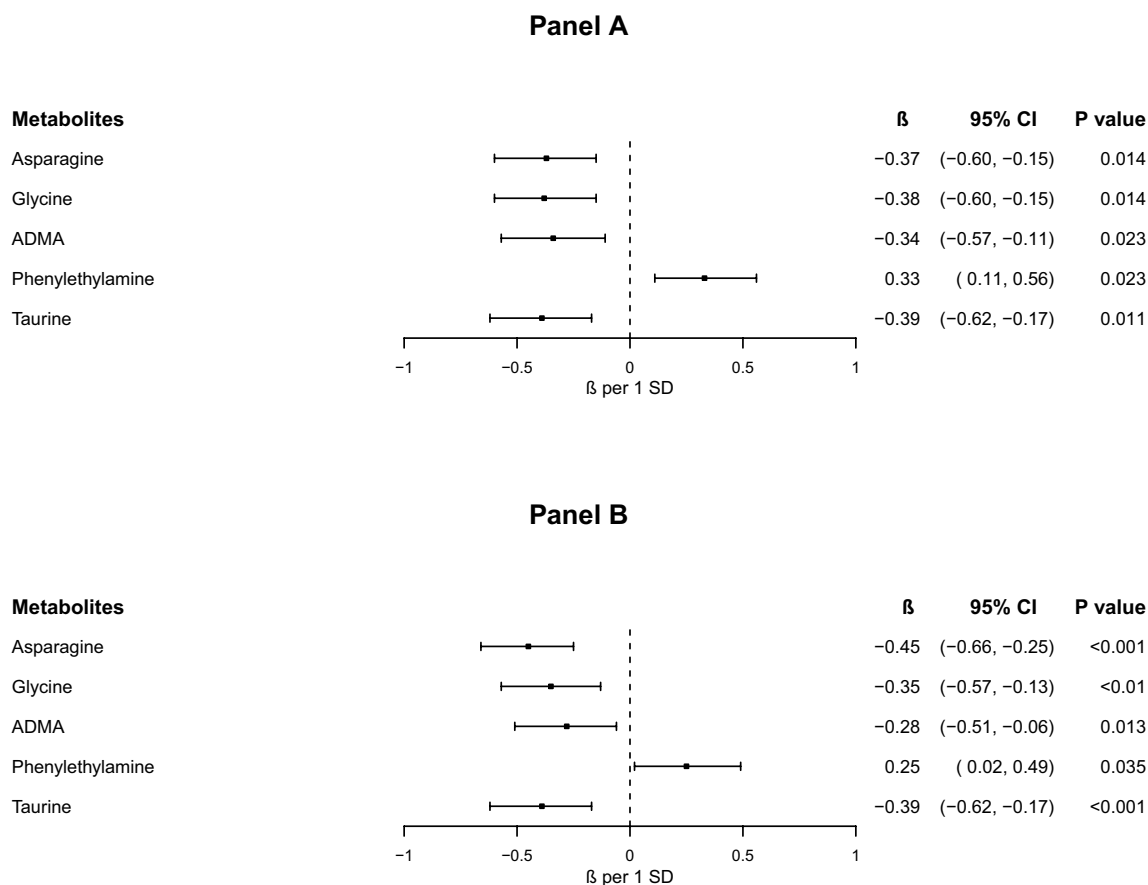
Panel A

Metabolites		ß	95% CI	P value
Asparagine		−0.37	(−0.60, −0.15)	0.014
Glycine		−0.38	(−0.60, −0.15)	0.014
ADMA		−0.34	(−0.57, −0.11)	0.023
Phenylethylamine		0.33	(0.11, 0.56)	0.023
Taurine		−0.39	(−0.62, −0.17)	0.011

−1   −0.5   0   0.5   1
ß per 1 SD

Panel B

Metabolites		ß	95% CI	P value
Asparagine		−0.45	(−0.66, −0.25)	<0.001
Glycine		−0.35	(−0.57, −0.13)	<0.01
ADMA		−0.28	(−0.51, −0.06)	0.013
Phenylethylamine		0.25	(0.02, 0.49)	0.035
Taurine		−0.39	(−0.62, −0.17)	<0.001

−1   −0.5   0   0.5   1
ß per 1 SD

Fig. 1 Associations between GALNT2 expression levels and serum metabolites. β (per 1 SD increase *GALNT2* expression) and 95% CIs were estimated in univariate (**A**) and in multivariate (**B**) regression models, adjusting for age at recruitment, sex, smoking habit, BMI, HbA1c, eGFR, diabetes duration, and ongoing treatment

After a stepwise (forward–backward) analyses aimed at taking into account the correlations among metabolites from the same family, the aminoacid glycine (P value = 0.0014) and two biogenic amines phenylethylamine (P value = 0.0065) and taurine (P value = 0.0011) remained associated with *GALNT2* expression levels.

Finally, none of the three metabolites correlated with HbA1c (data not shown) nor influenced the observed correlation between HbA1c and *GALNT2* expression (β ± SE and P values moving to −0.190 ± 0.064, P value = 0.004; −0.165 ± 0.067; P value = 0.017 and −0.203 ± 0.063; P value = 0.0018 after adjusting for glycine, phenylethylamine and taurine, respectively).

Discussion

Our study investigated whether in patients with type 2 diabetes *GALNT2* expression is characterized by a specific signature belonging to several metabolite families, including acylcarnitines, amino acids, biogenic amines, glycerophospholipids and sphingolipids. We also investigated the association between *GALNT2* expression and several clinical variables. Firstly, *GALNT2* expression was independently and negatively correlated with HbA1c levels, a finding that may well be secondary to the reported positive effect of *GALNT2* on insulin sensitivity [6]. This link is also suggested by the negative association between *GALNT2* and BMI, which however did not survive a multivariable model comprising several additional clinical variables. The expression of *GALNT2* was also independently associated with serum levels of the aminoacid glycine and arginine and the biogenic amines phenylethylamine, taurine and ADMA. When collinearity within the same metabolite family was taken into account, only glycine, taurine and phenethylamine remained associated with GALNT2 expression levels. Interestingly, the association between *GALNT2* expression and HbA1c was not modified taking into account these three latter metabolites, thus suggesting they do not mediate the positive effect of *GALNT2* on glucose control.

Previous studies have highlighted that glycine, is consistently and negatively associated with reduced insulin

sensitivity [33, 34], impaired glucose homeostasis [33, 35–39] and liver steatosis [38]. In addition, low glycine levels have been reported to predict prospectively the development of type 2 diabetes [36, 37, 39–41]. Also plasma taurine is reduced in subjects with metabolic syndrome [42], diabetes [43, 44] and obesity [45, 46] as well as in obese animals [47]. All these previous evidences on the role of glycine and taurine make our present correlative findings compatible with the belief that *GALNT2* is involved in insulin sensitivity and resistance [6]. On the other hand, we acknowledge that their interpretation is not straightforward. Indeed, if the positive effect of *GALNT2* on insulin sensitivity were mediated by the above-mentioned metabolites, one would expect an association with glycine and taurine in the opposite direction to that observed in our study (i.e., positive rather than negative correlation). This makes unlikely that glycine or taurine mediate the positive effect of *GALNT2* on glucose control as also suggested by the observation that the association between *GALNT2* and HbA1c does not change much after adjusting for these two metabolites. It can therefore be hypothesized that the counterintuitive associations we here report represent a homeostatic mechanism in which *GALNT2* upregulation acts as a fine tuner to counteract insulin-resistance induced (or simply marked) by low levels of glycine and taurine. Conversely, no published data are available on circulating phenethylamine levels in different conditions related to metabolic abnormalities. Interestingly, fecal phenethylamine levels, derived from bacterial fermentation of amino acids in the gut, are correlated positively with glucose intolerance and negatively with improved diet-induced insulin sensitivity [48] while urinary phenylethylamine levels were higher in obese women as compared to their normal/underweight counterparts [49]. These reports suggest that phenethylamine also plays a role in several clinically relevant insulin resistance phenotypes. Unfortunately, it is not known whether and how fecal and urinary phenethylamine levels are correlated with serum levels, thus making difficult the interpretation of the positive association we observed between *GALNT2* expression and circulating phenethylamine. In all, we do acknowledge that the associations of GALNT2 expression levels with HbA1c and several circulating metabolites may imply more than a single and unambiguous interpretation and, consequently, does not allow, yet, to define a clear metabolic signature linking GALNT2, circulating metabolites and clinical features related to insulin resistance.

Among limitation of our study, we do recognize that expression data in peripheral white blood cells may not mirror those of other tissues, including the most important ones for glucose homeostasis maintenance. On the other hand, this cell model has been successfully used in *cis*-eQTL, *trans*-eQTL analyses from the eQTLGen consortium (https://www.eqtlgen.org/) aimed at understanding the genetic architecture underlying complex traits including insulin resistance-related abnormalities [50]. Furthermore, we recognize that the small sample size of our study impacts statistical power, thus making it possible that we missed additional associations between *GALNT2* expression and circulating levels of other metabolites as well as subtle effects of the associated metabolites on the role of *GALNT2* on HbA1c and other clinical features (i.e., false-negative results).

In conclusion, our data indicate for the first time that in type 2 diabetes the expression of *GALNT2* is associated with several serum metabolites. This association needs to be further investigated. To understand in depth its role in mediating the effect of GALNT2 on insulin sensitivity, glucose control and other clinical features in people with diabetes. If our current findings are confirmed and deepened by other studies to gain a better comprehension of the molecular effects of GALNT2 on insulin sensitivity, this will likely become instrumental in the discovery of hitherto unknown pathogenic nodes that can be targeted with new therapies in patients with insulin resistance and related anomalies.

Declarations

Conflict of interest The authors have no conflict of interest to declare.

Ethical statement This study involves human participants and was approved by Institutional Ethic Committee Fondazione Casa Sollievo della Sofferenza. Participants gave informed consent to participate in the study before taking part.

References

1. Reaven GM (1988) Banting lecture 1988: role of insulin resistance in human disease. Diabetes 37(12):1595–607. https://doi.org/10.2337/diab.37.12.1595

2. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB (2005) Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 112(20):3066–3072. https://doi.org/10.1161/CIRCULATIONAHA.105.539528

3. WHO (2017) Noncommunicable diseases, progress monitor

4. Schjoldager KT, Narimatsu Y, Joshi HJ, Clausen H (2020) Global view of human protein glycosylation pathways and functions. Nat Rev Mol Cell Biol 21(12):729–749. https://doi.org/10.1038/s41580-020-00294-x

5. Steentoft C, Vakhrushev SY, Joshi HJ et al (2013) Precision mapping of the human O-GalNAc glycoproteome through SimpleCell technology. EMBO J 32(10):1478–1488. https://doi.org/10.1038/emboj.2013.79

6. Antonucci A, Marucci A, Trischitta V, Di Paola R (2022) Role of GALNT2 on insulin sensitivity, lipid metabolism and fat homeostasis. Int J Mol Sci 23(2):1. https://doi.org/10.3390/ijms23020929

7. Marucci A, Cozzolino F, Dimatteo C et al (2013) Role of GALNT2 in the modulation of ENPP1 expression, and insulin signaling and action GALNT2: a novel modulator of insulin signaling. BBA Mol Cell Res 1833(6):1388–1395. https://doi.org/10.1016/j.bbamcr.2013.02.032

8. Marucci A, Antonucci A, De Bonis C et al (2019) GALNT2 as a novel modulator of adipogenesis and adipocyte insulin signaling. Int J Obes (Lond) 43(12):2448–2457. https://doi.org/10.1038/s41366-019-0367-3

9. Roberts F, Zhu D, Farquharson C, Macrae VE (2019) ENPP1 in the regulation of mineralization and beyond. Trends Biochem Sci 44(7):616–628. https://doi.org/10.1016/j.tibs.2019.01.010

10. Maddux BA, Sbraccia P, Kumakura S et al (1995) Membrane glycoprotein PC-1 and insulin resistance in non-insulin-dependent diabetes mellitus. Nature 373(6513):448–451. https://doi.org/10.1038/373448a0

11. Maddux BA, Goldfine ID (2000) Membrane glycoprotein PC-1 inhibition of insulin receptor function occurs via direct interaction with the receptor alpha-subunit. Diabetes 49(1):13–19. https://doi.org/10.2337/diabetes.49.1.13

12. Maddux BA, Chang YN, Accili D, McGuinness OP, Youngren JF, Goldfine ID (2006) Overexpression of the insulin receptor inhibitor PC-1/ENPP1 induces insulin resistance and hyperglycemia. Am J Physiol Endocrinol Metab 290(4):E746–E749. https://doi.org/10.1152/ajpendo.00298.2005

13. Di Paola R, Caporarello N, Marucci A et al (2011) ENPP1 affects insulin action and secretion: evidences from in vitro studies. PLoS ONE 6(5):e19462. https://doi.org/10.1371/journal.pone.0019462

14. Dimatteo C, Marucci A, Palazzo A et al (2013) Role of somatomedin-B-like domains on ENPP1 inhibition of insulin signaling. Biochim Biophys Acta 1833(3):552–558. https://doi.org/10.1016/j.bbamcr.2012.10.017

15. Khetarpal SA, Schjoldager KT, Christoffersen C et al (2016) Loss of function of GALNT2 lowers high-density lipoproteins in humans, nonhuman primates, and rodents. Cell Metab 24(2):234–245. https://doi.org/10.1016/j.cmet.2016.07.012

16. Kathiresan S, Melander O, Guiducci C et al (2008) Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 40(2):189–197. https://doi.org/10.1038/ng.75

17. Lee YH, Nair S, Rousseau E et al (2005) Microarray profiling of isolated abdominal subcutaneous adipocytes from obese vs non-obese Pima Indians: increased expression of inflammation-related genes. Diabetologia 48(9):1776–1783. https://doi.org/10.1007/s00125-005-1867-3

18. Li X, Zhang Y, Zhang M, Wang Y (2020) GALNT2 regulates ANGPTL3 cleavage in cells and in vivo of mice. Sci Rep 10(1):1–9. https://doi.org/10.1038/s41598-020-73388-3

19. Schjoldager KTBG, Vester-Christensen MB, Bennett EP et al (2010) O-glycosylation modulates proprotein convertase activation of angiopoietin-like protein 3: possible role of polypeptide GalNAc-transferase-2 in regulation of concentrations of plasma lipids. J Biol Chem 285(47):36293–36303. https://doi.org/10.1074/jbc.M110.156950

20. Willer CJ, Sanna S, Jackson AU et al (2008) Newly identified loci that influence lipid concentrations and risk of coronary artery disease. Nat Genet 40(2):161–169. https://doi.org/10.1038/ng.76

21. Willer CJ, Mohlke KL (2012) Finding genes and variants for lipid levels after genome-wide association analysis. Curr Opin Lipidol 23(2):98–103. https://doi.org/10.1097/MOL.0b013e328350fad2

22. Teslovich TM, Musunuru K, Smith AV et al (2010) Biological, clinical and population relevance of 95 loci for blood lipids. Nature 466(7307):707–713. https://doi.org/10.1038/nature09270

23. Roman TS, Marvelle AF, Fogarty MP et al (2015) Multiple hepatic regulatory variants at the GALNT2 GWAS locus associated with high-density lipoprotein cholesterol. Am J Hum Genet 97(6):801–815. https://doi.org/10.1016/j.ajhg.2015.10.016

24. Marucci A, di Mauro L, Menzaghi C et al (2013) GALNT2 expression is reduced in patients with Type 2 diabetes: possible role of hyperglycemia. PLoS ONE 8(7):e70159. https://doi.org/10.1371/journal.pone.0070159

25. Almon RR, DuBois DC, Lai W, Xue B, Nie J, Jusko WJ (2009) Gene expression analysis of hepatic roles in cause and development of diabetes in Goto-Kakizaki rats. J Endocrinol 200(3):331–346. https://doi.org/10.1677/JOE-08-0404

26. Zilmer M, Edmondson AC, Khetarpal SA et al (2020) Novel congenital disorder of O-linked glycosylation caused by GALNT2 loss of function. Brain 143(4):1114–1126. https://doi.org/10.1093/brain/awaa063

27. Chen J, Guan L, Liu H, Liu Q, Fan P, Bai H (2021) GALNT2 gene variant rs4846914 is associated with insulin and insulin resistance depending on BMI in PCOS patients: a case-control study. Reprod Sci 28(4):1122–1132. https://doi.org/10.1007/s43032-020-00380-7

28. Yang Q, Vijayakumar A, Kahn BB (2018) Metabolites as regulators of insulin sensitivity and metabolism. Nat Rev Mol Cell Biol 19(10):654–672. https://doi.org/10.1038/s41580-018-0044-8

29. Scarale MG, Mastroianno M, Prehn C et al (2022) Circulating metabolites associate with and improve the prediction of all-cause mortality in type 2 diabetes. Diabetes 71(6):1363–1370. https://doi.org/10.2337/db22-0095

30. Vandesompele J, De Preter K, Pattyn F et al (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. https://doi.org/10.1186/gb-2002-3-7-research0034

31. Bennett DA (2001) How can I deal with missing data in my study? Aust N Z J Public Health 25(5):464–469

32. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodol) 57(1):289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

33. Thalacker-Mercer AE, Ingram KH, Guo F, Ilkayeva O, Newgard CB, Garvey WT (2014) BMI, RQ, diabetes, and sex affect the relationships between amino acids and clamp measures of insulin action in humans. Diabetes 63(2):791–800. https://doi.org/10.2337/db13-0396

34. Newgard CB, An J, Bain JR et al (2009) A branched-chain amino acid-related metabolic signature that differentiates obese and

lean humans and contributes to insulin resistance. Cell Metab 9(4):311–326. https://doi.org/10.1016/j.cmet.2009.02.002

35. Palmer ND, Stevens RD, Antinozzi PA et al (2015) Metabolomic profile associated with insulin resistance and conversion to diabetes in the Insulin Resistance Atherosclerosis Study. J Clin Endocrinol Metab 100(3):E463–E468. https://doi.org/10.1210/jc.2014-2357

36. Floegel A, Stefan N, Yu Z et al (2013) Identification of serum metabolites associated with risk of type 2 diabetes using a targeted metabolomic approach. Diabetes 62(2):639–648. https://doi.org/10.2337/db12-0495

37. Guasch-Ferré M, Hruby A, Toledo E et al (2016) Metabolomics in prediabetes and diabetes: a systematic review and meta-analysis. Diabetes Care 39(5):833–846. https://doi.org/10.2337/dc15-2251

38. Felig P, Marliss E, Cahill GF (1969) Plasma amino acid levels and insulin secretion in obesity. N Engl J Med 281(15):811–816. https://doi.org/10.1056/NEJM196910092811503

39. Wang-Sattler R, Yu Z, Herder C et al (2012) Novel biomarkers for pre-diabetes identified by metabolomics. Mol Syst Biol 8:615. https://doi.org/10.1038/msb.2012.43

40. Wang TJ, Larson MG, Vasan RS et al (2011) Metabolite profiles and the risk of developing diabetes. Nat Med 17(4):448–453. https://doi.org/10.1038/nm.2307

41. Ferrannini E, Natali A, Camastra S et al (2013) Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. Diabetes 62(5):1730–1737. https://doi.org/10.2337/db12-0707

42. Sun S, He D, Luo C et al (2021) Metabolic syndrome and its components are associated with altered amino acid profile in chinese han population. Front Endocrinol (Lausanne) 12:795044. https://doi.org/10.3389/fendo.2021.795044

43. Sak D, Erdenen F, Müderrisoglu C et al (2019) The relationship between plasma taurine levels and diabetic complications in patients with type 2 diabetes mellitus. Biomolecules 9(3):1. https://doi.org/10.3390/biom9030096

44. De Luca G, Calpona PR, Caponetti A et al (2001) Taurine and osmoregulation: platelet taurine content, uptake, and release in type 2 diabetic patients. Metabolism 50(1):60–64. https://doi.org/10.1053/meta.2001.19432

45. Rosa FT, Freitas EC, Deminice R, Jordão AA, Marchini JS (2014) Oxidative stress and inflammation in obesity after taurine supplementation: a double-blind, placebo-controlled study. Eur J Nutr 53(3):823–830. https://doi.org/10.1007/s00394-013-0586-7

46. Lee MY, Cheong SH, Chang KJ, Choi MJ, Kim SK (2003) Effect of the obesity index on plasma taurine levels in Korean female adolescents. Adv Exp Med Biol 526:285–290. https://doi.org/10.1007/978-1-4615-0077-3_36

47. Tsuboyama-Kasaoka N, Shozawa C, Sano K et al (2006) Taurine (2-aminoethanesulfonic acid) deficiency creates a vicious circle promoting obesity. Endocrinology 147(7):3276–3284. https://doi.org/10.1210/en.2005-1007

48. Zhai L, Xiao H, Lin C et al (2023) Gut microbiota-derived tryptamine and phenethylamine impair insulin sensitivity in metabolic syndrome and irritable bowel syndrome. Nat Commun 14(1):4986. https://doi.org/10.1038/s41467-023-40552-y

49. Long SE, Jacobson MH, Wang Y et al (2022) Longitudinal associations of pre-pregnancy BMI and gestational weight gain with maternal urinary metabolites: an NYU CHES study. Int J Obes (Lond) 46(7):1332–1340. https://doi.org/10.1038/s41366-022-01116-0

50. Võsa U, Claringbould A, Westra HJ et al (2021) Large-scale cis- and trans-eQTL analyses identify thousands of genetic loci and polygenic scores that regulate blood gene expression. Nat Genet 53(9):1300–1310. https://doi.org/10.1038/s41588-021-00913-z

51. Levey AS, Stevens LA, Schmid CH et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150(9):604–612. https://doi.org/10.7326/0003-4819-150-9-200905050-00006