SUPPLEMENTARY DATA

Supplementary Table S1. Primer sequences

Purpose	Direction	Sequence
Amplification and resequencing of p.Lys355Gln	Forward	GGCTCCATTTTATTGATTCCA
	Reverse	ATGCTTCCTCCCATTTGC
Cloning of human *SLC19A2*	Forward	CCGCTCGAGGCCACCATGGATGTGCCCGGCCC
	Reverse	GCGACCGGTGGTGAAGTGGTTACTTGAGAACTTGATTGTGGATCTTCC
Site directed mutagenesis	Forward	GCAGTTGGTTATATACAAATATCCTGG
	Reverse	CCAGGATATTTGTATATAACCAACTGC
qPCR (human *SLC19A2*)	Forward	GGCCGGACAAGAACCTGAC
	Reverse	ACACAGGAAACAGTAGCACCA
qPCR (mouse *SLC19A2*)	Forward	GGATGGGAGGACATTGAGTC
	Reverse	TCCACAGGACTCTGAACACC
qPCR (mouse *SLC19A3*)	Forward	TGGTCAAGTCGAGCTATTCG
	Reverse	GTGTCTATGCCGAACACCAG
qPCR (mouse *TBP*)	Forward	AAGGGAGAATCATGGACCAG
	Reverse	CCGTAAGGCATCATTGGACT

SUPPLEMENTARY DATA

Supplementary Table S2. Variants in genes causing syndromic forms of diabetes or neonatal diabetes

Gene	Protein	Mutation	Kindred
WFS1	Wolfram Syndrome 1	p.D85N p.G604S p.C355Y	F2 F17 F17
ALMS1	Alstrom Syndrome Protein 1	p.V381I p.I443V	F11 F22
SLC19A2	Thiamine transporter 1	p.K355Q	F23

SUPPLEMENTARY DATA

Supplementary Table S3. Additional variants identified in family F23.

Gene	AAChange	SIFT [cutoff<0.05] (1)	PolyPhen2 [scale 0 to 1] (2)	LRT [scale 0 to 1] (3)	MutationTaster [scale 0 to 1] (4)	GERP [cutoff≥ 3] (5)	Phenocopy subject [age at diagnosis]	Non-penetrant subject [age]
SMURF1	NM_001199847:c.A1070G:p.K357R	0.61	0.014	1	0.999	5.09	I-1 [35]	I-2 [60], II-5 [40]
SEMA4A	NM_001193302:c.A4G:p.I2V	0	0.137	0.996	0.728	4.08	-	II-5 [40]
SARDH	NM_001134707:c.C731T:p.T244M	0.03	0.538	0.999	0.287	4.86	I-1 [35], II-1 [28]	I-2 [60], II-5 [40]
HECW2	NM_020760:c.A1868G:p.E623G	0.23	0.913	0.959	0.144	5.05	I-1 [35]	II-5 [40]
AGMO	NM_001004320:c.G1297T:p.V433F	0.04	0.068	0.759	0.440	1.43	I-1 [35]	-

Supplementary Table S4. Software prediction algorithms used to assess the p.Lys355Gln mutation effect on THTR1 protein

Software prediction algorithm	Score	Effect predicted
SIFT (1)	0.059 (cutoff = 0.05)	Tolerated
PROVEAN (6)	-2.69 (cutoff=-2.5)	Deleterious
PolyPhen2 (2)	0.95	probably damaging
MutationTaster (4)	0.96 (scale 0 to 1)	disease causing
LTR (3)	1 (scale 0 to 1)	Deleterious
Mupro (7)	Confidence score = -0.867 (scale -1 to 1)	Decrease Stability

SUPPLEMENTARY DATA

Supplementary Table S5. Characteristics of islet donors

Donor UNOS ID	Gender	Ethnicity/Race	Age (years)	BMI	Diabetic donor status
ABD1375	Female	White	47	25.0	No
ZHD244	Male	Hispanic/Latino	49	31.3	No
ABJU206	Female	White	52	31.4	No
XHW271	Female	White	50	26.4	No
ABLM090	Male	Hispanic/Latino	58	31.2	No
ACAF132A	Female	White	36	42.7	No
YJO424	Male	Caucasian	49	28.2	T2D
AAFS251	Male	Asian	49	23.9	T2D
Xhk168a	Female	White	38	37.8	T2D
YIC101	Male	White	48	35.8	T2D
AAFS251	Male	Asian	49	23.9	T2D
ABFE184	Female	White	44	32.8	T2D

References

1. Ng PC, Henikoff S: SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31:3812-3814
2. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR: A method and server for predicting damaging missense mutations. Nat Methods 2010;7:248-249
3. Chun S, Fay JC: Identification of deleterious mutations within three human genomes. Genome Res 2009;19:1553-1561
4. Schwarz JM, Rodelsperger C, Schuelke M, Seelow D: MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010;7:575-576
5. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S: Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol 2010;6:e1001025
6. Choi Y, Chan AP: PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics 2015;31:2745-2747
7. Cheng J, Randall A, Baldi P: Prediction of protein stability changes for single-site mutations using support vector machines. Proteins 2006;62:1125-1132

SUPPLEMENTARY DATA

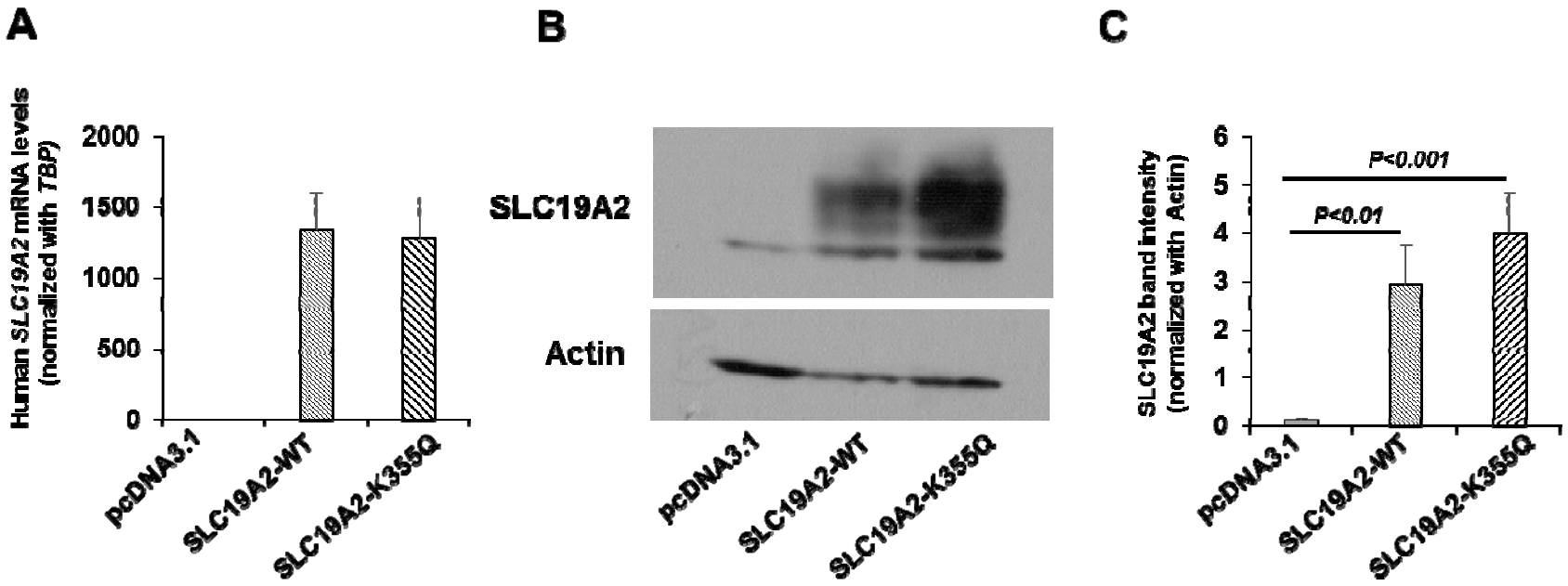
Supplementary Figure S1. SLC19A2 expression levels.

The pcDNA3.1 plasmid construct carrying coding sequence of the human SLC19A2 gene (NM_006996; OHu18269C, Genscript) was used as a template for generation of SLC19A2-K355Q mutant clone by means of site-directed mutagenesis. MIN6-m9 cells were transfected with either pcDNA3.1 empty vector, SLC19A2-WT, or SLC19A2-K355Q construct. Total RNA and protein were extracted from transfected cells at 48 hours after transfection.

A. Relative SLC19A2 mRNA expression levels. Reverse transcription and qPCR were performed using the SuperScript® III Platinum® SYBR® Green One-Step qPCR Kit (Life Technologies) on an ABI 2900HT thermocycler with primers specific to human *SLC19A2* gene. Results were normalized to mouse Tata Box Binding Protein (TBP) expression levels.

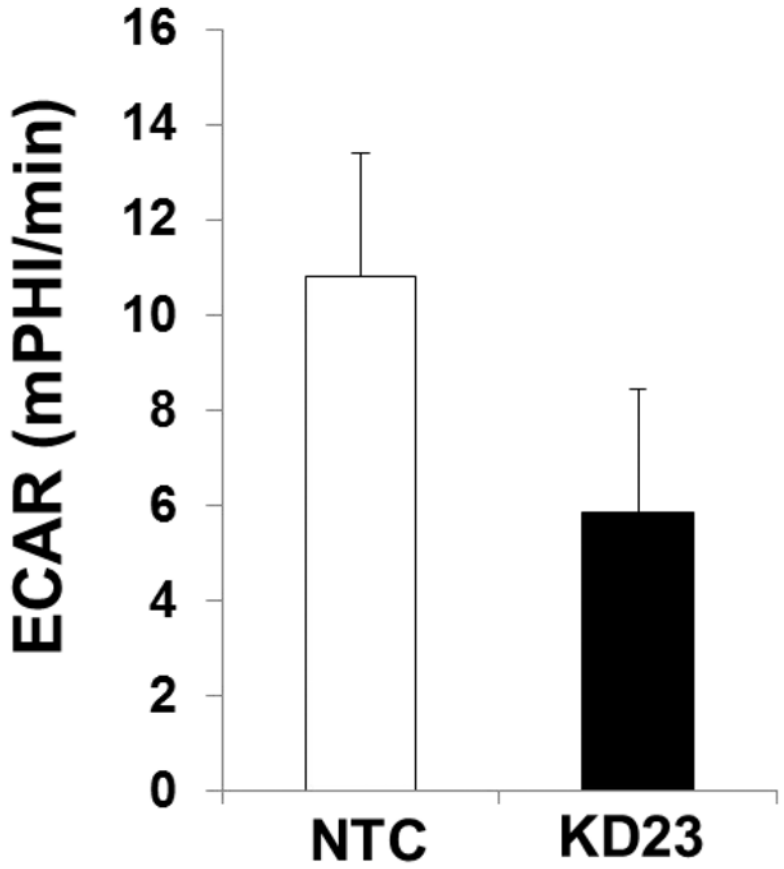
B. SLC19A2 protein levels. Concentrations of protein extracts from transfected MIN6-m9 cells were measured using a BCA protein assay kit (Pierce). Extracts were subjected to Western blotting with primary antibodies overnight at 4°C. Mouse and human anti-THTR-1 and anti-β-actin were from Sigma (#HPA016599) and Cell Signaling (#4970), respectively.

C. Relative SLC19A2 protein levels. Band intensity of SLC19A2 and actin were quantitated by ImageJ-NIH (https://imagej.nih.gov/ij/).



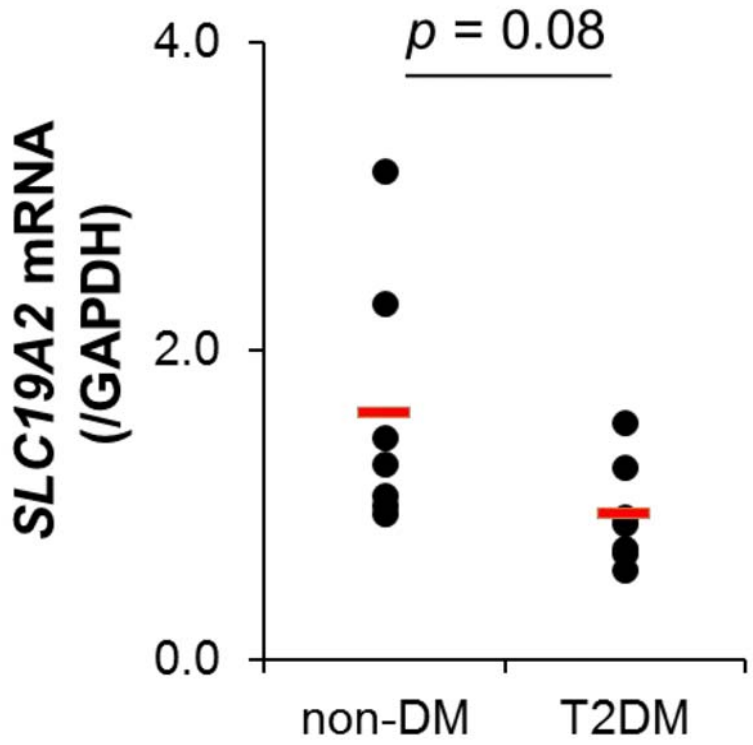
SUPPLEMENTARY DATA

Supplementary Figure S2. Glycolytic activity of MIN6-m9 cells indicated by extracellular acidification rate (ECAR).
Cells were suspended in DMEM containing 5.5 mM glucose and seeded onto Seahorse 24-well XF Cell Culture Microplates at the density of 50,000 cells/well. ECAR were measured by means of extracellular flux analysis with the Seahorse instrument (Seahorse Bioscience) at 18-24 hours after seeding. Data are presented as mean ± SE of 3 independent experiments.



SUPPLEMENTARY DATA

Supplementary Figure S3. Analysis of SLC19A2 mRNA levels in islets isolated from human subjects.
Islets from non-diabetic (n=6) and T2D (n=6) human subjects were obtained from the Integrated Islet Distribution Program. All studies and protocols used were approved by the Joslin Diabetes Center's Committee on Human Studies (CHS#5-05). Upon receipt, islets were cultured overnight in Miami Media #1A (Cellgro). Total RNA was extracted using RNeasy Mini Kit (QIAGEN). One mg RNA was reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative PCR was performed in an ABI 7900HT system, using SYBR Green Supermix (Bio-Rad). GAPDH or b-actin were used as an internal control.



SUPPLEMENTARY DATA

Supplementary Figure S4. Immunostaining of SLC19A2 and insulin of endocrine pancreas from mice model.
Representatives immunostaining of mouse pancreas (n=3). Mouse pancreata were analyzed by immunostaining using anti-insulin (green), anti-SLC19A2 (red). Nuclei were stained with DAPI (blue). Pink boxes indicating areas shown at higher magnification in the right panel. Scale bar=20μm.