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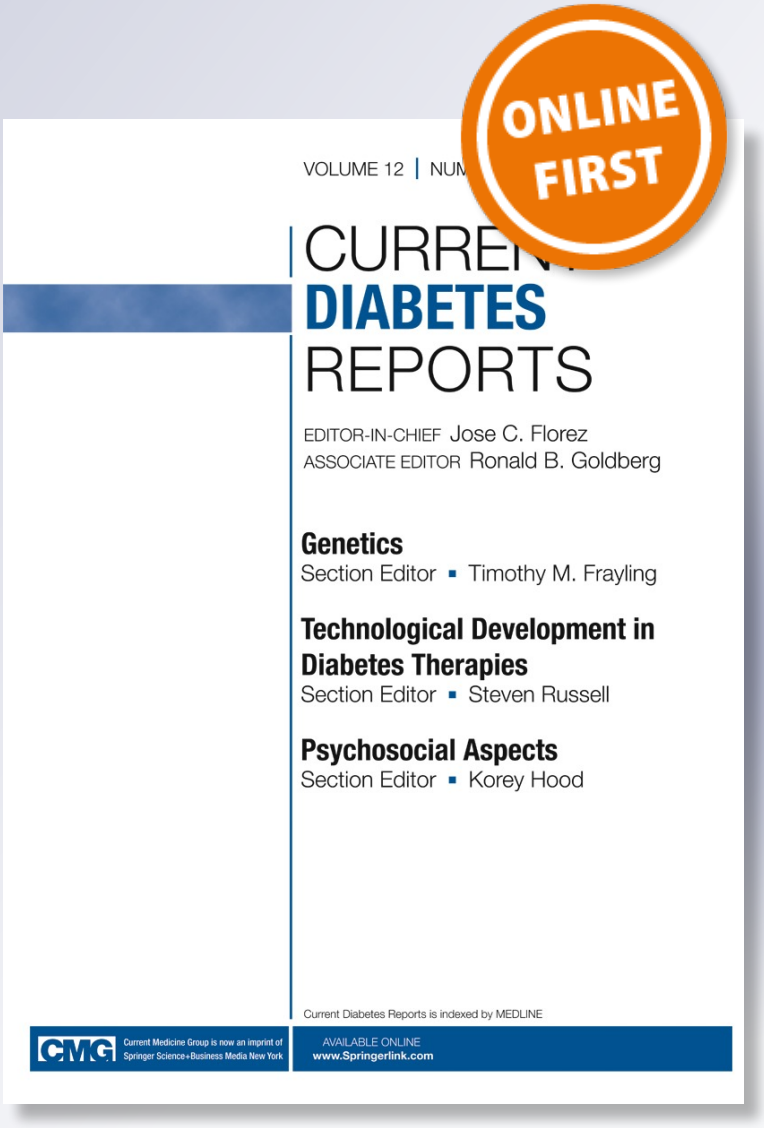

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Epigenetics of Insulin Resistance: An Emerging Field in Translational Medicine

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Abstract In this article, we review the current knowledge of and recent insights into the role of epigenetic factors in the development of insulin resistance (IR), with emphasis on peroxisome proliferator-activated receptor gamma coactivator 1α (*PPARGC1A* or *PGC1α*) methylation on fetal programming and liver modulation of glucose-related phenotypes. We discuss the pathogenesis of IR beyond the integrity of β-cell function and illustrate the novel concept of *mitochondrial epigenetics* to explain the pathobiology of metabolic-syndrome-related phenotypes. Moreover, we discuss whether epigenetic marks in genes of the circadian rhythm system are able to modulate insulin/glucose-related metabolic functions and place hypoxia inducible factor 1 α (HIF1α) as a part of the master *CLOCK* gene/protein interaction network that might modulate IR. Finally, we highlight relevant information about epigenetic marks and IR so that clinicians practicing in the community may envision future areas of medical intervention and predict putative biomarkers for early disease detection.

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Introduction

The concept of insulin resistance (IR), which was initially introduced for the characterization of patients with impaired glucose tolerance and type 2 diabetes (T2D) [1], is now largely recognized as the hallmark metabolic disturbance that links the clinical disorders clustered in the metabolic syndrome (MS), including central obesity, arterial hypertension, prothrombotic and proinflammatory states, ovarian polycystosis, and nonalcoholic fatty liver disease (NAFLD), among others such as hyperuricemia. In fact, IR is regarded as the unifying pathophysiologic process in the MS.

It was through decades of IR scientific research that its pathogenesis was found to be associated with the interplay between lifestyle factors, such as increased nutrient availability, overnutrition, and decreased physical activity, in the context of a genetic predisposing background. In fact, much of the effort to understand the pathobiology of IR has been focused on the search of gene variants that not only influence the development of the disease, as shown in recent large-scale studies [2–6], but also contribute to variation in IR by changes in hepatic glucose metabolism [7].

Nevertheless, even with a comprehensive survey of the entire genome by genome-wide and candidate gene association studies, many crucial aspects remain inconclusive, and many key questions remain unanswered because the identified loci explain less than 10 % of the IR population variance and the best characterized genetic associations with T2D correspond to loci involved in the β-cell biology [8]. Thus, other factors have been implicated in the pathogenesis of IR: epigenetic mechanisms, which not only explain the complex interaction between

genes and environment, but also introduce the putative role of regulation of gene transcription and chromosome organization in the pathobiology of the disease. More important, epigenetic mechanisms give a biologically plausible explanation for how environmental stimuli and nutritional cues modulate IR and introduce the concept of IR as a dynamic process that may operate in a tissue-specific manner, capable of being reverted by pharmacological or lifestyle interventions.

Thus, in this overview, we review the current knowledge of and recent insights into the role of epigenetic factors in the development of IR, with emphasis on the influence of peroxisome proliferator-activated receptor gamma coactivator 1α (*PPARGC1A* or *PGC1α*) on both fetal programming and *in uterus* metabolic environment and on liver modulation of glucose-related phenotypes. In addition, we discuss the systemic effects of IR beyond the integrity of β-cell function and postulate a "nonpancreatic approach" to understanding T2D pathogenesis. Furthermore, we introduce a novel concept in order to understand the impact of lifestyle intervention on IR-related phenotypes: mitochondrial epigenetics. Finally, we discuss whether epigenetic marks in the genes of the circadian rhythm system are able to modulate insulin/glucose-related metabolic functions.

The Uterine Environment and Epigenetic Marks and Their Role in Adult MS: *PPARGC1A* and Master Metabolic Regulator

After decades of epidemiological research, the medical community is aware of the strong impact of altered intrauterine growth on the development of adult metabolic and cardiovascular disorders. Interestingly, birth weight has been the hallmark feature that connects impaired early-life exposure to nutrients with adult chronic diseases, such as T2D, coronary heart disease, and arterial hypertension [9–14]. This concept was inspired by the Barker hypothesis [15], which postulated that to adapt to a limited supply of nutrients, fetuses make permanent changes in their physiology and metabolism. Barker called these changes *metabolic programming*, which is the origin of metabolic diseases in later life. Interestingly, the initial description, which was understood as the "small baby syndrome hypothesis," was further extended to abnormal fetal growth that involves the two extremes of birth weight, restricted and excessive growth, both of which may originate from alterations in the uterine metabolic milieu [16].

Several animal and human studies have demonstrated the impact of periconceptional exposure to an adverse nutritional environment on DNA methylation of candidate genes, as recently revised [17, 18].

Surprisingly, findings about some interesting candidate genes (*INSIGF*, *GNASAS*, *MEG3*, *IL10*, *LEP*, *ABCA1*, and *IGF2* genes) in a series of remarkable human studies performed on individuals who were prenatally exposed to famine during the Dutch hunger winter in 1944–1945 could not be consistently replicated [19–21], suggesting either that other loci may be involved in fetal metabolic programming or that, perhaps, genomic DNA isolated from the whole blood is not suitable enough for understanding fetal metabolic programming.

Hence, we have hypothesized that exploration of epigenetic marks in DNA isolated from the umbilical cord would be extremely informative, not only because this tissue represents the link between mother and fetus during pregnancy, but also because umbilical cord stromal cells are capable of differentiating into almost all adult cells, including hepatocytes, epithelial cells, neurons, and mesenchyme-derived cells, such as osteocytes, chondrocytes, and adipocytes [22]. Thus, we focused on the impact of birth weight and mother's prepregnant characteristics on umbilical cord DNA methylation in master metabolic genes and explored the status of differential DNA methylation on the promoter of TFAM (mitochondrial transcription factor A, a gene involved in mitochondrial function), *PPARGC1A* (involved in adaptive thermogenesis, glucose and fat oxidation in muscle and fat tissues, and gluconeogenesis in the liver), and *PPARG* (a master gene that controls adipogenesis and insulin signaling) [23]. Interestingly, we observed that promoter methylation of *PPARGC1A* in the umbilical cord of newborns was positively correlated with maternal body mass index (BMI) [23], suggesting that fetal metabolic programming starts very early and maternal metabolic status is capable of influencing an offspring's metabolism throughout epigenetic regulation of active "metabolic genes." It is worth mentioning that *PPARGC1A* promoter methylation in the umbilical cord was modified by the presence of two A risk alleles at the polymorphism rs8050136 in the *FTO* gene (associated with fat mass and obesity), which might indicate that *FTO* acts by altering DNA methylation of metabolic-relevant genes [23].

We next questioned the role of *PPARGC1A* and its ability to connect the maternal environment to the metabolic programming of the progeny and development of adult MS. To answer this question, we carried out an animal study involving rats fed either a high-fat diet (HFD) or a standard chow diet during gestation and lactation and then explored the metabolic effects in adult life [24]. We demonstrated that IR- and MS-related phenotypes in adult life were critically modulated by "liver programming," which implicated changes in mitochondrial DNA (mtDNA) copy number and transcriptional activity of the coactivator *Ppargc1a* [24]. In fact, we observed that liver transcriptional activity of *Ppargc1a* strongly modulates, in a sex-specific manner, glucose homeostasis (liver expression of mRNA *Ppargc1a* was found to be inversely correlated with IR by homeostasis model assessment [HOMA-IR]) and organ fat accumulation in adult life after exposure to a nutritional insult [24]. Our experimental data regarding mtDNA copy number could also be translated to humans, since we observed that

mtDNA content decreased in newborns with abnormal weight, in comparison with infants with appropriate weight for gestational age [25].

Interestingly, circulating leptin was strongly, positively, and linearly correlated with newborn gestational-age-adjusted body weight, and hence, it did not explain the association of abnormal newborn weight with the phenotypes later in life. In addition, newborn body weight could be predicted by the prior offspring, possibly reflecting the fact that this is a reproducible pattern in a family and even from generation to generation.

Two remarkable studies introduced the role of epigenetic marks in the human placenta and observed that lower DNA methylation levels in the promoter of *ADIPOQ* of the placenta were correlated with higher maternal glucose levels during the second trimester of pregnancy [26]. The same group also showed that placental DNA methylation levels of the leptin gene were correlated with glucose levels [27]. Altogether, these observations suggest that to obtain clues about human fetal metabolic programming, researchers should examine tissues that are strong surrogates of the babies' future metabolic program, such as the placenta and umbilical cord. In addition, although it remains to be proven, it seems that modifying the mother's BMI may be an achievable goal for preventing adult diseases in the offspring.

On the other hand, histone modifications and fetal chromatin structure changes associated with MS-related phenotypes still remain poorly unexplored, and there is scarce information regarding human studies. Conversely, more data have been obtained from animal studies; for example, experimental evidence from rodent models showed that methylation levels of histone H3 at lysine residue 4 (H3K4Me2) in candidate gene promoters, such as *Igf1* (insulin-like growth factor 1), are modulated by gestational food restriction and are associated with metabolic abnormalities and obesity [28]. Similarly, gestational protein restriction in pregnant mice was found to be associated with histone modifications in the promoter region of *Igf2* (decrease in H3K4Me3 and H4K20Me3 and increase in H3K9Me3 and H3K27Me3), leading to changes in the mRNA liver transcription level of the fetus [29]. Furthermore, *in uterus* exposure to HFD was observed to program the gluconeogenic capacity of offspring through histone modifications in the *Pck* (phosphoenolpyruvate carboxykinase 1) gene locus in the offspring liver [30].

Interestingly, it was shown that *in uterus* exposure to a caloric-dense HFD induces site-specific alterations in fetal hepatic H3 acetylation, which significantly modify gene expression, leading to obesity and IR [31].

Remarkable findings were recently published demonstrating that *in uterus* exposure to maternal HFD in primates disrupts fetal hepatic circadian gene expression by acetylation of fetal histone H3 at lysine 14 (H3K14Ac) of the neuronal PAS domain protein 2 gene promoter, a paralog of the *Clock* transcription factor [32•]. Finally, Fu et al. recently showed that intrauterine growth restriction modifies epigenetic characteristics of the rat hepatic insulin growth factor-1 gene (*Hgf1*) along the length of the whole gene and that many changes persist postnatally [33].

Thus, altogether, these findings strongly suggest that the molecular basis of the fetal origin of MS-related diseases is associated with epigenetic modifications in the liver epigenome and that "liver metabolic imprinting" during fetal life may be involved in the pathogenesis of adult complications, such as IR- and MS-related phenotypes.

Therefore, clinicians should think about early intervention before pregnancy to reduce the alarming figures of T2D and obesity in children and the adult population, because the epigenetic marks, once triggered during fetal development, seem to be a hardly reversible process.

The Liver Epigenome and IR: From Nuclear to Mitochondrial DNA Methylation

Human physiology has shown that the liver is a key player in the control of glucose homeostasis; in fact, the pathogenesis of peripheral IR is strongly associated with the ability of the liver to suppress endogenous glucose production. Clinical data have consistently shown that fatty liver is insulin resistant and that liver fat is significantly and linearly correlated with all components of the MS independently of obesity [34]. Moreover, it was also shown that intrahepatic fat, but not visceral fat, is linked to metabolic complications of obesity [35]. Hence, we questioned whether fatty liver and IR may be linked by tissue-specific epigenetic modifications that act as a potential modifier of both the conditions. To answer this question, we studied the pattern of DNA methylation of *PPARGC1A* and *TFAM* promoter associated CpG dinucleotides in liver biopsies of patients with NAFLD at different histological disease stages [36••]. We observed that the methylation levels of *PPARGC1A* promoter CpGs were correlated with HOMA-IR and plasma fasting insulin levels and that liver abundance of *PPARGC1A* mRNA was inversely correlated with the methylation levels of *PPARGC1A* promoter CpGs, independently of disease severity [36••]. In addition, NAFLD was associated with reduced liver mtDNA copy number, and liver mtDNA abundance was inversely correlated with HOMA-IR, plasma fasting insulin, glucose, and *PPARGC1A* promoter methylation status [36••].

In this complex scenario, liver mitochondria seem to be critically involved in the control of systemic metabolic functions. Interestingly, decreased mitochondrial DNA copy number is an early manifestation observed in adolescents with IR [37]. In fact, not only mitochondrial dysfunction [38], but also mitochondrial dynamics [39] and biogenesis were found to be associated with T2D and IR [40–43]. Thus,

we explored a novel hypothesis regarding the possibility of "mitochondrial epigenetic" factors to regulate mitochondrial function, and we tested the role of liver mtDNA methylation in the modulation of MS-intermediate phenotypes. This hypothesis was inspired by the recent findings that confirmed a CpG-dependent interaction of a novel mitochondrial isoform of DNMT1 (mtDNMT1) with the human mitochondrial genome [44••]. Surprisingly, we observed that the hepatic status of *MT-CO1* methylation modulates the levels of high-density lipoprotein (HDL) cholesterol, showing that DNA methylation of *MT-CO1* is inversely correlated with plasma HDL levels [45••]. In addition, a significant association between the level of liver *MT-CO1* methylation and BMI was also found, suggesting that the liver epigenome is highly involved in the control of metabolic stressors, IR, and cardiovascular disease. In addition, NAFLD severity, a hallmark of IR, was observed to be associated with decreased *MT-ND6* gene expression, probably due to an increased DNA methylation of the gene [45••]. To our knowledge, this is the first time that an epigenetic mark in the mitochondrial genome has been found to be associated with a human complex disease.

Therefore, clinicians should note that fatty liver is not an innocent bystander condition of the MS, because molecular changes occurring in the steatotic liver strongly influence the systemic metabolic scenario and are associated with cardiovascular disease [46]. Hence, monitoring of this condition should be implemented to prevent the development of long-term consequences of IR.

Dysregulation of Glucose Homeostasis, Epigenetic Marks, and Clinical Consequences

The failure to respond to physiological levels of insulin leads to a global dysregulation of glucose homeostasis. In some target tissues, such as heart, kidney, endothelial cells, and retina, hyperinsulinemia and hyperglycemia are responsible for alterations of organ morphology and function, as was reviewed recently [47]. In fact, the clinical evidence strongly suggests that the structural changes observed in target tissues are linked to changes in their transcriptional program that may be explained by epigenetic regulation. This hypothesis is consistent with recent evidence from experimental studies showing that hyperglycemia induces deacetylation and dephosphorylation of histone H3 in the heart and kidneys of diabetic rats, resulting in alteration of extracellular matrix gene expression and, consequently, functional and structural impairment [48•]. Moreover, microarray data have revealed that the expression of stress-responsive genes is modulated by hyperglycemia by reversible histone modifications in 3T3-L1 preadipocytes [49]. In a diabetic rodent model, continued hypermethylation of CpG sites at the regulatory region of the catalytic subunit of the mitochondrial DNA replication enzyme POLG was found to affect its binding to the mtDNA, compromising its transcriptional activity, which might explain the progression of diabetic retinopathy [50•]. In addition, chronic hyperglycemia was noted to be associated with changes in thioredoxin-interacting protein expression, an endogenous inhibitor of antioxidant thioredoxin induced by H3K9 acetylation, suggesting its critical role in ocular inflammation and endothelial dysfunction in diabetic retinopathy [51]. The effect of hyperglycemia on peripheral endothelial cells was also linked to modulation of acetylated H3K9/K14 [52] and was found to involve H3K4 methyltransferase, Set7 [53].

Furthermore, evidence from human studies is also available. For example, we evaluated the DNA methylation status of the transcription factor A mitochondrial (*TFAM*) promoter in peripheral blood mononuclear cells of adolescents with features of MS and observed an inverse correlation with IR, considering either metabolic quantitative traits (fasting insulin, as well as glucose levels and HOMA index) or IR as a dichotomous condition [54]. In fact, the ratio of the promoter methylated/unmethylated DNA was also noted to be inversely correlated with obesity [54].

In addition, it was shown that DNA methylation of the insulin promoter is increased in patients with T2D, and it was found to be negatively correlated with insulin gene expression in human pancreatic islets [55], a scenario that aggravates glycemic control in the presence of IR.

Altogether, these molecular studies reinforce the concept of "metabolic or glycemic memory," which suggests that glucose-induced epigenetic events are responsible for hyperglycemic complications [56, 57]. The importance of this concept is the novel idea that epigenetic marks can persist and perpetuate the glucose effect on regulation of gene expression even when cells/tissues return to normoglycemia, suggesting that epigenetic events, once activated, can be passed to subsequent cell generations [56, 57]. A clear example of this concept was recently published, showing that persistence of hyperglycemia induces global DNA hypomethylation that correlates with aberrant gene expression for a subset of loci in regenerating tissue [58]. In this scenario, an important question of whether glucose variability would be an independent predictor of end-organ damage remains to be answered.

Finally, as was previously reported, bile acid and glucose metabolism are strongly related [59]; in fact, in streptozocin-induced diabetic mice and genetically obese ob/ob mice, hyperglycemia was found to increase histone acetylation status on the cytochrome P450 enzyme cholesterol 7 α-hydroxylase (*CYP7A1*) gene promoter, leading to elevated basal *Cyp7a1* expression and an enlarged bile acid pool with altered bile acid composition [60]. Indeed, activation of nuclear farnesoid-X receptor (FXR) by bile acid may

modulate the expression of *PEPCK* and, consequently, gluconeogenesis and lipid and glucose metabolism [61] through the inhibition of the hepatic nuclear factor 4 alpha (*HNF4a*), a well-known T2D-associated gene [62]. In addition, bile acids activated TGR5 (also known as GPBAR1, M-BAR, and BG37), a member of the rhodopsin-like superfamily of transmembrane GPCRs, may favor, through the release of incretins, metabolic control [63]. The action of bile acids on epigenetic marks should be further explored, although some indications already exist [59, 64].

Thus, clinicians should consider early detection of hyperglycemia and early intervention to avoid irreversible changes in the transcriptional program of target tissues that negatively impact on their structure and function.

The Circadian Rhythm and Modulation of IR by Epigenetic Modifications

It is well known that the major function of the circadian rhythm system is the internal cycling of physiologic and metabolic events. In fact, there is robust clinical evidence showing that deregulation of the circadian rhythmicity results in pathophysiological changes resembling MS and fat accumulation [65–70], and a comprehensive review has been published recently [71]. Interestingly, candidate gene association studies have shown that variants and related haplotypes in the master regulator of the circadian rhythm, the *CLOCK* gene, are involved in the genetic susceptibility to MS-related phenotypes, as demonstrated by us earlier [72–74] and subsequently further restated by others [75].

Do Epigenetic Modifications Have Any Role in the Pathobiological Connection Between Circadian Rhythm and IR? Prediction of Unexplored Pathways and Areas of Future Clinical Research

The master endogenous clock located in the central nervous system and the molecular oscillators in the peripheral tissues receive light information to entrain the internal clock to the 24-h day; thus, its programming could be driven by epigenetic modifications. Regardless of the biological plausibility, the master circadian gene *CLOCK* has intrinsic histone acetyltransferase (HAT) activity, and this enzymatic function contributes to chromatin-remodeling events implicated in the circadian control of gene expression; in addition, *CLOCK* primarily acetylates histones H3 and H4 [76].

On the other hand, plenty of evidence from in vitro and experimental studies confirms the association between circadian timing and epigenetic code, which was thoroughly reviewed recently [77–79]. Conversely, the evidence from human studies about the role of epigenetic modulation on circadian genes and metabolic control is still scarce. Interestingly, a recent human pilot study demonstrated differential promoter methylation in the core circadian genes in shift workers, showing that long-term shift work resulted in hypomethylation of *CLOCK* and hypermethylation of *CRY2* [80]. Rotating shift work has been found to be associated with many components of the MS, and the interaction of variants of the *CLOCK* and other genes may aggravate the disorder [65, 74].Thus, it is tempting to speculate that subjects exposed to circadian misalignment of jet lag and shift work sleep disruption are susceptible to developing IR and MS as a consequence of modulation of the metabolic epigenome—particularly, the liver epigenome.

The master clock is located in the hypothalamic suprachiasmatic nucleus; nevertheless, the molecular machinery governed by the peripheral clocks present in tissues, such as the liver, is critical for the regulation of metabolic homeostasis. For example, recent findings showed that insulin sensitivity is modulated by changes in histone acetylation in the liver, and genomic recruitment of histone deacetylase 3 (HDAC3) by Rev-erbα directs a circadian rhythm of histone acetylation and gene expression required for normal hepatic lipid homeostasis [81]. Interestingly, we found that regardless of the diet, hepatic expression of Rev-erbα was elevated in an experimental model of cardiovascular disease, the spontaneously hypertensive rat [82]. In addition, mice with liver-specific depletion of *Hdac3* were found to have higher insulin sensitivity without any changes in insulin signaling or body weight, when compared with wild-type mice [83].

Besides the genes that classically integrate the master *CLOCK* transcription network, there are interesting putative pathways, which are still poorly explored in the clinical setting, that might be involved in the pathogenesis of IR. The predicted functional partners of *CLOCK* are shown in Fig. 1. Remarkably, in silico prediction demonstrated that hypoxia inducible factor 1 (HIF1) is associated not only with IR and regulation of β-cell function [84], but also with a protein substrate of HDACs [85]. Furthermore, *PPARGC1A* coactivator-1, which is rhythmically expressed in the liver, was observed to indirectly modulate *HIF1a* signaling to adapt mitochondrial demands [86]. Most important, *Ppargc1*-deficient mice showed aberrant expression of *CLOCK* and metabolic genes [87]. It is worth mentioning that in silico explorations of gene and protein relationships should be considered hypothesis generating until confirmed by experimental data.

While an overlap between hypoxic and circadian pathways has been described earlier [88], experimental evidence from mice showed that exposure to hypoxia led to increased *PER1* and *CLOCK* protein levels in mice [89], while the association between *CLOCK*, hypoxia, and IR is still unexplored. However, as was reported recently by us, upregulation of *Hif1α* in the liver is involved in MS-related

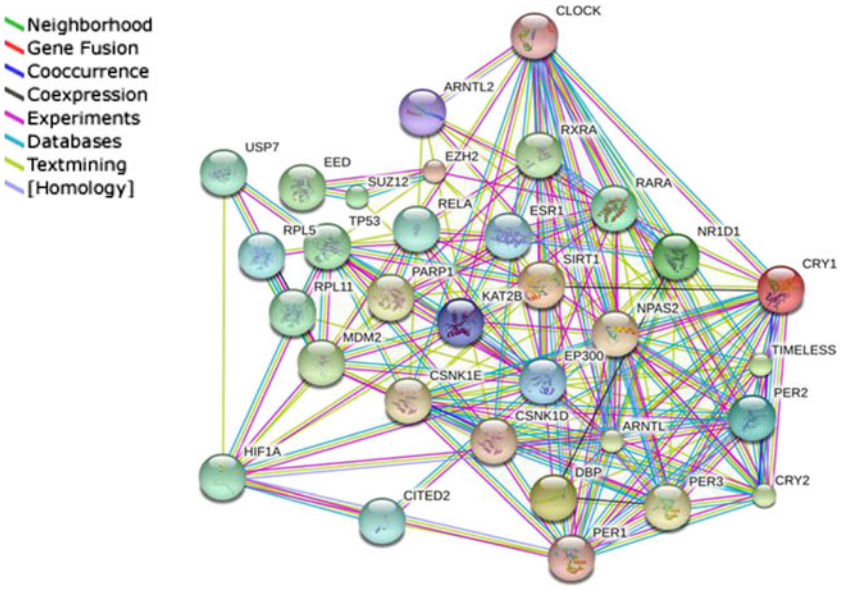

Fig. 1 In silico prediction of CLOCK gene–protein interactions. Network prediction was performed by STITCH resource (http://string-db.org/). CRY1, cryptochrome 1 (photolyase-like); SIRT1, sirtuin (silent mating type information regulation 2 homolog) 1 (S. cerevisiae); NAD-dependent protein deacetylase; DBP, D site of albumin promoter (albumin D-box) binding protein; TIMELESS, timeless homolog (Drosophila); NR1D1, nuclear receptor subfamily 1, group D, member 1; functions as a constitutive transcriptional repressor. RARA, retinoic acid receptor, alpha; PER2, period homolog 2 (Drosophila); EP300, microRNA 1281, functions as histone acetyltransferase and regulates transcription via chromatin remodeling. KAT2B, K(lysine) acetyltransferase 2B, functions as a histone acetyltransferase (HAT) to promote transcriptional activation. ARNTL2, aryl hydrocarbon receptor nuclear translocator-like 2; CLOCK, clock homolog (mouse); PER1, period homolog 1 (Drosophila); EZH2, enhancer of zeste homolog 2 (Drosophila); CSNK1D, casein kinase 1, delta; NPAS2, neuronal PAS domain protein 2; CSNK1E, casein kinase 1, epsilon; PER3, period homolog 3 (Drosophila); PARP1, poly (ADP-ribose) polymerase 1; ARNTL, aryl hydrocarbon receptor nuclear translocator-like; CRY2, cryptochrome 2 (photolyase-like); MDM2, Mdm2 p53 binding protein homolog (mouse); RXRA, retinoid X receptor, alpha; EED, embryonic ectoderm development; SUZ12, suppressor of zeste 12 homolog (Drosophila); TP53, tumor protein p53; RPL11, ribosomal protein L11; HIF1A, hypoxia inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor); USP7, ubiquitin specific peptidase 7; RELA, v-rel reticuloendotheliosis viral oncogene homolog A (avian); CITED2, Cbp/p300-interacting transactivator; RPL5, small nucleolar RNA, C/D box 21; ESR1, estrogen receptor 1

phenotypes and cardiovascular disease [24]. Indeed, through meta-analysis (1,072 patients from 11 studies), we found that NAFLD as a hallmark of IR is significantly associated with obstructive sleep apnea (OSA). Indeed, OSA patients carry an increase of 13 % of ALT and 4.4 % of AST levels and an almost threefold risk of having liver fibrosis when they have NAFLD, which is 2.6-fold more frequent in OSA patients (unpublished data).

Conclusion

Epigenetic changes that involve master regulators of metabolic functions such as *PPARGC1A* have broader implications, from the modulation of uterine environment to tissue-specific modifications of the transcriptional program associated with IR and metabolic syndrome.

Likewise, mitochondrial function and deregulation of oxidative phosphorylation (OXPHOS) in target tissues, as initially reported by Mootha and coworkers in skeletal muscle [90••], significantly impact on the glucose-related metabolism and peripheral IR [36••]. Remarkably, mitochondrial epigenetics is a novel mechanism for understanding the pathobiology of complex diseases such as NAFLD that are involved in the systemic abnormalities associated MS and intermediate phenotypes [45••].

In fact, reduction of *PPARGC1A* transcript levels in metabolically active tissues such as skeletal muscle and the liver strongly modify either insulin secretion [90••] or peripheral IR [36••], respectively.

Furthermore, posttranslational modifications of PPARGC1A may impact on the modulation of IR by enhancing mitochondrial biogenesis and OXPHOS capacity and modulating fatty-acid oxidation.

On the other hand, accumulating evidence indicates that circadian desynchronization and/or alterations in circadian clock gene function have a strong impact on the maintenance of metabolic homeostasis and cardiovascular function. Therefore, clinical investigators should consider the need for implementation of large prospective studies evaluating early therapeutic interventions in subjects chronically exposed to a rotating shift work schedule before irreversible

epigenetic modifications reprogram of the *CLOCK*- related genes and global metabolic homeostasis. Interestingly, some commonly used drugs, such as statins (such as rosiglitazone) and natural compounds (such as estradiol, royal jelly, genisterin, etc.), may have some roles in histone acetylation and methylation, with some acting on *ESR1* (estrogen receptor 1, already mentioned in Fig. 1).

Finally, caution should be taken when using some drugs with unexpected actions on chromatin. An excellent example is valproic acid, a commonly used antiepileptic drug, which may develop MS components as an important side effect [91], possibly related to the fact that valproic acid has proven to be an inhibitor of HDACs [92].

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- • Of importance
- •• Of major importance

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