# Epigenetic dysregulation in pancreatic islets and pathogenesis of type 2 diabetes

Most type 2 diabetes is thought to be the result of an interaction between genetic and environmental factors. However, the genetic components discovered to date can explain only a small proportion of the observed heritability. The 'missing heritability' might be accounted for by rare variants, gene–environment interactions and epigenetics.

Epigenetics is the study of potentially heritable traits that cannot be explained by changes in the underlying deoxyribonucleic acid (DNA) sequence; that is, a change in phenotype without a change in genotype. The term also refers to the changes themselves: covalent modification of DNA, protein or ribonucleic acid, resulting in changes to the function and/or regulation of these molecules. Epigenetic changes occur in regular and natural ways, but can also be influenced by several factors including age, environment and disease state. Epigenetic mechanisms include DNA methylation, histone modification, chromatin remodeling and non-coding ribonucleic acid-associated gene silencing. Among them, DNA methylation is the best known epigenetic process involving the transfer of a methyl group ($CH_3$) onto the C5 position of the cytosine to form 5-methylcytosine. The majority of this kind of methylation occurs on cytosines that precede a guanine nucleotide (CpG sites).

Among all diseases, the evidence linking epigenetic processes with cancer has been most extensively studied. Recently, several studies focused on the relationship between epigenetic changes and type 2 diabetes. Volkmar et al.[1] reported the epigenetic dysregulation in pancreatic islets from patients with type 2 diabetes. They carried out comprehensive DNA methylation profiling in islets by using the Infinium HumanMethylation27 BeadChip (~27,000 CpG sites representing ~0.1% of the CpG sites in the human genome), and found 276 CpG sites corresponding to 254 genes with differential DNA methylation in diabetic islets. Deyeh et al.[2] also carried out a more comprehensive DNA methylation analysis by using the Infinium HumanMethylation450 BeadChip (~480,000 CpG sites representing ~1.7% of the CpG sites in the human genome), and identified 1,649 CpG sites corresponding to 853 genes and 561 intergenic regions with differential DNA methylation in diabetic islets. They also showed that 102 of differentially methylated genes, including *CDKN1A*, *PDE7B*, *SEPT9* and *EXOC3L2*, were differentially expressed in diabetic islets. However, these studies covered just ~1.7% of CpG sites in the human genome. Thus, the role of epigenetics in the pathogenesis of type 2 diabetes remains largely unknown.

In a recent report, Volkov et al.[3] carried out a more comprehensive and unbiased methylation analysis on human pancreatic islets and found novel differentially methylated regions (DMRs) in diabetic islets. They carried out whole-genome bisulfite sequencing (WGBS) in pancreatic islets from six individuals with type 2 diabetes and eight non-diabetic controls. Although WGBS is expensive, it offers the most comprehensive DNA methylation profile on a single nucleotide resolution. Their analysis covered ~83% of all genomic CpG sites. The average methylation level was 76% with a bimodal distribution: the highest peak at 90% and the second peak at 1.4%. CpG sites located in introns and exons showed the highest degree of methylation (~78%), whereas those around the transcription start site, such as the promoter and the first exon of protein-coding genes, had the lowest degree of methylation (10%–30%). Integration of their WGBS data with histone modification data generated in human islets by the Roadmap Epigenomics Consortium[4] showed that histone modifications associated with active chromatin correlated with a lower degree of methylation (12% for histone H3 lysine 9 acetylation and 8% for histone H3 lysine 4 trimethylation), whereas those associated with repressive chromatin had a higher degree of methylation (50% for histone H3 lysine 27 trimethylation and 80% for histone H3 lysine 9 trimethylation). In addition, combining the WGBS data with published chromatin immunoprecipitation assays with sequencing data[5] showed that binding sites of islet-specific transcription factors (*PDX1*, *FOXA2*, *NKX6.1* and *NKX2.2*) had a lower degree of methylation (21%–44%). Based on the WGBS data and ribonucleic acid-sequencing data obtained from the same islet samples, they investigated the relationship between DNA methylation and gene expression levels. Interestingly, CpG sites located around the transcription start site of protein-coding non-transcribed genes were hypermethylated, whereas those located in exon and intron regions of protein-coding non-transcribed genes showed significantly lower methylation levels than those located in exon and intron regions of protein-coding transcribed genes.

By using BSmooth software (available at: http://rafalab.jhsph.edu/bsmooth),[6] DMRs were defined as regions of three or more consecutive differentially methylated sites with an average methylation difference ≥5% between the control and type 2 diabetes groups. They identified 25,820 DMRs, among which ~13,700 showed increased and ~12,100 decreased levels of methylation in diabetic islets.

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Two of the most significant DMRs including 164 and 105 CpG sites were located in *PDX1* gene, strongly supporting the previous finding of increased methylation and decreased expression of *PDX1* in diabetic islets (Figure 1). Glucose increases *Pdx1* methylation in mouse clonal β-cells,[7] and a hyperglycemia-associated single-nucleotide polymorphism in *PDX1* alters *PDX1* methylation status in human islets.[8] These findings together show that epigenetic modifications of *PDX1* could contribute to the development and pathogenesis of type 2 diabetes.

Among the DMRs, 159 corresponded to 43 of 65 candidate genes for type 2 diabetes,[9] including *ADCY5*, *KCNQ1* and *TCF7L2*. Interestingly, binding sites for islet-specific transcription factors, enhancer regions and different histone marks were enriched in the DMRs. In fact, 457 genes annotated to DMRs, including

*NR4A3*, *PARK2*, *PID1*, *SLC2A2* (*GLUT2*) and *SOCS2*, were differentially expressed in diabetic islets. These genes are enriched for pathways including type 2 diabetes and metabolism, showing that epigenetic changes in islets would result in transcriptional changes that might contribute to altered metabolism and the pathogenesis of type 2 diabetes.

Furthermore, they tried to elucidate whether genes with DMRs and differential expression in diabetic islets have functional roles in insulin secretion. Overexpression of *Nr4a3*, *Pid1* and *Socs2* resulted in decreased fold change of glucose-stimulated insulin secretion (secretion at 16.7 mmol/L glucose vs 2.8 mmol/L glucose), whereas knockdown of *Park2* diminished insulin secretion from INS-1 832/13 rat clonal β-cells, the phenotype similar to that in individuals with type 2 diabetes. This study provides comprehensive islet methylome

profiles of controls and diabetes patients, highlighting the contribution of epigenetic dysregulation in islets to the pathogenesis of type 2 diabetes.

Is information on islet methylome profile useful for the prediction and prevention of type 2 diabetes? As pancreatic islets cannot be non-invasively assessed, it is difficult to apply the islet methylome information for a clinical situation. One solution is use of non-invasively obtained biomarkers that reflect islet methylome profiles. Recently, Bacos *et al.*[10] reported a potential of blood-based biomarkers that correlate with methylation changes in human islets. They first carried out a comprehensive DNA methylation analysis by using the above-mentioned Infinium HumanMethylation450 BeadChip in pancreatic islets of 87 non-diabetic individuals, aged 26–74 years. They found 241 methylation sites (corresponding to 154 genes and intergenic regions) that showed significant age-dependent changes. Interestingly, methylation levels of all 241 sites increased with age. Methylation levels of 32 sites correlated with the expression of respective genes, including *FHL2*, *ZNF518B*, *GNPNAT1* and *HLTF*, and knockdown of these four genes showed functional effects on clonal β-cells. Then, they examined whether age-related methylation changes in blood cells reflect the changes in pancreatic islets. By comparing with data from a previous study of methylation in leukocytes of 421 individuals aged 14–94 years,[11] 139 of the 241 sites showing age-associated methylation changes in islets also showed age-associated changes in leukocytes. The methylation levels of all 139 sites increased with age in both islets and leukocytes. The 139 sites correspond to 83 genes, including *KLF14*, *FHL2*, *FAM123C* and *ZNF518B*. More interestingly, they also examined the association of methylation status in both islets and blood with insulin secretory function, and with future diabetes development. The data suggest that blood-based epigenetic markers correlate with insulin secretory function, and might predict the future capacity to secrete insulin and risk of future diabetes.
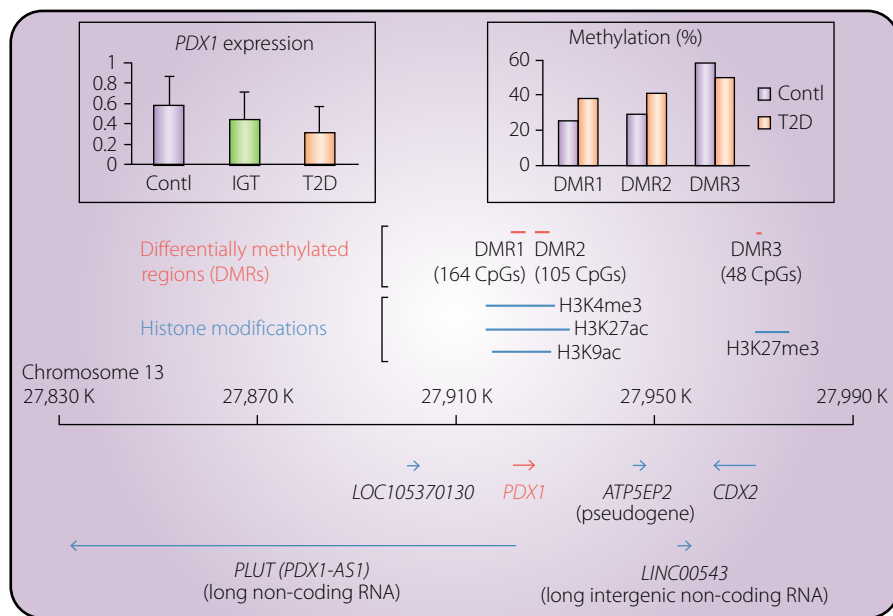


**Figure 1** | Epigenetic modifications around *PDX1*. Differentially methylated regions (DMRs; red bars) and histone modifications (blue bars) are shown around the *PDX1* region on human chromosome 13. *PDX1* expression levels in control participants (Contl), participants with impaired glucose tolerance (IGT) and participants with type 2 diabetes (T2D) are shown on the upper left panel.[7] Average methylation levels of DMRs of control and type 2 diabetes participants are shown on the upper right panel.[3] Histone modifications associated with active chromatin[4]: histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 lysine 9 acetylation (H3K9ac). Histone modifications associated with active enhancers: histone H3 lysine 27 acetylation (H3K27ac). Histone modifications associated with repressive chromatin: histone H3 lysine 27 trimethylation (H3K27me3).

The relationship between epigenetic changes and type 2 diabetes has just begun to be studied recently. The relationship in other ethnic groups, especially Asian populations including Japanese, needs to be elucidated. In particular, blood-based biomarkers that reflect methylation status in pancreatic islets might be useful for prediction, prevention and treatment of type 2 diabetes.

## DISCLOSURE

The author declares no conflict of interest.

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