(Epi)genomic heterogeneity of pancreatic islet function and failure in type 2 diabetes

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ABSTRACT

Background: Pancreatic islets of Langerhans are heterogeneous tissues consisting of multiple endocrine cell types that carry out distinct yet coordinated roles to regulate blood glucose homeostasis. Islet dysfunction and specifically failure of the beta cells to secrete adequate insulin are known precursors to type 2 diabetes (T2D) onset. However, the exact genetic, (epi)genomic, and environmental mechanisms that contribute to islet failure, and ultimately to T2D pathogenesis, require further elucidation.

Scope of review: This review summarizes efforts and advances in dissection of the complex genetic underpinnings of islet function and resilience in T2D pathogenesis. In this review, we will highlight results of the latest T2D genome-wide association study (GWAS) and discuss how these data are being combined with clinical measures in patients to uncover putative T2D subtypes and with functional (epi)genomic studies in islets to understand the genetic programming of islet cell identity, function, and adaptation. Finally, we discuss new and important opportunities to address major knowledge gaps in our understanding of islet (dys)function in T2D risk and progression.

Major conclusions: Genetic variation exerts clear effects on the islet epigenome, regulatory element usage, and gene expression. Future (epi)genomic comparative analyses between T2D and normal islets should incorporate genetics to distinguish patient-specific from disease-specific differences. Incorporating genotype information into future analyses and studies will also enable more precise insights into the molecular genetics of islet deficiency and failure in T2D risk, and should ultimately contribute to a stratified view of T2D and more precise treatment strategies. Islet cellular heterogeneity continues to remain a challenge for understanding the associations between islet failure and T2D development. Further efforts to obtain purified islet cell type populations and determine the specific genetic and environmental effects on each will help address this. Beyond observation of islets at steady state conditions, more research of islet stress and stimulation responses are needed to understand the transition of these tissues from a healthy to diseased state. Together, focusing on these objectives will provide more opportunities to prevent, treat, and manage T2D.

Keywords Type 2 diabetes; Pancreatic islets; (Epi)genomics; Genetics; Environment; Dysfunction

1. TYPE 2 DIABETES: A DISEASE OF MANY FACES

Like all other forms of diabetes, type 2 diabetes (T2D) is fundamentally a genetic disease. It is a complex, polygenic disorder with substantial environmental contributions and multiple gene—environment interactions. It manifests when insulin resistance unmask intrinsic flaws in islet sensory and/or secretion machinery or impaired resilience and compensation mechanisms, ultimately leading to beta cell failure and death and insufficient insulin secretion. In contrast to monogenic forms of diabetes, which overwhelmingly alter protein-encoding DNA sequences, genome-wide association studies (GWAS) have revealed that the large majority of DNA sequence changes linked to T2D reside in non-coding regions of the genome. Recent studies have highlighted the genetic variability of T2D through quantitative (epi)genomic and clinical measures of human islet function. As (epi)genomic editing technologies such as CRISPR-Cas9 become more widespread in the field, further studies will be able to perturb specific (epi)genetic elements in various islet cell line and whole animal models to bridge the gap between diabetes genetic susceptibility and phenotypes. Examination of patient clinical measurements of islet function alone also provide substantial insights into T2D heterogeneity. One study that performed a topological analysis of 2,551 T2D patients and their corresponding clinical data identified 3 subtypes of T2D with each group showing a distinct and strong association with either diabetic microvascular complications, cardiovascular disease, or neurological diseases and allergies, respectively [1]. Three years later, Ahlqvist and colleagues analyzed a cohort of 8,980 Eastern European individuals with diabetes and subdivided patients into 5 distinct groups with different disease progression and clinical complications [2]. The first subgroup of patients (n = 577) was labeled as severe autoimmune diabetes based on a high prevalence of auto-antibodies for zinc transporter 8A (ZnT8A). Group two (severe insulin deficient diabetes; n = 1575) was characterized by poor metabolic control (highest glycated hemoglobin (HbA1c) levels) and insulin deficiency, while...
individuals in group three (severe insulin resistant diabetes; n = 1373) had higher BMI and insulin resistance. The fourth group (mild obesity related diabetes; n = 1942) possessed the highest average BMI and individuals in the fifth group (mild age-related diabetes; n = 3513) had the latest average age of disease onset. Notably, individuals from groups 1 and 2 had an increased prevalence of ketoacidosis at diagnosis and poor sustained insulin use. Patients in group 2 were also at a high risk for diabetic retinopathy while group 3 patients had a higher risk for kidney disease and diabetic complications (e.g., stroke, etc.). Further collection of patient clinical data and stratification into different subgroups of T2D can help guide medical professionals to design more successful and precise medical solutions. Distinct sets of T2D risk variants were also enriched in each of these clusters, suggesting these may represent genetically distinct T2D subtypes.

2. T2D GENETICS, SUBTYPES, AND MODULES

In late August 2018, the DIAMANTE consortium reported the identification of 243 T2D risk loci, 135 of which were newly discovered in the latest T2D GWAS meta-analysis of almost 900,000 individuals (74,124 cases and 824,006 controls) [3]. They identified 403 signals in 243 T2D risk loci, with approximately two-thirds (n = 151) containing one association signal and the remaining comprising 2–10 distinct ones. Importantly, the approximately three-fold increase in effective sample size enabled the identification of ‘credible’ sets of putative functional SNPs in each locus using genetic fine-mapping approaches. These efforts reduced the putative functional variants to less than 50 for over half of the association signals. For 101 loci, they refined the credible set of SNPs to 10 or fewer variants. Qualitatively, this study underscored conclusions from previous work by the T2D-GENES and GOT2D consortia on the potential regulatory nature of these T2D-associated sequence changes [4], as the large majority of these credible set SNPs overlap non-coding regions of the genome. Most of the T2D risk alleles are considered to act at least in part through islets, given that these overlap with active regulatory elements in LCLs, including active histone tail modification. These sequences overlapped epigenetic hallmarks of active regulatory regions in LCLs, including active histone tail modifications (such as H3K27ac), open chromatin sites, and ENCODE cis-regulatory elements (REs) controlling islet gene expression. Multiple groups completed epigenome profiling of islets at the level of chromatin accessibility, histone post-translational modifications, specific islet transcription factor binding, and DNA methylation to identify putative regulatory regions and specific cis-REs [7–13]. Integration of these epigenomic maps and overlap with T2D GWAS SNP locations revealed a significant and specific enrichment in active regulatory regions, most notably in islet-specific stretch enhancer chromatin states [8] (http://theparkerlab.org/tools/isletseq/) or enhancer clusters [9] (http://www.isletregulome.org/isletregulome/), than would be expected by chance. Naturally, this led to the hypothesis that GWAS variants alter cis-RE expression levels.

3. FUNCTIONALIZING GENETIC VARIATION OF ISLET FUNCTION AND FAILURE

Given their non-coding locations in the genome, it seemed plausible that at least a subset of T2D GWAS SNPs might alter the use or activity of cis-regulatory elements (REs) controlling islet gene expression. Complementary in vitro and in vivo approaches have been developed and employed to nominate putative causal variant(s) among a set of genetically linked SNPs. Traditional reporter assays such as luciferase have been used as in vitro tools to test putative promoter or enhancer sequences for regulatory activity and to successfully identify expression-modulating effects of select candidate T2D GWAS SNPs, usually at the scale of one to tens of test sequences at the most. Unfortunately, they are too expensive and time consuming to systematically assess the hundreds to thousands of T2D credible set SNPs for their ability to modulate reporter activity. Massively parallel reporter assays (MPRA) allow one to create pooled libraries of thousands of DNA sequences and test for their effects on reporter gene expression in a single transfection. MPRA can be used both to test relative regulatory element activity of distinct sequences and to assess and compare the gene regulatory effects of GWAS SNPs (Figure 1). Barcoded reporter gene (e.g., gfp) transcripts are sequenced and quantified by RNA-sequencing. To identify sequences possessing cis-RE activity, barcode counts in the RNA-sequencing data are compared to those in the original plasmid library. Moreover, expression-modulating effects of GWAS SNP effects on cis-RE activity can be identified by comparing RNA-seq barcode counts of the DNA sequences containing the T2D risk and non-risk alleles. To date, most MPRA studies have tested the ability of selected DNA sequences to enhance the transcriptional activity of a minimal promoter, but there is potential to apply more sophisticated techniques to test combinatorial enhancer-promoter sequence effects.

Although these assays have not been conducted yet with T2D GWAS/credible set SNPs, their utility has been demonstrated for the study of other traits and diseases. For example, Tewhey et al. tested approximately 30,000 SNPs associated with expression differences in lymphoblastoid cell lines (LCLs) [14]. 12% of these sequences significantly altered reporter activity, with 95% of those exhibiting increased, enhancer, activity. These sequences overlapped epigenetic hallmarks of active regulatory elements in LCLs, including active histone tail modifications (such as H3K27ac), open chromatin sites, and ENCODE
LCL transcription factor binding sites. As anticipated, these active sequences were enriched for DNase hypersensitive sites that were unique to LCLs and not those for other cell types. Together, these data suggest that MPRA are capable of predicting DNA sequence regulatory activity relevant to in vivo contexts and warrant their use as a tool to identify putative functional variants among the hundreds to thousands of T2D credible set SNPs.

Alternatively, SNP effects on in vivo regulatory element use can be deciphered from (epi)genomic profiles if the individual from which they were generated was genotyped. This information can be leveraged in two ways. If these profiles are generated from multiple genotyped individuals, chromatin accessibility quantitative trait locus (caQTL) analysis can be performed to identify SNP alleles that alter chromatin accessibility (as depicted in Figure 1). Khetan et al. recently completed ATAC-seq analysis of human islets from 19 donors to identify approximately 150,000 open chromatin regions (OCRs) genome-wide [15]. caQTL analyses identified 2949 OCRs whose accessibility was modulated by the genotype of a SNP residing within it. caQTL analysis can be performed to identify SNP alleles that alter chromatin accessibility (as depicted in Figure 1). 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eQTLs may be due to (1) limited power to detect eQTLs with these sample sizes; (2) the distinct locations of the majority of detected eQTL (promoters) compared to GWAS SNPs (enhancers); or the possibility that many T2D GWAS SNPs modulate physiologic or pathologic islet responses. eQTL meta-analyses of the gene expression from hundreds of islets across multiple groups should help resolve these questions. Techniques that interrogate the 3-dimensional architecture of human islets and beta cells have revealed important regulatory features and uncovered extensive enhancer—enhancer, enhancer-promoter, and promoter-promoter links. Initial insights were derived from targeted approaches such as circular chromatin conformation capture (4C) to identify regions interacting with promoters of the INs gene [18,19] or those of other select genes, such as the transcription factors PDX1 and ISL1 [9]. These targeted approaches revealed extensive intra-chromosomal looping interactions with multiple putative enhancers, some at ranges up to hundreds of kilobases to megabases away. When Xu and colleagues blocked INs promoter activity, both contacts with and expression of the interacting genes SYT8 and ANO1 decreased [19]. Interestingly, both of these genes promote insulin secretion, which led the authors to suggest a model wherein physical association of genes involved in insulin metabolism with the highly transcribed INs locus stimulates their expression. Surprisingly, 4C-seq in human EndoC-βH1 beta cells detected contacts between the INs promoter and gene loci on multiple other chromosomes, including both type 1 and type 2 diabetes GWAS loci [20]. As the technical vs. biological nature of trans-chromatin interactions and their meanings is still debated, it will be exciting to see if the existence or dynamics of interactions between these GWAS loci and INs in these potential transcription factories contribute to the underlying molecular mechanisms of their association with either type 1 or type 2 diabetes in people. Nonetheless, these studies of a handful of specific sites reveal surprising intricacies and potential functional importance of transcriptional regulation in islet cell nuclei. More broadly, the ‘all-by-all’ Hi-C approach was recently applied to define the genome-wide location of chromatin interactions and define chromatin territories/neighborhoods in pancreatic islets from one individual and EndoC-βH1 [21]. The majority (>90%) of Hi-C loops detected were shared with other cell types. Moreover, the anchor points of these shared loops were significantly enriched for CTCF and CTCFL motifs, which suggests that the large majority of chromatin neighborhoods are dictated by the relatively limited flexibility in conformations to pack the chromatin into each cell type’s nucleus. In contrast, approximately 10% (n = 1,078) of interactions were unique to EndoC and islets, such as those in the ZnT8 transporter-encoding SLC30A8 locus, and were enriched for beta cell-specific transcription factor binding site motifs [21]. These large-scale Hi-C chromatin neighborhoods in EndoC-βH1 were further refined into hundreds of actively transcribed promoter-promoter, promoter-enhancer, and enhancer—enhancer interactions that were revealed by RNA PolII ChIA-PET [21]. RNA PolII looping sites were notably enriched for beta-cell transcription factors and brought into close proximity genes associated with glucose sensing and insulin secretion. Surprisingly, some loci exhibited an extensive, highly connected network of chromatin interactions, often consisting of >5 distinct connections. These included gene loci encoding transcription factors associated with beta cell identity and development (e.g., PDX1, ISL1, NKX6-1, MAFB, and MIR-375) as well as glucose sensing and insulin metabolism and secretion (e.g., PCSK1, RIMBP2, RGS7, and CDC42) [21]. Overlap of T2D-associated GWAS SNPs with any ChiA-PET interactions was modest, however, suggesting that (1) the sensitivity of PolII ChiA-PET technology was insufficient; (2) PolII-mediated looping with GWAS SNP-containing cis-REs differs significantly between primary adult human islets and EndoC-βH1, which appears to retain some epigenomic signatures of its fetal pancreas origin looping; and/or (3) the looping effect(s) of some GWAS SNP-containing cis-REs may be condition-specific, as has been shown in other cell types [22,23]. Additionally, EndoC-βH1 genotypes may not have permitted assessment of certain GWAS SNPs impacts on cis-RE usage as only 20–30% of associated loci were heterozygous in the cell line. The large majority of disease variants were homozygous in EndoC-βH1 for the non-risk allele. Thus, if the risk allele creates or activates a cis-RE, it will not be detected in this particular individual. Nonetheless, these analyses nominated T2D-associated SNPs mapping to target genes CEP41 and C11orf54 with consistent alterations on cis-RE activity and gene expression in islets and EndoC-βH1 [21]. Several studies have demonstrated that the physiology of EndoC-βH1 cells, including insulin secretion and response to glucose, closely resembled that of islets [24,25]. However, these cells are not responsive to cytokines [26] suggesting there are significant differences in some of their functional properties. Further studies of specific pathways and processes should therefore exercise caution when extending results found in EndoC-βH1 into primary human beta cells. Integration of islet and corresponding cell line multi-genomic datasets with genome-wide genetic variation information, and designing tools to make this information accessible in an interactive, searchable format such as in a web application (https://shinyapps.jax.org/endoc-islet-multi-omics/), will be crucial for nominating further T2D gene targets.

4. PRECISE CELLULAR GENOMICS

Elucidating the molecular mechanisms that lead to beta cell dysfunction and T2D pathogenesis has been a major focus of diabetes research for decades. However, advances in single cell genomic profiling techniques have led to greater understanding of non-beta cell type transcriptional regulation and suggest that they may play important roles in hallmark features of beta cell insufficiency and failure linked to T2D genetic risk and pathophysiology. Single cell transcriptome analysis of human islet cells indicate that multiple monogenic diabetes genes are highly expressed in beta cells (e.g., PDX1, PAX4, INS, HNF1A, and GCK) [27]. However, other non-beta cell types express genes mutated in monogenic diabetes (such as PAX6 and RXF6), congenital hyperinsulinemia (HADH, UCP2) and those implicated as T2D GWAS target/effectors genes [28]. Recent study of type 1 diabetic (T1D) human islets has provided surprising insights into alpha cell biology. In T1D islets, the alpha cell proportions remain relatively unchanged despite abnormal glucagon secretion [29]. This dysregulated glucagon secretion is instead accompanied by decreased expression of important islet transcription factors including ARX, MAFB, and RXF6 and increased expression of stress response factors such as ATF4, ERN1, and HSPA5 [29] suggesting that changes in alpha cell identity may ultimately lead to their dysfunction. Analysis of normal and T2D islet single cells with simultaneous RNA-seq and patch clamping (patch-seq) also revealed subpopulations of alpha cells with varying enrichment for ER stress response genes (e.g., DDIT3, CHOP, PPI1R15A) [30]. Interestingly, this transcriptomic heterogeneity was consistent in normal and T2D islets and associated with variability in alpha cell electrophysiological measures; ER stressed alpha cells had lower cellular size and Na⁺ peak current. Prior single cell transcriptomic analyses have also noted subpopulations of ER-stressed beta cells [31,32] which implicates the dysfunction of both alpha and beta cells in diabetes pathogenesis. Similarly, the integrity of beta and alpha cell functions seem to be
dependent on each other, as under hypoglycemic conditions, T2D islets show reduced insulin, C-peptide, and glucagon secretion [33]. Additionally, during a glycemic clamp experiment, an increase in glucagon secretion was positively correlated with beta cell function suggesting that signaling between the two islet cell types is crucial for maintaining glucose homeostasis. Studies of delta cells in Sst-Cre transgenic mouse models [34–36] reveal that timely regulation of insulin secretion is controlled by various delta-cell specific pathways. Induction of the ghrelin receptor (Ghsr) in delta cells was correlated with enhanced somatostatin release and ultimately reduced insulin and glucagon secretion [35,36]. Furthermore, the peptide hormone Unc3 was shown to be co-released with insulin from beta cells to activate type 2 corticotropin-releasing hormone receptor (Crhr2) on delta cells in an alternate pathway that promotes somatostatin release and negatively regulates insulin levels [34]. Delta cells are also notably enriched for G protein-coupled receptors (e.g., GLP1R, GIPR, GPR120) which exert careful control over metabolism [37]. These receptors are also common therapeutic targets of T2D, suggesting that treatment and management of the disease should not neglect delta cell functionality and/or survival.

Efforts to characterize the epigenomes of each islet cell type are emerging and revealing new insights of cellular fate and differentiation. Two groups have performed open chromatin profiling of purified beta and alpha cell fractions [10,12] and identified between 1850 and 3999 beta and 5316-27,000 alpha-specific peaks. These cell-specific regions were enriched for transcription factor motifs implicated in cell development and were enriched for diabetes-associated SNPs. Arda and colleagues also suggest that the beta cell epigenome is plastic and capable of being derived from other endocrine and exocrine precursor cells. Discrepancies in the numbers of cell-specific peaks determined by both groups are likely due to the cell surface markers used to enrich for each. CD26/DPP4, used by Arda et al., is a strong positive selector for alpha cells, which then enables negative selection for beta and other minor cell populations. However, this method of enrichment for beta cells will not remove contaminating delta and PP/gamma cells. Continued development of new tools and markers for islet cell enrichment, such as NTPDase3 [38] should continue to help us to understand changes elicited by genetic and environmental factors in each distinct cell type.

Iterative proteomic screens in human islets are also proving useful for identifying putative cell-specific surface markers for isolation [39], wherein beta and delta cell populations were obtained by co-enrichment for CD9 and CD56. Challenges currently remain to exclusively enrich for the minor islet cell types (delta, gamma/PP), thus strategies that negatively select for these cells may be needed. Study of the rarer gamma/PP cells, which constitute roughly <1–5% of the total islet volume, remain limited due to the lack of known cell-surface markers for enrichment and purification (Figure 2). Whole islet analyses are unable to capture cell type-specific changes and therefore preclude analysis of their potential roles in T2D genetics and pathophysiology. Given the clear and extensive genotype effects on cis-RE usage [13,15] and gene expression [11,16,17] in islets, more extensive analysis of sorted cell types from multiple individuals is warranted to define a representative set of islet cell-specific REs and distinguish condition-specific from genotype-driven effects on their use and activity.

5. ISLET RESPONSES; MOVING BEYOND STEADY STATE MEASUREMENTS

To date, the overwhelming majority of studies including and assessing genetic variation have proﬁled the steady state patterns of epigenetic modifications and gene expression in islets or their constituent cell types. Others have compared how these steady state measures differ between T2D and non-diabetic (ND) individuals [13,16,40–44]. Surprisingly, these studies, especially transcriptome analyses, have identiﬁed only modest alterations despite clear phenotypic differences in Hba1c and other metabolic traits in T2D vs. ND donors. This suggests that alterations in transcriptional regulation may not contribute to T2D pathogenesis, or that these (epi)genomic comparative studies are not effectively capturing the alterations associated with islet (patho)physiologic decline or T2D onset. Genomic assays such as RNA-seq provide only a snapshot of tissues’ or cell types’ transcriptomes at a given point in time. Genes that are important for islet function and resilience (e.g., Gene A) and genes whose expression induces islet failure (e.g., Gene C) would be detected in a comparative analysis between islets at healthy and T2D states (Figure 3). In contrast, genes that are temporarily induced by the initiation of islet stress or in the compensation or pre-diabetic stages (e.g., Gene B) before decline towards disease state would be missed.

Furthermore, T2D is a complex disease with dynamic ranges of severity and secondary health complications across individuals. Thus, comparing single snapshots of gene expression in T2D individuals at different stages of islet health and disease progression may simply lead to obfuscation. Longitudinal studies of in vivo epigenetic and gene expression changes in islets of severe, early onset (db/db) or polygenic, late-onset (Tallyho, NZO) [45–47] diabetic mouse models may be the only practical solution to identify the temporal nature of these changes and identify the molecular features of islet dysfunction, compensation, and failure in T2D pathogenesis. Indeed, longitudinal analyses of aging islets in mice identiﬁed DNA methylation changes in key genomic regions associated with beta cell proliferation and metabolism [48]. These ﬁndings suggest that changes in the islet (epi)genome and transcriptome may also be dynamic during the course of T2D development and progression. Alternatively, in vitro, it may be possible to subject human islets to diabetic-like conditions through the use of inﬂammatory cytokines and/or oxidative and ER stress. Already, studies from a few groups have demonstrated clear differences in islet gene expression, including the modulation of putative T2D target genes, during stimulatory or stress responses, and certain epigenetic and gene expression features in islets are only revealed upon these in vitro or in vivo exposures, such as glucose-stimulated insulin secretion, palmitate, inﬂammatory cytokines or other response defects [49–53]. Examining the transcriptomic and (epi)genomic changes of human islets under these various stressors over time may provide greater knowledge of the epigenetic and gene expression changes preceding islet stress, failure, and ultimately diabetes onset.

6. FROM CORRELATION TO CAUSATION: MODELING T2D VARIANT EFFECTS AND TARGET GENE FUNCTION

Together, the studies highlighted above combine to unveil important information necessary to translate T2D GWAS statistical associations into biological knowledge of the genes and mechanisms underlying T2D risk and progression: the putative functional variant(s), the gene or genes they regulate, whether the T2D risk alleles activate or inactivate their targets, and the cell types in which they elicit these effects. The final, and most critical, step is to take these data, generate testable hypotheses, and create accurate, or perhaps more importantly, relevant models to determine the molecular, cellular, and physiologic functions of the T2D-associated target genes and thereby close the association-biology gap.
A variety of cellular and animal models have been developed and applied over the past few years to experimentally manipulate cis-regulatory elements and their target gene function as it related to beta cell/islet function, glucose homeostasis, and T2D pathogenesis. CRISPR/Cas9 has revolutionized our ability to modify genomes and epigenomes almost at will. Unsurprisingly, CRISPR (epi)genome editing tools can and have been used to target putative T2D target genes or cis-REs in beta cell lines and assess their functions. As some of the T2D risk variants have been linked to increased cis-RE activity and target gene expression, such as the C2CD4A/B locus, the CRISPR activation systems, comprising a catalytically dead Cas9 (dCas9) protein linked to epigenetic activator proteins such as the histone acetyltransferases p300/CBP or synthetic activation module (SAM), will be essential tools to model the molecular and cellular effects of the T2D risk variant.

Review
ADCY5 locus provides an example of how the complementary approaches described above can converge to translate the T2D risk variant statistical associations into consistent phenotypes and increased biological knowledge. Among several putative functional SNPs in this T2D-associated locus, one (rs11708067) overlaps an islet enhancer and alters islet chromatin accessibility [13,15] and is linked to reduced T2D risk allele expression and insulin content was substantially reduced in INS-1 (832/13) clones in which this cis-RE was deleted using CRISPR/Cas9 compared to wildtype clones [55]. Finally, ADCY5 silencing in human islets impaired glucose signaling and glucose-stimulated insulin secretion. Other loci, such as TCF7L2, illustrate a challenge of studying ‘enhanceropathies’. The genetic and molecular pathophysiology seems more complicated, with potentially pleiotropic effects. The rs7903146 T2D-associated SNP overlaps an islet open chromatin region (OCR) and the risk ‘T’ allele is associated with increased TCF7L2 expression and decreased insulin content and secretion in human islets [59]. However, TCF7L2 is expressed in multiple metabolically active tissues, and tissue-specific deletions implicate distinct roles for Tcf7l2 function in liver and in both pancreatic islet alpha and beta cells to maintain glucose homeostasis. Some of these complications likely reflect the differing functional impacts of mutations in the regulatory element vs. the protein-coding gene. SHH provides an extreme but informative example of this [60]. SHH is used iteratively during development for fundamental patterning decisions in different tissues, and Shh whole body knockouts are embryonic lethal. However, homozygous deletion of a distal limb enhancer sequence >1 million nucleotides away from the Shh promoter produces mice with no limbs, and single nucleotide changes in the equivalent Shh distal limb enhancer in humans cause polydactyly. We anticipate that direct modeling of the ‘enhanceropathies’ by (1) explicitly removing the cis-RE or switching SNP alleles within it using genome editing or (2) epigenetically modulating its activity will help to resolve the pleiotropic and contradictory effects of certain GWAS loci, such as TCF7L2.

Figure 3: Challenges with identifying gene expression alterations in type 2 diabetes. Gene expression measurements from RNA-seq data typically represent only a snapshot of tissues’ or cell types’ transcriptome at a given point in time. In recent comparative analyses of islet intact and single cell transcriptomes from T2D and ND individuals, relatively few genes are significantly altered despite the clear phenotypic differences between them. This may suggest that the mechanisms that precede islet failure and T2D pathogenesis are post-transcriptional and cannot be detected in conventional RNA-seq analyses. However, it is also possible that the putative paths of these genes’ alterations over the course of islet physiological decline and T2D development are simply being missed. Genes that are important for islet function and resilience (e.g., Gene A) and those whose expression directly induces or is the consequence of islet failure (e.g., Gene C) may be detected in a comparative analysis between islets at healthy and decompensated states. However, response genes that are temporarily induced by islet stress (e.g., Gene B) would not be detected in this comparison.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Stratification of genetic risk and progression in T2D and precise understanding of the molecular, cellular, and physiologic effects of T2D risk variants are key objectives moving forward. Studies to date have provided important insights into and surprises about fundamental features of islet gene regulatory programs and the genetics of T2D. The extensive effect of genetic variation on islet cis-RE use and gene expression that has been uncovered, even under steady state, emphasizes the importance of sampling multiple individuals for a given process that is to be studied and of evaluating and incorporating genetic variation into future human islet studies to identify representative, not ‘reference’, islet responses to stimuli and diabetogenic stressors. Genetic and environmental factors appear to be impinging on the same territory of the genome to elicit their (potentially deleterious) epigenetic effects on islet cell identity and function, namely distal enhancer elements. Incorporation of genotype information for each human islet sample will allow us to decode the genetic programming of islet environmental responses and identify T2D SNPs that enhance or suppress these responses. Together with improving techniques to purify and obtain distinct islet cell populations, we can better
understand each cell type’s genomic architecture and better characterize their roles in islet resilience and failure. Experimental manipulation of the regulatory elements and/or the target genes identified by (epi)genomic approaches described above and modeling the putative pathways and processes they implicate in human islet cell lines (e.g., EndoC-βH1-H3) is essential to progress from correlation to causation. Similarly, transitioning from “the” mouse (C57BL/6) to multiple mouse models for insights into the effects of naturally occurring genetic variation on islet function and physiology [61] and for manipulation of key genomic elements should also help characterize the dynamic range of islet behavior and response.

T2D is a heterogeneous, complex, and progressive disorder, as multiple subtypes have been identified and associated with different genetic risk and clinical outcome profiles. Future islet genomics studies that focus on identifying the distinct subgroups of individuals with distinct genes/pathways that are disrupted and/or contributing to islet (dys)function at basal and/or responsive states are needed. Furthermore, priority should be given to profiling more islets from pre-diabetic and T2D individuals to characterize the transition between basal to stressed to T2D state and determine if there are intermediate signatures for islet failure and T2D onset. Together, this multi-pronged approach toward studying T2D genetics and islet pathophysiology will help identify additional targets and opportunities for intervention that can be exploited for more precise and effective preventative, treatment, and management options for T2D.

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**CONFLICT OF INTEREST**

None declared.

**REFERENCES**

[1] Li, L., Cheng, W.-Y., Glicksberg, B.S., Gottlesman, O., Tamler, R., Chen, R., et al., 2015. Identification of type 2 diabetes subgroups through topological analysis of patient similarity. Science Translational Medicine 7(311):311ra174.

[2] Ahlvqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., et al., 2018. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. The Lancet Diabetes & Endocrinology 6(5):361–369.

[3] Mahajan, A., Tallinn, D., Thurner, M., Robertson, N.R., Torres, J.M., Rayner, N.W., et al., 2018. Fine-mapping type 2 diabetes loci to single-variant resolution using high-density imputation and islet-specific epigenome maps. Nature Genetics 50(11):1505–1513.

[4] Fuchsberger, C., Flannick, J., Teslovich, T.M., Mahajan, A., Agarwala, V., Gautilon, K.J., et al., 2016. The genetic architecture of type 2 diabetes. Nature 536(7614):41–47.

[5] Udder, M.S., Kim, J., von Grothuuss, M., Bonás-Guarch, S., Cole, J.B., Chiu, J., et al., 2018. Type 2 diabetes genetic loci informed by multi-trait associations point to disease mechanisms and subtypes: a soft clustering analysis. Public Library of Science Medicine 15(8):e1002654.

[6] Wood, A.R., Jonsson, A., Jackson, A.U., Wang, N., van Leeuwen, N., Palmer, N.D., et al., 2017. A genome-wide association study of NGTT-based measures of first-phase insulin secretion refines the underlying physiology of type 2 diabetes variants. Diabetes 66(8):2296–2309.

[7] Stitziel, M.L., Sethupathy, P., Pearson, D.S., Chines, P.S., Song, L., Erdos, M.R., et al., 2010. Global epigenomic analysis of primary human pancreatic islets provides insights into type 2 diabetes susceptibility loci. Cell Metabolism 12(5):443–455.

[8] Parker, S.C.J., Stitziel, M.L., Taylor, D.L., Orozco, J.M., Erdos, M.R., Akiyama, J.A., et al., 2013. Chromatin stretch enhancer states drive cell-specific gene regulation and harbor human disease risk variants. Proceedings of the National Academy of Sciences of the United States of America 110(44):17921–17926.

[9] Pasquali, L., Gautlon, K.J., Rodriguez-Segui, S.A., Mularoni, L., Miguel-Escalada, I., Akerman, I., et al., 2014. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. Nature Genetics 46(2):136–143.

[10] Ackermann, A.M., Wang, Z., Schug, J., Naji, A., Kaestner, K.H., 2016. Integration of ATAC-seq and RNA-seq identifies human alpha cell and beta cell signature genes. Molecular Metabolism 5(3):233–244.

[11] Varshney, A., Scott, L.J., Welch, R.P., Erdos, M.R., Chines, P.S., Narisu, N., et al., 2017. Genetic regulatory signatures underlying islet gene expression and type 2 diabetes. Proceedings of the National Academy of Sciences of the United States of America 114(9):2301–2306.

[12] Arda, H.E., Tsai, J., Rosli, Y.R., Giresi, P., Bottino, R., Greenleaf, W.J., et al., 2018. A chromatin basis for cell lineage and disease risk in the human pancreas. Cell Systems 7(3):310–322.e4.

[13] Thurner, M., van de Bunt, M., Torres, J.M., Mahajan, A., Nylander, V., Bennett, A.J., et al., 2018. Integration of human pancreatic islet genomic data refines regulatory mechanisms at type 2 Diabetes susceptibility loci. Elife 7.

[14] Trewhey, R., Kollari, D., Park, D.S., Liu, B., Winnicki, S., Reilly, S.K., et al., 2016. Direct identification of hundreds of expression-modulating variants using a multiplexed reporter assay. Cell 165(6):1519–1529.

[15] Khetan, S., Kursawé, R., Youn, A., Lawlor, N., Jillette, A., Marquez, E.J., et al., 2018. Type 2 diabetes-associated genetic variants regulate chromatin accessibility in human islets. Diabetes 67(11):2466–2477.

[16] Fadista, J., Vikman, P., Laakso, E.O., Mollet, I.G., Escalada, I., Akerman, I., et al., 2018. Pancreatic islet enhancer clusters enriched in type 2 diabetes risk-associated variants. Nature Genetics 46(2):136–143.

[17] van de Bunt, M., Fox, J.E.M., Dai, X., Barrett, A., Grey, C., Li, L., et al., 2015. Transcription expression data from human islets links regulatory signals from genome-wide association studies for type 2 diabetes and glycemic traits to their downstream effectors. Public Library of Science Genetics 11(12):e1005694.

[18] Xu, Z., Wei, G., Chepelev, I., Zhao, K., Felsenfeld, G., 2011. Mapping of INS promoter interactions reveals a role for transcription. Nature Structural & Molecular Biology 18(3):372–378.

[19] Xu, Z., Leffevre, G., Manivannan, T., Claire, M.B.F.S., Riddick, G., Felsenfeld, G., 2014. Mapping of long-range INS promoter interactions reveals a role for calcium-activated chloride channel ANO1 in insulin secretion. Proceedings of the National Academy of Sciences of the United States of America 111(47):16760–16765.
Jian, X., Felsenfeld, G., 2018. Insulin promoter in human pancreatic β cells contacts diabetes susceptibility loci and regulates genes affecting insulin metabolism. Proceedings of the National Academy of Sciences of the United States of America 115(20):E4633–E4641.

Lawlor, N., Márquez, E.J., Orchard, P., Narisu, N., Shamim, M.S., Thibodeau, A., et al., 2019. Multiomic profiling identifies cis-regulatory networks underlying human pancreatic β cell identity and function. Cell Reports 26(3):788–801.e6.

Phanstiel, D.H., Van Bortle, K., Spacek, D., Hess, G.T., Shamim, M.S., Machol, I., et al., 2017. Static and dynamic DNA loops form AP-1-bound activation hubs during macrophage development. Molecular Cell 67(6):1037–1048.e6.

Li, P., Mitra, S., Spotski, R., Oh, J., Liao, W., Tang, Z., et al., 2017. STAT5-mediated chromatin interactions in super enhancers activate IL-2 highly inducible genes: functional dissection of the Il2ra gene locus. Proceedings of the National Academy of Sciences of the United States of America 114(46):12111–12119.

Andersson, L.E., Valfat, B., Bagge, A., Sharroyko, V.V., Nicholls, D.G., Ravassard, P., et al., 2015. Characterization of stimulus-secretion coupling in the human pancreatic EndoC-βH1 beta cell line. Public Library of Science one 10(3):e0120879.

Terao, H., Lenzen, S., 2017. Dynamics of insulin secretion from EndoC-βH1 β-cell pseudopods in response to glucose and other nutrient and nonnutrient secretagogues. Journal of Diabetes Research 2017:2309630.

Oleson, B.J., McGraw, J.A., Broniowska, K.A., Annamalai, M., Chen, J., 2012. The Jackson laboratory, NONcNZO10Lt/J and TALLYHO/JngJ. Journal of Diabetes Research 2012:165327.

Li, J., Klughammer, J., Farlik, M., Penz, T., Spittler, A., Barbieux, C., et al., 2016. Single-cell transcriptomes reveal characteristic features of human pancreatic islet cell types. European Molecular Biology Organization Reports 17(2):178–187.

Lawlor, N., George, J., Bolsiety, M., Kursawe, R., Sun, L., Sivakamasundari, V., et al., 2017. Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. Genome Research 27(2):208–222.

Brissova, M., Haliyur, R., Saunders, D., Shrestha, S., Dai, C., Blodgett, D.M., et al., 2018. β cell function and gene expression are compromised in type 1 diabetes. Cell Reports 22(10):2667–2676.

Camunas-Soler, J., Dai, X., Hang, Y., Bautista, A., Lyon, J., Suzuki, K., et al., 2019. Pancreas patch-seq links physiologic dysfunction in diabetes to single-cell transcriptomic phenotypes. BioRxiv 555110.

Baron, M., Veres, A., Wolok, S.L., Faust, A.L., Gaulouy, R., Vetere, A., et al., 2016. A single-cell transcriptomic map of the human and mouse pancreases reveals inter- and intra-cell population structure. Cell Systems 3(4):346–360 e4.

Muraro, M.J., Dharmadhikari, G., Grün, D., Groen, N., Dielen, T., Jansen, E., et al., 2016. A single-cell transcriptome atlas of the human pancreas. Cell Systems 3(4):385–394.e3.

Mumme, L., Breuer, T.G.K., Rohrer, S., Schenker, N., Menge, B.A., Holst, J.J., et al., 2017. Defects in α-cell function in patients with diabetes due to chronic pancreatitis compared with patients with type 2 diabetes and healthy individuals. Diabetes Care 40(10):1314–1322.

van der Meulen, T., Donaldson, C.J., Cáceres, E., Hunter, A.E., Cowing-Zitron, C., Pound, L.D., et al., 2015. Urocortin3 mediates somatostatin-dependent negative feedback control of insulin secretion. Nature Medicine 21(7):769–776.

DiGuccio, M.R., Mawla, A.M., Donaldson, C.J., Noguchi, G.M., Vaughan, J., Cowing-Zitron, C., et al., 2016. Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets. Molecular Metabolism 5(7):440–458.

Adraenenssens, A.E., Svendsen, B., Lam, B.Y.H., Yao, G.S.H., Holst, J.J., Reimann, F., et al., 2016. Transcriptomic profiling of pancreatic alpha, beta and delta cell populations identifies delta cells as a principal target for ghrelin in mouse islets. Diabetologia 59(10):2156–2165.

Reimann, F., Gribble, F.M., 2016. G protein-coupled receptors as new therapeutic targets for type 2 diabetes. Diabetologia 59(2):229–233.

Saunders, D.C., Brissova, M., Phillips, N., Shrestha, S., Walker, J.T., Aramandla, R., et al., 2019. Ectonucleoside triphosphate diphosphohydrolase-3 antibody targets adult human pancreatic β cells for in vitro and in vivo analysis. Cell Metabolism 29(3):745–754.e4.

Sharivkin, R., Walker, M.D., Soen, Y., 2015. Functional proteomics screen enables enrichment of distinct cell types from human pancreatic islets. Public Library of Science One 10(2):e0115100.

Khetan, S., Kursawe, R., Youn, A., Lawlor, N., Jillette, A., Marquez, E.J., et al., 2018. Type 2 diabetes associated genetic variants regulate chromatin accessibility in human islets. Diabetes 67(11):2466–2477.

Taneera, J., Lang, S., Sharma, A., Fadista, J., Zhou, Y., Ahlgvist, E., et al., 2012. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metabolism 16(1):122–134.

Olsson, A.H., Volkov, P., Bacos, K., Dayah, T., Hall, E., Nilsson, E.A., et al., 2014. Genome-wide associations between genetic and epigenetic variation influence miRNA expression and insulin secretion in human pancreatic islets. Public Library of Science Genetics 10(1):e1004735.

Volkov, P., Bacos, K., Ofir, J.K., Esquerra, J.L.S., Eliasson, L., Rönn, T., et al., 2017. Whole-genome bisulfite sequencing of human pancreatic islets reveals novel differentially methylated regions in type 2 diabetes pathogenesis. Diabetes 66(4):1074–1085.

Solimena, M., Schulte, A.M., Marselli, L., Ehehslt, F., Richter, D., Kleberg, M., et al., 2018. Systems biology of the IMIDIA biobank from organ donors and pancreatotomised patients defines a novel transcriptomic signature of islets from individuals with type 2 diabetes. Diabetologia 61(3):641–657.

Leiter, E.H., Strobel, M., O’Neill, A., Schultz, D., Schile, A., Reifnyder, P.C., 2013. Comparison of two new mouse models of polygenic type 2 diabetes at the jackson laboratory, NONcZD10Lt/J and TALLYHO/JngJ. Journal of Diabetes Research 2013:165327.

Kluth, O., Matzke, D., Schulze, G., Schwenk, R.W., Joost, H.-G., Schürmann, A., 2014. Differential transcriptome analysis of diabetes-resistant and -sensitive mouse islets reveals significant overlap with human diabetes susceptibility genes. Diabetes 63(12):4230–4238.

Joost, H.-G., Schürmann, A., 2014. The genetic basis of obesity-associated type 2 diabetes (diabetesis) in polygenic mouse models. Mammalian Genome: Oficial Journal of the International Mammalian Genome Society 25(9–10):401–412.

Avrahami, D., Li, C., Zhang, J., Schug, J., Avrahami, R., Rao, S., et al., 2015. Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. Cell Metabolism 22(4):619–632.

Ezizik, D.L., Sammeh, M., Buckenroth, C., Bottu, G., Sisino, G., Igillo-Esteve, M., et al., 2012. The human pancreatic islet transcriptome: expression of candidate genes for type 1 diabetes and the impact of pro-inflammatory cytokines. Public Library of Science Genetics 8(3):e1002552.

Cnop, M., Abdulkarim, B., Bottu, G., Cunha, D.A., Igoillo-Esteve, M., Eizirik, D.L., Sammeth, M., Bouckenooghe, T., Bottu, G., Sisino, G., Igoillo-Esteve, M., et al., 2012. A systems genetics approach identifies genes and pathways for type 2 diabetes in human islets. Cell Metabolism 16(1):122–134.

Avrahami, D., Li, C., Zhang, J., Schug, J., Avrahami, R., Rao, S., et al., 2015. Aging-dependent demethylation of regulatory elements correlates with chromatin state and improved β cell function. Cell Metabolism 22(4):619–632.
[52] Hall, E., Dekker Nittert, M., Volkov, P., Malmgren, S., Mulder, H., Bacos, K., et al., 2018. The effects of high glucose exposure on global gene expression and DNA methylation in human pancreatic islets. Molecular and Cellular Endocrinology 472:57–67.

[53] Nammo, T., Udagawa, H., Funahashi, N., Kawaguchi, M., Uebanso, T., Hiramoto, M., et al., 2018. Genome-wide profiling of histone H3K27 acetylation featured fatty acid signalling in pancreatic beta cells in diet-induced obesity in mice. Diabetologia 61(12):2608–2620.

[54] Joung, J., Konermann, S., Gootenberg, J.S., Abudayyeh, O.O., Platt, R.J., Brigham, M.D., et al., 2017. Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Nature Protocols 12(4):828–863.

[55] Roman, T.S., Cannon, M.E., Vadlamudi, S., Buchkovich, M.L., Wolford, B.N., Welch, R.P., et al., 2017. A type 2 diabetes-associated functional regulatory variant in a pancreatic islet enhancer at theADCY5locus. Diabetes 66(9):2521–2530.

[56] Kycia, I., Wolford, B.N., Huyghe, J.R., Fuchsberger, C., Vadlamudi, S., Kursawe, R., et al., 2018. A common type 2 diabetes risk variant potentiates activity of an evolutionarily conserved islet stretch enhancer and increases C2CD4A and C2CD4B expression. The American Journal of Human Genetics 102(4):620–635.

[57] Klann, T.S., Black, J.B., Challapali, M., Safi, A., Song, L., Hilton, I.B., et al., 2017. CRISPR-Cas9 epigenome editing enables high-throughput screening for functional regulatory elements in the human genome. Nature Biotechnology 35(6):561–568.

[58] Hodson, D.J., Mitchell, R.K., Marselli, L., Pullen, T.J., Gimeno Brias, S., Semplici, F., et al., 2014. ADCYS couples glucose to insulin secretion in human islets. Diabetes 63(9):3009–3021.

[59] Zhou, Y., Park, S.-Y., Su, J., Bailey, K., Ottosson-Laakso, E., Shcherbina, L., et al., 2014. TCF7L2 is a master regulator of insulin production and processing. Human Molecular Genetics 23(24):6419–6431.

[60] Visel, A., Rubin, E.M., Pennacchio, L.A., 2009. Genomic views of distant-acting enhancers. Nature 461(7261):199–205.

[61] Keller, M.P., Gatti, D.M., Schueler, K.L., Rabaglia, M.E., Stapleton, D.S., Simecek, P., et al., 2018. Genetic drivers of pancreatic islet function. Genetics 209(1):335–356.