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Human islets expressing *HNF1A* variant have defective β cell transcriptional regulatory networks

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**Graphical abstract**
Human islets expressing HNF1A variant have defective β cell transcriptional regulatory networks

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Using an integrated approach to characterize the pancreatic tissue and isolated islets from a 33-year-old with 17 years of type 1 diabetes (T1D), we found that donor islets contained β cells without insulitis and lacked glucose-stimulated insulin secretion despite a normal insulin response to CAMP-evoked stimulation. With these unexpected findings for T1D, we sequenced the donor DNA and found a pathogenic heterozygous variant in the gene encoding hepatocyte nuclear factor-1α (HNF1A). In one of the first studies of human pancreatic islets with a disease-causing HNF1A variant associated with the most common form of monogenic diabetes, we found that HNF1A dysfunction leads to insulin-insufficient diabetes reminiscent of T1D by impacting the regulatory processes critical for glucose-stimulated insulin secretion and suggest a rationale for a therapeutic alternative to current treatment.

Introduction

The clinical diagnosis of diabetes, reflected by hyperglycemia, is straightforward; however, identifying the underlying molecular mechanism(s) is often challenging and sometimes not possible. These challenges are further confounded by well-documented heterogeneity of type 2 diabetes and with heterogeneity of type 1 diabetes (T1D) increasingly being recognized (1, 2). Partly, this is because the molecular defect(s) for most forms of diabetes is not known and partly because the molecular phenotyping of tissues involved in human diabetes is inadequate and limited. Critical human tissue and cellular samples relevant to diabetes are challenging to collect, sometimes not accessible, or limited by tissue processing that precludes functional analysis and the application of new technologies. For example, technical barriers inadvertently be misleading. For example, gene expression responses to inflammation in mice appear to have incomplete predictive clinical value and correlate with only a minority of human gene expression changes (3). Similarly, some rodent models of human monogenic diabetes do not fully reflect the altered glucose homeostasis observed in humans. For example, heterozygous mutations in the key pancreatic islet transcription factor hepatocyte nuclear factor-1α (HNF1A), which causes the most common form of monogenic diabetes, does not mimic the human disease in mouse models, leaving the pathophysiologic effect of HNF1A genetic variants on the human pancreatic islet incompletely understood (4–6).

To overcome these translational barriers and improve preclinical modeling of human disease, renewed emphasis and new approaches to study human tissue have led to the development of collaborative human tissue repositories or accessible databases such as the Network of Pancreatic Organ donors with Diabetes (nPOD), the Genotype Tissue Expression Project (GTEX), and the Human Islet Research Network (HIRN). Studies of human pancreatic islets have illustrated important similarities and differences from rodent islets in endocrine cell composition and arrangement, innervation, vasculature, and function (7–9).

Using infrastructure to study pancreatic islets and tissue from donors with diabetes in conjunction with the donors’ deidentified medical records, investigators are working to better understand the changes in the pancreatic islet in diabetes (10–12). In this report, we describe unexpected functional and molecular findings from the pancreas of an individual with the clinical diagnosis translation into clinically relevant information and may even inadvertent...
responded normally to high glucose coupled with the phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX). Moreover, glucagon secretion from donor islet α cells had an abrogated response to potent α cell stimuli such as low glucose (1.7 mM) and epinephrine (1 μM) and, strikingly, showed an inhibitory response to membrane depolarization by KCl (Supplemental Figure 1, E, F, and H). Donor pancreas islet innervation and vasculature, important for coordinated islet function in vivo (8, 14), were normal (Supplemental Figure 1I).

Because of these unexpected histological and functional findings, we sequenced the donor DNA for variants associated with monogenic diabetes and uncovered a heterozygous, disease-associated variant in a conserved region of the POUH DNA binding domain of HNF1A (c.779C>T, p.Thr260Met) (15) (Supplemental Table 2 and Supplemental Figure 1J). Variants in HNF1A comprise the most common form of maturity-onset diabetes of the young 3 (termed MODY3) (16). HNF1AT260M displayed compromised DNA binding. Nuclear HNF1A protein was detected in both the exocrine and endocrine compartments of the donor pancreas, with normal expression in β cells and α cells (Figure 2A and Supplemental Figure 2, A and B). The DNA binding capacity of the altered HNF1A T260M protein, as assessed by electrophoretic mobility shift assay (EMSA), was severely compromised compared with HNF1A WT protein (Figure 2B and Supplemental Figure 2, C–E). Accordingly, HNF1AT260M had little to no ability to stimulate MAFA region 3 enhancer-driv-
Reporter activity relative to HNF1A WT in cotransfection assays with SEM. See complete unedited blots in the supplemental material. Predicted to result in the loss of this interaction by destabilizing Arg-263 and subsequently DNA binding. Results of control samples are expressed as mean ± SEM. Molecular modeling of the HNF1A T260 variant in PyMOL predicts that the hydroxyl group (red) on threonine 260 (Thr-260) stabilizes arginine 263 (Arg-263) by hydrogen bonding to nitrogen (blue). Arg-263 H-bonds to the DNA backbone of the fifth adenosine of the HNF1A consensus recognition motif (5′-CTTGGTTAATAATTCACCAGA-3′) in control conditions (18). A missense mutation from threonine to methionine at position 260 is predicted to result in the loss of this interaction by destabilizing Arg-263 and subsequently DNA binding. Results of control samples are expressed as mean ± SEM. See complete unedited blots in the supplemental material.

Figure 2. Expression and functional characterization of HNF1A+/T260M variant. (A) Analysis of donor’s native pancreatic tissue for HNF1A compared with controls (n = 4 donors; ages 10–55 years) revealed HNF1A protein in donor β cells. Scale bar: 50 μm. (B) Electrophoretic mobility shift assay (EMSA) shows that the HNF1A+/T260M variant has impaired DNA binding, with loss of the HNF1A-specific DNA binding complex (arrow) in Myc-tagged HNF1A+/T260M–transfected HeLa cells compared with Myc-tagged HNF1A WT. Specificity of this complex (arrow) was shown by exclusive elimination of these species by adding either Myc antibody (Myc-Ab) or unlabeled oligonucleotide (WT Oligo) containing the HNF1A consensus recognition motif, but not a mutated form of this oligonucleotide (Comp Oligo). Moreover, HNF1A antibody (HNF1A-Ab) only supershifted (s.s.) this complex. All samples in B include oligonucleotide labeled with 32P as described in the supplemental material. Asterisk indicates nonspecific complexes. NT, nontransfected HeLa cells. One representative experiment of 3 is shown. (C) Molecular modeling of the HNF1A+/T260M variant in PyMOL predicts that the hydroxyl group (red) on threonine 260 (Thr-260) stabilizes arginine 263 (Arg-263) by hydrogen bonding to nitrogen (blue). Arg-263 H-bonds to the DNA backbone of the fifth adenosine of the HNF1A consensus recognition motif (5′-CTTGGTTAATAATTCACCAGA-3′) in control conditions (18). A missense mutation from threonine to methionine at position 260 is predicted to result in the loss of this interaction by destabilizing Arg-263 and subsequently DNA binding. Results of control samples are expressed as mean ± SEM. See complete unedited blots in the supplemental material.

HNF1A+/T260M β cells have preserved markers of β cell identity, but changes in processes critical for GSIS. RNA-sequencing and transcriptional profiling of purified HNF1A+/T260M β cells (Figure 3, A and B) showed relatively preserved expression of INS mRNA and transcription factor markers of β cell identity (PDX1, NKX2.2, and NKX6.1), which was confirmed by protein expression analysis (Supplemental Figure 2H). However, decreased expression of other transcription factors associated with mature β cell function (i.e., MAFA, SIX3, and RXF6; refs. 17, 19, and 20, respectively) suggests that the HNF1A+/T260M variant impacts transcriptional regulatory networks required for β cell function rather than maintaining identity. Decreased expression of known (e.g., MLXIPL, HNF4A, PKM, OGDH, PPP1R1A, G6PC2, and TMEM27) (21) and previously undescribed HNF1A targets (IAPP, ABCC8, KCNJ11, TMEM37, SYNGR4, and FOXRED2) likely contributes to the loss of GSIS identified by islet perfusion (Figure 3C). Notably, most voltage-gated calcium channels, such as L-type and P/Q-type, were not changed in HNF1A+/T260M β cells, but ATP-sensitive channels were decreased (ABCC8, KCNJ11, KCNJ8, and FXYD2). Pathway analysis of HNF1A+/T260M β cells revealed changes in glucose metabolism and ATP production important in glucose-mediated insulin secretory processes as well as in core cellular pathways such as gene transcription, intracellular protein transport (i.e., synthesis, ubiquitination, and exocytosis), cell stress response, and cell signaling (Figure 3D and Supplemental Tables 5 and 7). Approximately 50% of the genes differentially expressed in HNF1A+/T260M β cells were also altered in donor α cells (Supplemental Figure 3, A–G), suggesting that HNF1A dysfunction is a common effector in both cell types (Supplemental Tables 5–7). We also noted that other processes such as amino acid nutrient sensing and metabolism, cell cycle regulators, and cell adhesion/motility were altered in HNF1A+/T260M islet cells (SLC38A4, GLUL, IGFBP5, and CREB3L1).
changes in core metabolic functions, such as gene transcription, protein synthesis and degradation, unfolded protein response, and intracellular and cell-cell communications in \( \text{HNF1A}^{+/T260M} \) \( \beta \) cells. From this data set, we propose that this class of loss-of-function variants in \( \text{HNF1A} \) leads to insulin-insufficient diabetes, not by significant loss of \( \beta \) cell mass, but rather by impacting \( \beta \) cell transcriptional regulatory networks (\( \text{HNF4A}, \text{MAFA}, \text{RFX6}, \text{SIX3}, \text{FOXA2}, \) and \( \text{MLXIPL} \)) that results in impairment of \( \beta \) cell pathways necessary for a normal insulin response to glucose (Figure 4).

Furthermore, by investigating hormone secretion in isolated pancreatic islets, we discovered that depolarization by KCl, which directly stimulates hormone secretion by activating voltage-dependent calcium channels, was impaired in \( \text{HNF1A}^{+/T260M} \) \( \alpha \) and \( \beta \) cells, in contrast to results from mouse models (4). Interestingly, elevated basal insulin secretion was observed in islets from this donor, consistent with decreased expression of genes associated with glucose sensitivity of insulin secretion (\( \text{G6PC2} \) and \( \text{SLC37A4} \)) (24). Our data also revealed a previously unrecognized role for \( \text{HNF1A} \) in \( \alpha \) cell function, as \( \text{HNF1A}^{+/T260M} \) impacted expression of many shared genes involved in hormone-regulated secretion (Supplemental Figure 3C). Transcriptomic analysis also uncovered \( \text{HNF1A} \)-regulated gene targets in \( \beta \) cells, such as \( \text{PPPIR1A} \) and \( \text{RFX6} \), and pathways, like protein synthesis and amino acid metabolism. In addition, a
**Figure 4. Model of HNF1A dysfunction in human β cells.** From these results, we propose that dysfunction of HNF1A leads to decreased expression of direct targets, which encompass both enzymatic and gene regulatory products, producing broad changes in transcriptional regulation, glucose metabolism, and hormone secretion. These processes ultimately lead to β cell dysfunction and result in clinical manifestation of insulin-insufficient diabetes.

The number of genes differentially regulated in HNF1A+/−T260M β cells included those identified in β cell subpopulations by Dorell and colleagues (HCN4, SPP1, KCNJ8, RFX6, SIX3, PPP1R1A, FAM159B, and G6PC2), suggesting that HNF1A may participate in the development of these β cell populations (25).

Preserved β cell mass in a pancreas with 17 years of MODY3 highlights the importance of clinical identification and intervention even years after the diagnosis of diabetes. Low-dose sulfonylurea therapy produces effective glycemic control in some individuals even years after the diagnosis of diabetes. Low-dose sulfonylurea therapy produces effective glycemic control in some individuals even years after the diagnosis of diabetes. Low-dose sulfonylurea therapy produces effective glycemic control in some individuals even years after the diagnosis of diabetes.

**Methods**

Detailed methods are in the supplemental materials. RNA-sequencing data were deposited in the NCBI's Gene Expression Omnibus (GEO) database: GSE106148 (control α cells), GSE116559 (control β cells), and GSE120299 (HNF1A+/−T260M donor α and β cells).

**Statistics.** Values are shown as mean ± standard error of the mean (SEM) for control samples. Data from a sample size of n = 1 for the donor precluded formal statistical analysis.

**Study approval.** The Vanderbilt University IRB declared that studies on deidentified human pancreatic specimens do not qualify as human subject research.

**Author contributions**

RH, RWS, MB, and ACP conceived and designed the research. RH, XT, MS, JL, DCS, RA, GP, SDR, and RB performed experiments. RH, XT, MS, SS, RDB, NP, SEL, DMH, LHP, RWS, MB, and ACP analyzed data and interpreted results. RH prepared figures. RH drafted the manuscript. RH, DMH, RWS, MB, and ACP edited and revised the manuscript. RH, XT, MS, JL, DCS, RA, GP, SDR, RB, NP, SEL, RDB, DMH, LHP, RWS, MB, and ACP approved the final version of the manuscript.

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