Epigenetic regulation of insulin action and secretion – role in the pathogenesis of type 2 diabetes

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Abstract. Ling C (Lund University, Scania University Hospital, Malmö, Sweden). Epigenetic regulation of insulin action and secretion – role in the pathogenesis of type 2 diabetes (Review). J Intern Med 2020; 288: 158–167.

The prevalence of type 2 diabetes (T2D) is rapidly increasing worldwide. Obesity, physical inactivity and ageing increase the risk of T2D. Epigenetic modifications can change due to environmental exposures and may thereby predispose to disease. This review aims at summarizing recent advances in epigenetics related to T2D, with a special focus on impaired insulin action and secretion in humans. There will be an emphasis on analyses in human tissues; both from T2D case-control cohorts and intervention studies. Current data support an important role for epigenetics in the pathogenesis of T2D. Numerous studies have found differential DNA methylation and gene expression in skeletal muscle, adipose tissue, the liver and pancreatic islets from subjects with T2D compared with nondiabetic controls. For example, PDX1 has increased DNA methylation and decreased expression in pancreatic islets from patients with T2D compared with nondiabetic controls. Nongenetic risk factors for T2D such as ageing, unhealthy diets and physical activity do also impact the epigenome in human tissues. Interestingly, physical activity altered DNA methylation of candidate genes for T2D such as THADA in muscle and FTO, KCNQ1 and TCF7L2 in adipose tissue. There is also a strong interaction between genetic and epigenetic factors that together seem to affect T2D. mQTL studies in human adipose tissue and pancreatic islets showed that SNPs associated with DNA methylation levels in numerous sites. Several of these SNPs are also associated with T2D. Recent data also support that DNA methylation of some sites in blood may be developed into biomarkers that predict T2D since methylation of, for example TXNIP, ABCG1 and SREBF1 associated with future T2D. Future studies should use this information for development of new therapies and biomarkers and thereby improve prediction, prevention and treatment of T2D and its complications.

Keywords: ATAC-seq, chromatin structure, diet, DNA methylation, Epigenetics, exercise, histone modifications, insulin action, insulin resistance, insulin secretion, precision medicine, Type 2 diabetes.

Introduction

Type 2 diabetes (T2D) is characterized by chronic hyperglycaemia as a consequence of impaired insulin action and secretion. Insulin-resistance results in reduced glucose uptake in skeletal muscle and adipose tissue in combination with elevated hepatic glucose production and secretion. As a consequence, there is an increased demand of insulin secretion from pancreatic β-cells. However, when pancreatic islets eventually fail to meet the increased need for insulin secretion, T2D develops.

Many people in the world have a sedentary lifestyle, including unhealthy diets and physical inactivity. In addition, the world’s population is getting older. As a consequence, the prevalence of obesity and T2D is rapidly increasing worldwide. Today, there are more than 422 million people with T2D and the number is expected to rise to 592 million in 2035 [1,2]. T2D is a complex and polygenic disease. Genome-wide association studies (GWAS) have shown that many genetic variants with small effect sizes predispose to T2D [3,4]. Ageing, obesity, high-energy diets, lack of physical exercise and an impaired intrauterine environment represent non-genetic risk factors for T2D. Gene-environment interactions, potentially through epigenetic mechanisms, may also affect the disease pathogenesis [5,6]. Indeed, over the last fifteen years there have
been an increasing number of studies supporting a key role for epigenetics in the development of diabetes [7-13]. The goal of this review is to summarize current view of whether epigenetics regulates insulin action and secretion (Fig. 1). Also, it aims to answer whether epigenetics plays a role in the pathogenesis of type 2 diabetes in humans (Fig. 1). Finally, can epigenetics be of any clinical use and provide new therapeutic targets for diabetes? There is a special focus on DNA methylation.

Epigenetics was coined by Conrad Waddington, to link developmental biology and genetics and he set up an epigenetics research unit to study these connections [14]. Moreover, Waddington and Hadorn related genes, gene action to development. In 1969, Griffith and Mahler proposed that DNA methylation and demethylation might contribute to long-term memory in the brain [15]. In 1975, two papers suggested that DNA methylation could have effects on gene expression and these studies proposed that changes in methylation could explain changes in expression. Here, Riggs addressed the issue of X-chromosome inactivation. Histone modifications are also considered to be epigenetic marks, and these marks have been studied for almost 50 years [16]. Since those early days, the research field has grown and currently more than 32 000 publications are found when the search-term ‘epigenetics’ is used. Today, researchers have identified hundreds of histone modifications that are considered epigenetic marks and a large number of enzymes that regulate both DNA methylation and histone modifications have been studied [6,17]. DNMT1, DNMT3a and 3b are methyltransferases that add methyl groups to a cytosine followed by a guanine, a so called CpG site. DNMT1 is known to copy the methylome during replication and when this is not sufficient, passive demethylation can occur. TET enzymes (TET1, TET2 and TET3) convert methyl groups into hydroxyl methylation and may thereby contribute to active demethylation. DNA methylation was initially thought to be a silencing mark, where increased methylation was associated with decreased expression. However, today it is established that it depends on the genomic location and increased methylation in gene body has been associated with increased expression [12]. Moreover, noncoding RNAs have also been considered as epigenetic mechanisms [18]. Epigenetics seems to control cell-specific gene expression, imprinting, X-chromosome activation and cell differentiation. Dysregulation of epigenetic mechanisms may therefore contribute to numerous disease, including T2D [19]. The different epigenetic marks regulate the genome in a complex interplay that needs further investigations.

**Does epigenetics affect insulin action and secretion?**

Insulin resistance and impaired insulin secretion are hallmarks of T2D. Understanding these features could provide valuable insights into the disease. Numerous epigenetic modifications have been found in target tissues for insulin, for example skeletal muscle, adipose tissue and liver using T2D case-control cohorts [8-10,20-25]. These modifications include altered DNA methylation of candidate genes for T2D such as PPARG, KCNQ1, TCF7L2 and IRS1. Altered DNA methylation has also been found in adipose tissue as well as in myoblasts and myotubes of obese insulin-resistant versus nonobese subjects [26-28]. Furthermore, a recent study reported epigenetic and metabolic changes in skeletal muscle during the improvement of insulin sensitivity after metabolic surgery in obese humans [29]. Most of these studies do not show if epigenetic modifications directly affect insulin action but they report epigenetic alterations in insulin-resistant people. However, a few of these studies performed functional follow-up experiments of genes with epigenetic alterations. For example, Davegardh et al. [28] studied IL32
(encoding the cytokine Interleukin (IL)-32), which showed a large increase in expression together with altered methylation in human myotubes compared with myoblasts. Silencing this gene in human myoblasts and myotubes demonstrated that IL-32 regulates myogenesis, insulin sensitivity and ATP levels in muscle cells. In addition, IL32 transgenic mice had lower insulin response and muscle weight. In a different study, You et al. [25] demonstrated that DNA methyltransferase 3a (Dnmt3a) is necessary to mediate insulin resistance in mouse and human adipocytes. Adipose-specific Dnmt3a knock-out mice were protected from diet-induced insulin resistance without accompanying changes.
in adiposity. However, further studies that dissect whether epigenetic mechanisms cause insulin resistance in humans are needed.

Several studies have investigated the link between epigenetics and insulin secretion in humans [7,11,12,30-38]. These include case-control studies where DNA methylation was analysed in islets from human donors with T2D and nondiabetic controls (Table 1). The initial studies used a candidate gene approach and studied methylation of PPARGC1A, INS and PDX1 [7,30,31]. DNA methylation was increased and gene expression decreased for all these genes in islets from donors with T2D. Of note, Barres et al. [8] did also find increased methylation and decreased expression of PPARGC1A in skeletal muscle from subjects with T2D. Moreover, when PPARGC1A (encoding PGC1α, a transcriptional co-activator) was silenced in human islets, insulin secretion was decreased. We also found seven differentially methylated regions (DMRs) annotated to PDX1 in T2D islets when whole-genome bisulfite sequencing (WGBS) was used, which further support an important role of epigenetic regulation of this key transcription factor in diabetes [12]. PDX1 encodes a transcription factor which regulates development of β-cells. It also controls INS expression in mature β-cells. Mice lacking Pdx1 develop diabetes and humans with mutations in PDX1 get a monogenic form of diabetes (MODY4) [39-41]. There was a strong negative correlation between PDX1 expression and promoter/enhancer methylation [31]. Luciferase experiments further showed that increased methylation of the enhancer region directly reduced the transcriptional activity of PDX1 [31].

Array and sequenced based approaches have been used to find differential methylation in T2D islets [11,12,32]. These studies clearly showed that there is a link between epigenetics and insulin secretion. They identified some novel candidates with altered DNA methylation and gene expression in T2D, for example CDKN1A, PDE7B, EXOSC3L2, PID1, SOCS2, PARK2 and NR4A3. Functional follow-up experiments in clonal β-cells demonstrated that all these genes regulated insulin secretion [11,12]. Known candidate genes for T2D, identified by GWAS, such as PPARG, KCNQ1, TCF7L2, IRS1, ADCYS and FTO also had altered methylation in T2D islets [11].

Recent studies have also analysed the chromatin structure in human islets from donors with T2D and nondiabetic controls using ATAC-seq [35,42]. Interestingly, the DMRs annotated to PDX1 are located in open chromatin regions (OCRs). Moreover, both studies found SNPs associated with T2D located in OCRs supporting their functional role. Whilst Khetan et al. found 1515 OCRs, Bysani et al. found 1078 OCRs which differed in islets from T2D versus control donors. Several of these peaks are annotated to candidate genes for T2D such as HHEX, GLIS3, MTNR1B and PARK2. Several of these genes are known to affect insulin secretion. New functional follow-up experiments showed that silencing of SLC16A7, which encodes a pyruvate transporter that has increased expression and enrichments of ATAC-seq peaks in T2D islets, resulted in increased glucose-stimulated insulin secretion [42].

Although these studies in human islets and clonal β-cells support a direct role of epigenetics in the impaired insulin secretion seen in diabetes, further studies are needed to demonstrate causality.

Do nongenetic risk factors for T2D affect epigenetics?

The prevalence of T2D increases with ageing. We demonstrated already in 2007 that age affects both DNA methylation and expression of a candidate gene for T2D, NDUFb6, in skeletal muscle from elderly compared with young people [5]. NDUFb6 belongs to the OXPHOS genes that are downregulated in muscle from subjects with T2D [43]. Another OXPHOS gene, COX7A1, does also have increased methylation and decreased expression in muscle of elderly people and this gene is downregulated in T2D muscle [43,44]. More recent studies have used an array-based approach to study the association between age and DNA methylation in human pancreatic islets, adipose tissue and the liver [26,45,46]. Interestingly, these studies demonstrate that age alters DNA methylation of the same locus, for example CpG sites annotated to KLF14, ELOVL2 and FHL2, in numerous target tissues for T2D. The data in human pancreatic islets also suggest that the epigenetic changes that take place with ageing may be protective against T2D [45]. Moreover, Horvath suggested that the age-related alterations in DNA methylation can be used as an epigenetic clock [47].

A healthy lifestyle reduces the prevalence of T2D and may prevent diabetes in people with increased disease risk [48]. Physical activity and different diets may affect the epigenome and thereby the risk
of disease. We and others have shown that physical activity alters the DNA methylation pattern in human skeletal muscle and adipose tissue [20,49,50]. Several studies have also investigated the effects of different diets on the human epigenome. These diet interventions include short-term high-fat diet overfeeding, 36-hours fasting and a randomized control trial comparing the effects of saturated and polyunsaturated fat [[51-58]. The epigenetic changes induced by exercise and different diets may indeed affect the disease risk and some of the identified changes took place in candidate genes for T2D and were associated with differential gene expression.

Many people with T2D have increased levels of lipids and glucose in their circulation, which have negative effects on islet function [59]. Our group has studied DNA methylation, gene expression, insulin secretion and apoptosis in human pancreatic islets treated with different diabetogenic exposures such as (i) high levels of glucose, (ii) high levels of palmitate and (iii) both high levels of glucose plus palmitate for 48 h [36,37,38]. All three exposures resulted in impaired glucose-stimulated insulin secretion. However, only exposure to both high levels of glucose plus palmitate resulted in increased apoptosis. Exposure to high glucose had the smallest effect on gene expression and DNA methylation, whilst high glucose plus palmitate had the biggest effect (Fig. 2). Interestingly, GLRA1 showed reduced expression and differential methylation in islets from all three exposure groups. GLRA1 encodes a glycine receptor that acts as a ligand-gated ion channel. This receptor is also downregulated in islets from T2D donors and the expression correlates negatively with HbA1c levels [60]. Glycine is an amino acid that is involved in several metabolic pathways, such as glutathione synthesis and regulation of one-carbon metabolism [61]. Obesity and T2D are associated with lower circulating glycine levels. Silencing of GLRA1 in clonal β-cells resulted in reduced glucose-stimulated insulin secretion and exposing β-cells to glycine and taurine (glycine receptor agonist) increased insulin secretion [37]. Of note, CDKN1A was upregulated and showed decreased methylation in islets exposed to high levels of palmitate, as well as to both high levels of glucose plus palmitate [36,38]. As mentioned earlier, this gene does also have decreased methylation and is upregulated in T2D islets [11]. Insulin secretion and proliferation were decreased when CDKN1A was overexpressed [11]. Numerous other genes differentially expressed and methylated by these diabetogenic exposures had similar regulation in T2D islets [11,36-38]. These data suggest that environmental exposures can induce epigenetic changes, followed by altered gene expression that may predispose to impaired insulin secretion and T2D. However, the number of studies analysing DNA methylation in human pancreatic islets still remains limited and we summarize numerous of these in Table 1.

Do genetic and epigenetic interactions contribute to T2D?

Genome-wide association studies have identified several hundred SNPs associated with T2D [4]. Of note, ~25% of all SNPs in the human genome introduce or remove a CpG site (so called CpG-SNPs) and since DNA methylation in humans takes place on a cytosine before a guanine, common genetic variation directly affects every individual’s methylome. Dayeh et al. showed in 2013 that 48% of identified T2D-associated SNPs are CpG-SNPs that directly affect the DNA methylation pattern in human pancreatic islets [62]. These include the TCF7L2, KCNQ1, PPARG, ADCY5 and IRS1 locus. Numerous methylation quantitative trait locus (mQTL) studies have also investigated the genome-wide interaction between SNPs and DNA methylation in cis and trans in target tissues for T2D [63,64,65]. Here, thousands of SNPs were found to be associated with DNA methylation in human adipose tissue and pancreatic islets. Numerous of these SNPs had previously been found to be associated with T2D using GWAS. Overall, it is clear that there is a strong interaction between genetics and epigenetics. This interaction is likely to affect the risk of T2D.

Do epigenetic modifications cause T2D?

Some studies have taken on the challenging task to examine if epigenetic mechanisms may cause T2D. These studies have used both mathematical modelling such as causal inference tests (CITs) and Mendelian randomization analyses as well as experimental approaches including epigenetic editing [64-67] (Fig. 3a).

The CITs were based on mQTL analyses performed in human pancreatic islets and adipose tissue [64,65]. This method tests if a SNP may cause a phenotype via DNA methylation (methylation mediated) or if it is reactive (methylation consequential) or independent (Fig. 3b). CIT identified SNP-CpG pairs where methylation in human islets and
adipose tissue is the potential mediator of gene expression, insulin secretion and insulin resistance, respectively. For example, SNPs annotated to CAMK1D have a causal role on HOMA-IR (measure of insulin sensitivity) mediated via DNA methylation in adipose tissue. SNPs annotated to this gene have also been associated with T2D and it encodes a calcium/calmodulin-dependent protein kinase [68]. Interestingly, another mQTL, where DNA methylation in human islets potentially mediates the causal association between a SNP and insulin secretion, was annotated to PTPRN2, which encodes a protein that is an autoantigen in type 1 diabetes [65,69]. These data support a causal role of methylation on impaired insulin secretion and action and thereby on T2D.

Cardona et al. found that methylation of a CpG site at CPT1A has a possible direct causal role in T2D when they used Mendelian randomization analyses [66]. CPT1A encodes an enzyme regulating the carnitine-dependent transport across the mitochondrial inner membrane and its deficiency results in reduced fatty acid beta-oxidation. Taylor et al. did also use this method to test the link between SNPs, methylation and gene expression and metabolic phenotypes [70].

Epigenetic editing is based on a DNA recognition domain fused to a catalytic domain of a chromatin-modifying enzyme that enables site-specific targeted demethylation or methylation of DNA as well as site-specific changes in histone modifications [71]. Several guide systems exist including transcription activator-like effector (TALE), zinc-finger proteins and Clustered regularly interspaced palindromic repeats (CRISPR) – catalytically inactive Cas9 (dCas9) (CRISPR-dCas9) RNA-guided DNA targeting. Only a few studies have used this approach in cell types of importance for diabetes [67,72,73]. Ou et al. [67] used the TALE-TET1 system to demethylate the imprinted control region 2 (ICR2) near CDKN1C. This resulted in altered methylation, decreased expression of CDKN1C, a

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Fig. 2 Illustration of how exposure to high glucose and palmitate affects human pancreatic islets in three studies by Hall et al.
cell cycle inhibitor and increased replication of pancreatic β-cells. Liu et al. [72] used targeted demethylation of the distal MyoD enhancer by dCas9-Tet1 and managed to reprogram fibroblasts into myoblasts. Moreover, using this approach, Kameswaran et al. identified an intronic enhancer that seems to regulate allele-specific expression at the imprinted DLK1-MEG3 locus [73]. Combining this approach with the identification of epigenetic changes in humans will help answering whether identified changes of specific genomic regions cause T2D-associated phenotypes such as insulin resistance and decreased insulin secretion.

Can epigenetics be of clinical use in diabetes?

There are two areas where epigenetics may be of clinical use in T2D. Novel blood-based epigenetic biomarkers may be used to predict risk for diabetes and related complications. Moreover, inhibitors targeting epigenetic enzymes may be potential future therapies for T2D.

Several studies have shown that DNA methylation in blood is associated with future risk of T2D using prospective population-based cohorts [13,66,74,75]. Methylation of the ABCG1, PHOSPHO1, TXNIP and CPT1 locus could be replicated in independent prospective cohorts. Data from some studies also suggest that blood-based DNA methylation may be used as biomarkers to predict complications in diabetic patients. Agardh et al. found that methylation of, for example AHRR and GLRA1 was associated with future retinopathy. Chen et al. studied methylation in blood from people with diabetes and tested if it was associated with metabolic memory, the prolonged beneficial effects of intensive versus conventional therapy during the Diabetes Control and Complications Trial (DCCT) on the progression of microvascular outcomes in the long-term follow-up Epidemiology of Diabetes Interventions and Complications (EDIC) Study [76]. They showed that methylation differences during the DCCT persist at certain loci associated with glycemia for several years during
the EDIC Study, which support an epigenetic explanation for metabolic memory. Although these studies did not develop blood-based epigenetic biomarkers that predict diabetes or its complications, the data support further development of such biomarkers for clinical use in precision medicine.

Several studies have investigated whether inhibitors of epigenetic enzymes may affect insulin secretion and action. Studies from our group and Thomas Mandrup-Poulsen’s group have used inhibitors of histone deacetylases (HDACs) and lysine demethylases and studied their impact on islet function ([77–84]). These studies support a protective β-cell function and show improved insulin secretion when cells are exposed to these inhibitors. Interestingly, treating human islets from T2D donors with a HDAC inhibitor improved insulin secretion [78]. These results should be followed up.

Conclusion and future perspective

Current data clearly support an important role for epigenetics in the development of T2D through impaired insulin secretion and action. Human T2D case-control studies and intervention studies in non-diabetic people found epigenetic alterations of candidate genes (e.g. PDX1, CDKN1A and GLRA1) which seem to contribute to the disease. Moreover, data from CIT in mQTL studies, Mendelian randomization analyses of DNA methylation data in T2D prospective cohorts and epigenetic editing studies support an important role for epigenetics in the development of T2D. Interestingly, several studies have generated DNA methylation data in blood from prospective cohorts and these studies showed that methylation of some sites associated with future T2D and its complications. Future studies should focus on proving causality between epigenetics and T2D, and these should also test if new T2D therapies targeting epigenetic mechanisms could be developed. The field of blood-based epigenetic biomarkers seems promising and such tools may be developed and used for precision medicine resulting in better prediction, prevention and treatment of T2D.

Acknowledgements

This work was supported by grants from the Novonordisk foundation, Swedish Research Council, Region Skåne (ALF), ERC-Co Grant (PAINTBOX, No 725840), H2020-Marie Skłodowska-Curie grant agreement No 706081 (EpiHope), Härt Lund fonden, EFSD, Exodiab, Swedish Foundation for Strategic Research for IRC15-0067, Swedish Diabetes Foundation and, Albert Pålsson Foundation. The funders had no role in preparation of the manuscript.

Conflict of interest

The author declares that she has no conflict of interest in relation to this publication.

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