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ORIGINAL ARTICLE

The co-activator-associated arginine methyltransferase 1 (CARM1) gene is overexpressed in type 2 diabetes

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Abstract

Purpose We examined the expression of a panel of epigenetic enzymes catalyzing histone tails post-transcriptional modifications, together with effectors of metabolic and inflammatory alterations, in type 2 diabetes.

Methods Cross-sectional, case–control study of 21 people with type 2 diabetes and 21 matched controls. Total RNA was extracted from white cells and reverse transcribed. PCR primer assays for 84 key genes encoding enzymes known to modify genomic DNA and histones were performed. Western blot was performed on lysates using primary antibodies for abnormally expressed enzymes. Hormones and cytokines were measured by multiplex kits. A Bayesian network was built to investigate the relationships between epigenetic, cytokine, and endocrine variables.

Results Co-activator-associated aRginine Methyltransferase 1 (CARM1) expression showed a five-fold higher median value, matched by higher protein levels, among patients who also had increased GIP, IL-4, IL-7, IL-13, IL-17, FGF basic, G-CSF, IFN-γ, and TNFα and decreased IP-10. In a Bayesian network approach, CARM1 expression showed a conditional dependence on diabetes, but was independent of all other variables nor appeared to influence any.

Conclusions Increased CARM1 expression in type 2 diabetes suggests that epigenetic mechanisms are altered in human diabetes. The impact of lifestyle and pharmacological treatment on regulation of this enzyme should be further investigated.

Keywords Type 2 diabetes · Histone methylation · Epigenetics · CARM1

Abbreviations

CARM1	Co-activator-associated aRginine Methyltransferase 1
FGF	Fibroblast growth factor
G-CSF	Granulocyte colony-stimulating factor
GIP	Gastric inhibitory polypeptide or glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide 1
IFN-γ	Interferon gamma
IP-10	Interferon-γ-inducible protein 10
IL-1ra	Interleukin-1 receptor antagonist
IL-1β	InterLeukin 1 beta
IL-4	InterLeukin 4
IL-5	InterLeukin 5
IL-6	InterLeukin 6
IL-7	InterLeukin 7
IL-9	InterLeukin 9
IL-10	InterLeukin 10
IL-12	InterLeukin 12
IL-13	InterLeukin 13
IL-17	InterLeukin 17
MCP-1	Monocyte chemoattractant protein-1

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MIP-1a	Macrophage inflammatory protein 1a
NFkB	Nuclear factor kappa-light-chain-enhancer of activated B cells
PAI-1	Plasminogen activator inhibitor-1
PDGF	Platelet-derived growth factor
TNF-α	Tumor necrosis factor alpha
VEGF	Vascular endothelial growth factor

Introduction

Clinical, epidemiological, and experimental evidence indicates that environmental factors impact on the natural history of type 2 diabetes [1] and may induce changes in the function of genes affecting major diabetes traits. However, while the contribution of most known genetic markers to the total risk of developing type 2 diabetes appears minimal, more recent evidence supports an important role for epigenetic mechanisms in the etiology of this disorder [2]. In murine models, exposure of parents to environmental hits known to cause epigenetic changes reprograms insulin sensitivity as well as beta-cell function in the progeny [3], indicating that some of these changes can be transmitted through generations, while specific human epigenotypes may predict adiposity and type 2 diabetes with much greater power than any polymorphism so far identified [4].

Since, over the years, we have been investigating the environmental dimensions involved in lifestyle changes in people with type 2 diabetes [5–7], we examined the expression levels of a panel of epigenetic enzymes catalyzing histone tails post-transcriptional modifications in blood samples of patients with type 2 diabetes and matched non-diabetic controls. In addition, we investigated a number of circulating effectors of metabolic and inflammatory alterations related to type 2 diabetes to search for possible associations with the expression of such enzymes.

Research, design, and methods

The study had a cross-sectional, case–control approach. Twenty-one patients with type 2 diabetes were randomly selected from the database of the Diabetes Clinic of the Department of Medical Sciences at Turin University. Inclusion criteria were: age 40–70, treatment by diet alone or oral glucose-lowering agents, and at least 1-year attendance in the clinic. Exclusion criteria included current insulin treatment, known psychiatric conditions, and neoplastic or other severe diseases with potential impact on the epigenetic machinery. Twenty-one non-diabetic healthy individuals carefully matched by age and sex acted as controls. Anthropometric, clinical, blood chemistry, epigenetic, and endocrine variables were measured. Clearance had been obtained from the institutional Ethics Committee (Comitato Etico Interaziendale A.O.U. Città della Salute e della Scienza di Torino—A.O. Ordine Mauriziano—A.S.L. Città di Torino—File CEI 177, Protocol N. 0018935 of 11 March 2011), and all patients and controls gave their informed consent, according to the principles of the Helsinki Declaration.

Blood was taken by clean venipuncture, avoiding stasis, and immediately processed. Plasma glucose, HbA1c, total and HDL cholesterol, tryglyceride, and serum creatinine were measured by standard techniques. LDL cholesterol was calculated by Friedewald's formula [8]. Serum insulin was measured by a fully automated two-site, solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 2000, Siemens Healthcare Diagnostics). Insulin resistance was calculated as HOMA-IR index [9].

RNA samples and real-time RT-PCR

Total RNA was extracted from white blood cells immediately after venipuncture following cell isolation, using the E.Z.N.A. blood RNA kit (OMEGA Bio-Tek Inc., Norcross, GA, USA), according to the manufacturer's instructions. After quantification with NanoDrop2000 spectrophotometer (Thermo Scientific, Waltham, MA), 1 µg of total RNA was reverse transcribed using Superscript III reverse Transcriptase (Invitrogen, Waltham, MA, USA) and analyzed by the Human Epigenetic Chromatin Modification Enzymes PCR Array (QIAGEN; http://sabiosciences.com/rt_pcr_product/HTML/PAHS-085Z.html), according to the manufacturer's instructions.

The PCR array is a set of optimized Real-Time PCR primer assays on 96-well for 84 key genes encoding enzymes known or predicted to modify genomic DNA and histones plus five housekeeping genes. Controls are also included on each array for genomic DNA contamination, RNA quality, and general PCR performance. The results were analyzed using software provided by the manufacturer (http://pcrdataanalysis.sabiosciences.com/pcr/array-analysis.php). The differences in gene expression were then validated by quantitative Real-Time PCR, performed in triplicate using iQ SYBR Green Supermix on iCycler Real-Time detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Differentially regulated genes resulted to be over or under the arbitrary chosen cutoff (fold changes > 1.5 vs. controls). Among them, only *CARM1* gene expression, which is associated to histone methyltransferases, was significantly upregulated in T2DM patients compared with controls.

For copy number analysis, the Real-Time PCR amplification products for CARM1 and Gapdh, used as internal control, were cloned into the pGEM-T easy vector (Promega, Madison, WI, USA), and calibration curves were

made from serial ten-fold dilutions of plasmid DNAs as described previously [10]. The equations drawn from the graph of the standard curves were used to calculate the precise number of specific cDNA molecules present in the samples (copy molecules). Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) was used to design specific primers, which were then purchased from Sigma-Aldrich (St. Louis, MO, USA). The SYBR green primers used are as follows:

CARM1 human *Forward*:	5′-CCTTTAGCCAACACG GGGAT-3′5′-CTAGCTCCC
CARM1 human *Reverse*:	GTAGTGCATGG-3′
GAPDH human *Forward*:	5′-CGCTCTCTGCTCCTCC TGTTC-3′
GAPDH human *Reverse*:	5′-TTGACTCCGACCTTCA CCTTCC-3′

Immunoblot procedure

For Western blot analysis, cells were solubilized for 30 min at 4 °C with lysis buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 10 mM EDTA, 10 mM $Na_2P_2O_7$, 2 mM Na_3VO_4, 100 mM NaF, 10% glycerol, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 10 g/ml aprotinin), as previously described [11]. Lysates were centrifuged at 12,000 × *g* for 20 min and the supernatant fractions were frozen at −80 °C until used. For Western blotting, 50 µg of lysate proteins were heated at 95 °C in Laemmli buffer [12]. Proteins were separated by 10% SDS-PAGE and then transferred to 0.45-mm Immobilon-P membranes (Millipore, Bedford, MA, USA). Immunoblots were performed using primary antibodies for CARM1 (Cell Signaling Technology—# 4438, 1:1000) and NF-kB p65 (Santa Cruz Biotechnology, INC—sc-8008, 1:1000), with 14-3-3θ as the loading control (sc-732, 1:10,000). Immunodetected proteins were visualized using an enhanced chemiluminescence kit (BioRad, Hercules, CA, USA). Densitometric analysis was performed using ImageJ software.

Adiponectin, adipsin, GIP, GLP-1, glucagon, ghrelin, leptin, resistin, and visfatin were measured by the Bioplex Pro Assays (Bio-Rad, Hercules, CA), according to the manufacturer's protocol. Results are expressed as picograms per milliliter.

Determination of cytokine and growth factor circulating levels

Serum samples were processed for the concentrations of IL-1ra, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p70), IL-13, IL-15, IL-17a, basicFGF, eotaxin, G-CSF, GM-CSF, IFN-γ, IP-10, MCP-1, MIP-1alpha, MIP-1beta, RANTES, TNF-α, PDGF-BB, and VEGF using the Bioplex Multiplex Human Cytokine, Chemokine, and Growth Factor kit (Bio-Rad, Hercules, CA), according to the manufacturer's protocol. Results are expressed as picograms per milliliter.

Statistical analyses

Continuous data are presented as medians and interquartile ranges. Discrete data are given as counts and percentages. Chi-square test or, where appropriate, Fisher exact test were performed to compare the groups of categorical data; the Mann–Whitney U test was used to compare continuous data.

A Bayesian network was built to investigate the relationships between epigenetic, citokine, and endocrine variables. Since the normality distribution of continuous variables was not satisfied, Hartemink Information-preserving Discretization was carried out. Normality distribution was tested by the Shapiro–Wilk test. Both the structure and numerical parameters were learned entirely from the data. The Greedy Thick Thinning score-based algorithm was performed [13] to build the graph. Given the structure of the network, Expectation–Maximization algorithm [14] was applied for the learning parameters task, i.e., learning the conditional probability tables.

Analyses were carried out using R versions 3.3 and Genie version 2.0.

Power analysis showed that 21 + 21 sample size allowed a statistical power of 81% to detect a difference of 25% between the groups in the primary outcome, with type I error $\alpha = 5\%$ and a Bonferroni correction for 100 multiple tests.

Results

Table 1 shows the clinical data of the patients and healthy controls. The latter had lower BMI and higher levels of total and LDL cholesterol. Plasma glucose and serum glucagon were higher in the patients, whereas serum insulin and HOMA-IR index did not differ between the groups. It should be noted in this respect that all 21 patients were on metformin and 10 also on pioglitazone, both insulin-sensitizing drugs, and most of them on statins and other lipid-lowering drugs.

Table 2 shows the expression and protein levels of CARM1 and the concentrations of circulating hormones and chemokines measured. CARM1 expression showed a five-fold higher median value, matched by higher CARM1 protein levels, among the patients who also had higher circulating GIP, IL-4, IL-7, IL-13, IL-17, FGF basic, G-CSF, IFN-γ, and TNFα and markedly decreased IP-10.

Table 1 Clinical characteristics of patients and controls

	Type 2 diabetes	Non-diabetic controls	*p*
N	21	21	–
Gender	M = 11, F = 10	M = 11, F = 10	–
Age (years)	63.00 [59.00, 68.00]	65.00 [60.00, 68.00]	0.724
Diabetes duration (years)	17.00 [14.00, 20.00]	–	–
Family history for DM	**18**	**9**	**0.01**
Smokers (No/Yes/Former)	8/7/6	13/2/6	0.138
Occupation (H/R/B/S/A/W/O)[a]	3/12/4/1/0/1/0	0/10/1/1/2/2/5	0.055
Body mass index	27.00 [24.00, 31.00]	24.00 [23.00, 27.00]	**0.043**
Hypertension (Yes/No)	15/6	6/15	0.014
Plasma glucose (mg/dl)	**149.60 [126.70, 169.90]**	**79.10 [75.90, 89.20]**	**<0.001**
Total cholesterol (mg/dl)	**193.00 [157.00, 211.00]**	**216.00 [199.00, 226.00]**	**0.016**
HDL cholesterol (mg/dl)	74.00 [63.00, 94.00]	92.00 [74.00, 103.00]	0.078
LDL cholesterol (mg/dl)	**123.00 [89.00, 154.00]**	**166.00 [123.00, 189.00]**	**0.005**
Triglyceride (mg/dl)	99.00 [69.60, 140.20]	106.60 [93.50, 133.30]	0.372
Creatinine (mg/dl)	0.93 [0.70, 1.16]	0.87 [0.76, 1.11]	0.910
Serum insulin (uIU/ml)	6.67 [2.54, 9.52]	8.06 [5.98, 10.00]	0.186
HOMA-IR	2.48 [0.90, 3.21]	1.57 [1.26, 2.22]	0.242
C-Peptide (ng/ml)	1439.66 [991.52, 1704.28]	1511.43 [1210.55, 2118.97]	0.274
Glucose-lowering medication (Met/Pio/DPP4i/Sulpho)[b]	21/10/11/2	–	–
Lipid-lowering medication (Statins/Ezetimibe/Fenofibrate/Other)	10/2/2/1	–	–

All data are expressed as median and [IQR]

[a]*H* housewife, *R* retired, *B* blue-collar, *S* self-employed, *A* artisan, *W* white-collar, *O* other

[b]*Met* metformin, *Pio* pioglitazone, *DPPr4i* DPP4 inhibitor, *Sulpho* sulphonylurea

Bold values indicate statistically significant differences bewteen groups

Figure 1 shows the Bayesian network used to analyze the possible interplay among the variables measured. The dichotomy type 2 diabetes/health was directly linked to plasma glucose levels, CARM1 expression, and leptin. A second independent node, centered on IL-1b, proceeded to IL-6, TNFα, and, through the latter, IFN-γ. TNFα also associated with most variables that differed between patients and controls of this study: IL-7, IL-4, and IL-17. In turn, type 2 diabetes/healthy, leptin, MCP1, PAI-1, G-CSF, and FGFb showed conditional dependence on IL-7. In summary, CARM1 expression showed conditional dependence on the presence/absence of diabetes, but was independent of all other variables nor did it appear to influence any.

Discussion

Emerging evidence indicates that susceptibility to type 2 diabetes may be affected by epigenetic mechanisms involving the action of histone-modifying enzymes [15], and that environmental hits deregulating such mechanisms may contribute to the pathogenesis of the disease. This is, in our knowledge, the first report on increased expression of co-activator-associated arginine methyltransferase 1 (CARM1), also designated as protein arginine methyltransferase 4 (PRMT4), in patients with type 2 diabetes. This finding originated from the analysis of differential expression of a panel of 84 genes coding for enzymes involved in epigenetic regulation of cell metabolism in age-matched and gender-matched samples of healthy subjects and patients with type 2 diabetes. We then employed a Bayesian network approach to investigate the inter-relationships among CARM1 expression, clinical variables, and circulating levels of hormones and citokines known to be abnormally represented and/or potentially involved in upstream or downstream pathophysiological pathways in type 2 diabetes. Since most patients were on insulin-sensitizing and lipid-lowering medication, the variables related to insulin resistance and lipid profile were not included in the analysis.

Table 2 Results

	Type 2 diabetes	Non-diabetic controls	*p*
CARM1 expression (CN ratio)	**0.21 [0.05, 0.50]**	**0.04 [0.04, 0.05]**	**<0.001**
CARM1 protein (arbitrary units)	**0.99 [0.92, 1.25] (*n* = 17)**	**0.72 [0.63, 0.79] (*n* = 9)**	**0.002**
NFkB protein (arbitrary units)	0.56 [0.54, 0.88] (*n* = 17)	0.58 [0.53, 0.76] (*n* = 9)	0.914
Adiponectin (Ug/ml)	15.31 [8.02, 20.46]	10.01 [7.92, 16.46]	0.387
Adipsin (Ug/ml)	1.13 [0.88, 1.47]	1.34 [1.17, 1.51]	0.168
GIP (pg/ml)	**781.06 [637.22, 898.87]**	**595.09 [479.46, 685.88]**	**0.001**
GLP-1 (pg/ml)	1073.41 [1047.60, 1163.00]	1023.16 [979.24, 1081.28]	0.090
Glucagon (pg/ml)	**1964.84 [1926.75, 2028.42]**	**1905.57 [1822.36, 1976.59]**	**0.031**
Ghrelin (pg/ml)	5398.11 [5014.30, 6266.91]	5804.53 [4828.24, 6482.14]	0.763
Leptin (pg/ml)	8037.69 [5353.22, 16613.55]	10550.51 [6044.20, 24658.11]	0.571
Resistin (pg/ml)	5764.29 [4540.62, 6924.18]	6111.50 [4917.19, 9626.81]	0.274
Visfatin (ng/ml)	13.24 [10.75, 14.70]	11.41 [10.79, 12.77]	0.227
PAI-1 (ng/ml)	113.81 [92.49, 123.47]	103.80 [92.96, 117.64]	0.715
PDGF-bb (pg/ml)	2572.91 [2187.63, 3258.04]	2594.47 [1814.05, 3178.25]	0.554
TNF-α (pg/ml)	**81.02 [75.41, 89.44]**	**69.81 [61.43, 72.61]**	**0.012**
VEGF (pg/ml)	72.17 [49.48, 114.47]	79.94 [50.83, 110.03]	0.772
IL-1ra (pg/ml)	169.79 [163.56, 188.61]	151.16 [129.66, 204.42]	0.151
IL-1ß (pg/ml)	8.38 [7.74, 8.71]	7.31 [6.88, 8.81]	0.203
IL-4 (pg/ml)	**11.32 [10.54, 12.00]**	**9.80 [9.28, 10.13]**	**0.002**
IL-5 (pg/ml)	52.46 [47.40, 56.17]	50.79 [45.67, 53.29]	0.302
IL-6 (pg/ml)	8.26 [7.78, 9.39]	8.42 [6.98, 9.55]	0.801
IL-7 (pg/ml)	**36.48 [34.02, 39.50]**	**29.02 [27.75, 32.79]**	**<0.001**
IL-9 (pg/ml)	100.15 [93.57, 109.15]	95.37 [88.51, 104.05]	0.399
IL-10 (pg/ml)	21.70 [17.66, 27.36]	20.24 [14.58, 28.58]	0.615
IL-12(p70) (pg/ml)	51.71 [39.61, 74.57]	57.71 [34.68, 71.23]	0.860
IL-13 (pg/ml)	**27.40 [25.37, 28.92]**	**22.82 [20.27, 27.40]**	**0.028**
IL-17 (pg/ml)	**181.72 [165.02, 195.92]**	**160.02 [151.68, 170.03]**	**0.008**
FGF basic (pg/ml)	**75.94 [70.37, 84.05]**	**64.65 [55.73, 73.87]**	**0.003**
G-CSF (pg/ml)	**66.02 [63.96, 72.18]**	**55.66 [47.27, 63.96]**	**<0.001**
IFN-γ (pg/ml)	**163.67 [153.95, 177.99]**	**139.08 [134.05, 153.95]**	**0.004**
IP-10 (pg/ml)	**353.24 [299.79, 458.09]**	**817.07 [627.57, 1123.42]**	**<0.001**
MIP-1a (pg/ml)	7.31 [5.95, 7.77]	9.05 [5.45, 11.06]	0.385
MCP-1 (pg/ml)	24.32 [19.78, 30.79]	22.19 [15.92, 28.00]	0.159
Eotaxin (pg/ml)	166.53 [128.79, 224.58]	127.20 [114.36, 184.29]	0.199

All data are expressed as median and [IQR]

Bold values indicate statistically significant differences bewteen groups

CARM1 enhances transcriptional activation by nuclear receptors by interacting with the co-activator p160 and cAMP response element-binding protein-binding protein (CBP), and methylation of histone H3 at arginine 17 (H3-R17) [16]. CARM1 is reported to act as co-activator for transcription factor nuclear factor-kappaB (NFkB), enhancing its activity in a CBP (p300)-dependent manner [17]. However, NFkB did not differ in the patients and controls of this study, despite increased CARM1 expression and protein (Table 2), suggesting that other mechanisms may be activated by this enzyme in peripheral white blood cells.

CARM1 is a possible key regulator of glucose-induced insulin secretion from pancreatic β cells via methylation of H3R17 [18] and a critical component of glucose metabolism in the liver, where it is necessary for cAMP-mediated activation of the genes coding for rate-limiting gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase [19]. High-glucose-induced CARM1 expression was reported to enhance apoptosis of human retinal pigment epithelial cells via H3R17 dimethylation, suggesting a role in the pathogenesis of diabetic eye disease [20, 21]. However, the same authors reported that ubiquitination-dependent CARM1 degradation may

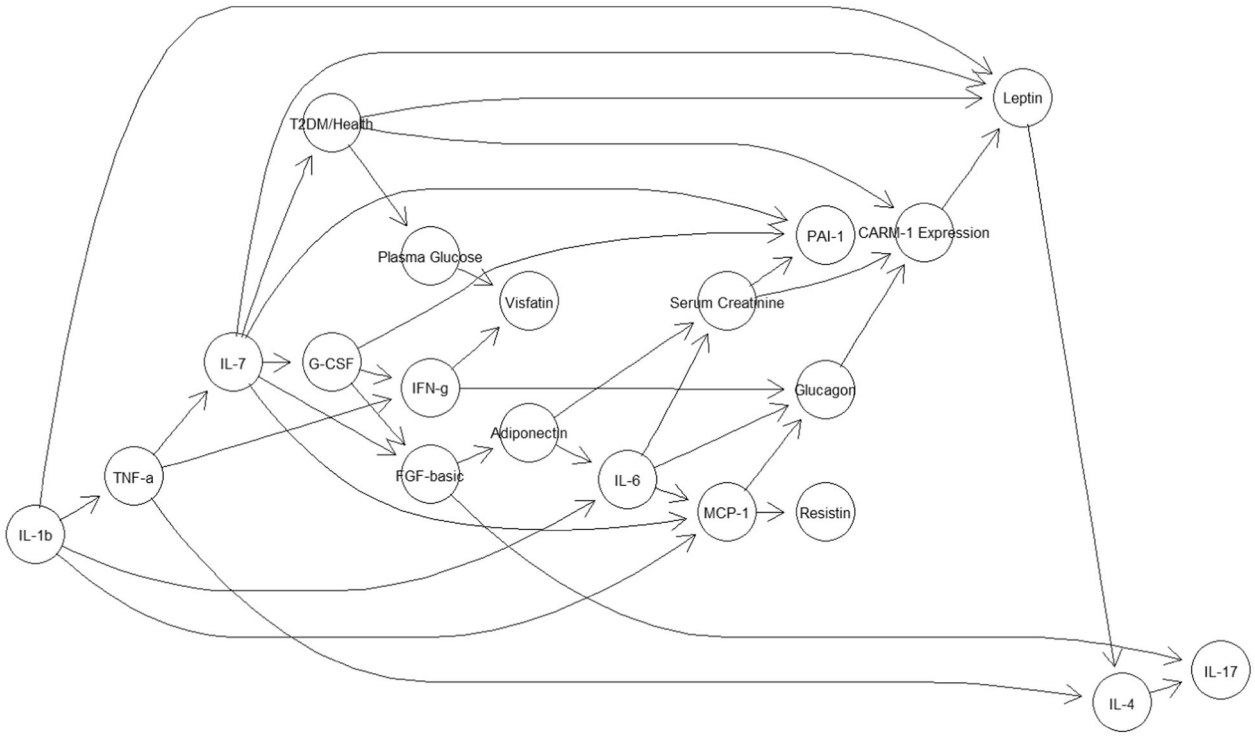

Fig. 1 Bayesian network depicting the relationships among CARM-1 expression, clinical variables, and circulating levels of hormones and cytokines abnormally represented in type 2 diabetes

promote podocyte apoptosis via Notch1 activation [22], suggesting a role also in diabetic nephropathy.

Histone arginine methylation by CARM1 may play a role in autophagy, a highly conserved self-digestion process essential to maintain homeostasis and viability in conditions of nutrient starvation [23], further suggesting its engagement when cell metabolism is dysregulated. In addition, the methyltransferase activity associated with CARM1 may be required for gene expression programming in glycogen metabolism in skeletal muscle and human glycogen storage diseases [24]. CARM1 upregulation was linked to modulation of adipocyte metabolism in visceral adipose tissue [25], hepatocyte proliferation [26], and presentation of arginine (di)methylated HLA class I peptides for recognition by T cells [27]. An association with phenotypical alcohol consumption in rodents represents the only possible link so far reported between CARM1 and behavioral/recreational aspects [28].

Our Bayesian network analysis suggests that the independent dichotomy type 2 diabetes/health is directly associated with 3 nodes: blood glucose, CARM1 expression, and leptin. Another node, IL-1b, is pivotal to TNF-α, Leptin, IL4, and IL6. In turn, TNF-α is linked to IFN-γ, both directly and indirectly, via IL7 and G-CSF. Interestingly, the involvement of IL-1b, IL6, TNF-α, and IFN-γ in low-grade inflammation associated with type 2 diabetes and obesity [29] are well known. In turn, these cytokines may conditionally influence disease state via IL7, whereas a direct influence of disease state on CARM1 expression does not appear mediated by plasma glucose.

IL-7 is important for B and T cell development. It mediates glucose utilization in lymphocytes through transcriptional regulation of the hexokinase II gene [30] and an increased tyrosine phosphorylation/nitrosylation ratio was reported in vitreous samples from patients subjected to vitrectomy for severe proliferative diabetic retinopathy [31]. In our Bayesian network (Fig. 1) IL-7 is an important node leading to 6 other nodes: type 2 diabetes/health, leptin, MCP1, PAI-1, and two other cytokines increased in the patients of this study, FGF basic, G-CSF, and, through the latter, IFN-γ. FGF basic is involved in fibrogenesis and may play a role in the pathogenesis of diabetic nephropathy [32] and peripheral artery disease [33]. G-CSF controls the production, differentiation, and function of granulocytes but no specific involvement has been hypothesized for this molecule in the pathogenesis of diabetes or its complications. IFN-γ, a member of the type II interferon class that triggers cellular responses to microbial and viral infections, may be involved in the apoptosis of beta cells in type 2 diabetes [34] and, together with IL-17, in their autoimmune destruction in type 1 diabetes [35].

IL-4 is produced by activated T cells and stimulation of its receptor is thought to result in activation of Jak1, Jak3, and/or Fes tyrosine kinases. IL-4 stimulation was shown to

mediate tyrosine phosphorylation of the substrates IRS-1 and 4PS [36]. Increased levels of IL-4 were reported in patients with severe proliferative diabetic retinopathy [37].

In our Bayesian network (Fig. 1), IL-4 and FGF-beta were linked to IL-17, a proinflammatory cytokine produced by activated T cells which regulates the activity of NF-kB and mitogen-activated protein kinases. IL-17 enhances the expression of IL6 and cyclooxygenase-2, and the production of nitric oxide. High levels of this cytokine are associated with chronic inflammatory diseases such as rheumatoid arthritis, psoriasis, and multiple sclerosis. In the Rotterdam study, high levels of IL-17 were associated with 24% decreased incidence of type 2 diabetes [38], but its role remains unclear. Roohi et al. reported no association [39], another study found better metabolic control in patients with newly diagnosed type 2 diabetes and reduced IL-17 levels [40], and a cross-sectional survey reported inverse associations of serum IL17 with type 2 diabetes and retinopathy [41].

Finally, interferon-γ-inducible protein 10 (IP-10) is a chemokine that may contribute to the pathogenesis of several autoimmune diseases, including type 1 diabetes [42]. A role for IP-10 in cardiovascular disease, atherosclerosis, aneurysm formation, and myocardial infarction has been reviewed recently [43]. It was suggested that an additional benefit of incretin-based therapy may involve GLP-1 receptor-mediated signals suppressing the expression of IP-10, which binds to Toll-like receptor 4 and impairs beta-cell function and viability in diabetes [44], and this may be consistent with the fact that about half of our patients were on DPP4 inhibitors and had markedly decreased IP-10, despite increased IFN-γ.

The reduced number of patients is a limitation of this study. Although statistical power analysis supports the ability of the sample to detect the difference measured in CARM1 expression, the possibility of detecting differences in the other variables studied and/or applying classical multivariate analysis remains limited. This applies to a number of potential modulators of epigenetic mechanisms, including for instance diabetes duration and control. On the other hand, we measured a vast array of epigenetic enzymes and run a Bayesian analysis including a large number of effectors potentially involved in the presence and pathogenesis of type 2 diabetes. Another limitation is that the patients were treated by insulin-sensitizing and lipid-lowering drugs, so that an influence of these agents on CARM1 and/or the other variables measured cannot be ruled out. However, since no information is available on the necessary duration of drug withdrawal, a period of washout was neither feasible nor ethical in this exploratory phase of the study. Another limitation is that all markers were evaluated in circulating white blood cells, and whether their expression profile is comparable to that in the liver, adipose, muscle, or other tissues relevant to type 2 diabetes remains to be further investigated.

The observation of increased expression of CARM1 in type 2 diabetes should be considered preliminary and further investigations should recruit larger numbers of newly diagnosed, drug-naive type 2 diabetes individuals, and involve them in prospective studies that will also evaluate the impact of lifestyle and/or pharmacological interventions on the epigenetic machinery.

Submission declaration

These data were accepted for oral presentation at the 118th Congress of the Italian Society of Internal Medicine (SIMI) in Rome on the 27–29 October 2017.

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Author contributions M.P. and M.T. conceived the study, researched data, and drafted the manuscript. C.A., F.B., P.F., S.M., L.A., M.C., and P.U. participated in data acquisition. F.B., P.B., and F.C. contributed to analysis and interpretation of data and reviewed/edited the manuscript. G.G. and M.D. contributed to the discussion and reviewed/edited the manuscript. All authors gave their final approval to this version of the manuscript.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent to inclusion in the study was obtained from all individual participants included in the study.

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