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Integrating Transcriptome and Epigenome: Putting Together the Pieces of the Type 2 Diabetes Pathogenesis Puzzle

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The complex pathophysiological process of type 2 diabetes (T2D) involves perturbation of gene expression, leading to derangement of multiple physiological processes in tissues that are important in glucose homeostasis (1). Interactions of environmental factors with genetic and epigenetic variants are likely to play an important role; however, the relative contribution of each component to gene expression linked to T2D pathogenesis could differ among individuals. Understanding the molecular mechanisms underlying this interaction is crucial for developing novel therapeutic and preventative strategies for T2D.

Incomplete concordance of T2D in monozygotic (MZ) twins (considered genetically identical) is indicative that nongenetic/environmental factors can play a prominent role in T2D susceptibility and pathogenesis (2). During pre- and postnatal life, environmental factors (including dietary factors) in each tissue microenvironment interact with cellular genetic regulatory architecture and may modulate gene expression. Environmental factors also may modulate gene expression by causing epigenetic modifications of the genome, including DNA methylation (Fig. 1). Thus, tracing molecular events through layers of biological information, including DNA methylation, gene expression, genotype, and physiological data, is required for solving the puzzle of the etiology of T2D.

The role of DNA methylation in cancer and some rare syndromes is well established but defining its contribution in T2D and related common metabolic disorders remains challenging (3–5). Studies that used DNA from human blood cells provide some supporting evidence (6–8). However, because of the tissue-specific nature of DNA methylation, analyzing tissues important for glucose homeostasis may be required to reveal its broader role in T2D susceptibility and pathogenesis (9). A limited number of epigenetic studies from human tissues (including adipose, muscle, and pancreatic islets) provide some evidence for association of differential CpG methylation with glucose homeostasis traits, obesity, metabolic syndrome, and T2D (10–13). However, findings from these studies need to be replicated. A recent study in adipose tissue from 648 female twins expanded understanding of DNA methylation–mediated transcriptional regulation, but a need for disease-specific studies remains (14).

Leveraging the availability of subcutaneous adipose tissue samples from a unique cohort of MZ twins discordant for T2D, a study published in this issue by Nilsson et al. (15) integrates epigenomic and transcriptomic data to identify the role of DNA methylation in modulating expression of genes involved in T2D pathogenesis. T2D in discordant MZ twins can be largely attributed to the nonshared environmental factors between twin pairs, and the strength of this innovatively designed study is identifying environmentally modulated epigenetic variations that may perturb expression of genes in adipose tissue.

In the study by Nilsson et al. (15), the adipose tissue genome-wide expression profile of 12 discordant MZ twin pairs revealed 197 significantly differentially expressed transcripts ($P < 0.05$ and $q < 0.15$) with an average expression difference of 15.3% (range 5–43%) between diabetic and nondiabetic co-twins. Interestingly, gene-set enrichment analysis detected glycan biosynthesis and metabolism pathway genes as most strongly enriched among the transcripts upregulated in the T2D subjects. Along with this novel finding, the authors also detected genes in several previously reported pathways (16), including upregulation of immune and inflammation pathway genes

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COMMENTARY

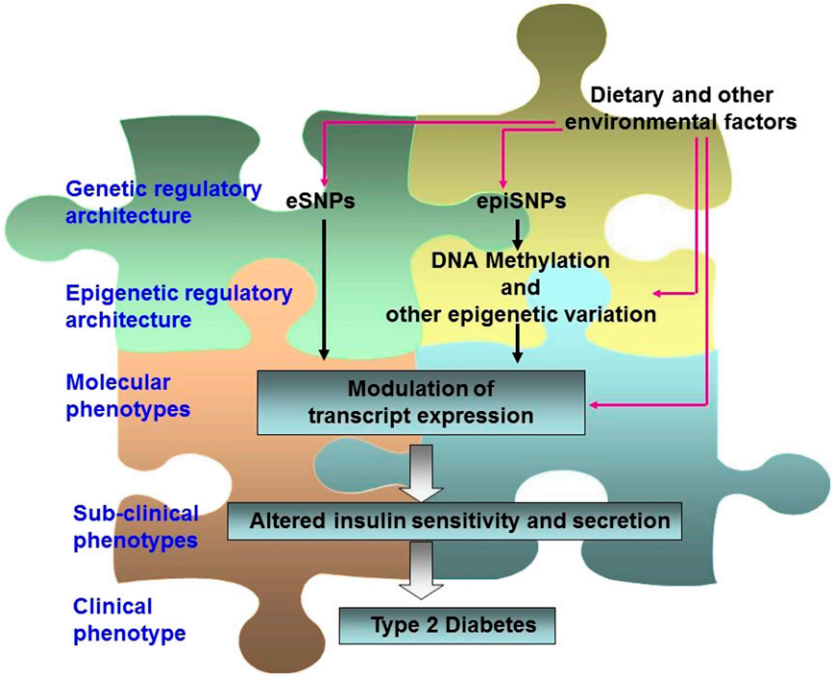

Figure 1—A causal model of T2D pathogenesis. Environmental factors (including dietary factors) may modulate the expression of genes by interacting with cellular genetic regulatory architecture or by causing epigenetic modifications of the genome, including DNA methylation. Perturbation of gene expression in tissues important for glucose homeostasis causes derangement of multiple physiological processes and leads to the pathogenesis of T2D. An integrative functional 'omics paradigm that traces molecular events through layers of biological information is required for solving the puzzle of the etiology of T2D. epiSNP, methylation and other epigenetic regulatory single nucleotide polymorphism (SNP); eSNP, expression regulatory SNP.

and downregulation of genes involved in oxidative phosphorylation, branched-chain amino acids, and carbohydrate and lipid metabolism among people with T2D. Intriguingly, in concordance with the decrease in oxidative phosphorylation in adipose tissue, for the first time they demonstrated a decrease in mitochondrial DNA content in adipose tissue of people with T2D, which may indicate a decrease in mitochondria. As the authors used samples isolated from whole adipose tissue, it is not clear if these derangements are localized to adipocytes or whether they occur in other cell types of adipose tissue.

The authors performed genome-wide CpG site DNA methylation analysis in a discovery cohort of 14 MZ twin pairs and a replication cohort of unrelated people with T2D and normal glucose tolerance. They detected multiple differentially methylated sites, including 1,410 sites that overlapped between the two cohorts. However, the absolute difference in DNA methylation in these CpG sites between patients with diabetes and nondiabetic individuals was small; most sites showed a 1–7% point difference in β-value. Genes that exhibit differential methylation were enriched for several biologically relevant pathways, including inflammation and glycan metabolism. These pathways also showed differential modulation at the transcript level. Further, the authors showed correlations between transcript expression and DNA methylation in CpG sites of 103 genes with differential expression in T2D co-twins. In line with the emerging themes from the epigenomics literature, this study showed both positive and negative correlations of CpG site DNA methylation with transcript expression (14). Negatively correlated CpG sites were enriched in gene promoter regions, whereas positively correlated sites were enriched in gene bodies and 3′UTRs. Thus, despite the limited statistical power of this study, integrating transcriptomic and methylomic data appears to be a useful approach in revealing novel transcriptional regulatory mechanisms, some of which may operate through epigenetic modulation in causing T2D.

Dynamic regulation of DNA methylation in mammalian cells, including those of humans, is primarily achieved through a cyclic enzymatic cascade comprised of cytosine methylation (by DNA methyltransferases), iterative oxidation of methyl groups (by ten-eleven translocation proteins [TETs]), and restoration of unmodified cytosines (by either replication-dependent dilution or DNA glycosylase-initiated base excision repair) (17). This enzymatic cascade is susceptible to environmental factors, including changes in nutrient intake and metabolism (which modulate the level of cosubstrates and cofactors in cells), and plays an important role in epigenetic variability (18). Such dynamic events are difficult to follow in cross-sectional studies (19). However, Nilsson et al. (15) identified decreased levels of *TET1* expression and modulation of other genes in this pathway in T2D subjects.

It is now appreciated that a substantial portion of the epigenetic process operates within the context of genomic sequence (20,21). The stronger correlations of DNA methylation in adipose tissue of MZ twins compared with same-sex dizygotic twins in the study by Nilsson et al. (15) support this view. Interestingly, compared with all sites, CpG sites that are differentially methylated in T2D subjects show stronger heritability for DNA methylation, indicating stronger genetic regulation of DNA methylation for these disease-associated sites. Thus, further studies that integrate both genotypic information and environmental risk factor assessments (e.g., dietary intake) will be useful in improving our understanding of T2D-associated modulation of gene expression.

In summary, the findings of this integrative genomic study by Nilsson et al. (15) support the role of DNA methylation in modulating expression of genes in several biological pathways that may be causally involved in T2D. Despite the correlative nature of the findings from this cross-sectional study, its extensive catalog of T2D-associated molecular changes will be valuable in developing many novel hypotheses for future studies to trace the causal chain of molecular events involved in T2D pathogenesis. Epigenetic analyses in longitudinal or interventional cohorts (including diet, lifestyle, and pharmaceutical interventions to improve glucose homeostasis) may help to elucidate the role of DNA methylation in the molecular events leading to T2D. Utilization of next-generation sequencing techniques (including RNA-seq, whole-genome sequencing, and whole-genome bisulfite sequencing) for the generation of multiscale 'omics' data may be useful in characterizing the gene-expression regulatory network associated with T2D pathogenesis at the nucleotide level. Finally, before we can translate this knowledge in clinical applications, the remaining chasm between correlation and causality needs to be resolved by experiments using in vitro cell models or suitable animal models.

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