Epigenetic disturbances in obesity and diabetes: Epidemiological and functional insights

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ABSTRACT

Background: Obesity and type 2 diabetes (T2D) are major public health issues worldwide, and put a significant burden on the healthcare system. Genetic variants, along with traditional risk factors such as diet and physical activity, could account for up to approximately a quarter of disease risk. Epigenetic factors have demonstrated potential in accounting for additional phenotypic variation, along with providing insights into the causal relationship linking genetic variants to phenotypes.

Scope of review: In this review article, we discuss the epidemiological and functional insights into epigenetic disturbances in obesity and diabetes, along with future research directions and approaches, with a focus on DNA methylation.

Major conclusions: Epigenetic mechanisms have been shown to contribute to obesity and T2D disease development, as well as potential differences in disease risks between ethnic populations. Technology to investigate epigenetic profiles in diseased individuals and tissues has advanced significantly in the last years, and suggests potential in application of epigenetic factors in clinical monitoring and as therapeutic options.

Obesity and type 2 diabetes (T2D) are major public health issues worldwide, and put a significant burden on the healthcare system. Genetic variants, along with traditional risk factors such as diet and physical activity, could account for up to approximately a quarter of disease risk. Epigenetic factors have demonstrated potential in accounting for additional phenotypic variation, along with providing insights into the causal relationship linking genetic variants to phenotypes.

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DNA methylation, a key regulator of gene expression and molecular phenotype, is one of the most well-characterized epigenetic modifications, involving the conversion of cytosine to 5-methylcytosine via the covalent transfer of a methyl group to the fifth carbon position of cytosine [19]. This epigenetic mark remains stable during cell division and acts as a form of cellular memory, regulating a wide range of cellular activities including transcription and chromosomal stability, and plays a crucial role in embryonic development, genomic imprinting and X-chromosome inactivation. Methylation patterns are established and modified by specific DNA methyltransferases, such as DNMT1 which transfers patterns of methylation to a newly synthesized strand after DNA replication [20]. DNA methylation is also functionally linked in transcription to histone modification, another major epigenetic regulator in mammalian cells [17]. Together, these two epigenetic processes may provide the underlying mechanism for the stable propagation of gene activity from one generation of cells to the next (Figure 1) [21]. In addition, microRNAs (miRNA) and other small RNAs also provide post-transcriptional regulation for gene and protein expression [22].

Evidence has consistently pointed to the role of epigenetic modification in response to environmental exposure. For example, it is well established that dietary manipulation in Avy mice such as a methyland poor diet impacts upon its coat colour, weight and propensity to develop diabetes and cancer [23]. In rats, maternal protein restriction has been shown to lead to impaired glucose tolerance and insulin resistance in the adult offspring, accompanied by changes in expression for genes involved in insulin-signalling [24]. Similarly, intrauterine growth restriction (IUGR) in rats has resulted in histone code modifications repressing glucose transporter 4 (glut4) expression in the offspring and widespread alterations of DNA methylation in pancreatic islets, and a resultant, increased risk of T2D [25]. Similarly, in humans, maternal under-nutrition and low birth-weight have been associated, following IUGR, with increased risk of T2D in the offspring, with changes in DNA methylation at the HNF4A gene locus, a known T2D susceptibility locus [26–28]. Data from the Dutch Hunger Winter Families study has also demonstrated that individuals exposed to famine in utero during World War II have less methylation of the IGF2 gene in blood DNA and tended to develop obesity later in life at higher rates compared to unexposed same-sex siblings [29].

### 3. DNA METHYLATION IN OBESITY AND TYPE 2 DIABETES

Aberrant DNA methylation has been found to be associated with various complex human diseases, in particular with metabolic disturbances such as obesity and T2D [30–37]. Using the comprehensive array-based relative methylation (CHARM) analysis in 74 individuals, Feinberg and colleagues reported a total of 227 variably methylated regions across the genome enriched for developmental genes. Among these regions, half were stable within individuals for more than 10 years on average, of which four showed consistent covariation with BMI, and were located in or near genes previously implicated in regulating body weight or T2D [30]. Taking an epigenome-wide approach with the Illumina Infinium HumanMethylation450 (450k) array, using peripheral blood samples with a modest sample size of 48 obese and 48 lean African-American youths, Xu et al. found >20,000 differentially methylated CpG sites at a false discovery rate (FDR) of less than 0.05, accompanied by significant enrichment of genes identified by previous GWAS to be associated with obesity as well as T2D and other related diseases such as hypertension and dyslipidemia [31].

In European individuals, Dick et al. reported an increase in methylation at the HIF3A locus to be associated with increase in BMI of adults, both in blood and in adipose tissue. The authors also observed a significant inverse correlation between methylation and gene expression of HIF3A in adipose tissue, providing further support for the role of hypoxia inducible transcription factor pathways in obesity [32]. This association of methylation changes in HIF3A was also corroborated by another study conducted in ∼2,000 African American adults from the Atherosclerosis Risk in Communities (ARIC) study, suggesting that disturbances in DNA methylation associated with adiposity traits are likely stable across tissue type and ethnicity [33]. He and colleagues reported that DNA methylation changes that impact upon adolescent body weight in healthy individuals coincided with methylation patterns at genes previously implicated in obesity, suggesting that the observed changes in methylation profiles occurring early in life at adolescence may likely impact upon increased risk for cardiometabolic diseases later in adulthood [37].

Wahl et al. demonstrated that BMI is associated with widespread changes in DNA methylation in blood across Europeans and Indian Asians (n ∼ 10,000), with these changes largely a consequence rather than cause of adiposity [35]. The identified methylation loci were not only enriched for functional genomic features in multiple tissues and identified genes involved in important pathways such as lipid and lipoprotein metabolism, but also identified gene expression signatures across multiple loci. In addition, a methylation risk score calculated across the loci associated with BMI was strongly predictive of future T2D. Interestingly, it has also been separately shown that approximately half of all T2D associated SNPs affect DNA methylation in blood, further suggesting that aberrant methylation is part of a causal pathway towards development of T2D [38].

To investigate the contribution of DNA methylation to T2D risk in Indian Asians and its contribution to the increased risk relative to Europeans,
4. ALTERNATIVE EPIGENETIC MECHANISMS IN OBESITY AND TYPE 2 DIABETES

4.1. Histone modification

Despite its strong functional link to DNA methylation, there exists less conclusive evidence in the role that histone modification plays with respect to obesity and T2D. To date, there are only a handful of observational studies in T2D, conducted on a small number of subjects. Significantly higher levels of histone H3K9me2 were reported around the interleukin-1A promoter and PTEN coding regions in monocytes from T2D subjects relative to controls [47], along with elevated histone H3 acetylation at the tumor necrosis factor-alpha (TNF-α) and cyclooxygenase-2 (COX-2) gene promoter regions [48]. In addition, subjects with prevalent T2D displayed Set7-dependent monomethylation of H3K4me1 in the NF-κB promoter region, potentially contributing to underlying vascular dysfunction [49]. In an effort to further understand the dynamics of histone marks underlying obesity and T2D, Nie and colleagues undertook a mass spectrometry-based label-free and chemical stable isotope labeling quantitative proteomic approach to systematically profile liver histone post-translational modifications in a prediabetic high-fat diet-induced obese (DIO) mouse model, and reported fifteen histone marks differing in abundance in DIO mouse liver compared with liver from chow-fed mice in label-free quantification, and six histone marks in stable isotope labeling quantification. Interestingly, metformin was able to reverse DIO-stimulated histone H3K36me2, providing support that the histone modification is potentially associated with T2D development [50].

4.2. MicroRNA (miRNA)

MicroRNAs (miRNAs) are a class of small non-coding RNAs of 20–24 nt in length involved in the regulation of gene expression at the post-transcriptional level [51]. miRNAs act by degrading their target miRNAs and/or inhibiting their translation, and are involved in the maintenance of normal cellular physiology and regulation of numerous biological processes including cell proliferation and differentiation. In contrast to research on the contribution of histone modifications to obesity and T2D, there has been significantly more work done with respect to the role of miRNAs. Heneghan et al. found unique miRNA expression profiles for omentum and subcutaneous adipose tissues, along with significantly different levels of two miRNAs (miR-17-5p and miR-132) in omental adipose tissue between obese and non-obese subjects. The authors also observed that the miRNA expression in both omental fat and blood form obese individuals correlated with a range of adiposity and glycemic traits, including BMI, fasting blood glucose, and glycosylated hemoglobin [52].

In plasma, for both adults and children, circulating levels of miRNAs are associated with obesity and other anthropometric measurements, including percentage fat mass and waist/hip circumference [53]. There is a substantial overlap in the panel of miRNAs reported, namely in increased levels of miR-140-5p and -142-3p, and decreased levels of miR-532-5p, -125b, -221, -130b and -423-5p. These miRNAs were identified from the comparison of morbidly obese with lean individuals [53], and in the comparison of lean versus obese children [53]. In another recent study that investigated plasma miRNAs in insulin resistance phenotypes in females with and without obesity across 175 miRNAs, ~60% of the miRNAs were found to be significantly different between controls and at least one obesity phenotype (adjusted P ≤ 0.05), of which two of the miRNAs (miR-378a and miR-122) were observed to be perturbed in metabolically relevant tissues.
such as visceral adipose tissue and pericardial fat in a murine model of obesity [54]. Comparison of miRNA profile of skeletal muscle tissue between prevalent T2D individuals versus controls reported widespread changes, of which 15% of the differentially expressed miRNAs already showed changes in individuals with impaired glucose tolerance, indicative of early involvement of these miRNAs in T2D development [55]. To explore the potential of circulating miRNA profiles in T2D patients as a convenient biomarker, Zampetaki and colleagues carried out a systematic strategy of comprehensive miRNA profiling via an array-based approach on pooled samples. The authors further identified and validated a plasma miRNA signature of five miRNAs (miR-15a, miR-28-3p, miR-29b, miR-126 and miR-223) that displayed a characteristic deregulation in T2D patients, with this change in miRNA profile already observable 5–10 years before disease onset [56]. Other studies that undertook either a microarray profiling approach or a more targeted qPCR strategy in serum, peripheral blood mononuclear cells and whole blood have discovered additional miRNA markers associated with T2D and other glycaemic traits [57–65]. In particular, de Candia et al. recently quantified circulating miRNAs in incident diabetic individuals, allowing them to differentiate, for the first time, between pre-diabetic subjects that progress to T2D or not based on miRNA profiles [66]. Interestingly, a number of these microRNAs also significantly correlated with measures of cholesterol metabolism.

5. FUNCTIONAL INSIGHTS AND POTENTIAL MECHANISMS

DNA methylation at CpG sites regulates gene expression and mediates biological response to environmental exposures. Obesity has been suggested to be a form of systemic, low-grade inflammatory state in adipose tissue, characterized by proinflammatory macrophage infiltration and oxidative stress, which promotes insulin resistance [67]. In view of the critical role that hypoxia plays in the regulation of inflammation and reactive oxygen species production [67–72], it is natural to hypothesize that hypoxia and cellular hypoxic responses may provide mechanistic insight into the causal mechanisms underlying obesity, inflammation, and insulin resistance. Indeed, in fat tissue, a direct role of hypoxia in triggering adipose tissue dysfunction underlying obesity, both in adipose and non-adipose cells, has been demonstrated across in vivo and in vitro studies, as well as in animal models [73–76]. To date, there have been three HIF-α subunits identified, namely HIF-1α, -2α, and -3α. The role of HIF-1α and HIF-2α plays in obesity and glyceric traits such as insulin resistance has been extensively characterized. The two isoforms mediate adaptation and survival to hypoxia through activation of genes involved in angiogenesis, glucose uptake and glycolysis, with non-overlapping targets [67]. Interestingly, differential methylation in HIF3A, which encodes the less well interrogated subunit HIF-3α, has been identified in methylation studies for obesity in both adults and children, and even neonates [32,33,77–79].

Taking a Mendelian Randomization approach, Wahl et al. provided evidence supporting the view that the observed changes in DNA methylation across majority of the 187 identified loci are likely a consequence, and not the cause of adiposity [35]. The methylation loci were enriched for sites of open chromatin in multiple tissues, consistent with the presence of constitutive cis enhancers. In addition, the candidate genes at these loci consisted of genes previously reported to be involved in lipid metabolism, amino acid and small molecule transport, and inflammation, as well as metabolic, cardiovascular, respiratory and neoplastic disease, including ABCG1, which was also identified in earlier EWASs for T2D [34,46]. The only CpG site found in the study that suggested a potential causal role of methylation on BMI was cg26663590. Methylation levels at the locus measured at baseline was predictive of weight gain in longitudinal population studies. This locus also contains the gene encoding SH2B1, which has been previously linked with obesity in GWAS, and reported to be involved in energy and glucose homeostasis [80,81].

In the T2D EWAS conducted across Indian Asian and European incident T2D cases, in addition to ABCG1, significant association was identified for differential methylation and risk of future T2D at four other epigenetic loci, namely PHOSPHO1, SOC3S, SREBF1, and TXNIP [34]. Consistent with being an early marker for impaired glucose homeostasis, TXNIP expression was found to be highly sensitive to glucose concentration, while methylation changes at ABCG1, PHOSPHO1, SOC3S and SREBF1 were associated with measure of adiposity and T2D-related traits such as BMI, waist circumference, insulin concentrations, and HOMA-IR.

Although the exact mechanisms accounting for these early changes in methylation levels prior to T2D onset are unclear, it is worth noting that apart from PHOSPHO1, a bone specific phosphatase with a recognized role in bone mineralization [82], the remaining four genes lie within key pathways underlying T2D and associated metabolic disturbances. ABCG1 is involved in macrophage cholesterol and phospholipid transport, promotes cholesterol efflux to HDL, and regulates cellular lipid homeostasis in various cell types, including pancreatic β-cells. Abcg1–/− mice have impaired glucose tolerance and insulin secretion with normal insulin sensitivity [83], while ABCG1 expression has been shown to be downregulated in humans with diabetes and upregulated by the use of insulin sensitizing agents [84]. SOC3S, a cytokine-inducible negative regulator of cytokine signaling, is a major negative regulator of insulin signaling, and has been implicated in the pathogenesis of obesity and associated metabolic abnormalities. SOC3S expression is increased in skeletal muscle in the setting of diet-induced and genetic obesity, inflammation, and hyperlipidemia, along with impaired systemic and muscle-specific glucose homeostasis and insulin action in the case of muscle-specific overexpression of SOC3S despite unchanged body weight [85]. On the other hand, SOC3S–/− mice demonstrate protection against obesity-induced hyperinsulinemia and insulin resistance [86]. SREBF1 is the master transcriptional regulator of hepatic lipogenesis. Insulin activates SREBF1 by increasing SREBP1 transcription, and the processing of SREBF1 from an inactive membrane-bound precursor to a soluble fragment capable of translocating to the nucleus to activate transcription. SREBF1 is decreased in insulin-deficient states such as fasting, but increased in feeding, obesity and insulin resistance [87]. TXNIP, a key component of pancreatic β-cell biology, nutrient sensing, energy metabolism, and regulation of cellular redox, has its expression highly induced by glucose through activation of the carbohydrate response element-binding protein, which binds the TXNIP promoter [88]. TXNIP downregulates GLUT1, a major transmembrane glucose transporter, thereby acting as a negative feedback loop to regulate glucose entry and mitochondrial oxidative stress. In fact, TXNIP is one of the most glucose-responsive genes expressed in human islets, acting as a mediator of glucotoxic β-cell death in animal models. TXNIP downregulation has a protective effect against obesity-induced diabetes by preventing β-cell apoptosis and preserving β-cell mass [89]. Apart from the above candidate genes, functional annotation of aberrantly methylated genes identified from an EWAS on pancreatic islets from T2D and non-diabetic donors, along with additional RNAi experiments highlighted pathways implicated in β-cell survival and function, as well as cellular dysfunction and adaptation to stressors [44]. A separate study on rat islets and clonal β-cells also found Hdac7...
expression to result in impaired mitochondrial function and insulin secretion [90], whereby HDAC7 was one of the differentially methylated loci identified from human islet EWAS [91].

In terms of the role of miRNAs, obesity-induced overexpression of miR-143 has been shown to inhibit insulin-stimulated AKT activation and glucose homeostasis in mice, while mice deficient for the miR-143-145 cluster are protected from the development of obesity-associated insulin resistance. Together, these provide support that the dysregulation of post-transcriptional gene silencing contributes to the development of obesity-induced insulin resistance [92].

MIR-126, first discovered by Zampetaki et al. in the comparison miRNA profiles between prevalent T2D cases and control within the population-based Bruneck study [56], plays a key role in endothelial cells and contributes to the maintenance and repair of vascular integrity and angiogenesis [93]. This finding was subsequently corroborated by Zhang et al., where it was found to be the only microRNA with a significantly reduced expression in patients with T2D compared to normoglycemic individuals, but also in individuals with impaired fasting glucose relative to normal subjects, suggesting a role in the development of T2D [94].

6. EXISTING CHALLENGES AND FUTURE DIRECTIONS

Recent advancements in technology has now rendered it possible to investigate the link between DNA methylation and various human phenotypes in a high-throughput fashion. To date, most of the published EWASs were performed on methylation arrays. Although the development of methylation arrays has made DNA methylation analyses much more affordable, these arrays remain largely inefficient, covering less than 3% of the CpG sites in the human genome, even on the latest Illumina MethylxomeEPIC Beadchip [95]. In addition, as the contents of these arrays were determined by expert panels, the selected CpG sites present a biased representation of the genome.

In contrast, whole genome bisulfite sequencing (WGBS) is able to reveal methylation status at each cytosine across the whole genome, with approximately 95% of all CpG sites in the human genome assessable via WGBS [95]. In fact, despite the value of higher resolution and unbiased coverage, the widespread utilization of WGBS has been hindered primarily by its high cost and the large DNA input required, compounded by the extensive computational power and expertise necessary for its accurate interpretation. The value of WGBS in the study of diabetes has been previously demonstrated by Jeon et al. who found a 10 kb stretch in the MS2 gene displaying methylation differences strongly related to hyperglycemia in islet preparations from two donors with T2D and 16 non-diabetic donors [96], and Volkov et al. who identified >25,000 differentially methylated regions in islets from individuals with T2D covering loci with known islet function and binding sites previously identified by ChIP-seq for islet-specific transcription factors as well as enhancer regions [97]. With maturation of the next generation sequencing (NGS) technology and improvements in library preparation methods [98], both the cost of WGBS and the DNA input amount necessary have greatly reduced, rendering this technology increasingly affordable for usage in EWAS and other studies. In our benchmarking study where we systematically compared the performance of three WGBS library preparation methods with low DNA input requirement (Swift Biosciences Accel-NGS, Illumina TrueSeq and Qiagen Qiaseq) on two-state-of-the-art sequencing platforms (Illumina NovaSeq and HiSeq X), we demonstrated that the Swift Accel-NGS library preparation method presented the best overall data quality, along with similar performance between NovaSeq and HiSeq X platforms [99]. When we assessed concordance between data generated by WGBS and methylation arrays, we discovered systematic biases between WGBS and methylation arrays, with lower precision observed for WGBS across all depths investigated (10–120x). To achieve a level of precision broadly comparable to the methylation array, a minimum coverage of 100x is recommended. Given the differential cost for sequencing at different depths, this has important implications for the design of future WGBS studies, in particular population-based studies on large cohorts. For example, to detect a difference of 1% in methylation levels between cases and controls, as was observed in previous T2D EWAS, a sample size of ~4,500 will be needed on the methylation array, and ~5,000 for WGBS at 100x coverage. For an average detectable difference of ~6% as reported to be observed in pancreatic islets, a sample size of 158 will suffice at 100x, or 384 at a more modest 30x coverage (Figure 2).

One major drawback of WGBS is the bisulfite conversion step itself, in view of the harsh chemical reaction that degrades up to 99% of the input DNA. This is particularly challenging for clinical application, in view of the limited amount of available input material. Liu et al. successfully developed a bisulfite-free method that directly interrogates 5-methylcytosine (5 mC) and 5-hydroxymethylcytosine (5hmC) at single-base resolution [100]. The method claims to achieve higher sequencing quality for cytosines and guanine base pairs, along with more even coverage of the genome and shorter computational time for analysis. Given the lower DNA input necessary, this method for single-base resolution whole genome sequencing of the epigenome could be the next step forward in epigenetic studies.

7. POTENTIAL THERAPEUTICS

Epigenetic-based therapy, or “epidrugs” as coined by Berdasco and Esteller in their recent review, is one potential focus area for translational epigenetics research [101]. Although all the epidrugs approved for clinical use today are in the field of oncology, it is clear from this that epigenetic-based therapies are currently being explored in preclinical studies as well as in clinical trials. It is also worth noting that although the currently approved epidrugs consist of only DNA methyltransferase inhibitor (DNMTi) or histone deacetylase inhibitor (HDACi), other alternative targets are being investigated, including methyltransferase inhibitors (HMTi) and histone demethylase inhibitors (HDMi).

One potential candidate for the prevention of T2D is HDAC7 which encodes a histone deacetylase (HDAC). In an EWAS on islets from 15 T2D and 34 non-diabetic donors, HDAC7 was found to be
hypomethylated and overexpressed in islets from donors with T2D [91]. A follow-up study in in rat islets and clonal β-cells subsequently found increased Hdac7 expression to impact upon impaired mitochondrial function and insulin secretion, suggesting that changes in HDAC7 methylation and expression may perturb β-cell function [90]. Therefore, it was suggested that HDAC inhibitors could serve as a potential novel therapy by restoring the observed defects described above in Hdac7-overexpressing β-cells [102]. In addition, Set7, a histone methyltransferase, has also been suggested as a novel therapeutic approach to prevent atherosclerotic vascular disease in T2D patients, in view of the impact on vascular dysfunction by Set7-induced epigenetic changes in T2D [49].

MiRNAs could also serve as potential therapeutic agents for obesity and/or T2D. Although their current use is again restricted to cancer, there are on-going clinical trials that are evaluating miRNA mimetics in other pathologies. One example is a mimetic of miR-34a, which is inhibited in most cancers and has been demonstrated to inhibit fat browning in obesity in part by suppressing the browning activators fibroblast growth factor 21 (FGF21) and SIRT [103]. This miR-34a mimetic is currently in Phase 1 clinical trial [104].

Last but not least, demethylating agents might play a critical role in therapeutic options for the treatment and prevention of obesity, T2D and associated complications. The two demethylating agents currently in clinical use are 5-azacytidine and decitabine, both of which are approved only for the treatment of specific forms of myelodysplastic syndrome and acute myeloid leukemia [105]. However, given the broad demethylating properties of both agents and their corresponding high cytotoxicity when incorporated into DNA, it is likely safe to say that these therapies will not be applicable in chronic non-life threatening diseases such as T2D and its associated complications. Less toxic and more-targeted demethylating therapies are needed before it will become possible to incorporate demethylating agents in routine weight management and diabetes treatment.

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CONFLICT OF INTEREST

None declared.

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