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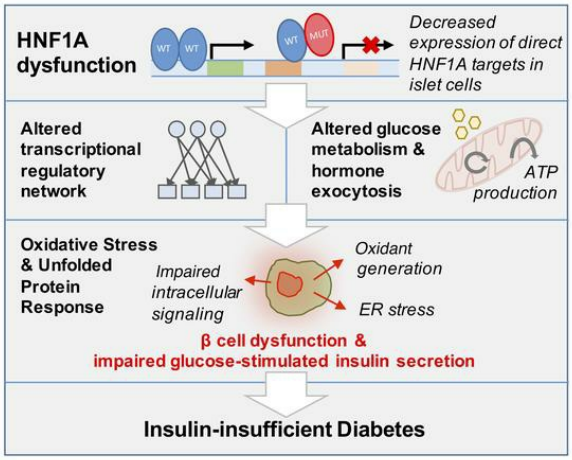
Graphical abstract



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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 prevent sampling of the human pancreas, as it cannot be safely and routinely biopsied in living individuals and rapidly undergoes autodigestion postmortem, hindering adequate molecular diagnostic and clinical phenotyping of the human pancreatic islet in all forms of diabetes.

To overcome such limitations, experimental models have been used to discover critical contributions to our understanding of human physiology and disease. However, in several instances, widely used model systems appear limited in their translation into clinically relevant information and may even 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

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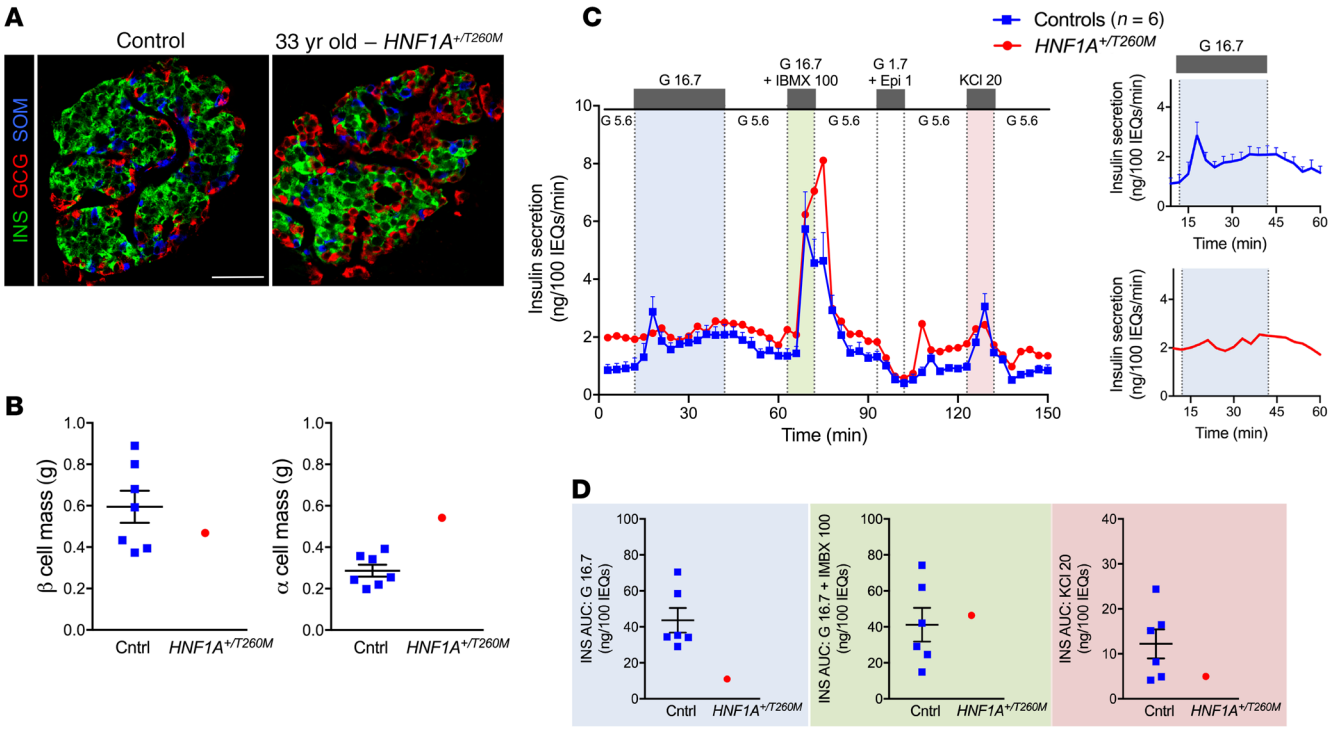

Figure 1. Histological and functional analysis of *HNF1A*$^{+/T260M}$ pancreas and islets. (**A**) Example of expression of insulin (INS), glucagon (GCG), and somatostatin (SOM) in donor's native pancreatic tissue compared with control. Scale bar: 50 μm. (**B**) β and α cell mass (grams) in *HNF1A*$^{+/T260M}$ pancreas compared with controls (n = 7 donors; ages 10–55 years). Each data point represents the average mass across the combined pancreatic head, body, and tail regions. (**C**) Insulin secretion in islets isolated from the *HNF1A*$^{+/T260M}$ pancreas compared with controls (n = 6 donors; ages 8–55 years) and normalized to overall islet cell volume (expressed as islet equivalents, IEQs). Islets were teated with 5.6 mM glucose (G 5.6); 16.7 mM glucose (G 16.7); 16.7 mM glucose plus 100 μM isobutylmethylxanthine (G 16.7 + IBMX 100); 1.7 mM glucose plus 1 μM epinephrine (G 1.7 + Epi 1); or 20 mM potassium chloride (KCl 20) at the indicated times. Insets show average insulin response of controls and *HNF1A*$^{+/T260M}$ donor to 30-minute stimulation with 16.7 mM glucose. (**D**) Integrated insulin secretion was calculated as the area under the curve (AUC) for the indicated secretagogue (shaded to correspond to color-matched regions of perifusion trace in panel **C**). Results of control samples are expressed as mean ± SEM.

of T1D, thus highlighting how systematic analysis of rare human samples can provide critical insight into human disease and potentially lead to new approaches to therapy.

Results and Discussion

As part of studies of the pancreas and islets from individuals with T1D (10–12), we were surprised to find that analysis of sections from the head, body, and tail regions of one donor's pancreas showed that all islets contained insulin-positive β cells (Figure 1A and Supplemental Figure 1A and insets, and Supplemental Tables 1, 3, and 4; supplemental material available online with this article; https://doi.org/10.1172/JCI121994DS1), but lacked insulitis typical of T1D (infiltration of CD45$^+$ cells) (13).

Donor pancreas had normal β cell mass, but β cells were functionally impaired. The donor pancreas had β and δ cell mass within the normal range, with slightly elevated α cell mass (Figure 1B and Supplemental Figure 1B) and an increased α/β cell ratio (Supplemental Figure 1, C, G, and H). No β cell apoptosis (TUNEL) or proliferation (Ki67) was detected (Supplemental Figure 1D). In a dynamic perifusion system, isolated donor islets had normal insulin content but had higher basal insulin secretion, lacked biphasic glucose-stimulated insulin secretion (GSIS), and had a decreased secretory response to KCl-mediated membrane depolarization (Figure 1C and inset, Figure 1D, and Supplemental Figure 1G). Despite the lack of GSIS, the donor's islets 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 POU_H 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).

HNF1A^{T260M} 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, HNF1A^{T260M} had little to no ability to stimulate *MAFA* region 3 enhancer-driv-

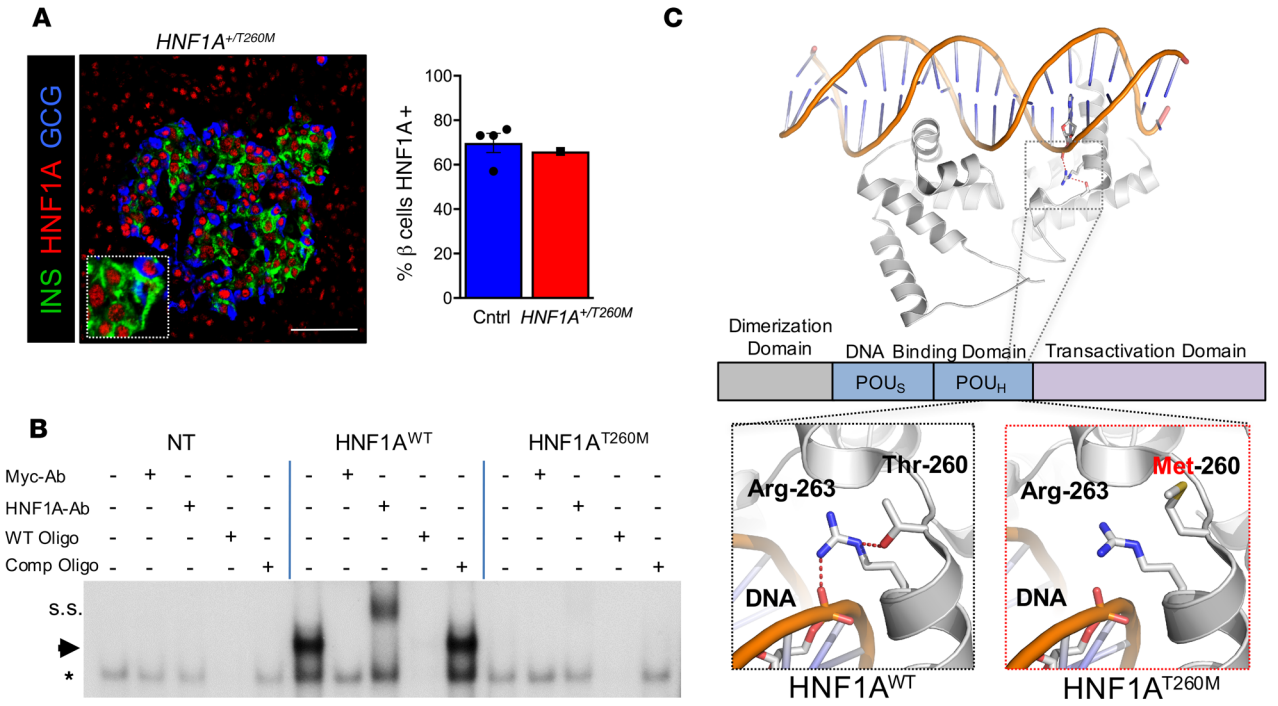

Figure 2. Expression and functional characterization of HNF1A^{T260M} variant. (**A**) Analysis of donor's native pancreatic tissue for HNF1A compared with controls (n = 4 donors; ages 10–55 years) revealed HNF1A protein in donor β cells. Scale bar: 50 μm. (**B**) Electrophoretic mobility shift assay (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 ^{32}P 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^{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.

en reporter activity relative to HNF1A^{WT} in cotransfection assays (Supplemental Figure 2, F and G). Notably, each of these proteins was expressed at similar levels, and WT activation was dependent on HNF1A-site binding, as described previously (17). Furthermore, HNF1A^{T260M} decreased HNF1A^{WT} activation in a dose-dependent manner, providing evidence that the dominant-negative action of HNF1A^{T260M} is due to dimerization with HNF1A^{WT} (Supplemental Figure 2, F and G). Moreover, protein modeling predicted that disrupted DNA binding results from the missing hydrogen donor at position 260 in the variant protein, which destabilizes the DNA-binding Arg-263 residue (Figure 2C), yet leaves the distinct dimerization domain in the transcription factor intact (18).

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 *RFX6*; 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 perifusion (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*).

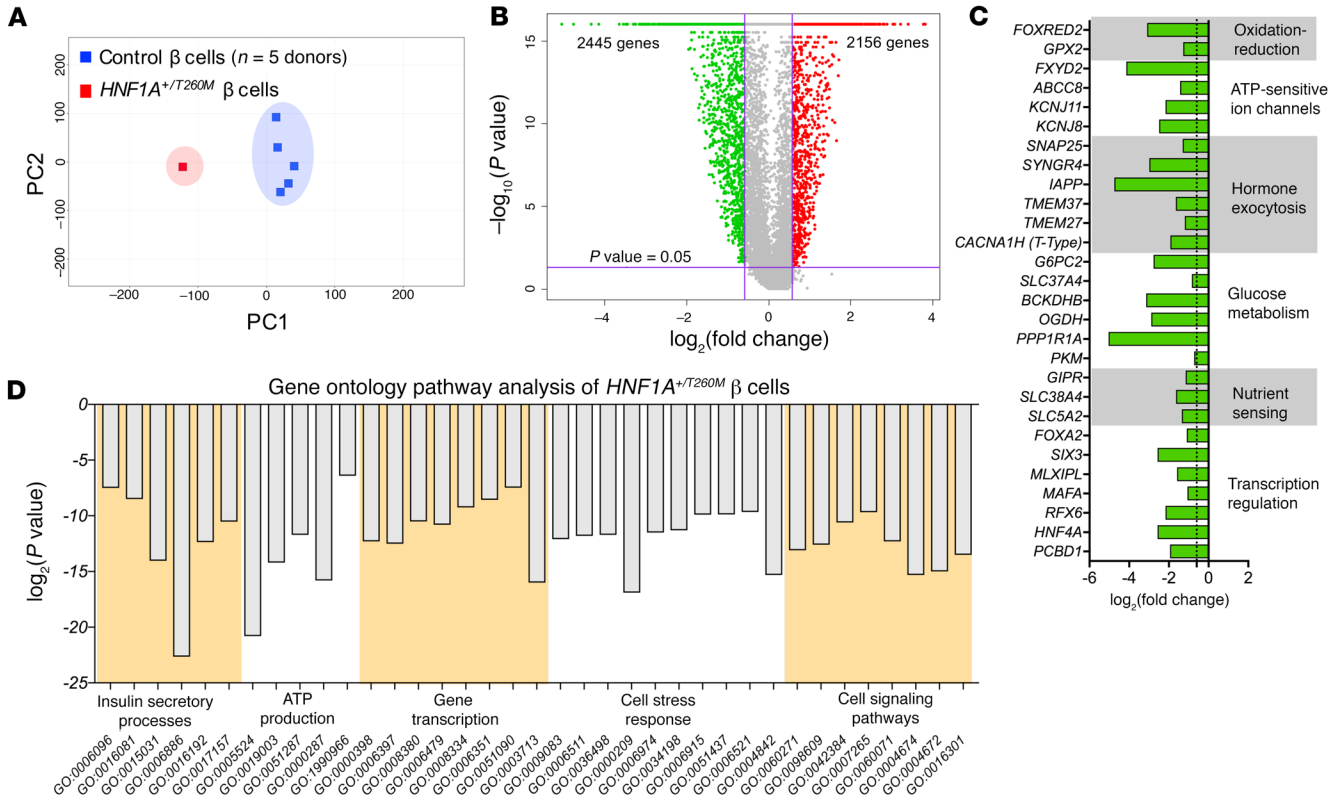

Figure 3. Transcriptomic analysis of *HNF1A*$^{+/T260M}$ β cells. (**A**) Principal component analysis (PCA) plot depicts clustering of control β cells (n = 5 donors; ages 26–55 years) (31) separate from *HNF1A*$^{+/T260M}$ β cells. (**B**) The volcano plot demonstrates transcripts differentially expressed between control and *HNF1A*$^{+/T260M}$ β cells (red, upregulated gene expression; green, downregulated gene expression). Differential expression was calculated based on fold change (FC) ≥1.5 with a P-value cutoff of <0.05 for calculated Z score. (**C**) Genes of interest and HNF1A targets are significantly downregulated in *HNF1A*$^{+/T260M}$ β cells. The vertical dotted line represents FC = 1.5 times the threshold; $P < 0.05$ for all values shown. (**D**) Significant processes identified by Gene Ontology (GO) term-enrichment analysis are grouped and displayed by their P value on a $\log_2$ scale.

This report highlights how molecular and functional findings in unique human samples, even in a single case, can contribute to our understanding of physiology and disease pathogenesis. Levels of *HNF1A* gene transcript in the human pancreas are substantially lower compared with mouse (22), such that mouse models of heterozygous *HNF1A* do not phenocopy the human disease (4–6). Missense mutations in the *HNF1A* dimerization and DNA binding domains account for the majority of described pathogenic *HNF1A* variants (23). Our modeling predicted that the T260M change would impair DNA binding of HNF1A, rendering this transcriptional factor nonfunctional, which was demonstrated by EMSA analysis. The dose-dependent decrease in transcriptional activity in WT HNF1A–dependent *MAFA* gene activation by HNF1A^{T260M} suggests that the dimerization between these proteins leads to impaired DNA binding activity and reduced HNF1A target gene expression in individuals carrying this variant.

In one of the first direct studies of human islets from an individual with a heterozygous, missense variant in the *HNF1A* locus, we show that this *HNF1A*$^{+/T260M}$ donor had relatively normal β cell mass and maintained many key markers of β cell identity but lacked an insulin secretory response to glucose challenge. This insulin secretory deficit was accompanied by alterations in genes encoding pathways of glucose metabolism and ATP production, which were also coupled with changes in core metabolic functions, such as gene transcription, protein synthesis and degradation, unfolded protein response, and intracellular and cell-cell communications in *HNF1A*$^{+/T260M}$ β cells. From this data set, we propose that this class of loss-of-function variants in *HNF1A* leads to insulin-insufficient diabetes, not by significant loss of β cell mass, but rather by impacting β cell transcriptional regulatory networks (*HNF4A*, *MAFA*, *RFX6*, *SIX3*, *FOXA2*, and *MLXIPL*) that results in impairment of β 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 *HNF1A*$^{+/T260M}$ α and β 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 (*G6PC2* and *SLC37A4*) (24). Our data also revealed a previously unrecognized role for HNF1A in α cell function, as *HNF1A*$^{+/T260M}$ impacted expression of many shared genes involved in hormone-regulated secretion (Supplemental Figure 3C). Transcriptomic analysis also uncovered HNF1A-regulated gene targets in β cells, such as *PPP1R1A* and *RFX6*, and pathways, like protein synthesis and amino acid metabolism. In addition, a

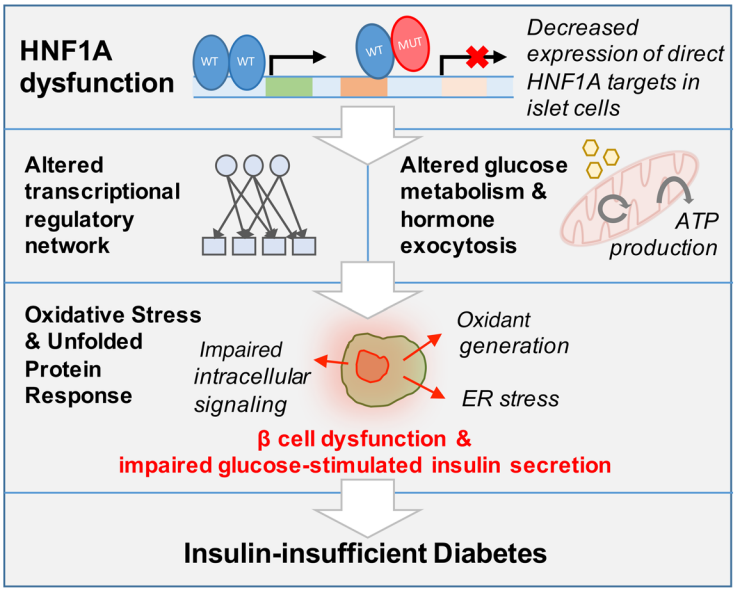

Figure 4. Model of HNF1A dysfunction in human β cells. From these results, we propose that dysfunction of HNF1A leads to decreased expression of direct targets, which encompass both enzymatic and gene regulatory products, producing broad changes in transcriptional regulation, glucose metabolism, and hormone secretion. These processes ultimately lead to β cell dysfunction and result in clinical manifestation of insulin-insufficient diabetes.

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 with MODY3 (26) by stimulating this existing β cell reservoir; however, our data provide rationale for a therapeutic alternative to current treatment. Sulfonylureas likely have clinical efficacy by initiating membrane depolarization with potassium channel closure and bypassing effects from impaired ATP production, producing insulin responses comparable to control subjects (27). However, individuals with MODY3 are more sensitive to sulfonylureas (28), which may lead to hypoglycemia and limit this treatment; this might result in part from impaired glucagon secretion related to α cell depolarization (Supplemental Figure 1E). The islet perifusion data from this donor suggest that targeting cAMP-dependent pathways of insulin secretion, such as with glucagon-like peptide 1 (GLP-1) agonists, would have advantages over sulfonylureas, as this pathway of insulin secretion is preserved and accompanied by an intact glucagon response, thus lowering the risk of hypoglycemia in such MODY3 patients (29) (Figure 1C and Supplemental Figure 1E).

Clinical and pathogenic heterogeneity in clinically diagnosed T1D is now increasingly apparent with the ability to study affected human pancreatic tissue (13, 30). Clinical features of many MODY phenotypes, which make up 1%–5% of all diabetes cases, can be easily mistaken for T1D. Lack of islet-related humoral autoantibodies, significant family history of insulin-deficient diabetes, and/or a low renal threshold for glucose should prompt genetic testing for *HNF1A* variants and other monogenic forms of diabetes. Because of this, in collaboration with the Vanderbilt Institutional Review Board (IRB), we are working to communicate our findings to the deidentified donor's family and recommend diagnostic MODY genetic testing in potentially affected family members. Overall, this report shows how integrating clinical information with molecular and cellular analyses identified what appeared to be T1D was in fact part of a broader spectrum of insulin-deficient diabetes and provides translational insight into an incompletely understood form of human 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, SS, 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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