Cell Metabolism
Article

A Common Variant in *TFB1M* Is Associated with Reduced Insulin Secretion and Increased Future Risk of Type 2 Diabetes

Thomas Koeck,[1] Anders H. Olsson,[1] Marloes Dekker Nitert,[1] Vladimir V. Sharoyko,[1] Claes Ladenvall,[1] Olga Kotova,[1] Erwin Reiling,[2] Tina Rönn,[1] Hemang Parikh,[1] Jalal Taneera,[1] Johan G. Eriksson,[3,4] Metodi D. Metodiev,[5] Nils-Göran Larsson,[5] Alexander Balhuizen,[1] Holger Luthman,[1] Alena Stančáková,[6] Johanna Kuusisto,[6] Markku Laakso,[6] Pernille Poulsen,[7,8] Allan Vaag,[7] Leif Groop,[1] Valeriya Lyssenko,[1] Hindrik Mulder,[1,9] and Charlotte Ling[1,9,*]

[1]Department of Clinical Sciences, Lund University Diabetes Centre, CRC, Scania University Hospital, 205 02 Malmö, Sweden
[2]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden, The Netherlands
[3]National Institute for Health and Welfare, Helsinki, Finland
[4]Department of General Practice and Primary Health Care, University of Helsinki, Helsinki, Finland
[5]Max Planck Institute for Biology of Ageing, Cologne D-50931, Germany
[6]Department of Medicine, University of Eastern Finland, 70210 Kuopio, Finland
[7]Steno Diabetes Center, 2820 Gentofte, Denmark
[8]Novo Nordisk, DK-2860 Soeborg, Denmark
[9]These authors contributed equally to this work
*Correspondence: charlotte.ling@med.lu.se
DOI 10.1016/j.cmet.2010.12.007

SUMMARY

Type 2 diabetes (T2D) evolves when insulin secretion fails. Insulin release from the pancreatic β cell is controlled by mitochondrial metabolism, which translates fluctuations in blood glucose into metabolic coupling signals. We identified a common variant (rs950994) in the human transcription factor B1 mitochondrial (*TFB1M*) gene associated with reduced insulin secretion, elevated postprandial glucose levels, and future risk of T2D. Because islet *TFB1M* mRNA levels were lower in carriers of the risk allele and correlated with insulin secretion, we examined mice heterozygous for *Tfb1m* deficiency. These mice displayed lower expression of TFB1M in islets and impaired mitochondrial function and released less insulin in response to glucose in vivo and in vitro. Reducing TFB1M mRNA and protein in clonal β cells by RNA interference impaired complexes of the mitochondrial oxidative phosphorylation system. Consequently, nutrient-stimulated ATP generation was reduced, leading to perturbed insulin secretion. We conclude that a deficiency in TFB1M and impaired mitochondrial function contribute to the pathogenesis of T2D.

INTRODUCTION

Type 2 diabetes (T2D) is a multifactorial and polygenic disorder, characterized by impaired insulin secretion from pancreatic β cells and insulin resistance in peripheral tissues. Insulin secretion is controlled by fuel metabolism in pancreatic β cells. When plasma glucose levels rise, glucose is transported into the β cells and metabolized, generating metabolic coupling signals that trigger and amplify exocytosis (Henquin, 2009). In target tissues of insulin, the hormone controls glucose metabolism, including regulation of mitochondrial metabolism. In view of these fundamental principles, it is of no surprise that mitochondrial dysfunction has been implicated both in impaired insulin secretion and action. These pathogenic processes both precede manifest T2D. In fact, patients with mitochondrial dysfunction caused by mutations in mitochondrial DNA (mtDNA) often develop impaired insulin secretion (Goto et al., 1990; Reardon et al., 1992; van den Ouweland et al., 1992). Experimental studies have demonstrated that insulin secretion is critically dependent on mtDNA expression in pancreatic β cells (Silva et al., 2000). While several genes with important functions in mitochondrial oxidative phosphorylation (OXPHOS) show reduced expression in patients with T2D, only a few polymorphisms in these genes have been associated with the disease (Ling et al., 2007a, 2008; Mootha et al., 2003; Rönn et al., 2008, 2009).

The OXPHOS system, including the electron transport system (ETS) and ATP synthase, is located in the inner mitochondrial membrane. It consists of multiprotein complexes with approximately 90 known subunits encoded by both the nuclear and mitochondrial genomes (Scarpulla, 2008). The mitochondrial genome, mtDNA, encodes 13 of the protein subunits present in the ETS and ATP synthase as well as two rRNAs and 22 tRNAs. Transcription of mtDNA requires a specialized machinery, including factors necessary for promoter recognition, such as mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFB2M) (Falkenberg et al., 2002; Litonin et al., 2010; Metodiev et al., 2009; Scarpulla, 2008; Sologub et al., 2009). Mitochondrial transcription factor B1 (TFB1M) is a paralogue of TFB2M, but is probably not a transcription factor (Litonin et al., 2010; Metodiev et al., 2009). Instead, TFB1M is an essential methyltransferase that dimethylates two highly conserved adenines present in a conserved stem-loop structure at the 3′ end of the 12S rRNA (Metodiev et al., 2009). The

Table 1. Clinical Characteristics of Participants in the Diabetes Genetics Initiative, DGI; the Prevalence, Prediction and Prevention of Diabetes, Botnia PPP; the Helsinki Birth Cohort Study; the METabolic Syndrome In Men Study, METSIM; and the Malmö Preventive Project, MPP

	DGI (Controls)	DGI (T2D)	Botnia PPP	Helsinki Birth Cohort Study	METSIM	MPP
n (male/female)	1467 (707/760)	1464 (741/723)	4553 (2134/2419)	1618 (713/905)	6602 (6602/—)	16061 (10416/5645)
Age (years)	58.8 ± 10.1	64.4 ± 10.2	48.4 ± 15.6	61.6 ± 3.0	57.1 ± 6.9	45.5 ± 6.9
BMI (kg/m^2)	26.6 ± 3.7	28.5 ± 4.5	26.3 ± 4.3	27.1 ± 4.3	26.8 ± 3.8	24.3 ± 3.3
Fasting plasma glucose (mmol/l)	5.3 ± 0.5	9.5 ± 3.1	5.28 ± 0.57	5.49 ± 0.56	5.70 ± 0.49	5.45 ± 0.56
Glucose 30 min (mmol/l)	8.3 ± 1.5	13.3 ± 2.9	8.35 ± 1.55	9.10 ± 1.61	8.79 ± 1.43	9.97 ± 1.90
Glucose 120 min (mmol/l)	5.6 ± 1.3	14.7 ± 5.1	5.28 ± 1.64	6.88 ± 1.71	6.04 ± 1.68	6.39 ± 1.65
Fasting insulin (mU/l)	5.2 (4.3)	9.1 (9.0)	5.4 (4.2)	7.8 (6.5)	6.4 (5.7)	—
Insulin 30 min (mU/l)	50.2 (48.9)	28.0 (35.7)	50.3 (38.4)	62.0 (50.2)	52.6 (46.8)	—
Insulin 120 min (mU/l)	36.2 (29.7)	45.0 (50.3)	24.1 (26.7)	56.2 (56.6)	34.6 (42.6)	—
Insulinogenic index	14.1 (14.8)	3.5 (5.2)	15.8 (16.0)	15.9 (15.9)	16.2 (16.6)	—
Insulin sensitivity index (ISI)	8.6 (6.6)	3.8 (3.6)	8.0 (6.1)	5.0 (4.0)	6.2 (5.3)	—

Data are expressed as mean ± SD or median (interquartile range [IQR]). See also Table S2.

modification of 12S rRNA is essential for the integrity of the small mitochondrial ribosomal subunit and mitochondrial translation is abolished in its absence (Metodiev et al., 2009). TFB1M is alternatively known as dimethyladenosine transferase 1 mitochondrial (mDMAT1) in protein databases.

In view of the important role of ATP production in metabolic homeostasis, we examined whether polymorphisms in genes of the OXPHOS system and the mtDNA transcription and translation machinery are associated with β cell dysfunction, insulin resistance and/or T2D. To this end, we examined data from a genome-wide association study (GWAS), the Diabetes Genetics Initiative (DGI) (Saxena et al., 2007). We identified a common variant (rs950994) in the human *TFB1M* gene, which was associated with an impaired insulin response to glucose, elevated glucose levels during an oral glucose tolerance test (OGTT), increased future risk of T2D, and reduced islet *TFB1M* expression. Accordingly, we proceeded to study mice with a heterozygous deficiency of *Tfb1m* and clonal β cells, where *Tfb1m* had been silenced to mimic the human situation. We found that reduced levels of TFB1M caused impaired OXPHOS and consequently decreased insulin secretion. This study provides conclusive genetic and functional evidence that the *TFB1M* gene is associated with increased risk of T2D by causing impaired insulin secretion.

RESULTS

Identification of a Genetic Locus Associated with Quantitative Metabolic Traits and T2D

To unravel genetic loci associated with β cell dysfunction, insulin resistance, and/or T2D in or adjacent to genes of the OXPHOS system and/or the mtDNA transcription and translation machinery, we examined data from a GWAS, the DGI (Saxena et al., 2007) (Table 1). We identified a common variant (rs950994) in the gene encoding *TFB1M*, which was associated with decreased insulin secretion at 30 min (insulinogenic index, β = −0.17 ± 0.050, p = 0.0007), elevated 2 hr glucose levels during an OGTT (β = 0.12 ± 0.042, p = 0.007) (Table 2), and a nominally increased risk of T2D (odds ratio [OR] = 1.13, 95% confidence interval [CI] = 1.02–1.25, p = 0.017) in DGI. rs950994 is located in intron 2 of the *TFB1M* gene (Figure 1A and Figure S1, available online).

Insulin Secretion and Postprandial Glucose Levels In Vivo

Next, we examined whether the association of *TFB1M* rs950994 with impaired insulin secretion could be replicated in additional cohorts (Tables 1 and 2). In accordance with the effect of rs950994 in DGI, A-allele carriers of rs950994 showed decreased insulinogenic index in the Botnia Prevalence, Prediction and Prevention of Diabetes (Botnia PPP) study (β = −0.045 ± 0.018, p = 0.01); this effect was stronger in elderly individuals (age > median 50 yr, β = −0.085 ± 0.025, p = 0.001) (Table 2 and Table S1). In the METabolic Syndrome in Men (METSIM) study and the Helsinki Birth Cohort Study (HBCS), rs950994 did not exert a significant effect on insulin secretion (Table 2).

Given that the METSIM study only included males, we asked whether gender could affect the association of *TFB1M* rs950994 with impaired insulin secretion. It is not uncommon that gender is a determinant of genetic associations (Galimberti et al., 2008; Joyner et al., 2009; Kolz et al., 2009; Miyashita et al., 2007; Rimol et al., 2010), and metabolism in itself is known to be influenced by sex hormones. Indeed, the A-allele of rs950994 was associated with decreased insulinogenic index in females (Botnia PPP: β = −0.065 ± 0.024, p = 0.008; DGI: β =−0.20 ± 0.06, p = 0.0005), but not in males (Botnia PPP: p = 0.46 and DGI: p = 0.10) (Table 2). In line with this finding, gender-stratified meta-analysis of all studied cohorts supported an association between rs950994 and insulinogenic index in females (p = 0.006, $P_{heterogeneity}$ = 0.059), but not in males (p = 0.79, $P_{heterogeneity}$ = 0.072) (Figures S2A and S2B). Similar to the gender effect of rs950994 on insulin secretion, an association of rs950994 with postprandial (2 hr) glucose levels was only observed in females from Botnia PPP (β = 0.019 ± 0.009, p = 0.03) and DGI (β = 0.13 ± 0.07, p = 0.06) (Table 2). A meta-analysis further strengthened the association between rs950994 and 2 hr plasma glucose

Table 2. *TFB1M* rs950994 in Relation to Postprandial Glucose Levels and Insulin Secretion in Nondiabetic Individuals of the Studied Cohorts

		Genotypes				Additive Model		
Study	Phenotype	GG	GA	AA	RA	Beta	SEM	p value
DGI (n = 894)	Insulinogenic index	15.9 (18.4)	14.4 (14.8)	12.5 (11.0)	0.28	−0.17	0.050	**0.0007**
(n = 1286)	Glucose 2 hr (mmol/l)	5.60 ± 1.26	5.67 ± 1.29	5.96 ± 1.21		0.12	0.042	**0.007**
DGI (females, n = 471)	Insulinogenic index	17.9 (20.4)	14.0 (15.2)	13.0 (12.6)	0.28	−0.20	0.057	**0.0005**
(n = 674)	Glucose 2 hr (mmol/l)	5.75 ± 1.20	5.86 ± 1.30	6.08 ± 1.15		0.13	0.071	0.062
DGI (males, n = 423)	Insulinogenic index	14.1 (17.7)	14.9 (14.1)	11.0 (10.4)	0.28	−0.098	0.060	0.10
(n = 612)	Glucose 2 hr (mmol/l)	5.42 ± 1.32	5.48 ± 1.25	5.82 ± 1.29		0.098	0.083	0.24
Botnia PPP (n = 4290)	Insulinogenic index	16.5 (17.0)	15.5 (15.6)	14.6 (13.8)	0.31	−0.045	0.018	**0.010**
	Glucose 2 hr (mmol/l)	5.20 ± 1.64	5.24 ± 1.62	5.34 ± 1.66		0.011	0.007	0.10
Botnia PPP (females, n = 2260)	Insulinogenic index	17.3 (17.8)	16.3 (16.6)	14.7 (14.2)	0.31	−0.065	0.024	**0.008**
	Glucose 2 hr (mmol/l)	5.28 ± 1.49	5.37 ± 1.56	5.51 ± 1.47		0.019	0.009	**0.030**
Botnia PPP (males, n = 2026)	Insulinogenic index	15.8 (16.2)	14.8 (14.6)	14.3 (13.3)	0.30	−0.019	0.025	0.46
	Glucose 2 hr (mmol/l)	5.13 ± 1.79	5.09 ± 1.67	5.15 ± 1.84		0.004	0.011	0.68
HBCS (n = 1561)	Insulinogenic index	16.0 (15.6)	15.8 (16.3)	14.0 (15.1)	0.27	0.0003	0.029	0.99
	Glucose 2 hr (mmol/l)	6.86 ± 1.74	6.84 ± 1.66	7.13 ± 1.62		0.013	0.010	0.19
HBCS (females, n = 866)	Insulinogenic index	16.7 (15.7)	15.6 (16.3)	15.0 (15.2)	0.27	0.006	0.039	0.87
	Glucose 2 hr (mmol/l)	6.89 ± 1.66	7.01 ± 1.64	7.25 ± 1.65		0.019	0.013	0.14
HBCS (males, n = 695)	Insulinogenic index	15.7 (15.3)	15.9 (16.2)	12.4 (13.3)	0.27	0.001	0.043	0.99
	Glucose 2 hr (mmol/l)	6.81 ± 1.82	6.64 ± 1.66	6.99 ± 1.57		0.007	0.016	0.66
METSIM (n = 5400)	Insulinogenic index	15.6 (15.5)	16.2 (16.9)	16.0 (17.9)	0.31	0.019	0.015	0.20
	Glucose 2 hr (mmol/l)	6.10 ± 1.71	6.06 ± 1.65	6.21 ± 1.76		0.004	0.006	0.53
MPP (n = 8778)	Glucose 2 hr (mmol/l)	6.40 ± 1.64	6.37 ± 1.66	6.39 ± 1.68	0.32	−0.022	0.024	0.35
MPP (females, n = 3096)	Glucose 2 hr (mmol/l)	7.28 ± 1.59	7.23 ± 1.57	7.30 ± 1.58		−0.019	0.042	0.66
MPP (males, n = 5681)	Glucose 2 hr (mmol/l)	5.93 ± 1.47	5.90 ± 1.50	5.87 ± 1.50		−0.016	0.029	0.58

Data are expressed as mean ± SD or as median (IQR). β (SEM) are from linear regression adjusted for age, sex, and BMI. RA is the risk allele frequency. p values < 0.05 are shown in bold. See also Figure S2.

in females (β = 0.019 ± 0.007, p = 0.008, $P_{heterogeneity}$ = 0.175) (Figure S2C), while no association was observed in males (p = 0.43) (Figure S2D).

Insulin Sensitivity In Vivo

Mitochondrial metabolism has also been implicated in insulin sensitivity in peripheral tissues (Ling et al., 2004, 2007a; Rönn et al., 2008, 2009). Therefore, we tested whether the *TFB1M* variant rs950994 influenced insulin sensitivity in vivo during a hyperinsulinemic euglycemic clamp in twins. Here, female A-allele carriers showed reduced insulin-stimulated glucose uptake (Rd) during the clamp (G/G: 12.6 ± 0.5, n = 43 versus A/A + A/G: 10.4 ± 0.4, n = 50, p = 0.003), while no association was observed in male twins (G/G: 10.5 ± 0.5, n = 40 versus A/A + A/G: 10.3 ± 0.5, n = 56, p = 0.65). However, there was no significant association between rs950994 and insulin sensitivity index (ISI) during the OGTT in the studied cohorts, regardless of gender (Table S2).

Risk of T2D

After having established that the *TFB1M* single nucleotide polymorphism (SNP) rs950994 is associated with T2D-related traits, we asked whether the *TFB1M* SNP rs950994 could predict future T2D. To this end, we examined 16,061 Swedish subjects in a prospective study, the Malmö Preventive Project (MPP); 2,063 of the individuals developed diabetes during the median 25-year follow-up period (Table 1). The frequency of the risk A-allele of SNP rs950994 was higher in individuals who developed T2D compared to those who did not (34.1% versus 31.9%, p = 0.006). This yielded a modestly increased risk of T2D with an OR of 1.12 (95% CI = 1.04–1.20, p = 0.002). The observed effect on future risk of T2D was of a magnitude similar to the association with T2D observed in DGI (Saxena et al., 2007).

Furthermore, the *TFB1M* SNP rs950994 was an independent predictor of risk for T2D (OR = 1.11, 95% CI = 1.03–1.20, p = 0.005) in the multivariate regression analyses with a genetic risk score comprising 11 established genetic loci previously reported to be associated with increased risk of T2D in the MPP cohort (Lyssenko et al., 2008) (Table S3).

TFB1M Gene Expression and Insulin Secretion in Pancreatic Islets

To identify a mechanism whereby the risk variant of *TFB1M* contributes to the development of T2D, we examined whether rs950994 was associated with altered gene expression in human pancreatic islets by using quantitative real time-polymerase chain reaction (RT-PCR). Risk A-allele carriers of rs950994 showed a 24% reduction in *TFB1M* expression compared with GG carriers (n = 50, p = 0.005 for a dominant and p = 0.001 for an additive genetic model, respectively) (Figure 1B).

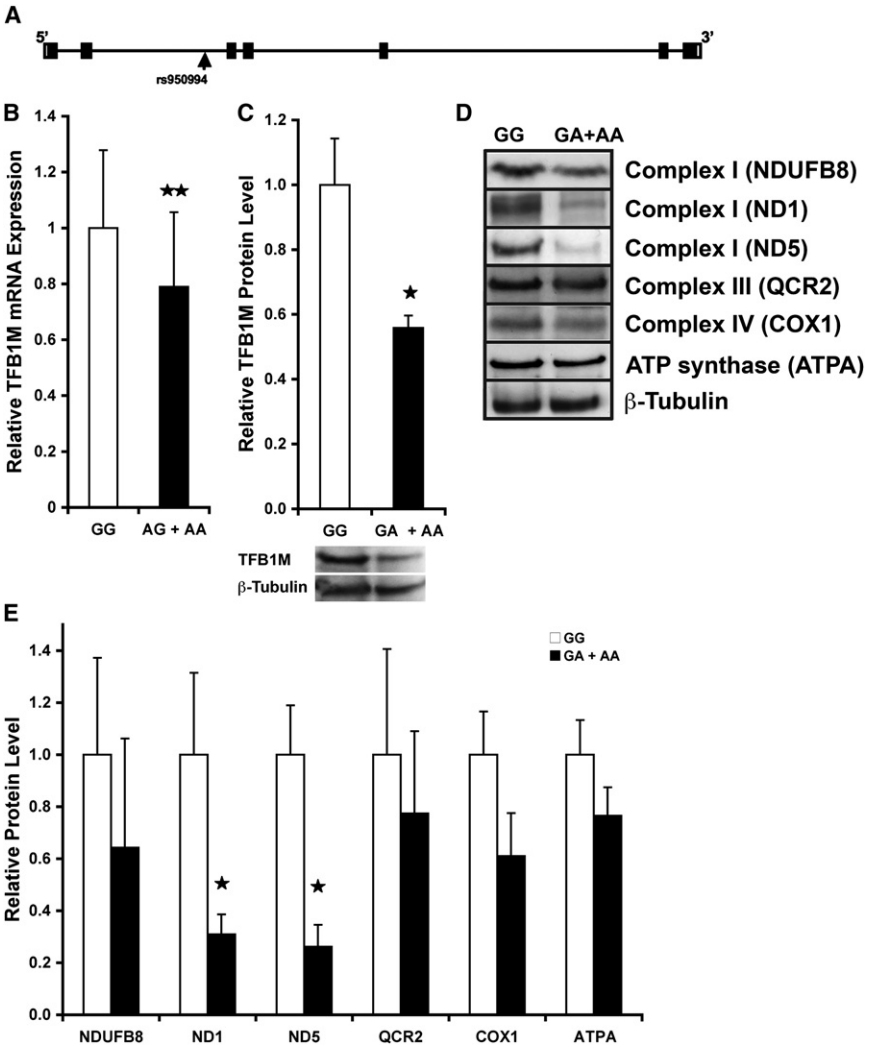

Figure 1. Effects of rs950994 on Human Pancreatic Islets

(A) Map of the human *TFB1M* gene; rs950994 (arrow) is located in intron 2 of the gene (see also Figure S1).

(B) *TFB1M* mRNA levels were lower in pancreatic islets from risk A-allele carriers (n = 24) compared with G/G carriers (n = 26) of *TFB1M* SNP rs950994 based on a dominant model (** < 0.01). Bars represent mean ± standard deviation (SD).

(C) Representative western blots and densitometric analysis of protein levels for TFB1M from four G/G carriers (white bar) and four risk A-allele carriers (black bars) of rs950994 (* < 0.05). Bars represent mean ± standard error of the mean (SEM).

(D) Representative western blots of protein levels for mitochondrially encoded ND1 (complex I), ND5 (complex I), and COX1 (complex IV) and nuclear-encoded NDUFB8 (complex I), QCR2 (complex III core protein 2), and ATPA (ATP synthase F1 subunit α) in pancreatic islets from four G/G carriers and four risk A-allele carriers of rs950994.

(E) Densitometric analysis of protein levels in pancreatic islets from four G/G carriers (white bar) and four risk A-allele carriers (black bars) of rs950994 (* < 0.05). Bars represent mean ± SEM.

Moreover, the mRNA levels of *TFB1M* correlated positively with glucose-stimulated insulin secretion (GSIS) in pancreatic islets from human donors challenged with glucose in vitro ($r = 0.40$, $p < 0.05$).

To examine whether reduced mRNA levels of *TFB1M* translate into reduced protein levels, we analyzed TFB1M and mitochondrially encoded subunits of complex I (ND1, ND5) and complex IV (COX1) as well as nuclear-encoded subunits of complex I (NDUFB8), complex III (QCR2), and ATP synthase (ATPA) in a subset of human islets. This subset of donors included four GG carriers and four A-allele carriers of rs950994; in islets from these risk-allele carriers, *TFB1M* mRNA levels were decreased by 44% (G/G: 1.46 ± 0.06, n = 4 versus A/G + A/A: 0.82 ± 0.19, n = 4, p = 0.02). Indeed, the protein levels of TFB1M (G/G: 1.00 ± 0.14 versus A/G + A/A: 0.55 ± 0.04, p = 0.02), ND1 (G/G: 1.00 ± 0.31 versus A/G + A/A: 0.31 ± 0.08, p = 0.02), and ND5 (G/G 1.00 ± 0.19 versus A/G + A/A 0.26 ± 0.08, p = 0.01) were significantly decreased in the A-allele carriers of this subset of donors, whereas those of NDUFB8 (p = 0.3), QCR2 (p = 0.3), COX1 (p = 0.16), and ATPA (p = 0.1) were not significantly changed (Figures 1C–1E). These findings suggest that reduced levels of *TFB1M* mRNA and protein in human islets are associated with reductions in mitochondrially encoded subunits of complex I.

TFB1M Deficiency, Insulin Secretion, and Mitochondrial Metabolism in Mice

To further elucidate the mechanisms whereby reduced expression of TFB1M may contribute to β cell failure and the development of T2D, we examined mice heterozygous ($Tfb1m^{+/-}$) for a germ-line deletion of *Tfb1m* (Metodiev et al., 2009). The rationale for this approach is that a reduction of TFB1M expression would mimic the situation in humans, whereas total deficiency is known to be embryonically lethal (Metodiev et al., 2009). Indeed, islets from $Tfb1m^{+/-}$ mice contained less TFB1M protein than their wild-type littermates (Figure 2A), confirming that these mice would be a suitable model to study the role of TFB1M in metabolic dysregulation.

First, the $Tfb1m^{+/-}$ mice were subjected to an intraperitoneal glucose tolerance test (IPGTT) to examine glucose tolerance and insulin secretion in vivo. We found that plasma glucose levels rose to higher levels in the $Tfb1m^{+/-}$ mice during the glucose challenge (16.1 ± 0.7 versus 18.7 ± 0.7 mM glucose, t = 10 min, p = 0.02; 16.5 ± 1.1 versus 19.5 ± 0.8 mM glucose, t = 30 min, p = 0.02; 14.2 ± 0.6 versus 16.2 ± 0.6 mM glucose, t = 60 min, p = 0.04; 1754 ± 65 versus 1984 ± 70, glucose-area under the curve [glc_{AUC}], p = 0.01 in control versus $Tfb1m^{+/-}$ mice, respectively; Figure 2B). At the same time insulin secretion tended to be lower, albeit not significantly different, in the

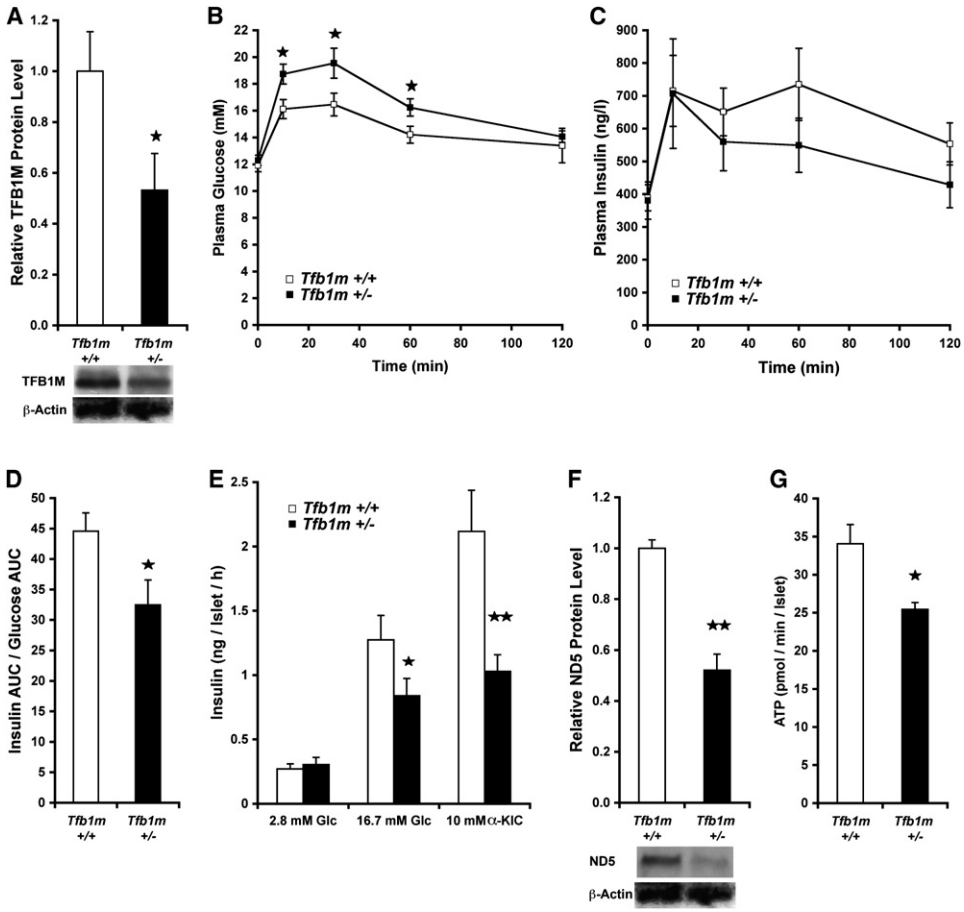

Figure 2. Reduced TFB1M Levels in Heterozygous Mice (*Tfb1m*$^{+/-}$) Result in Impaired Stimulated Insulin Secretion

(A) Pancreatic islets from heterozygous mice (*Tfb1m*$^{+/-}$) were used to test the effect of TFB1M deficiency. Fresh isolated pancreatic islets were subjected to analysis. Representative western blot and densitometric analysis of TFB1M protein levels in the *Tfb1m*$^{+/+}$ (n = 4) and *Tfb1m*$^{+/-}$ (n = 4) mice are shown.

(B and C) Plasma glucose (B) and (C) plasma insulin levels in the *Tfb1m*$^{+/+}$ (n = 10) and *Tfb1m*$^{+/-}$ (n = 11) mice during an IPGTT.

(D) The insulin-area-under-the-curve (ins_{AUC})/glucose-area-under-the-curve (glc_{AUC}) ratio for the *Tfb1m*$^{+/+}$ (n = 10) and *Tfb1m*$^{+/-}$ (n = 11) mice during the IPGTT reflects the capacity of the mice to release insulin in response to glucose.

(E) Insulin secretion in response to 2.8 and 16.7 mM glucose (Glc) (n = 12–15) and 10 mM α-KIC in the presence of 2.8 mM glucose (n = 13–15) was determined in a 1 hr static incubation of the pancreatic islets from *Tfb1m*$^{+/+}$ and *Tfb1m*$^{+/-}$ mice.

(F) Representative western blot from the same samples as for TFB1M in (A) (therefore with the same loading control) and densitometric analysis of ND5 protein levels (complex I, mitochondrially encoded) in pancreatic islets from *Tfb1m*$^{+/+}$ (n = 4) and *Tfb1m*$^{+/-}$ (n = 4) mice .

(G) Kinetics of ATP production were determined in digitonin-permeabilized islets from *Tfb1m*$^{+/+}$ (n = 6) and *Tfb1m*$^{+/-}$ (n = 6) mice in response to a mixture of pyruvate, glutamate, and malate. Bars represent mean ± SEM (* < 0.05; ** < 0.01). See also Table S4.

Tfb1m$^{+/-}$ mice compared with wild-type littermates (t = 120 min, p = 0.07; insulin-area under curve [ins_{AUC}], p = 0.12; Figure 2C). However, when total insulin secretion during the challenge (ins_{AUC}) was related to the rise in glucose (glc_{AUC}), we found that this ratio was decreased in *Tfb1m*$^{+/-}$ mice (44.5 ± 3.0 versus 32.5 ± 4.1, p = 0.03 in control versus *Tfb1m*$^{+/-}$ mice, respectively; Figure 2D). This implies that insulin secretion was insufficient to meet the demands of the glucose challenge in the *Tfb1m*$^{+/-}$ mice; hence, elimination of glucose was diminished. These results demonstrate that TFB1M deficiency is associated with glucose intolerance and impaired insulin secretion. If the *Tfb1m*$^{+/-}$ mice had a sufficient insulin secretory capacity, they would have compensated with increased insulin secretion.

To further understand whether this in vivo perturbation was caused by islet dysfunction and impaired mitochondrial function, we isolated islets from the genetically manipulated mice. We found that insulin secretion in response to glucose was attenuated in islets from the *Tfb1m*$^{+/-}$ mice (1.36 ± 0.19 versus 0.87 ± 0.14 ng insulin/islet/hr, p = 0.04 in control versus *Tfb1m*$^{+/-}$ mice, respectively; Figure 2E). Moreover, insulin secretion in response to α-ketoisocaproic acid (α-KIC), a secretagogue that directly stimulates mitochondrial metabolism, was markedly reduced (2.28 ± 0.32 versus 1.07 ± 0.13 ng insulin/islet/hr, p = 0.001 in control versus *Tfb1m*$^{+/-}$ mice, respectively; Figure 2E), while that in response to 35 mM KCl, a membrane-depolarizing agent, was unaffected (0.47 ± 0.20 versus 0.45 ± 0.12 ng insulin/islet/hr in the presence of 2.8 mM glucose in control

versus *Tfb1m*$^{+/-}$ mice, respectively). These data together suggest that impaired insulin secretion in *Tfb1m*$^{+/-}$ mice is caused by perturbed mitochondrial function. To confirm this, we analyzed levels of ND5 in isolated islets. Western blot analysis showed that the level of ND5 was lower in mitochondria from *Tfb1m*$^{+/-}$ mice (Figure 2F), reflecting the observation we made in islets from human risk-allele carriers (Figures 1D and 1E). Finally, we assayed the kinetics of ATP production, which is the final and essential product of mitochondrial metabolism and which controls insulin secretion via closure of the K_{ATP} channels. Indeed, the rate of ATP production in permeabilized isolated islets in response to a mixture of the mitochondrial metabolic substrates pyruvate, glutamate, and malate was significantly reduced in *Tfb1m*$^{+/-}$ mice (34.1 ± 2.5 versus 25.4 ± 0.9 pmol ATP/min/islet, p = 0.04 in control versus *Tfb1m*$^{+/-}$ mice, respectively; Figure 2G). We conclude that mimicking the deficiency of TFB1M occurring in the human in mice leads to glucose intolerance, impaired mitochondrial function, and perturbed fuel-stimulated insulin secretion.

Silencing of TFB1M in Clonal β Cells

To further model the situation in human and mouse islets and identify mechanisms whereby reduced expression of TFB1M may compromise mitochondrial function and insulin secretion, we silenced *Tfb1m* in the glucose-responsive 832/13 clonal β cell line with small interfering RNAs (siRNA). First, using RT-PCR and western blotting, we analyzed expression of TFB1M after silencing. We found a reduction in the *Tfb1m* mRNA level to 24.2% ± 0.6% (p = 0.0001; Figure 3A), and in protein level to 44.2% ± 1.8% of control (p = 0.0006; Figure 3B) after transfection with siRNA directed to *Tfb1m* for 96 hr. Similar to human and mouse islets, TFB1M deficiency in clonal β cells leads to a reduction in GSIS (181.5 ± 5.5 versus 110.0 ± 5.2 ng/mg/hr, p < 0.001 in control cells versus TFB1M-deficient cells, respectively; Figure 3C) as well as in insulin secretion in response to α-KIC (116.0 ± 9.3 versus 55.0 ± 5.2 ng/mg/hr, p = 0.001 in control cells versus TFB1M-deficient cells, respectively; Figure 3C). As in isolated mouse islets, the response to KCl was not significantly affected (Figure 3C), indicating that there was no unspecific perturbation of the exocytotic machinery or loss of insulin from the clonal β cells. The latter was confirmed by measurement of cellular insulin content after 96 hr of TFB1M knockdown (8.8 ± 0.4 versus 8.2 ± 0.5 μg/mg in control cells versus TFB1M-deficient cells, respectively).

Because the perturbation of insulin secretion stimulated by metabolic substrates in mouse islets and clonal β cells suggested that TFB1M deficiency disrupted only the mitochondrial control of stimulus-secretion coupling (Henquin, 2009) and given that TFB1M is thought to primarily control translation of mitochondrially encoded proteins (Metodiev et al., 2009), we further examined OXPHOS protein levels in mitochondria from the clonal β cells. Using a panel of selected antibodies to both mitochondrially and nuclear-encoded proteins for western blotting, we found decreased levels of OXPHOS proteins in β cells where TFB1M had been silenced (Figure 4A). While the levels of both assayed subunits of complex I, the mitochondrially encoded ND5, and the nuclear-encoded NDUFB8 were significantly reduced, only the mitochondrially encoded subunits of complex III, Cyt b, and of complex IV, COX1, showed significantly reduced protein levels in cells with TFB1M deficiency. This may be explained by the particularly crucial function of the mitochondrially encoded subunits during the modular assembly of complex I (Cardol et al., 2002; Chomyn, 2001; Perales-Clemente et al., 2010). Thus, reduced levels of both mitochondrially

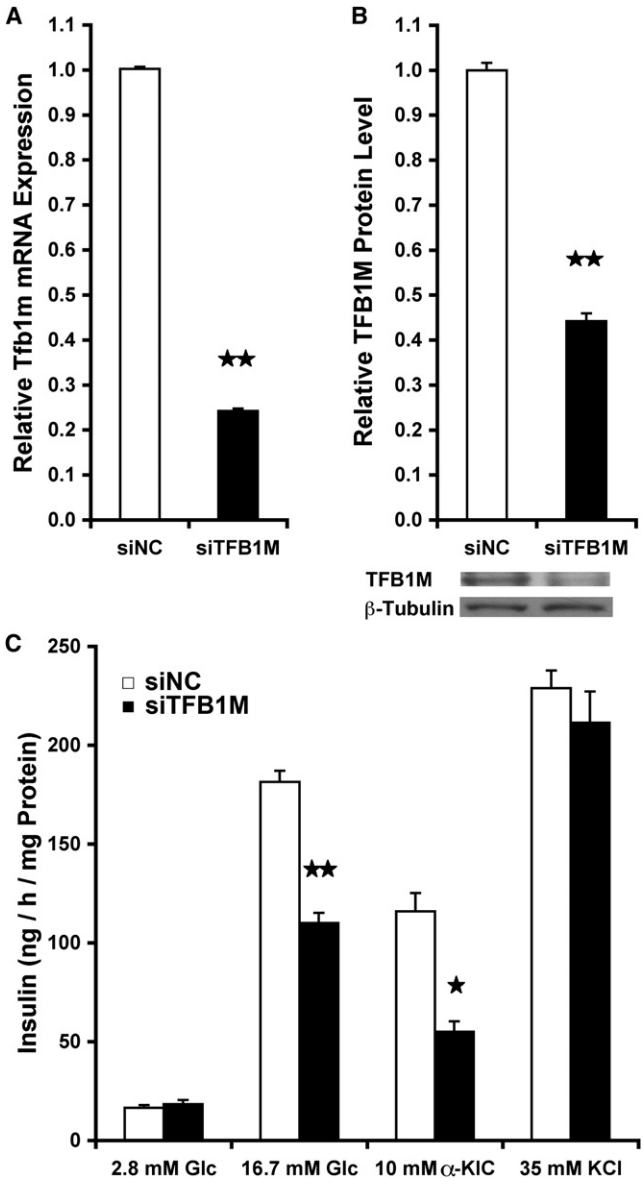

Figure 3. Reduced TFB1M levels in Clonal β Cells Result in Impaired Insulin Secretion

(A) Glucose-sensitive clonal β cells (INS 832/13) were used to examine the consequences of TFB1M deficiency. Ninety-six hours after transfection with siRNA to *Tfb1m*, cells were subjected to analysis. mRNA levels for *Tfb1m* were analyzed by quantitative polymerase chain reaction (Q-PCR) in control cells (siNC) and cells where Tfb1m had been silenced (siTFB1M) (n = 3).

(B) Densitometric analysis of western blots for TFB1M (n = 4).

(C) Insulin secretion in response to 2.8 and 16.7 mM glucose (Glc) (n = 10) and 10 mM α-KIC in the presence of 2.8 mM glucose (n = 4) and 35 mM potassium chloride (KCl) in the presence of 250 μM diazoxide (n = 3) was determined in a 1 hr static incubation of the 832/13 β cells. Bars represent mean ± SEM (* < 0.05; ** < 0.01). See also Table S1.

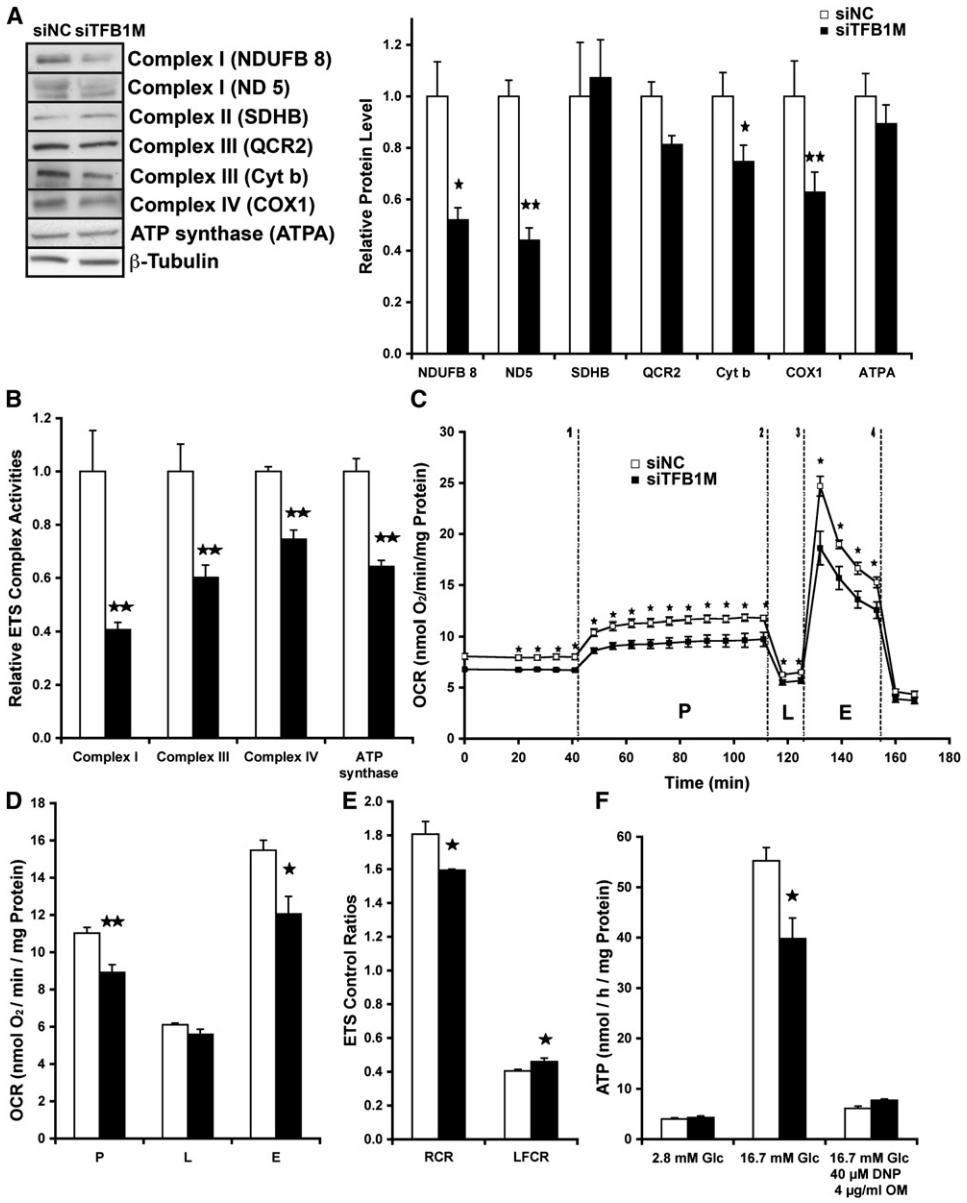

Figure 4. Reduced TFB1M Levels in Clonal β Cells Result in Impaired Mitochondrial OXPHOS and ATP Generation

(A) Clonal β cells (INS 832/13) were used to test the effect of TFB1M deficiency. Ninety-six hours after transfection with siRNA to *Tfb1m*, cells were subjected to analysis. White bars represent control (siNC) and black bars represent siTFB1M. Representative western blots and densitometric analysis of protein levels for NDUFB8 (complex I, nuclear encoded), ND5 (complex I, mitochondrially encoded), succinate dehydrogenase complex, subunit B, iron sulfur (SDHB, complex II, nuclear encoded), QCR2 (complex III core protein 2, nuclear encoded), Cyt b (complex III, mitochondrially encoded), COX1 (complex IV, mitochondrially encoded), and ATPA (ATP synthase F1 subunit α, nuclear encoded) (n = 4).

(B) Relative ETS complexes I, III, and IV and ATP synthase activities in cells where TFB1M had been silenced and normalized to the activities of each control (n = 8 for ETS complexes and 4 for ATP synthase).

(C) Analysis of oxygen consumption rates (OCR): Cells were preincubated at 2.8 mM glucose for 2 hr. Then OCRs were measured at 2.8 mM followed by measurements at 16.7 mM after glucose injection at time point 1. At time point 2, oligomycin, an inhibitor of ATP synthase was added; at time point 3, dinitrophenol, an uncoupler was added, followed by the addition of rotenone at time point 4, an inhibitor of complex 1. P, L, and E indicate the areas for further analysis (white squares represent siNC, black ones siTFB1M) (n = 4).

(D) Time-normalized area-under-the-curve analysis of OCR measurements calculating coupled OXPHOS capacity (P, excess ADP), proton-leak oxygen flux (L, ADP consumed), and uncoupled total electron transport capacity (area E, uncoupled).

(E) Calculated mitochondrial function parameters based on area-under- the-curve analysis: respiratory-control ratio (RCR = P/L) and leak-flux-control ratio (LFCR = L/E).

(F) Accumulation of cellular ATP was determined in 832/13 β cells upon a 1 hr stimulation by 2.8 mM glucose (Glc), 16.7 mM Glc, or 16.7 mM Glc in the presence of DNP and oligomycin (n = 4). Bars represent mean ± SEM. * < 0.05; ** < 0.01. See also Table S3.

encoded ND5 and nuclear-encoded NDUFB8 suggest that the assembly of complex I is disrupted because of the TFB1M deficiency. In contrast, protein levels of complex II, with all subunits encoded in the nucleus, were unaffected. Also, the citrate synthase activity was unchanged (122.8 ± 3.4 versus 118.6 ± 4.9 μmol citrate/min/mg in control cells versus TFB1M-deficient cells, respectively). This suggests that mitochondrial biogenesis was unaffected by TFB1M deficiency for 96 hr in the studied cell line.

The negative impact of TFB1M deficiency on respiratory proteins was further corroborated by activity determinations in ETS complexes I, III, and IV as well as of ATP synthase in mitochondria-enriched subcellular fractions. While activities of all these enzyme complexes were reduced when TFB1M had been silenced, complex I activity was, as predicted by the protein expression levels, most profoundly impaired by TFB1M knockdown (3.98 ± 0.26 versus 1.73 ± 0.15 μmol/min/mg, $p < 0.001$ in control cells versus TFB1M-deficient cells; Figure 4B).

Next, we examined the functional consequences of the observed OXPHOS protein and activity perturbations in TFB1M-silenced β cells. The measurement of O_2 consumption by cytochrome C oxidase (complex IV) is commonly used to assess the activity of mitochondrial ETS and OXPHOS. To this end, we measured O_2 consumption in the SeahorseXF24 Extracellular Flux Analyzer. The basal (2.8 mM glucose), stimulated (16.7 mM glucose; P) and uncoupled (40 μM 2,4-dinitrophenol [2,4-DNP]; E) O_2 consumption rates (OCR) were reduced by 18.3% ± 0.5%, 22.0% ± 0.2%, and 24.5% ± 2.7%, respectively, in cells where TFB1M had been silenced (Figure 4C). A time-normalized area-under-the-curve analysis further confirmed this conclusion. Here, we found that TFB1M deficiency caused significant decreases in the coupled OCR (area P: 11.0 ± 0.3 versus 8.9 ± 0.4 nmol O_2/min/mg, $p = 0.004$ in control cells versus TFB1M-deficient cells) and the uncoupled OCR (area E: 15.5 ± 0.5 versus 12.1 ± 0.9 nmol O_2/min/mg, $p = 0.01$ in control cells versus TFB1M-deficient cells) (Figure 4D). This analysis further revealed a decreased ADP-stimulated OCR, which was calculated as the difference between the coupled OCR, P, and the basal OCR (basal oxygen consumption without ADP addition or uncoupling), L (4.9 ± 0.4 versus 3.3 ± 0.2 nmol O_2/min/mg, $p = 0.01$ in control compared to TFB1M-deficient cells). The analysis also revealed a TFB1M-dependent decrease in the respiratory-control ratio (RCR) and an increase in the leak-flux-control ratio (LFCR), both indicating decreased coupling between electron transport and ATP synthesis (Figure 4E).

The final step in mitochondrial metabolism-linked OXPHOS is production of ATP, which is also established as the trigger of GSIS. Therefore, we analyzed whether the mitochondrial perturbations that we have described in TFB1M deficiency also affected the kinetics of mitochondrial ATP production in response to the same metabolic substrate mixture that was used for this analysis in mouse islets and the cellular ATP level at the end of GSIS (16.7 mM). Indeed, we found a 29.3% ± 0.5% decrease in the kinetics of mitochondrial ATP production (10.3 ± 0.2 versus 7.3 ± 0.3 nmol ATP/min/mg, $n = 4$, $p = 0.0002$ in control compared to TFB1M-silenced cells) and a 27.6% ± 4.8% decrease in the glucose-induced cellular ATP level ($p = 0.01$; Figure 4F) in the TFB1M-deficient cells. We confirmed that TFB1M knockdown did not affect the ATP levels in unstimulated cells and in cells treated with 2,4-DNP and oligomycin (Figure 4F). These compounds dissipate the mitochondrial membrane potential and block ATP synthase, respectively, thereby abolishing mitochondrial ATP synthesis. This indicates that the observed decrease in cellular ATP caused by TFB1M silencing is entirely because of impaired mitochondrial, and not glycolytic, ATP synthesis. Thus, in sum, the data show that impaired insulin secretion in TFB1M-deficient β cells is caused by perturbed OXPHOS.

TFB1M Gene Expression in Human Skeletal Muscle

Mitochondrial metabolism has also been implicated in control of insulin sensitivity and female A-allele carriers of rs950994 showed reduced Rd during a clamp. Therefore, we analyzed *TFB1M* mRNA expression in skeletal muscle from the twins. In agreement with the gender effect of rs950994 on insulin sensitivity during the clamp, female A-allele carriers showed reduced *TFB1M* expression in muscle (G/G: 0.26 ± 0.009, $n = 42$ versus A/A + A/G: 0.23 ± 0.008, $n = 39$, $p = 0.05$), whereas male twins did not (G/G: 0.29 ± 0.015, $n = 35$ versus A/A + A/G: 0.30 ± 0.013, $n = 49$, $p = 0.7$). Furthermore, while *TFB1M* expression was reduced by 13% ($p = 0.003$) in muscle from elderly (age 62.4 ± 0.2 yr) compared with young (age 28.0 ± 0.2 yr) female twins, no effect of age was seen in male twins ($p = 0.3$). We also found a positive association ($p = 0.01$) between insulin sensitivity in vivo and *TFB1M* expression in muscle (Table S4).

mtDNA Copy Number

Our study shows that reduced levels of TFB1M result in impaired OXPHOS. However, whether the mitochondrial number is changed in subjects carrying the risk allele of rs950994 remains unknown. We therefore tested whether rs950994 was associated with mtDNA copy number in blood cells from a subset of the Botnia cohort ($n = 467$). Although the effect size was small, mtDNA copy number was lower in A-allele carriers of rs950994 ($p = 0.009$) (Figure S3A). When stratifying the analysis for gender, rs950994 was associated with mtDNA copy number in females ($p = 0.005$), but not in males ($p = 0.34$) (Figure S3B).

DISCUSSION

Mitochondria are at center stage in cellular metabolism and hence play a critical role in the pathogenic processes leading to many diseases, particularly metabolic disorders (Goto et al., 1990; Kelley et al., 2002; Petersen et al., 2003, 2004; Reardon et al., 1992; van den Ouweland et al., 1992). Here, we present genetic, clinical, and experimental in vivo and in vitro evidence that a polymorphism in *TFB1M*, a nuclear-encoded factor involved in translational control in mitochondria (Falkenberg et al., 2002; Metodiev et al., 2009), is associated with impaired insulin secretion, elevated postprandial glucose levels, and T2D.

Mining the DGI GWAS for genes involved in OXPHOS led to identification of the association between rs950994 and T2D, and subsequently to the discovery that this polymorphism is associated with an increased future risk of T2D in the prospective MPP study. The effect of rs950994 on future risk of T2D is of a magnitude similar to the associations with T2D previously observed for approximately 20 SNPs (Lyssenko et al., 2008, 2009; Saxena et al., 2007). Together, these studies demonstrate

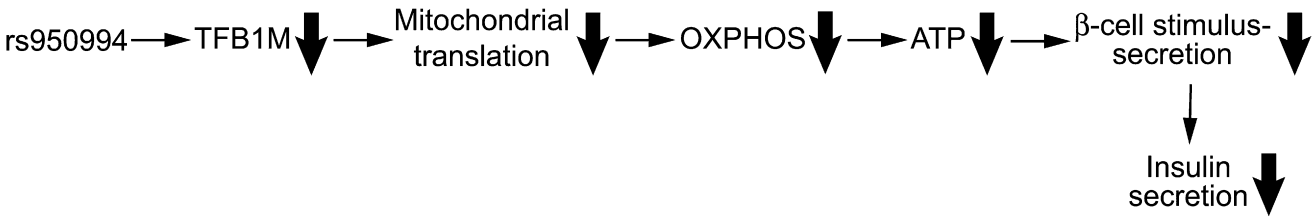

Figure 5. Model for a Possible Role of TFB1M in the Development of T2D

Our data suggest a model where the risk SNP confers lower TFB1M protein expression. Consequently, protein synthesis is impaired. As a consequence of reduced synthesis of mitochondrially encoded proteins, OXPHOS is restrained, reducing ATP production and hence stimulus-secretion coupling in the β cell is abrogated (see also Figure S3).

that T2D is a polygenic disease where multiple genetic variants play a role. rs950994 was also associated with elevated postprandial glucose levels during OGTTs in the DGI. The elevation of plasma glucose and association with T2D are probably accounted for by impaired insulin secretion, because the insulinogenic index was significantly reduced in risk-allele carriers of rs950994. However, a concomitant influence of impaired peripheral insulin action as demonstrated in female twin carriers of the risk allele cannot be excluded. The pathogenic role of TFB1M was further suggested by the fact that carriers of the risk allele showed a reduction in islet *TFB1M* expression and that expression of *TFB1M* correlated with the insulin response to glucose in isolated human islets as well as with protein levels of mitochondrially encoded OXPHOS complexes. Introducing the A of the risk allele instead of a G because of rs950994 disrupts several putative transcription factor binding sites in intron 2 of *TFB1M*, which may affect the expression of TFB1M. Accordingly, variable expression in different cell types of these putative transcription factors may explain why *TFB1M* mRNA expression was reduced in human pancreatic islets and skeletal muscle but unaltered in adipose tissue (data not shown).

To find further support for the role of *TFB1M* in the pathogenesis of T2D and to resolve pathogenic processes, we examined a mouse model for TFB1M deficiency. The *Tfb1m*$^{+/-}$ mice were chosen because they exhibited reduced, but not lacking, expression of TFB1M (Metodiev et al., 2009), which resembles the situation in human A-allele carriers of rs950994. Indeed, the *Tfb1m*$^{+/-}$ mice displayed many features similar to those found in the human cohorts with metabolic traits associated with *TFB1M* rs950994. All features of *Tfb1m*$^{+/-}$ mice assessed in this study point to impaired mitochondrial function as the cause of glucose intolerance via impaired insulin secretion. This is probably explained by insufficient regulatory actions of TFB1M on the translation of mitochondrially encoded OXPHOS protein subunits in islet β cell mitochondria.

To further elucidate the impact of TFB1M deficiency on mitochondrial function in β cells and therefore on pathogenic processes underlying the diabetogenic properties of the rs950994 polymorphism, we also performed in vitro studies in clonal β cells. These experiments were based on our observations that the risk-allele carriers exhibited reduced expression of *TFB1M* in human islets. Using siRNA to reduce TFB1M protein levels, we confirmed the association of multiple abnormalities in the OXPHOS system and therefore of impaired metabolism-coupled mitochondrial ATP generation with TFB1M deficiency. Our ETS complex activity measurements together with the western blot data propose that perturbations of complex I may play a particularly important role in TFB1M deficiency. However, although TFB1M deficiency resulted in a substantial decline in complex I protein and activity levels, the reductions in OCR, ATP production, and GSIS were less profound, albeit significant. This suggests that there is an overcapacity of the OXPHOS system, which prevents frank mitochondrial disease but not a restraining effect on β cell stimulus-secretion coupling. Alternatively, because complex II protein levels were unchanged upon TFB1M deficiency, the use of succinate as a substrate for the ETS may to some extent also compensate for the low complex I activity (Cimen et al., 2010). All together, the observed defects are likely to include not only changes in individual OXPHOS complexes but also the functional coordination of the whole system. Hence, insulin secretion in response to metabolic fuels (e.g., glucose and α-KIC) was impaired.

All our data collected from humans, mice, and clonal β cells converge into the recognition of a mitochondrial OXPHOS defect associated with the rs950994 polymorphism, which accounts for the impairment of insulin secretion. Our data suggest a model where this risk SNP confers lower TFB1M expression. Consequently, mitochondrial protein synthesis is impaired, restraining OXPHOS, and hence stimulus-secretion coupling in the β cell is abrogated (Figure 5). Indeed, we found that ATP levels, known to be the trigger of insulin secretion (Henquin, 2009), were reduced both in *Tfb1m*$^{+/-}$ mice and in clonal β cells where TFB1M had been silenced. This mirrors findings in islets from humans with T2D, which exhibit lower ATP levels and impaired hyperpolarization of the inner mitochondrial membrane (Anello et al., 2005), a process that normally occurs in response to elevated glucose. Also, recent studies in a mouse model of T2D, the MKR mouse, demonstrate the evolution of mitochondrial defects during the progression to diabetes (Lu et al., 2010).

A remarkable finding in our study is that the pathogenic effect of the genetic variant, to a large extent, seems to depend on gender. Accordingly, no effect of the rs950994 polymorphism was observed in the METSIM study, which was comprised of males only. Moreover, the association of the risk allele with insulinogenic index was lost in the male contingent both in the DGI GWAS and the Botnia PPP study when the subjects were stratified for gender. Similarly, the association of rs950994 with Rd was only found in the female twins. Although it is not uncommon that gender influences genetic associations (Joyner

et al., 2009; Miyashita et al., 2007; Rimol et al., 2010), future studies are needed to further dissect the molecular mechanisms underlying the gender-specific effects of rs950994. In addition, age also seems to affect the association of *TFB1M* with various parameters analyzed in the study. In agreement with previous studies, this emphasizes the important role of aging in the development of mitochondrial dysfunction and T2D (Larsson, 2010; Ling et al., 2004, 2007a; Rönn et al., 2008, 2009).

Clearly, mitochondrial metabolism also plays an important role for glucose homeostasis in target tissues of insulin (Kelley et al., 2002; Ritov et al., 2005). We have previously proposed that genetic and epigenetic regulation of mitochondrial function increase susceptibility for insulin resistance with increasing age (Ling et al., 2004, 2007a, 2007b; Rönn et al., 2008, 2009). Here, a dysfunctional phenotype in insulin target tissues was apparent but not pronounced. Although an association between rs950994 and Rd was found during clamp studies in female twins, associations of the risk allele with insulin sensitivity measures during OGTTs were lacking. This may be because of the fact that the clamp technique is more sensitive and specific than an OGTT for analyzing insulin sensitivity.

In summary, *TFB1M* can be added to the growing list of genes implicated in the risk of developing T2D. Its pathogenic role is not difficult to understand, given the critical role of mitochondrial function in control of insulin secretion from the pancreatic β cell and in the target tissues of the hormone. In fact, the dysregulation of mtDNA has previously been implicated in the pathogenesis of T2D, because genetic inactivation of *Tfam* in mouse β cells leads to impaired insulin secretion and diabetes (Silva et al., 2000). Moreover, deficiency of Pdx1, a crucial transcription factor in pancreatic β cells, causes mitochondrial dysfunction and defective insulin secretion in mice and clonal β cells because of suppression of *Tfam* expression (Gauthier et al., 2009).

EXPERIMENTAL PROCEDURES

Study Populations

The DGI GWAS comprises geographically matched controls and discordant sib-ships selected from Finland and Sweden (Saxena et al., 2007). For replication, the Botnia PPP, the HBCS, and the METSIM studies were used. For prospective analysis the MPP was employed. Human pancreatic islets were from deceased donors (24 female, 26 male), body mass index (BMI) 17.6–36.6 kg/m^2, aged 26–75 years. The twins were identified through The Danish Twin Register. All participants gave informed consent for the studies and the local ethics committees approved the protocols. The studies are described in more detail in Supplemental Experimental Procedures.

Animals

Wild-type (*Tfb1m*$^{+/+}$) and heterozygous (*Tfb1m*$^{+/-}$) male mice, 2–3 months of age (Metodiev et al., 2009), were housed at 22°C with a daylight period of 12 hr and fed diet and water ad libitum. All experiments were approved by the local Animal Ethics Committee.

Gene Expression

Total RNA was extracted from human muscle biopsies, islets, and clonal β cells. *TFB1M* mRNA levels were quantified by TaqMan Real-Time PCR as described in Supplemental Experimental Procedures.

RNA Interference

RNA interference in clonal INS-1 832/13 β cells is described in detail in Supplemental Experimental Procedures.

Intraperitoneal Glucose Tolerance Test

Intraperitoneal glucose tolerance tests were performed in fasted mice as described (Fex et al., 2007).

Insulin Secretion and Content

Cells transfected with siRNA for *Tfb1m* or islets from *Tfb1m*$^{+/+}$ and *Tfb1m*$^{+/-}$ mice were kept at 37°C for 1 hr in 2.8 or 16.7 mM glucose or in 2.8 mM glucose and 10 mM α-KIC. The buffer was removed and secreted insulin was determined. For insulin content, scraped cells were centrifuged and the supernatant diluted 10 times in acidified ethanol prior to measurement; for details, see Supplemental Experimental Procedures.

ATP and Citrate Synthase Activity Measurements

Cellular ATP content was measured with a luminescence assay. The rate of mitochondrial ATP production in digitonin-permeabilized mouse islets and clonal INS-1 832/13 cells was measured as previously described (Malmgren et al., 2009). A Citrate Synthase Assay Kit (Sigma, St. Louis, MO) was used according to instructions by the manufacturer. Enzymatic ATP hydrolysis was assessed by a high-sensitivity ATPase assay kit (Innova Biosciences, Babraham, Cambridge, UK) according to the instructions provided by the manufacturer.

Oxygen Consumption Rate

To determine cellular OCR in INS-1 832/13 β cells, we used the Seahorse XF24 Extracellular Flux Analyzer (Seahorse Bioscience, Billerica, MA), which measures OCR, as described (Malmgren et al., 2009). For details, see Supplemental Experimental Procedures.

ETS Complex Assays

Fifty micrograms of protein from mitochondria-enriched fraction pellets were used to assay the activities of the mitochondrial ETS complexes I, III, and IV. For details, see Supplemental Experimental Procedures.

Quantification of Mitochondrial DNA Content

Mitochondrial DNA content was assessed using a modification of the quantitative real-time PCR-based method described previously (Reiling et al., 2010). For details, see Supplemental Experimental Procedures.

Statistical Analysis

The statistical analyses are described in detail in Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four tables, three figures, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.cmet.2010.12.007.

ACKNOWLEDGMENTS

The DGI GWAS was supported by Novartis. Studies in Malmö were supported by the Swedish Research Council, Region Skåne, Malmö University Hospital, an EU grant (CEED3), the European Foundation for the Study of Diabetes (EFSD) and Linné grant (B31 5631/2006). Support was received from these foundations: Knut and Alice Wallenberg, Lundberg, Novo Nordisk, Söderberg, Hedlund, Bergvall, and Påhlsson. Human pancreatic islets were obtained from the Nordic Islet Transplantation Program courtesy of Professor Olle Korsgren, Uppsala University, Sweden. The METSIM Study and HBCS were supported by grants from the Academy of Finland and the Finnish Diabetes Research Foundation. The mtDNA assay was a generous gift from Dr. Leen M. 't Hart, Leiden University Medical Centre, Netherlands.

Received: August 20, 2009
Revised: June 12, 2010
Accepted: November 10, 2010
Published: January 4, 2011

REFERENCES

Anello, M., Lupi, R., Spampinato, D., Piro, S., Masini, M., Boggi, U., Del Prato, S., Rabuazzo, A.M., Purrello, F., and Marchetti, P. (2005). Functional and morphological alterations of mitochondria in pancreatic beta cells from type 2 diabetic patients. Diabetologia *48*, 282–289.

Cardol, P., Matagne, R.F., and Remacle, C. (2002). Impact of mutations affecting ND mitochondria-encoded subunits on the activity and assembly of complex I in Chlamydomonas. Implication for the structural organization of the enzyme. J. Mol. Biol. *319*, 1211–1221.

Chomyn, A. (2001). Mitochondrial genetic control of assembly and function of complex I in mammalian cells. J. Bioenerg. Biomembr. *33*, 251–257.

Cimen, H., Han, M.J., Yang, Y., Tong, Q., Koc, H., and Koc, E.C. (2010). Regulation of succinate dehydrogenase activity by SIRT3 in mammalian mitochondria. Biochemistry *49*, 304–311.

Falkenberg, M., Gaspari, M., Rantanen, A., Trifunovic, A., Larsson, N.G., and Gustafsson, C.M. (2002). Mitochondrial transcription factors B1 and B2 activate transcription of human mtDNA. Nat. Genet. *31*, 289–294.

Fex, M., Nitert, M.D., Wierup, N., Sundler, F., Ling, C., and Mulder, H. (2007). Enhanced mitochondrial metabolism may account for the adaptation to insulin resistance in islets from C57BL/6J mice fed a high-fat diet. Diabetologia *50*, 74–83.

Galimberti, D., Scalabrini, D., Fenoglio, C., De Riz, M., Comi, C., Venturelli, E., Cortini, F., Piola, M., Leone, M., Dianzani, U., et al. (2008). Gender-specific influence of the chromosome 16 chemokine gene cluster on the susceptibility to Multiple Sclerosis. J. Neurol. Sci. *267*, 86–90.

Gauthier, B.R., Wiederkehr, A., Baquié, M., Dai, C., Powers, A.C., Kerr-Conte, J., Pattou, F., MacDonald, R.J., Ferrer, J., and Wollheim, C.B. (2009). PDX1 deficiency causes mitochondrial dysfunction and defective insulin secretion through TFAM suppression. Cell Metab. *10*, 110–118.

Goto, Y., Nonaka, I., and Horai, S. (1990). A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. Nature *348*, 651–653.

Henquin, J.C. (2009). Regulation of insulin secretion: a matter of phase control and amplitude modulation. Diabetologia *52*, 739–751.

Joyner, A.H., J, C.R., Bloss, C.S., Bakken, T.E., Rimol, L.M., Melle, I., Agartz, I., Djurovic, S., Topol, E.J., Schork, N.J., et al. (2009). A common MECP2 haplotype associates with reduced cortical surface area in humans in two independent populations. Proc. Natl. Acad. Sci. USA *106*, 15483–15488.

Kelley, D.E., He, J., Menshikova, E.V., and Ritov, V.B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes *51*, 2944–2950.

Kolz, M., Johnson, T., Sanna, S., Teumer, A., Vitart, V., Perola, M., Mangino, M., Albrecht, E., Wallace, C., Farrall, M., et al; EUROSPAN Consortium; ENGAGE Consortium; PROCARDIS Consortium; KORA Study; WTCCC. (2009). Meta-analysis of 28,141 individuals identifies common variants within five new loci that influence uric acid concentrations. PLoS Genet. *5*, e1000504.

Larsson, N.G. (2010). Somatic mitochondrial DNA mutations in mammalian aging. Annu. Rev. Biochem. *79*, 683–706.

Ling, C., Poulsen, P., Carlsson, E., Ridderstråle, M., Almgren, P., Wojtaszewski, J., Beck-Nielsen, H., Groop, L., and Vaag, A. (2004). Multiple environmental and genetic factors influence skeletal muscle PGC-1alpha and PGC-1beta gene expression in twins. J. Clin. Invest. *114*, 1518–1526.

Ling, C., Poulsen, P., Simonsson, S., Rönn, T., Holmkvist, J., Almgren, P., Hagert, P., Nilsson, E., Mabey, A.G., Nilsson, P., et al. (2007a). Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. J. Clin. Invest. *117*, 3427–3435.

Ling, C., Wegner, L., Andersen, G., Almgren, P., Hansen, T., Pedersen, O., Groop, L., Vaag, A., and Poulsen, P. (2007b). Impact of the peroxisome proliferator activated receptor-gamma coactivator-1beta (PGC-1beta) Ala203Pro polymorphism on in vivo metabolism, PGC-1beta expression and fibre type composition in human skeletal muscle. Diabetologia *50*, 1615–1620.

Ling, C., Del Guerra, S., Lupi, R., Rönn, T., Granhall, C., Luthman, H., Masiello, P., Marchetti, P., Groop, L., and Del Prato, S. (2008). Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. Diabetologia *51*, 615–622.

Litonin, D., Sologub, M., Shi, Y., Savkina, M., Anikin, M., Falkenberg, M., Gustafsson, C., and Temiakov, D. (2010). Human mitochondrial transcription revisited: only TFAM and TFB2M are required for transcription of the mitochondrial genes in vitro. J. Biol. Chem. *285*, 18129–18133.

Lu, H., Koshkin, V., Allister, E.M., Gyulkhandanyan, A.V., and Wheeler, M.B. (2010). Molecular and metabolic evidence for mitochondrial defects associated with beta-cell dysfunction in a mouse model of type 2 diabetes. Diabetes *59*, 448–459.

Lyssenko, V., Jonsson, A., Almgren, P., Pulizzi, N., Isomaa, B., Tuomi, T., Berglund, G., Altshuler, D., Nilsson, P., and Groop, L. (2008). Clinical risk factors, DNA variants, and the development of type 2 diabetes. N. Engl. J. Med. *359*, 2220–2232.

Lyssenko, V., Nagorny, C.L., Erdos, M.R., Wierup, N., Jonsson, A., Spégel, P., Bugliani, M., Saxena, R., Fex, M., Pulizzi, N., et al. (2009). Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. Nat. Genet. *41*, 82–88.

Malmgren, S., Nicholls, D.G., Taneera, J., Bacos, K., Koeck, T., Tamaddon, A., Wibom, R., Groop, L., Ling, C., Mulder, H., and Sharoyko, V.V. (2009). Tight coupling between glucose and mitochondrial metabolism in clonal beta-cells is required for robust insulin secretion. J. Biol. Chem. *284*, 32395–32404.

Metodiev, M.D., Lesko, N., Park, C.B., Cámara, Y., Shi, Y., Wibom, R., Hultenby, K., Gustafsson, C.M., and Larsson, N.G. (2009). Methylation of 12S rRNA is necessary for in vivo stability of the small subunit of the mammalian mitochondrial ribosome. Cell Metab. *9*, 386–397.

Miyashita, A., Arai, H., Asada, T., Imagawa, M., Matsubara, E., Shoji, M., Higuchi, S., Urakami, K., Kakita, A., Takahashi, H., et al; Japanese Genetic Study Consortium for Alzeheimer's Disease. (2007). Genetic association of CTNNA3 with late-onset Alzheimer's disease in females. Hum. Mol. Genet. *16*, 2854–2869.

Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., et al. (2003). PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. *34*, 267–273.

Perales-Clemente, E., Fernández-Vizarra, E., Acín-Pérez, R., Movilla, N., Bayona-Bafaluy, M.P., Moreno-Loshuertos, R., Pérez-Martos, A., Fernández-Silva, P., and Enríquez, J.A. (2010). Five entry points of the mitochondrially encoded subunits in mammalian complex I assembly. Mol. Cell. Biol. *30*, 3038–3047.

Petersen, K.F., Befroy, D., Dufour, S., Dziura, J., Ariyan, C., Rothman, D.L., DiPietro, L., Cline, G.W., and Shulman, G.I. (2003). Mitochondrial dysfunction in the elderly: possible role in insulin resistance. Science *300*, 1140–1142.

Petersen, K.F., Dufour, S., Befroy, D., Garcia, R., and Shulman, G.I. (2004). Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N. Engl. J. Med. *350*, 664–671.

Reardon, W., Ross, R.J., Sweeney, M.G., Luxon, L.M., Pembrey, M.E., Harding, A.E., and Trembath, R.C. (1992). Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet *340*, 1376–1379.

Reiling, E., Ling, C., Uitterlinden, A.G., Van't Riet, E., Welschen, L.M., Ladenvall, C., Almgren, P., Lyssenko, V., Nijpels, G., van Hove, E.C., et al. (2010). The association of mitochondrial content with prevalent and incident type 2 diabetes. J. Clin. Endocrinol. Metab. *95*, 1909–1915.

Rimol, L.M., Agartz, I., Djurovic, S., Brown, A.A., Roddey, J.C., Kähler, A.K., Mattingsdal, M., Athanasiu, L., Joyner, A.H., Schork, N.J., et al; Alzheimer's Disease Neuroimaging Initiative. (2010). Sex-dependent association of common variants of microcephaly genes with brain structure. Proc. Natl. Acad. Sci. USA *107*, 384–388.

Ritov, V.B., Menshikova, E.V., He, J., Ferrell, R.E., Goodpaster, B.H., and Kelley, D.E. (2005). Deficiency of subsarcolemmal mitochondria in obesity and type 2 diabetes. Diabetes *54*, 8–14.

Rönn, T., Poulsen, P., Hansson, O., Holmkvist, J., Almgren, P., Nilsson, P., Tuomi, T., Isomaa, B., Groop, L., Vaag, A., and Ling, C. (2008). Age influences DNA methylation and gene expression of COX7A1 in human skeletal muscle. Diabetologia *51*, 1159–1168.

Rönn, T., Poulsen, P., Tuomi, T., Isomaa, B., Groop, L., Vaag, A., and Ling, C. (2009). Genetic variation in ATP5O is associated with skeletal muscle ATP50 mRNA expression and glucose uptake in young twins. PLoS ONE *4*, e4793.

Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., de Bakker, P.I., Chen, H., Roix, J.J., Kathiresan, S., Hirschhorn, J.N., Daly, M.J., et al; Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research. (2007). Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science *316*, 1331–1336.

Scarpulla, R.C. (2008). Transcriptional paradigms in mammalian mitochondrial biogenesis and function. Physiol. Rev. *88*, 611–638.

Silva, J.P., Köhler, M., Graff, C., Oldfors, A., Magnuson, M.A., Berggren, P.O., and Larsson, N.G. (2000). Impaired insulin secretion and beta-cell loss in tissue-specific knockout mice with mitochondrial diabetes. Nat. Genet. *26*, 336–340.

Sologub, M., Litonin, D., Anikin, M., Mustaev, A., and Temiakov, D. (2009). TFB2 is a transient component of the catalytic site of the human mitochondrial RNA polymerase. Cell *139*, 934–944.

van den Ouweland, J.M., Lemkes, H.H., Ruitenbeek, W., Sandkuijl, L.A., de Vijlder, M.F., Struyvenberg, P.A., van de Kamp, J.J., and Maassen, J.A. (1992). Mutation in mitochondrial tRNA(Leu)(UUR) gene in a large pedigree with maternally transmitted type II diabetes mellitus and deafness. Nat. Genet. *1*, 368–371.