Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism

FADISTA, João, et al.

Abstract

Genetic variation can modulate gene expression, and thereby phenotypic variation and susceptibility to complex diseases such as type 2 diabetes (T2D). Here we harnessed the potential of DNA and RNA sequencing in human pancreatic islets from 89 deceased donors to identify genes of potential importance in the pathogenesis of T2D. We present a catalog of genetic variants regulating gene expression (eQTL) and exon use (sQTL), including many long noncoding RNAs, which are enriched in known T2D-associated loci. Of 35 eQTL genes, whose expression differed between normoglycemic and hyperglycemic individuals, siRNA of tetraspanin 33 (TSPAN33), 5′-nucleotidase, ecto (NT5E), transmembrane emp24 protein transport domain containing 6 (TMED6), and p21 protein activated kinase 7 (PAK7) in INS1 cells resulted in reduced glucose-stimulated insulin secretion. In addition, we provide a genome-wide catalog of allelic expression imbalance, which is also enriched in known T2D-associated loci. Notably, allelic imbalance in paternally expressed gene 3 (PEG3) was associated with its promoter methylation and T2D status. Finally, RNA editing events [...]
Global genomic and transcriptomic analysis of human pancreatic islets reveals novel genes influencing glucose metabolism


¹Lund University Diabetes Centre, Department of Clinical Sciences, Skåne University Hospital Malmö, Lund University, 20502 Malmö, Sweden; ²Department of Information Engineering, University of Padova, 35131 Padova, Italy; and ³Department of Cell Physiology and Metabolism, University of Geneva, 1211 Geneva 4, Switzerland

Edited by Tak W. Mak, The Campbell Family Institute for Breast Cancer Research at Princess Margaret Cancer Centre, Ontario Cancer Institute, University Health Network, Toronto, Canada, and approved August 14, 2014 (received for review February 11, 2014)

Genetic variation can modulate gene expression, and thereby phenotypic variation and susceptibility to complex diseases such as type 2 diabetes (T2D). Here we harnessed the potential of DNA and RNA sequencing in human pancreatic islets from 89 deceased donors to identify genes of potential importance in the pathogenesis of T2D. We present a catalog of genetic variants regulating gene expression (eQTL) and exon use (sQTL), including many long noncoding RNAs, which are enriched in known T2D-associated loci. Of 35 eQTL genes, whose expression differed between normoglycemic and hyperglycemic individuals, sRNA of tetranspanin 33 (TSPAN33), 5′-nucleotidase, ecto (NT5E), transmembrane emp24 protein transport domain containing 6 (TMED6), and p21 protein activated kinase 7 (PAK7) in INS1 cells resulted in reduced glucose-stimulated insulin secretion. In addition, we provide a genome-wide catalog of allelic expression imbalance, which is also enriched in known T2D-associated loci. Notably, allelic imbalance in paternally expressed gene 3 (PEG3) was associated with its promoter methylation and T2D status. Finally, RNA editing events were less common in islets than previously suggested in other tissues. Taken together, this study provides new insights into the complexity of gene regulation in human pancreatic islets and better understanding of how genetic variation can influence glucose metabolism.

Type 2 diabetes (T2D) is an increasing global health problem (1). Although genome-wide association studies (GWAS) have yielded more than 70 loci associated with T2D or related traits (2, 3), they have not provided the expected breakthrough in our understanding of the pathogenesis of the disease. They have nonetheless pointed at a central role of the pancreatic islets and β-cell dysfunction in the development of the disease (4, 5). It therefore seems pertinent to focus on human pancreatic islets to obtain insights into the molecular mechanisms causing the disease (6, 7). Given that most SNPs associated with T2D lie in noncoding regions, the majority of causal variants are likely to regulate gene expression rather than protein function per se. Therefore, combination of DNA and RNA sequencing in the same individuals may help to disentangle the role these SNPs play in the pathogenesis of the disease (8). Although the human pancreatic islet transcriptome has been previously described (6, 9–18), using microarrays or RNA sequencing of a limited number of nondiabetic individuals, this has not allowed a more global analysis of the complexity of the islet transcriptome in T2D. Here we combined genotypic imputation, expression microarrays, and exome and RNA sequencing (Exome-Seq and RNA-Seq) in a large number of human pancreatic islets from deceased donors with and without T2D. This study identified a number of novel genes, including long intergenic noncoding RNAs (lncRNAs), whose expression and/or splicing influences insulin secretion and is associated with glycaemia. In addition, we provide a catalog of RNA editing and allelic-specific expression events in human pancreatic islets (SI Appendix, Fig. S1).

Results

Genes Showing Differential Expression Between Islets from Normoglycemic and Hyperglycemic Donors. To obtain a profile of gene expression variation in human islets, we sequenced the polyadenylated fraction of RNA from 89 individuals with different degrees of glucose tolerance, using 101 base pairs paired-end on an Illumina HiSeq sequencer (Dataset SI1 and SI Appendix, Fig. S1). Each individual transcriptome yielded, on average, 38.2 ± 4.4 (mean ± SD) million paired-end reads mapped to the human genome, with ~88% mapping to known exons from the RefSeq Gene database. Because any expression cutoff is arbitrary, we considered a gene to be expressed if it was observed in at least 5% of the samples. Applying this definition, we detected 91% of RefSeq genes. However, most of these genes are expressed at low levels, supporting the view of pervasive transcription and leakage in the human transcriptome (SI Appendix, Fig. S2) (19). Moreover, in support of previous results (20), we observed a good correlation between gene expression based on RNA-seq and microarrays in the 89 samples (r = 0.83; P < 0.0001) (SI Appendix, Fig. S3). To evaluate how well the RefSeq genes in our RNA-seq dataset are covered, we sequenced one sample at high depth of coverage (~150 million paired-end reads). As seen in SI Appendix, Fig. S4, our average sample coverage of 38.2 million paired-end reads is deep enough to detect the majority of known genes, transcripts, exons, and junctions. As expected, glucagon, insulin, and other

Significance

We provide a comprehensive catalog of novel genetic variants influencing gene expression and metabolic phenotypes in human pancreatic islets. The data also show that the path from genetic variation (SNP) to gene expression is more complex than hitherto often assumed, and that we need to consider that genetic variation can also influence function of a gene by influencing exon usage or splice isoforms (sQTL), allelic imbalance, RNA editing, and expression of noncoding RNAs, which in turn can influence expression of target genes.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Database deposition: The data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE50988).

1To whom correspondence may be addressed. Email: joao.fadista@med.lu.se or Leif.Groop@med.lu.se.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1402665111/-/DCSupplemental.
known pancreatic genes showed the highest expression (Fig. 1 and Dataset S1). To identify genes whose expression is influenced by glycermia (cause or consequence), we related gene expression to HbA1c, a measure of long-term glycemia, and compared expression in islets from donors with normal glucose tolerance (HbA1c ≤ 6.5%), impaired glucose tolerance (IGT; 6% ≤ HbA1c < 6.5%), and T2D (HbA1c ≥ 6.5%). By using a linear model adjusting for age and sex, we detected 1619 genes associated with HbA1c levels in both RNA-seq and microarrays (Database S1 and SI Appendix).

Briefly, genes were kept if both microarray and RNA-seq gene expression were nominally associated with HbA1c levels, with both nominal and permutation P values < 0.05. In addition, 271 genes showed specific exon associations, with HbA1c levels not detected at the gene level (Database S1). Of the genes associated with HbA1c levels, 70 were also associated with in vitro insulin secretion in human islets, further highlighting their role in glucose metabolism (Database S1). Of particular interest are the genes whose expression is associated with lower HbA1c levels and higher insulin secretion, such as RAS guanyl releasing protein 1 (RASGRP1) (6), transcription factor RFX3 (21), and nicotinamide nucleotide transhydrogenase (NNT) (22), all of which have been suggested to regulate insulin secretion (SI Appendix, Figs. S5–S7).

RFX3 has also been suggested to regulate the glucokinase promoter, and thereby its expression in a mouse insulinoma cell line MIN6 (21). In line with these findings, we observed a clear coexpression between the RFX3 and GCK genes in human islets (SI Appendix, Fig. S19). Of the established T2D and glycemic associated loci (2, 3, 23–28), whose gene expression proxies were associated with HbA1c levels, solute carrier family 30 (zinc transporter), member 8 (SLC30A8), glucose-6-phosphatase, catalytic, 2 (G6PC2), and proprotein convertase subtilisin/kexin type 1 (PCSK1) showed the highest expression using RNA-seq (Fig. 1B and Database S1). This is in line with our previous findings using microarrays (6).

SLC30A8, G6PC2, and PCSK1 also showed a strong positive correlation with glucagon expression (SI Appendix, Fig. S8). By using the RABT Cufflinks transcript assembly method (29), we also detected 445 potential novel genes with exon-exon junctions in addition to the existing GENCODE (30). UCSC transcriptional islet gene structure annotations (Database S1). Of these potential novel gene loci, 28 (6% of all genes) have coding potential, as assessed by the CPAT tool (31), and 391 (88%) are within 5 kb of known human islet active chromatin DNase, FAIRE, or H3K4me3 peaks (32–34), pointing to candidate nearby promoters for those genes (Database S1). One of these potential novel genes, although not showing any coding potential nor close to any known islet open chromatin mark, was also associated with HbA1c, and this new gene locus is in...
a ~10-kb region nominally significant in the MAGIC database for fasting glucose (23) (SI Appendix, Fig. S9).

**Effect of SNPs on Gene Expression (eQTLs) and Splicing (sQTLs) in Human Pancreatic Islets.** Because many SNPs are located in non-coding regions, suggesting they may influence gene expression, we analyzed whether any SNP genotyped in our islet samples and further imputed to the 1000 Genomes reference panel (35) would influence RNA-seq gene expression (eQTL) or exon use (sQTL) in cis (within 250 kb of the SNP). For analysis purposes, we identified a single best “sentinel” SNP for each gene or exon, defined as the SNP with the lowest P value per eQTL or sQTL gene. Applying these criteria and thresholds, we identified 616 cis eQTLs for known genes (Fig. 2A and Database S1), whereas 24 eQTLs were detected in previously unreported unknown transcribed loci (Database S1; Materials and Methods). Notably, 54% of these eQTLs would have been missed in a microarray because the gene is not probed on the array or shows low expression (SI Appendix, Fig. S10).

Our sample size permitted us to detect significant eQTLs at >90% power with an effect size of 0.5 (beta) or more (SI Appendix, Fig. S11). Notably, only in about half of the cases did the eQTL SNPs influence expression on the nearest gene. The strongest eQTLs were detected in the lactate dehydrogenase C (LDHC), RNA-synthetase 1 homolog B (Saccharomyces cervisiae) (TYW1B), and endoplasmic reticulum aminopeptidase 2 (ERAP2) genes (Fig. 2A). LDHC encodes the enzyme lactate dehydrogenase C, which catalyzes the glycolytic conversion of lactate to pyruvate. Although expression of another lactate dehydrogenase, LDHA, is suggested to be repressed in pancreatic β cells because of a minor role of anaerobic glycolysis in the adult β cell (36), LDHC is expressed at similar levels in α and β cells (17). Knock-down of LDHC and TYW1B in INS-1 cells using siRNA did not affect insulin secretion. ERAP2 has been ascribed a role in autoimmune and type 1 diabetes (37), and its eQTL sentinel SNP is in hard linkage disequilibrium (r² > 0.8) with the genome-wide significant GWAS SNP rs1019503 for glucose levels 2 h after an oral glucose challenge (3). Furthermore, we observed 371 splicing QTLs (sQTLs) not reflected by changes in expression at the gene level (Database S1 and SI Appendix, Fig. S13). There was eQTL and sQTL enrichment in regions of islet active chromatin, such as those characterized by DNase I hypersensitivity [Fisher exact test, P value < 2.2e−10 (odds ratio = 2.1) for eQTLs; and P value = 3.8e−11 (odds ratio = 1.9) for sQTLs], H3K4m3 [P value < 2.2e−10 (odds ratio = 3.1), for eQTLs and P value < 2.2e−10 (odds ratio = 2.3) for sQTLs], and FAIRE [P value < 2.1e−10 (odds ratio = 2.7) for eQTLs and P value = 0.02 (odds ratio = 1.9) for sQTLs] (32–34). There was no indication that these eQTLs and sQTLs were enriched in evolutionarily conserved sites.

Because GWAS for T2D only enabled identification of loci, rather than genes, we examined whether SNPs known to associate with T2D or related traits (glucose, insulin) would have a cis effect on gene expression or exon use. We found enrichment for GWAS T2D/glycemic trait loci in eQTLs (Fisher exact test, P value 4.1e−5; odds ratio = 5.0), with five GWAS SNPs showing a cis eQTL effect, and in the case of rs1535500, the effect was not on the nearest gene (Table 1 and SI Appendix, Fig. S14). Notably, of the 1,619 genes whose expression correlated with HbA1c, 35 (2%) had an eQTL (Database S1). We examined whether the eQTL SNPs in these genes were associated with insulin and glucose concentrations in the DIAGRAM and MAGIC databases (2, 3, 23–28). The sentinel eQTL SNP for sorting nexin 19 (SNX19), rs3751034, was nominally associated with HbA1c in MAGIC (P value < 0.01) (28). SNX19 has also been shown to regulate insulin secretion in a mouse pancreatic β-cell line (38). Finally, we tested whether the three eQTL genes [tetraspans 33 (TSPAN33), 5′-nucleotidase, ecto (NT5E), and transmembrane emp24 protein transport domain containing 6 (TMED6)] showing the strongest effect on HbA1c levels would also influence insulin secretion by disrupting their expression in INS-1 cells. We also tested p21 protein activated kinase 7 (PAK7), a gene associated with HbA1c levels in both RNA-seq and microarray but only detected as an eQTL gene by RNA-seq. Down-regulation of the expression of these genes was associated with significantly reduced glucose-stimulated insulin secretion (Fig. B2–D). Taken together, we present a list of SNPs influencing gene expression in human pancreatic islets with a likely role in regulating glucose homeostasis.

### Table 1. Genome-wide significant GWAS T2D/glycemic hits as eQTLs in human pancreatic islets

<table>
<thead>
<tr>
<th>SNP</th>
<th>Nearest gene</th>
<th>eQTL gene</th>
<th>eQTL P value</th>
<th>Allele change</th>
<th>eQTL direction</th>
<th>GWAS trait</th>
<th>GWAS effect allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1019503</td>
<td>ERAP2</td>
<td>ERAP2</td>
<td>7.1e−24</td>
<td>G &gt; A</td>
<td>+</td>
<td>2-h glucose (3)</td>
<td>A</td>
</tr>
<tr>
<td>rs2028299</td>
<td>AP3S2</td>
<td>AP3S2</td>
<td>1.1e−14</td>
<td>C &gt; A</td>
<td>—</td>
<td>T2D (24)</td>
<td>A</td>
</tr>
<tr>
<td>rs5059370</td>
<td>ABO</td>
<td>ABO</td>
<td>5.3e−08</td>
<td>T &gt; C</td>
<td>+</td>
<td>Disposition index (25)</td>
<td>C</td>
</tr>
<tr>
<td>rs1590963</td>
<td>MTNR1B</td>
<td>MTNR1B</td>
<td>8.6e−08</td>
<td>C &gt; G</td>
<td>+</td>
<td>T2D, fasting glucose (2, 26)</td>
<td>G</td>
</tr>
<tr>
<td>rs1535500</td>
<td>KCNK16</td>
<td>KCNK17</td>
<td>1.2e−06</td>
<td>G &gt; T</td>
<td>+</td>
<td>T2D (27)</td>
<td>T</td>
</tr>
</tbody>
</table>

---

**Fig. 3.** Expression analysis of the lincRNA LOC283177 in 89 human pancreatic islets. (A) The lincRNA LOC283177 has an eQTL (n = 89, significant at FDR < 1% and 10,000 permutations), (B) which is associated with HbA1c in the idlet donors (nominal and permutation P value < 0.05 after 10,000 permutations). Normal corresponds to normoglycemic donors (HbA1c ≤ 6.5%; n = 51), IGT corresponds to impaired glucose tolerant donors (6% ≤ HbA1c < 6.5%; n = 19), and T2D corresponds to diabetic donors (HbA1c ≥ 6.5%; n = 12). LOC283177 is coexpressed with the diabetic genes (C) MADD, (D) PAX6, (E) SYT11, and (F) associates with depolarization-evoked insulin exocytosis (Spearman correlation test significance at FDR < 1%).
eQTLs and HbaA1c Influence Expression of lincRNAs. lincRNAs have recently been ascribed a role in the regulation of gene transcription, including pancreatic islets (16). We identified 493 ReSeq lincRNAs expressed in the pancreatic islets, with 54 of those being influenced by eQTLs or sQTLs and/or related to HbaA1c levels (Database S1). Of these 54 lincRNAs, seven (13%) have also been reported in a study of lincRNAs in human pancreatic β cells (15). Of the 616 eQTLs we identified (Fig. 2A), 33 (5%) influenced the expression of lincRNAs, eight of which have also been reported in other tissues. Moreover, six (2%) of 371 sQTLs were seen within lincRNAs. Notably, 17 lincRNAs were significantly associated with HbaA1c levels, two of which also had an eQTL (LOC283177 and SNHG5) (Database S1). To obtain insight into putative target genes of these two lincRNAs, we performed a coexpression analysis linking their expression with all other genes in pancreatic islets. This analysis showed a strong coexpression of the MAP-kinase activating death domain (MADD), synaptotagmin 11 (SYT11), and paired box 6 (PAX6) genes with LOC283177 (Fig. 3). All these genes have been ascribed a key role in islet function. Synaptotagmin 11 (SYT11) is known to regulate exocytosis of insulin (39) and MADD proinsulin synthesis (25), and PAX6 is involved in development of pancreatic islets (40). In support of this, LOC283177 expression was directly associated with insulin exocytosis (Fig. 3F). The lincRNA ANRIL (also known as CDKN2B-AS1), located in a locus on chromosome 9p, has been associated with both T2D (2) and cardiovascular disease. Although eQTLs for ANRIL have been reported in human blood (41), we could not detect any eQTL for ANRIL in human islets or any coexpressed genes.

Allelic Expression Imbalance. Both allelic expression imbalance (AEI) and cis-QTL analysis detect genetic effects on gene transcription, although they frequently do not capture the same loci. Whereas eQTL and sQTL refer to the effect of a SNP on expression of the gene or specific exons/isoforms, respectively, AEI refers to imbalance between expression of maternal and paternal alleles and, consequently, can only be detected in the case of heterozygosity. To further elucidate the cis-regulatory potential in islets, we searched for genes showing allelic expression imbalance. We compared transcriptome and exome sequencing from the same individuals, using Fisher test to define significant deviation from the expected 50/50 allelic distribution for the SNPs. Thereby, we could detect 1,528 SNPs showing potential allelic imbalance in at least two samples at false discovery rate (FDR) <1% (Database S1). These encompass 1,102 genes, 14% of which have been previously suggested to be imprinted and/or showing imbalance of expression in other human tissues (Database S1). Only 3% of eQTLs and 0.5% of sQTLs were in strong linkage disequilibrium with an AEI SNP. We validated, with Sanger sequencing, an AEI in the MMP7 gene and showed that the missense variant rs10502001 is nominally associated with exocytosis of insulin (SI Appendix, Fig. S15). To detect allelic imbalance sites relevant to T2D, we filtered AEI sites ascertained in at least 50% of the samples and found PEG3 (paternally expressed gene 3). PEG3 is a gene known to be imprinted in other tissues and to change its methylation levels in murine oocytes of diabetic females (42), suggesting a link between allelic imbalance and imprinting/methylation. Notably, we show clear differences in the degree of methylation in a region of the PEG3 promoter, being hypomethylated in T2D islets, which do not have allelic imbalance (Fig. 4). These data indicate that differential methylation could be the cause of allelic imbalance, which in turn could influence susceptibility to T2D. Of SNPs associated with T2D or related glycemic traits, we found enrichment for allelic imbalance (Fisher exact test P value = 2.3e−06, odds ratio = 10.1, with eight showing evidence of allelic imbalance in solute carrier family 2, member 2 (SLC2A2), adaptor-related protein complex 3, sigma 2 subunit (AP3S2), thyroid adenoma associated (THADA), MADD, ERAP2, aminomethyltransferase (AMT), forkhead box A2 (FOXA2), and La ribonucleoprotein domain family, member 6 (LARP6) loci (Table 2).

RNA Editing. Finally, we assessed the frequency of RNA editing events in the pancreatic islet transcriptome, using a stringent pipeline to identify differences between DNA and RNA sequences, by comparing exome and RNA sequencing data (SI Appendix, Fig. S16). We found 65 loci showing potential RNA editing in at least two individuals overlapping 61 genes, two of which were in loci associated with T1D and T2D, GLI3 (44) and ZFAND3 (2) (Database S1). Seven of the RNA editing events have also been reported before. As previously observed (45), the majority of RNA editing events were localized in the 3′UTR region or downstream of genes (67%), suggesting that RNA editing might play a role in miRNA-mediated regulation of gene expression by altering miRNA target sites or by affecting degradation of RNA. As
Table 2. Allelic imbalance loci in high linkage disequilibrium with genome-wide significant GWAS T2D/glycemic trait hits

<table>
<thead>
<tr>
<th>AEI gene</th>
<th>AEI SNPs</th>
<th>Linkage disequilibrium $r^2 &gt; 0.8$ with GWAS top SNP</th>
<th>GWAS trait</th>
<th>GWAS effect allele</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC2A2</td>
<td>rs55679742, rs55989805</td>
<td>rs11920090</td>
<td>Fasting glucose (26)</td>
<td>T</td>
</tr>
<tr>
<td>AP3S2</td>
<td>rs20282899</td>
<td>rs1549318</td>
<td>Fasting proinsulin (25)</td>
<td>T</td>
</tr>
<tr>
<td>LARP6</td>
<td>rs3825970</td>
<td>rs1549318</td>
<td>Fasting proinsulin (25)</td>
<td>T</td>
</tr>
<tr>
<td>THADA</td>
<td>rs7587587</td>
<td>rs10203174</td>
<td>T2D (2)</td>
<td>C</td>
</tr>
<tr>
<td>MAD2</td>
<td>rs35233100</td>
<td>rs35233100</td>
<td>Fasting proinsulin (25)</td>
<td>C</td>
</tr>
<tr>
<td>ERAP2</td>
<td>rs2287988, rs2548538</td>
<td>rs1019503</td>
<td>2-h glucose (3)</td>
<td>A</td>
</tr>
<tr>
<td>AMT</td>
<td>rs6997</td>
<td>rs11715915</td>
<td>Fasting glucose (3)</td>
<td>C</td>
</tr>
<tr>
<td>FOXA2</td>
<td>rs6048192</td>
<td>rs613722</td>
<td>Fasting glucose (3)</td>
<td>G</td>
</tr>
</tbody>
</table>

Discussion

By combining RNA and exome sequencing of human pancreatic islets with in vitro and in vivo functional studies, we present novel insights into the molecular mechanisms by which impaired islet function can contribute to deregulated glucose metabolism. Coexpression analysis showed that expression of many genes correlated strongly with glucagon, not least SLC30A8 encoding the zinc transport protein ZnT8 (SI Appendix, Fig. S8). Rare loss-of-function variants in the SLC30A8 gene have recently been associated with lowering of blood glucose and protection from T2D (47), but the mechanism for this glucose-lowering effect has been unclear, especially as disruption of the SLC30A8 gene in mice has yielded the opposite phenotype: glucose intolerance (48).

The current data might thus shed some light on this paradox: the lower the expression in human pancreatic islets of SLC30A8, the lower the expression of glucagon. It remains to be shown whether carriers of these loss-of-function mutation carriers also show inappropriately low glucagon concentrations. Because most SNPs associated with T2D are intronic or intergenic, it has been assumed that most of them would influence expression, rather than function, of a gene. Although the nearest genes often have been suggested as targets, this has not previously been formally tested in human islets, which represent the culprit in the pathogenesis of T2D. Our current study in a large number of human islet cells allowed this analysis and showed enrichment of GWAS SNPs associated with T2D or glycemic traits in eQTLs (Table 1) and in genetic variants showing allelic imbalance (Table 2). Although we often assume that both parental alleles are expressed to the same degree, this was not the case for SNPs in 1,102 genes, including eight T2D-associated genes (Table 2). This could easily mask an association if the effect of the two parental alleles is bidirectional. We also found allelic imbalance to be often associated with DNA methylation. Genes found to be differentially methylated in human pancreatic islets of non-T2D versus T2D donors (49) were enriched to have allelic imbalance of expression in our dataset (Fisher exact test $P$ value = 6.8e-4; odds ratio = 1.5). Moreover, PEG3 was here detected to have its allelic imbalance associated with diabetic status (Fig. 4). PEG3 encodes for a zinc finger protein that may play a role in cell proliferation and p53-mediated apoptosis (50), which could be involved in the regulation of functional β-cell mass (51).

We also found several eQTLs and sQTLs associated with measures of β-cell function and glucose metabolism, most notably variation in the TMED6, NTSE, PAK7, and TSPAN33 genes, whose disruption in INS-1 cells resulted in impaired insulin secretion (Fig. 2 B–D). These and the other identified genes with an eQTL associated with in vitro and in vivo effects on glucose metabolism could be further explored as potential novel drug targets (Database S1). In addition, many eQTLs and sQTLs influenced expression of noncoding RNAs, many of which seem to target genes of importance for β-cell function. Among them, the lincRNA LOC283177 was found to be coexpressed with key genes implicated in islet function (PAK6, SYT11, and MAD2) (25, 39, 40), and its expression correlated with HbA1c levels and insulin exocytosis (Fig. 3). Finally, we also provide, to the best of our knowledge, the first genome-wide catalog of RNA editing events in human islets mostly related to A-to-G events, but our data also emphasize the need for validation rather than simply relying on RNA sequencing.

There are some limitations with the study we need to take into account. One caveat could be purity of human cadaver islets and differences in contribution of exocrine and endocrine tissue or different contribution of α and β cells between normoglycemic and hyperglycemic donors. We focused on whole islets, as sorting of islet cells would have limited the amount of tissue available for the different analyses. Furthermore, there is important additional information to gain from studying the microorgan islet as an entity, as shown by the expression of other pancreatic hormones and their coexpression. However, some information on cell-specific expression is available from three recent papers (16–18) on a small number of sorted β cells. As described in the Materials and Methods, Database S1, and SI Appendix, Fig. S20, there was no difference in purity between individuals with NGT, IGT, and T2D (Kruskal-Wallis rank sum test $P$ value = 0.83). In addition, the contribution of exocrine and endocrine tissue did not significantly differ between diabetic and non-diabetic islets, as indicated by expression of pancreatic-specific exocrine (alpha 2 amylase) and endocrine (glucagon in alpha cells, MAFA in beta cells, and somatostatin in delta cells) genes (SI Appendix, Fig. S21). Moreover, beta cell content, as measured by FACS β/α cells ratio, was also not significantly different among NGT, IGT, and T2D (Kruskal-Wallis rank sum test $P$ value = 0.14) (SI Appendix, Fig. S22). Acknowledging these limitations, only large enough numbers can outweigh the problems of heterogeneity and purity. To this end, the current study, to our knowledge, represents the largest collection of human islets published thus far. In conclusion, we provide a comprehensive catalog of novel genetic variants influencing gene expression in human pancreatic islets and metabolic phenotypes to facilitate diabetes research.

Materials and Methods

Detailed materials and methods, including all statistical analysis, are available in SI Appendix. Islets from 89 cadaver donors of European ancestry were provided by the Nordic Islet Transplantation Program and processed as previously described (6). Microarray analysis was performed using oligo (52) and sva (53) Bioconductor packages and processed with the standard Affymetrix protocol. Sample preparation for RNA-seq was performed using Illumina’s TruSeq RNA Sample Preparation Kit. Output reads were aligned to the human reference genome (hg19) with TopHat v2.0.2 (54), using Bowtie 0.10.2 (55). The deseq2 R package was used to count uniquely mapped reads in each exon (56). Gene and exon expression normalizations were then performed using the TMM method (57), and further normalization was applied by adjusting the expression to gene or exon length,
respectively. A linear model adjusting for age and sex as implemented in the R Matrix eQTL package (S8) was used to determine the expression of genes and exons with HBA1c class. Exome sequencing was performed using the Illumina exome sequencing protocols. Reads were aligned to the human genome (hg19) with BWA v0.6.2 (S9). Postalignment processing and SNP calling was done with GATK v.1.6.2 (S0). Allelic imbalance of expression was analyzed by Fisher exact test to calculate the proportion of reference/alternative alleles in the exome sequencing versus RNA-seq for each sample. RNA editing sites were called on autosomes in positions that were homozygous in the RNA-seq data. Genotyping was performed on the Illumina HumanOmniExpress 12x1 C Chips, and all of the samples passed standard genotyping QC metrics. Genotypes were imputed to 1000 Genomes data, using IMPUTE2 (S1) and SHAPEIT (S2). cis-eQTL and cis-QTL associations were computed between gene expression levels (eQTL) or exon expression levels (QTL) and all SNPs within 250 kb up- or downstream of each of these genes. We used a linear model adjusting for age and sex, as implemented in the R Matrix eQTL package (S8).

SUPPLEMENTAL INFORMATION

This Supplemental Information file includes Acknowledgments, Supplemental Materials and Methods, Supplementary References and Supplemental Figure legends for the Figures S1-S22 below.

Acknowledgments

This work was supported by grants from the Swedish Research Council (including project grants Dnr. 521-2010-3490 to L.G. and 521-2011-3386 to L.E., collaborative project grant Dnr. 2011-3315 to E.R., strategic research area grant EXODIAB Dnr. 2009-1039, and Linnaeus grant Dnr. 349-2006-237), as well as equipment grants from Wallenberg (KAW 2009-0243) and Lundberg Foundation (grant number 359). L.G. is supported from an Advanced Research Grant from the European Research Council (GENETARGET-T2D, GA 269045) and grants from Pfizer and the Novo Nordisk Foundation. L.E. is a senior researcher at the Swedish Research Council and received support from the Swedish Diabetes Foundation and Albert Påhlsson Foundation. J.E. is an EFSD-Lilly research fellow. In addition, the project was funded by an EU grant BetaBat (HEALTH-2011-277713). Human pancreatic islets were provided by the Nordic Network for Clinical Islet Transplantation by the courtesy of O. Korsgren, Uppsala, Sweden with financial support from EXODIAB and JDRF. Work by L.G. and C.W. was also supported by a grant from the Bo and Kerstin Hjelt Foundation. Furthermore, this research was supported by Fondazione CARIPARO ("RNA sequencing for quantitative transcriptomics" PhD Program), PRAT 2010 CPDA101217 ("Models of RNA sequencing data variability for quantitative transcriptomics"). We thank Britt-Marie Nilsson and Anna-Maria Veijanovska-Ramsay at Lund University for their technical assistance.
Supplemental Materials and Methods

Sample processing. Islets from 89 cadaver donors of European ancestry were provided by the Nordic Islet Transplantation Programme (http://www.nordicislets.org). All procedures were approved by the ethics committee at Lund University. Purity of islets was assessed by dithizone staining, while measurement of DNA content and estimate of the contribution of exocrine and endocrine tissue were assessed as previously described (6). The islets were cultured in CMRL 1066 (ICN Biomedicals) supplemented with 10 mM HEPES, 2 mM L-glutamine, 50 µg/ml gentamicin, 0.25 µg/ml Fungizone (GIBCO), 20 µg/ml ciprofloxacin (Bayer Healthcare), and 10 mM nicotinamide at 37 °C (5% CO2) for 1–9 days prior to RNA preparation. Total RNA was isolated with the AllPrep DNA/RNA Mini Kit following the manufacturer's instructions (Qiagen). RNA quality and concentration were measured using an Agilent 2100 bioanalyzer (Bio-Rad) and a Nanodrop ND-1000 (NanoDrop Technologies).

Microarray. Whole transcript microarray analysis was performed using GeneChip Human Gene 1.0 ST and processed with the standard Affymetrix protocol. The array data was then summarized and normalized with Robust Multi-array Analysis (RMA) method using the oligo package from BioConductor (52). Batch correction was done with COMBAT function from SVA package from BioConductor (53). Annotation was done using annotate package from BioConductor and hugene10sttranscriptcluster.db annotation data. Probesets were only kept if they matched uniquely to a gene in the latest hg19 human genome assembly. If more than one probeset matched a gene, one probeset at random was chosen in order to have only 1 probeset per gene. Finally, only probesets (or genes) mapped to the autosomes were kept.

RNA sequencing and analysis of gene and exon expression. Sample preparation was made using Illumina’s TruSeq RNA Sample Preparation Kit according to their recommendations using 1 ug of high quality total RNA. The target insert size was 300 bp and it was sequenced using a paired end 101 bp protocol on the HiSeq2000 platform (Illumina). Quality assessment was made pre- and post-sample preparation on the 2100 Bioanalyzer (Agilent). Illumina Casava v.1.8.2 software was used for base calling. Paired-end 101 bp length output reads were aligned to the human reference genome (hg19) with TopHat v.2.0.2 (54) using Bowtie v.0.12.8 (55). The TopHat parameters explicitly used are tophat -p 30 -G genes.gtf --library-type fr-unstranded -r 100 -F 0.05 --microexon-search. The annotated RefSeq GTF transcript and fasta genome files were from UCSC and were downloaded from http://cufflinks.cbcb.umd.edu/igenomes.html. Gene expression was measured as the normalized sum of expression of all exons. Exons were defined as non-overlapping unique exonic units, as described previously (56). The dexseq_count python script (http://www-huber.embl.de/pub/DEXSeq/analysis/scripts/) was used by counting uniquely mapped reads in each exon. Gene and exon expression normalizations were then performed using the TMM.
method (57), and further normalization was applied by adjusting the expression to gene or exon length, respectively. In addition, only the genes and exons that had reads mapped to them in at least 5% of the samples were kept. The Cufflinks tool v.1.3.0 (29) was used to detect novel gene loci. Novel intergenic gene loci were kept if they didn’t overlap any GENCODE v.12 gene (30), UCSC and Ensembl gene structures, had exon-exon junction reads mapped to them, had at least two exons with no Ns, and were expressed (non-null read coverage) in at least 5% of the samples. Coding potential of these novel intergenic loci was assessed with the CPAT tool (31).

**Differential expression of genes and exons between normoglycemic and hyperglycemic islets.** Samples were stratified based upon glucose tolerance estimated from HbA1c, i.e. donors with normal glucose tolerance (HbA1c < 6%, n=51), impaired glucose tolerance (IGT, 6% ≤ HbA1c < 6.5%, n=15), and T2D (HbA1c ≥ 6.5%, n=12) (63). A linear model adjusting for age and sex as implemented in the R Matrix eQTL package (58) was used to determine the expression of genes associated with glucose tolerance status. Genes were kept if both microarray and RNA-seq gene expression were nominally associated with HbA1c levels, with both nominal and permutation p-values < 0.05 (after performing 10,000 permutations). Known exons overlapping only one gene were classified as associated with HbA1c levels if the exon expression in RNA-seq was also confirmed at their exon-exon junction’s expression level, with both nominal and permutation p-values < 0.01 (after performing 10,000 permutations), and p-value/permutation p-value ratio ≤ mean ratio + 1 s.d.. Since most of the lincRNAs were not probed on the expression array, the list of lincRNAs associated with HbA1c levels was taken only from the RNA-seq data at a threshold of FDR<5%, and expressed in at least 5% of our samples. The same threshold was applied for the novel gene loci detected. Of note, the lincRNAs we have reported are all known RefSeq genes with known gene structures and annotations.

**Genotyping.** Genotyping was performed on the Illumina HumanOmniExpress 12v1 C chips and genotype calling was done with the Illumina Genome studio software. All the samples passed standard genotype QC (quality control) metrics: sample call rate >98%, only European ancestry assessed by principal component analysis comparisons with HapMap populations, gender matched, no relatedness, and no genome-wide heterozygosity outliers. SNPs were removed if SNP call rate < 98% and Hardy-Weinberg equilibrium test p-values < 5.7x10⁻⁷. Individual QC genotypes were imputed to 1000 Genomes data, using IMPUTE2 (61) and the March 2012 release of the 1000 Genomes Phase I panel (http://mathgen.stats.ox.ac.uk/impute/data_download_1000G_phase1_integrated.html). The program SHAPEIT (62) was used for the pre-phasing. Probabilistic genotypes were used for the subsequent analyses and after imputation, SNPs were filtered using a minor allele frequency (MAF) > 5% and an IMPUTE2 info value of >0.8.
**cis-eQTL and cis-sQTL analysis.** cis-eQTL and cis-sQTL analyses were carried out on samples from 89 individuals. Associations were computed between gene expression levels (eQTL), or exon expression levels (sQTL), and all SNPs within 250kb up- or downstream of each of these genes. We used a linear model adjusting for age and sex as implemented in the R Matrix eQTL package (58). Adjusting also for HbA1c did not significantly affect QTL results, so all the results are shown only with age and sex as covariates. The eQTLs and sQTLs were kept if the false discovery rate (FDR) was less than 1%, the QTL variants had rs IDs (for the sentinel variants), and if no smaller p-value was obtained after doing 10,000 permutations. A literature search (64-79) was performed to reveal whether the eQTLs observed in islets also were observed in other human tissues. Human pancreatic islets H3K4m3, FAIRE (moderate stringency FAIRE-seq site threshold from intersection of 3 islet samples) and DNase I hypersensitivity sites were annotated as such from recent studies (32-34). Evolutionarily conserved sites were defined has such if they were called conserved by both SiPhy (80) and GERP (81) programs, as annotated by Haploreg annotation tool (82). All enrichment analyses were carried out comparing the eQTL and sQTL SNPs plus SNPs in high LD ($r^2$>0.8) with them vs. all the SNPs tested, to avoid biasing enrichment to more densely genotype or imputed genomic regions.

**Exome sequencing and Allelic expression imbalance (AEI).** Exome sequencing was performed using Illumina exome sequencing protocols. To prepare the DNA for exome capture 1 ug of intact DNA was used as input for the TruSeq DNA sample preparation Kit v2 (Illumina), which was processed according to standard protocols. Briefly, DNA shearing was performed on the Covaris S2 with a target fragment size of 300 bp before end-repair, A-tailing and adaptor ligation. After DNA sample preparation, 500 ng of each sample was pooled together in libraries of a total of 5 samples before clustering with the TruSeq PE Cluster Kit v3 (Illumina). The libraries for 82 out of the 89 samples were then sequenced on the HiSeq2000 (Illumina) platform (paired end 101 bp protocol). Illumina Casava1.8.2 software was used for base calling. Paired-end reads were aligned to the human genome (hg19) with BWA v.0.6.2 (59) in paired-end mode with $-q$ 10 as a set parameter. Duplicated aligned reads were removed by Picard v.1.58 (http://picard.sourceforge.net), reads were then realigned and quality base scores were recalibrated using GATK v.1.6.2 (60). SNP calling was also done with GATK with parameters -T UnifiedGenotyper -baq RECALCULATE only under the TruSeq Exome targeted regions, and excluding regions of known segmental duplications, structural variants and repeats. We further restricted SNP calling to biallelic SNPs, with read depth > 14X, MAPQ0 < 1, homozygosity runs < 3 bp, mapping quality > 30, and QD (QualByDepth) > 2. The RNA-seq reads from the same 82 samples were also aligned with BWA but in single-end mode with $-q$ 15 as a set parameter and without removing potential duplicated reads. For each RNA-seq sample we called the genotypes that were detected as heterozygous SNPs in the exome sequencing. We then filtered out genomic positions where RNA-seq reads had less than 10X coverage and that both the reference and alternative alleles in the exome sequencing had less than 10X coverage. We then did a Fisher
exact test for the proportion of reference/alternative alleles in the exome sequencing vs. RNA-seq for each sample and kept only SNPs if the allelic imbalance was detected in at least 2 samples with a false discovery rate (FDR) p-value ≤ 0.01. False discovery rate (FDR) was calculated with the Benjamini & Hochberg method under the p.adjust function in R. Briefly, all the p-values retrieved from all the testable SNPs in each sample (after the filtering criteria written above) were sorted and FDR was applied to them for significance. We further filtered out SNPs overlapping known splice sites, that were not within RefSeq autosomal genes and were not present in dbSNP v.137 (with unique mapped position), as annotated by HaploReg (82). Genes with previous allelic imbalance or imprinting status were searched in literature (83, 84) (http://www.geneimprint.com/, http://www.otago.ac.nz/IGC).

**RNA editing.** RNA-seq reads from the 82 samples aligned with BWA were used for SNP calling with the same parameters and filters used for exome sequencing reads described above. For each exome sequenced sample we called the genotypes that were detected as SNPs in the RNA-seq data. RNA editing sites were called on autosomes in positions which were homozygous in the exome sequencing but heterozygous in the RNA-seq data in at least 2 samples. We further filtered out RNA editing variants with low quality and coverage < 15X; that were within +/- 10 bp of exon-exon junctions discovered in all 89 samples; overlapped known splice sites, more than one gene, present in dbSNP v.137, had HaplotypeScore > 13.0, ReadPosRankSum < -8.0, MQRankSum < -12.5, were within 100bp of each other; and were not in uniquely mapable 100mers regions. The RNA editing events were checked for novelty at DARNED database (85), a repository of RNA editing events in brain, blood and lymphoblastoid cell lines.

**Sanger sequencing analysis.** Validation of allelic imbalance and RNA editing was carried out by RT-PCR with subsequent Sanger sequencing. For reverse transcription SuperScript II RT was used with a mixture of random hexamer primers and dT<sub>18</sub> (Life Technologies); PCR was run using AmpliTaq Gold Master Mix (Life Technologies), and Sanger sequencing was performed by GATC Biotech. RNA editing was examined in the genes listed in Table S18; nucleotide position, primers used, and numbers of samples are indicated. Allelic imbalance was tested for the three variants listed in Table S19 in the number of heterozygous samples indicated. PCR was run using the programme: 6’ 96° - [96° 15’ - 55° 30’ - 72° 45’]∞ - 4∞∞. Sanger sequencing reads were analyzed with the Mutation Surveyor V3:97 software (SoftGenetics).

**RNA Interference (siRNA) and insulin secretion assay.** Clonal INS-1 832/13 β-cells were cultured as previously described (86) and transfected using a mixture of DharmaFECT® 1 (Dharmacon; Life Technologies) and the respective siRNAs. Different sets of siRNA sequences were purchased with siRNA identification numbers: s178860 and s178858 (TSPAN33), s132856 and s132854 (NT5E), s161202 and s161203 (PAK7) and s146175 (TMED6) (Ambion). For control purposes, a previously described control sequence Silencer® Negative Control #2 from Ambion was used. Cells were cultured in medium for 72 hours at 37°C in a humidified atmosphere
containing 95% air and 5% CO2 in the presence of 40 nM siRNA in 24-well cell culture microplates. Knockdown was assessed by RT-qPCR of the target genes as described above using the following Taqman® gene expression assays (Life Technologies): TSPAN33 (Rn01500778_m1), NT5E (Rn00665212_m1), PAK7 (Rn01746951_m1) and TMED6 (Rn01432785_m1). After transfection insulin secretion measurements were performed. Confluent plates containing transfected INS1-832/13 cells were washed twice with 1 mL pre-warmed Secretion Assay Buffer (SAB), pH 7.2 (114 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.16 mM MgSO4, 20 mM HEPES, 2.5 mM CaCl2, 25.5 mM NaHCO3 and 0.2% Bovine Serum Albumin) containing 2.8 mM glucose. The cells were then pre-incubated for two hours in new 2 mL SAB with 2.8 mM glucose. Afterwards, separate wells were incubated for 1 hour in 1 mL SAB containing either 2.8 mM or 16.7 mM glucose. Secreted insulin was measured from supernatant using Coat-a-Count Insulin radioimmunoassay kit (Siemens) and the values were normalized using total protein content individually for each well (BCA protein assay kit, Thermo Scientific).

Flow cytometry of islets cells. Human islets were dissociated to single cell suspension using Accutase (Life Technology). Dissociated islet cells were fixed and permeabilised prior of flow cytometric analysis of intracellular insulin and glucagon using anti-insulin and anti- glucagon antibodies (R&D Systems) conjugated with R-phycoerythrin and allophycocyanin respectively by the Lightning-Link technology (Innova Bioscience, Cambridge, United Kingdom). Flow cytometry data were acquired on a CyAN ADP (Beckman Coulter) and analyzed using FlowJo software (TreeStar, Ashland, OR, USA).

Accession numbers. Clinical information on the 89 islet donors, gene and exon annotation files, raw and processed files for their islet array and RNA-seq mRNA expression are deposited at GEO under the accession number GSE50398.
Supplementary References


Supplemental Figure legends

Figure S1. Overview of study design and main results. Different \textit{omics} platforms were used to assess a comprehensive spectrum of gene regulation in human pancreatic islets. RNA sequencing (RNA-seq) was used to detect known and novel genes expressed in at least 5\% of the 89 samples, and known exons and novel genes associated with glucose tolerance status. Known genes were reported to associate with glucose tolerance status if both Expression microarrays and RNA-seq detect them at nominal and permutation p-value<0.05. SNPs genotyped in our islet samples and further imputed to the 1000 Genomes reference panel were used in combination with RNA-seq to detect expression quantitative trait loci (eQTL) for known and novel genes, and splicing QTL (sQTL) for known exons at a 1\% false discovery rate (FDR) and 10,000 permutations. 35 genes had both eQTLs and associated linearly with glucose tolerance status. Allelic imbalance was detected by using Fisher exact test to compute significant deviations from the expected 50/50 allelic distribution when comparing Exome sequencing (Exome-seq) and RNA-seq for the same individuals (at 1\% FDR and detected in at least 2 samples). RNA editing was also detected by comparing exome and RNA sequencing data after a stringent pipeline (Materials and Methods and Supplemental Information).

Figure S2. Distribution of RNA-seq expression of known genes on 89 human pancreatic islet samples. Density of reads mapped to RefSeq genes with red vertical bars separating the 4 quartiles of expression.

Figure S3. Correlation between RNA-seq and microarray data on 89 human pancreatic islet samples. Spearman correlation between the normalized expression of genes detected in both platforms (Materials and Methods and Supplemental Information).

Figure S4. Fraction of RefSeq genes, transcripts, exons and junctions detected by RNA-seq as a function of cumulative reads mapped to these features. Black vertical line marks the average number of reads per sample (38.2 million paired-end reads) mapped to the human genome.

Fig. S5. Co-expression analysis of \textit{RASGRPI} (n=89). (A) \textit{RASGRPI} vs. \textit{GCG}. (B) \textit{RASGRPI} vs. \textit{INS}. (C) \textit{RASGRPI} vs. \textit{SST}. (D) \textit{RASGRPI} vs. Glucose tolerance status.

Fig. S6. Co-expression analysis of \textit{RFX3} (n=89). (A) \textit{RFX3} vs. \textit{GCG}. (B) \textit{RFX3} vs. \textit{INS}. (C) \textit{RFX3} vs. \textit{SST}. (D) \textit{RFX3} vs. Glucose tolerance status.

Fig. S7. Co-expression analysis of \textit{NNT} (n=89). (A) \textit{NNT} vs. \textit{GCG}. (B) \textit{NNT} vs. \textit{INS}. (C) \textit{NNT} vs. \textit{SST}. (D) \textit{NNT} vs. Glucose tolerance status.

Fig. S8. Co-expression analysis with glucagon gene (n=89). (A) \textit{SLC30A8} vs. \textit{GCG}. (B) \textit{PCSK1} vs. \textit{GCG}. (C) \textit{G6PC2} vs. \textit{GCG}.
**Figure S9.** Novel gene locus (chr12:43,504,654-43,507,028) (A) with evidence of sequence conservation and transcription, (B) associated with HbA1c levels in human pancreatic islets (Normal n=51; IGT n=15; T2D n=12), (C) and under a region nominally significant associated with fasting glucose in a previous study (23). Arrow provides the location of this new transcribed locus.

**Figure S10.** RNA-seq eQTL fraction detected to be nominally significant in the microarray data, stratified by gene expression quartiles (1 being the lowest and 4 the highest quartile).

**Figure S11.** Power to detect eQTLs as a function of sample size. This plot is calculated with the java applet at [http://homepage.stat.uiowa.edu/~rlenth/Power/](http://homepage.stat.uiowa.edu/~rlenth/Power/)

**Figure S12.** High linkage disequilibrium (LD) region around ERAP2 eQTL sentinel SNP (rs2910686) shows nominal significance with fasting glucose in MAGIC database (23).

**Figure S13.** Example of an sQTL not detected at gene level. (A) The sQTL is not detected at the gene expression level (p-value >0.05), (B) and only usage of exon 12 of BRD2 gene is associated with SNP rs114933220 (p-value = 9.5e-06). This gene has been linked to obesity and protection from type 2 diabetes.

**Figure S14.** Known type 2 diabetes (T2D) GWAS locus rs1535500 as eQTL for KCNK17. (A) The SNP rs1535500 is located in KCNK16, but (B) shows an eQTL effect on neighboring KCNK17 gene (p-value = 1.2e-06) (C) and not on KCNK16 (p-value > 0.05).

**Figure S15.** Example of allelic imbalance in the MMP7 gene validated by Sanger sequencing. (A) IGV browser with RNA-seq reads from samples in which the allelic imbalance locus rs10502001 was validated by Sanger sequencing. (B) The genotype for rs10502001 is associated with depolarization-evoked insulin exocytosis.

**Figure S16.** Overview of the pipeline for detecting RNA editing events in 82 human pancreatic islet samples. From the initial 89 samples we only had enough DNA and RNA for doing both RNA and Exome sequencing in 82 samples. Each of these 82 samples was then processed through this pipeline. To minimize the false positive rate we report only the RNA editing events detected in at least two individuals (Materials and Methods and Supplemental Information).

**Figure S17.** Distribution of RNA editing events (Materials and Methods and Supplemental Information).

**Figure S18.** RNA editing events in human pancreatic islets validated by Sanger sequencing. From 9 randomly chosen editing events, we could validate 3 out of 6 of the A-to-G events by Sanger sequencing, but none of the 3 non A-to-G events.

**Fig. S19.** RFX3 co-expression with glucokinase (GCK) (n=89).
**Fig. S20.** Islet purity for our 89 human pancreatic islet samples (assessed by dithizone staining) in relation to disease status. Kruskal-Wallis rank sum test was used to assess the association of gene expression with glucose tolerance status of the islet donors (Normal n=51; IGT n=15; T2D n=12).

**Fig. S21.** Expression of cell-type specific genes in relation to disease status. (A) **GCG** is an alpha-cell specific gene. (B) **MAFA** is a beta-cell specific gene. (C) **SST** is a delta-cell specific gene. (D) **AMY2A** is an exocrine specific gene. Kruskal-Wallis rank sum test was used to assess the association of gene expression with glucose tolerance status of the islet donors (Normal n=51; IGT n=15; T2D n=12).

**Fig. S22.** FACS beta/alpha cells ratio in relation to disease status of 49 islet donor samples (partially overlapped by our 89 islet donor samples used in our study). (A) Kruskal-Wallis rank sum test was used to assess the association of FACS beta/alpha cells ratio with glucose tolerance status (p-value = 0.1373) (Normal n=26; IGT n=14; T2D n=9). (B) The blue horizontal line separates the few T2D donors with high HbA1c (n=3, HbA1c ≥7.3%) that have an insulin/glucagon ratio less than any other Normal or IGT sample.
89 human pancreatic islet donors

**Expression microarray**
- 1619 known genes vs. HbA1c (array & RNA-seq)
- 271 known genes with exons vs. HbA1c (exon & junction data confirmation)
- 1 novel gene locus vs. HbA1c (FDR<1% & 10k perm)

**RNA-seq gene expression & exon usage**
- MAF ≥ 5% samples (18,567 known & 445 novel genes)
- 616 cis-eQTLs (known genes)
- 371 cis-sQTLs (known exons)
- 24 cis-eQTLs (novel gene loci) (FDR<1% & 10k permutations)

**Genotypic imputation**
- $r^2 \geq 0.8$ & MAF ≥ 5% (6.2 M variants)
- 1102 allelic imbalance genes (FDR<1% & ≥ 2 samples)
- 61 genes with RNA editing (≥ 2 samples)

**Exome-Seq**
- 35 known genes have eQTLs and associate with HbA1c
- 271 known genes with exons vs. HbA1c (exon & junction data confirmation)
Fig. S2

Log2(Normalized gene expression)

1st quartile 2nd 3rd 4th quartile

Frequency

-15 -10 -5 0 5 10

log2(Normalized gene expression)
Fig. S3

Mean expression values from RNA-seq and microarray (rho = 0.83)
Fig. S4

Detected features (compared with high-coverage sample)

Fraction of detected features

Million reads

- genes
- junctions
- transcripts
- exons
Fig. S10

% RNA-seq eQTLs detected in the array data

eQTL gene quartile of expression

1st quartile
2nd quartile
3rd quartile
4th quartile
Fig. S11

Power to detect eQTLs

Beta (effect size)
Fig. S14

Panel A: Chromosome 6 showing the location of rs1535500.

Panel B: Box plots showing KCNK17 expression levels for different copies of the rs1535500 minor allele.

Panel C: Box plots showing KCNK16 expression levels for different copies of the rs1535500 minor allele.

Legend:
- KCNK17: Blue bars
- KCNK16: Red bars

Gene: KCNK17
Common SNPs (137)
Fig. S15

A

rs10502001

RNAseq

Sanger

Islet:

22

31

51

54

72

81

B

p-value = 3.021e-03

Total exocytosis (F(Fp)/F)

Copies of the rs10502001 minor allele
Fig. S16

Exome-seq reads \(\xrightarrow{\text{BWA}}\) Human genome sequence (b37) \(\xleftarrow{\text{BWA}}\) RNA-seq reads

SNP calling with GATK Unified Genotyper

Discard dbSNPs and SNPs whose DNA and RNA share the same genotypes

Discard loci of known and novel splice sites, repeats, SVs, low qual, depth <15X, non homozygous DNA genotypes & overlapping more than one gene

BLAT filter – Discard loci with multiple genome hits at high sequence id

Discard SNPs with extreme editing degree

65 potential RNA edited loci in at least two individuals
RNA editing sites

RNA editing base change

AG: 35
AT: 1
CA: 1
CG: 1
CT: 1
GC: 1
GT: 1
TC: 17
TG: 2

Fig. S17
Validation of RNA editing by Sanger sequencing

**PPIA**

\[ A \rightarrow G \]

chr7 44 842 099

**DDX58**

\[ A \rightarrow G \]

chr9 32 456 316

**MAP6**

\[ A \rightarrow G \]

chr11 75 316 759
Fig. S20

A box plot showing islet purity (%) across three groups: Normal, IGT, and T2D. The box plot indicates that there is no significant difference in islet purity among the three groups, as evidenced by the p-value of 0.8347.
Fig. S21

A

GCG expression

Kruskal-Wallis rank sum test
p-value = 0.07

Glucose tolerance status

Normal IGT T2D

B

MAFA expression

Kruskal-Wallis rank sum test
p-value = 0.47

Glucose tolerance status

Normal IGT T2D

C

SST expression

Kruskal-Wallis rank sum test
p-value = 0.61

Glucose tolerance status

Normal IGT T2D

D

AMY2A expression

Kruskal-Wallis rank sum test
p-value = 0.30

Glucose tolerance status

Normal IGT T2D
Fig. S22

A

FACS beta/alpha cells ratio

Glucose tolerance status

Normal  IGT  T2D

B

FACS beta/alpha cells ratio vs. HbA1c (%)

5.0  5.5  6.0  6.5  7.0  7.5