

## Research Article

# PTEN-Induced Kinase 1 Regulates Mitochondrial Integrity and Insulin Secretion in Mouse Pancreatic $\beta$ -Cells

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## Abstract

PTEN-induced kinase 1 (PINK1) is linked to recessive familial Parkinson's disease (PD). PINK1 and Parkin (a PD-linked ubiquitin ligase) regulate mitochondrial quality control by promoting the degradation of depolarized mitochondria via mitophagy. In addition, PINK1 stimulates macro-autophagy (autophagy) by binding to Beclin-1. Mice and cells lacking PINK1 develop multiple mitochondrial defects that may be mediated by oxidative stress and abnormal mitophagy. Mitochondrial dysfunction is also implicated in the pathogenesis of type-2 diabetes (T2D), and several etiologic studies have shown a link between T2D and PD. Here we investigated the effects of PINK1 ablation on mitochondrial morphology and autophagy in mouse pancreatic  $\beta$ -cells. In addition, we studied how PINK1 deficiency and over-expression affects glucose-stimulated insulin secretion (GSIS) from pancreatic islets and MIN6 pancreatic  $\beta$ -cells. We show that loss of PINK1 in mice leads to mitochondrial enlargement/swelling in  $\beta$ -cells accompanied by an accumulation of autophagosomes and ubiquitinated protein inclusions. PINK1 deficiency impairs GSIS but not insulin production in pancreatic islets, while over-expression of PINK1 in MIN6  $\beta$ -cells increases GSIS. To our knowledge, this is the first demonstration that a familial PD-linked protein regulates mitochondrial integrity and GSIS in pancreatic  $\beta$ -cells. Our results provide a possible mechanistic explanation for the link between T2D and PD and implicate PINK1 as a risk factor in the development of diabetes. Through its ability to regulate mitochondrial quality control and GSIS in pancreatic  $\beta$ -cells PINK1 may be explored as a novel target for the treatment of T2D.

## INTRODUCTION

Mutations in PINK1 cause early-onset recessive Parkinson's disease (PD) [1-3]. PINK1 is a serine/threonine kinase with an N-terminal mitochondrial signal sequence [1]. Full-length PINK1 localizes to mitochondria, while an N-terminally cleaved form is present in the cytoplasm [4-6]. Consistent with this, PINK1 regulates both mitochondrial and cytosolic pathways to protect neurons against cell death [7-9]. PINK1 and Parkin cooperate to promote the selective degradation of depolarized mitochondria through mitophagy [10-12], a mechanism central to mitochondrial quality control. PINK1 also stimulates general autophagy by interacting with Beclin-1, which is independent of the PINK1 kinase activity [13]. In addition, PINK1 phosphorylates

the mitochondrial chaperone TRAP1, thereby protecting neurons against oxidative stress-induced apoptosis [8]. PINK1-deficient mice show age-dependent dopamine loss, impaired dopamine release and various abnormalities of mitochondrial function [14-16]. PINK1-deficient primary cells show respiratory defects and accumulation of enlarged and swollen mitochondria, possibly due to increased fusion of non-degraded, depolarized mitochondria with the normal mitochondrial network [17,18]. Finally, PINK1 protects cells against stress-induced apoptosis through enhancing signal transduction by the cell survival kinase Akt [9-19,20]. Although mutations in PINK1 have only been linked to PD, mitochondrial metabolism and autophagy are important to maintain homeostasis of cells and organs in

general. In particular, pancreatic  $\beta$ -cell function relies heavily on mitochondrial bioenergetics, and disturbances in mitochondrial function, autophagy and oxidative stress are implicated in the pathogenesis of diabetes [21-24]. Interestingly, several studies suggest that T2D is a risk factor for the development of PD [25,26], but the mechanistic links are poorly understood. Here, we investigated the effects of PINK1 ablation and over-expression on glucose-stimulated insulin secretion (GSIS) from pancreatic islets and cultured mouse MIN6 pancreatic  $\beta$ -cells. We show that loss of PINK1 impairs GSIS from pancreatic islets. In contrast, over-expression of PINK1 in MIN6 cells increases GSIS. In addition, we demonstrate that PINK1-deficient pancreatic  $\beta$ -cells (in sections of the pancreas) harbor significantly enlarged and swollen mitochondria and display accumulation of autophagosomes and ubiquitin-positive protein inclusions. Our results show that loss of PINK1 results in impaired GSIS from pancreatic  $\beta$ -cells and that this defect is associated with abnormal mitochondrial integrity and possibly autophagy. Mutations in PINK1 may increase the risk for diabetes and PINK1 may be explored as a novel target for the treatment of T2D.

## MATERIALS AND METHODS

### Animals

PINK1-deficient mice used in this study have been described elsewhere [14]. All work has been conducted according to national and international guidelines and has been approved by the Animal Care and Use Committee of the University of Kentucky. WT and PINK1-deficient littermates were analyzed at 4-5 months of age unless stated otherwise. Mice were housed under 12-hour light-dark cycles and had free access to food (Harlan #2918) and water.

### Analysis of PINK1 expression

Total RNA from 150-200 wildtype C57BL/6 mouse islets and from MIN6 cells was isolated and 0.5  $\mu$ g RNA was converted to cDNA (High Capacity cDNA Reverse Transcription kit, Applied Biosystems). Two  $\mu$ l cDNA reaction was used for PCR with intron-spanning primers complementary to exon-1 and exon-3 of the *Pink1* gene (35 cycles of 94°C/30s; 58°C/45sec and 72°C/1 min). As a negative control the cDNA synthesis step (reverse transcriptase) was omitted. PINK1 protein was detected in 5  $\mu$ m-thick paraffin sections of pancreata that were fixed in 4% paraformaldehyde at 4°C overnight. For immunohistochemistry, sections were de-waxed, incubated in citrate buffer (10mM, pH 6.0) for antigen retrieval in a preheated steamer for 20 min, blocked for 1 hour in blocking buffer (10% normal donkey serum, 0.2% Triton X-100, and 0.1% sodium azide in TBS) and stained in blocking buffer at 4°C overnight with rabbit anti-PINK1 antibody (1:100, NBP1-49678, Novus) and goat anti-insulin antibody (1:500, sc-7839, Santa Cruz). After washing sections were incubated for 1 hour in blocking buffer without Triton X-100 with donkey anti-rabbit IgG-Alexa 488 and donkey anti-goat IgG-Alexa 594 (both at 1:300 dilution, from Invitrogen). Sections were mounted in ProLong Gold medium (Invitrogen) and immediately imaged using a Leica TCS SP5 AOBs inverted confocal microscope at an emission wavelength of 488nm and 594nm.

### Isolation of islets and GSIS

Mouse islets were isolated via intraductal collagenase (Roche) digestion and Histopaque gradient (Histopaque-1077, Sigma) centrifugation by a method adopted by the Islet Procurement and Analysis Core, Diabetes Research and Training Center, Vanderbilt University Medical Center (Nashville, TN). Briefly, pancreata from mice were infused with 5ml of 0.5mg/ml collagenase in Hank's balanced salt solution (HBSS), surgically removed and collagenase digestion was continued for 10-15 min at 37°C and then stopped by addition of chilled HBSS containing 10% FBS. Islets were purified using a Histopaque gradient centrifugation step, individually picked by hand and cultured overnight in RPMI medium containing 5mM glucose, 10% (v/v) FBS, 100 U/ml penicillin and 100 $\mu$ g/ml streptomycin. For GSIS islets were transferred to culture inserts (Greiner Bio-One) in 12-well plates (25 islets per well) and equilibrated for 1 hour in buffer 1 (DMEM, 38mM sodium bicarbonate, 4mM L-glutamine, 1mM sodium pyruvate, 4.65mM HEPES and 1 g/l BSA) supplemented with 5mM (low) glucose. Basal insulin secretion was measured after 40 min incubation in buffer 1 supplemented with 5mM glucose. Thereafter the medium was replaced with buffer 1 containing 20mM (high) glucose and the insulin content in the medium measured again after 40 min. Total intracellular insulin was determined after disrupting the cells with acid-ethanol (75% ethanol, 0.2M HCl). Percent of secreted insulin was calculated as the ratio of secreted to intracellular insulin. All insulin measurements were done with the ultra-sensitive mouse insulin ELISA kit (Crystal Chem Inc.) according to the manufacturer's instructions.

### GSIS with transfected MIN6 cells

MIN6 cells were cultured in DMEM, 15% heat-inactivated FBS, 2mM L-glutamine, 45mM  $\beta$ -mercaptoethanol, 100 U/ml penicillin and 100  $\mu$ g/ml streptomycin. MIN6 cells (passage number <30, 75-80% confluent) were transfected with 1 $\mu$ g pCMVTNT PINK1 C-myc plasmid (Addgene plasmid 13314, deposited by Mark Cookson) or pEGFP-N1 (Clontech) using Lipofectamine 2000 (Invitrogen). Medium was changed 24 hours after transfection. 48 hours after transfection cells were washed once with Krebs's Ringer buffer containing 0.2% BSA (KRB-BSA) and 5mM glucose and then equilibrated in the same buffer for 1 hour. The medium was replaced with fresh KRB-BSA containing either 5mM (low) glucose or 20mM (high) glucose for 40 min. Insulin concentrations in medium were determined as above and normalized to total cell protein (measured with the BCA kit, Pierce).

### Analysis of mitochondrial morphology (electron microscopy)

Mice were euthanized by CO<sub>2</sub> inhalation. Pancreata were dissected immediately, rinsed with 0.1 M cacodylate buffer (pH 7.4) and immersed in freshly made 4% paraformaldehyde/3.5% glutaraldehyde/0.1M cacodylate buffer (pH 7.4) (Electron Microscopy Sciences). Pancreata were cut into small pieces (~1 mm<sup>3</sup>) and kept in fixation solution for 2 hours at 4°C. After four washes in 0.1M cacodylate buffer containing 5% sucrose (15 min each), tissue pieces were post-fixed in 1% osmium tetroxide for 90 min at 4°C, dehydrated in graded ethanol (from 50%-100%,

10 min at 4°C). Further dehydration was achieved by two 15-min incubations in absolute ethanol and two 15-min incubations in propylene oxide (PO) at RT. Tissues were infiltrated with 50% resin (tEpon)/50% propylene oxide with accelerator overnight at RT under a 60-watt lamp. The PO/resin mixture was poured off and tissues placed twice for 1 hour in fresh 100% resin. Tissues were embedded in BEEM capsules (Ted Pella Inc., CA) and allowed to polymerize at 60°C for 48 hrs. The beam capsules were snapped off and ultrathin sections of pancreata were prepared in the University of Kentucky Imaging Facility and imaged using a Philips Tecnai Biotwin-12 transmission electron microscope at the magnifications indicated in the figure legend. Mitochondrial size was quantified using NIH image J software (<http://imagej.nih.gov/ij>).

### Pancreas immunohistochemistry for LC3 and Ubiquitin

Procedures were the same as described above for detection of PINK1 expression. Antibodies used were rabbit anti-LC3 (1:50, Abcam ab58610) and rabbit anti-ubiquitin (1:100, Santa Cruz sc-9133). The anti-LC3 antibody recognizes both LC3I and LC3II isoforms, but LC3II is specific for autophagosomes and in IHC thus appears as punctae in cells.

### Data analysis

Data were analyzed by ANOVA and two-tailed *t*-test. Values of  $p < 0.05$  were considered statistically significant.

## RESULTS

### PINK1 deficiency impairs GSIS in pancreatic mouse islets

Mitochondria play an important function in the regulation of both neurotransmission [27] and insulin secretion [21–28]. Based on our previous studies showing mitochondrial defects *in vivo* and in several primary cell types of PINK1-deficient mice [14,17] we hypothesized that loss of PINK1 may also affect mitochondria in pancreatic  $\beta$ -cells, leading to impairments in GSIS. Using reverse-transcription PCR we first demonstrated that PINK1 mRNA is expressed in pancreatic islets and MIN6 cells, a mouse pancreatic  $\beta$ -cell line (Figure 1A). In addition, we showed that PINK protein is detectable in pancreatic  $\beta$ -cells by co-localization with insulin in sections of paraffin-embedded pancreas (Figure 1B). To test the hypothesis that PINK1 is important for  $\beta$ -cell function, we isolated primary islets from WT and PINK1<sup>-/-</sup> mice and performed GSIS assays. There was no significant difference in basal (5 mM glucose) insulin secretion between islets from WT and PINK1<sup>-/-</sup> mice and high (20 mM) glucose led to a significant increase in insulin release from islets of both genotypes (Figure 2). However, GSIS was significantly lower in islets isolated from PINK1<sup>-/-</sup> mice compared to WT islets (Figure 2, 60% reduction). Thus, PINK1 is required for maximum GSIS in mouse pancreatic islets.

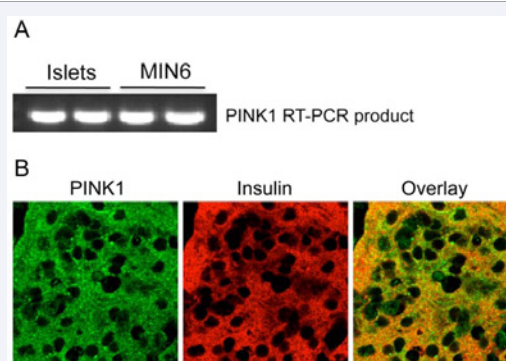
### PINK1 activates GSIS in MIN6 cells

To further investigate the role of PINK1 in GSIS, we utilized a gain-of-function approach where we measured GSIS in MIN6 cells transfected with either a plasmid carrying the PINK1 cDNA (MIN6-PINK1) or an EGFP control vector (MIN6-EGFP). Transfection efficiency was estimated at 40% (Figure 3A), and MIN6-PINK1

cells expressed ~4-fold higher levels of PINK1 mRNA compared to MIN6-EGFP cells as determined by quantitative real-time PCR (data not shown). Basal insulin secretion in MIN6-PINK1 and MIN6-EGFP cells was the same (Figure 3B, 5 mM glucose). Interestingly, over-expression of PINK1 increased GSIS to a level significantly higher than in MIN6-EGFP cells (Figure 3B, 20 mM glucose). Combined with the data shown above, these results suggest that PINK1 expression levels positively correlate with GSIS from pancreatic  $\beta$ -cells.

### Loss of PINK1 leads to mitochondrial swelling and cristae defects in pancreatic $\beta$ -cells

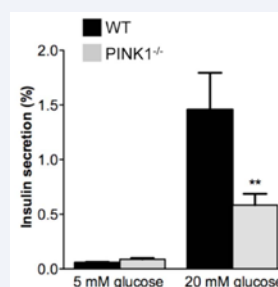
To study if PINK1 deficiency affects mitochondrial integrity in pancreatic  $\beta$ -cells, we analyzed mitochondrial morphology



**Figure 1** PINK1 is expressed in pancreatic islet cells and MIN6 cells.

(A) Expression of PINK1 mRNA in mouse pancreatic islets and the MIN6 pancreatic  $\beta$ -cell line. Total RNA was isolated and expression of *Pink1* mRNA was analyzed as described in Materials and Methods.

(B) Expression of PINK1 protein in  $\beta$ -cells of the mouse pancreas. Paraffin sections of normal mouse pancreas were stained with antibodies against PINK1 (green) and insulin (red) and imaged by confocal microscopy as described in Materials and Methods. Scale bar, 20  $\mu$ m.



**Figure 2** Loss of PINK1 reduces glucose-stimulated insulin secretion in pancreatic  $\beta$ -cells:

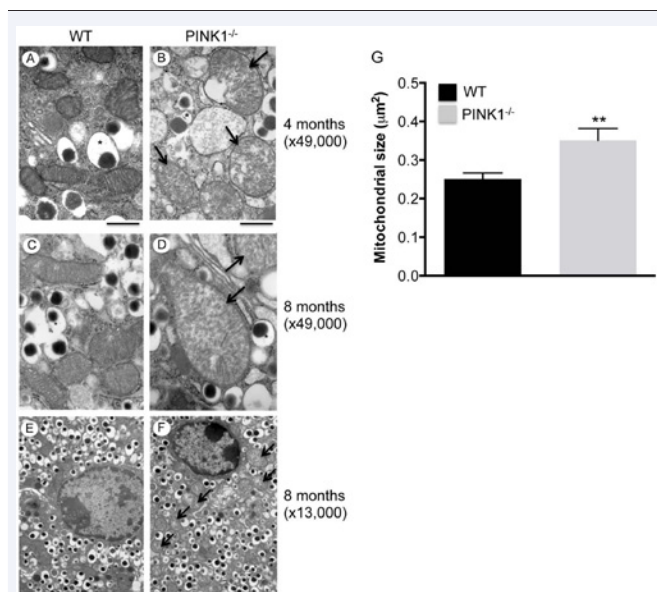
Insulin secretion from isolated pancreatic islets of 4–5 month-old WT and PINK1<sup>-/-</sup> mice (25 islets per animal). Islets were cultured overnight, incubated first for 40 min in buffer containing 5mM (low) glucose and then transferred to buffer with 20mM (high) glucose for 40 min. Insulin content in low-glucose and high-glucose medium was measured at the end of the 40 min incubation times. Secretion of insulin expressed as percentage of total cellular insulin (determined in acid ethanol-disrupted islets as described in Methods). Mean  $\pm$  SEM, \*\* $p < 0.01$ . Experiments were repeated two different times with similar results.



in pancreatic islets of WT and PINK1<sup>-/-</sup> mice using electron microscopy. Consistent with results in other cells [17] we found that the average size of mitochondria in  $\beta$ -cells from PINK1<sup>-/-</sup> mice is increased (Figure 4), with occasional accumulation of severely swollen mitochondria (arrows in Figure 4B, D and F). In addition, in many PINK1<sup>-/-</sup> mitochondria the cristae (folds of the inner membrane carrying the respiratory complexes) show reduced density and signs of fragmentation (arrows in Figure 4B and 4D). In contrast, the number of insulin granules was similar in WT and PINK1<sup>-/-</sup>  $\beta$ -cells (Figure 4E-F), in agreement with the normal intracellular insulin content and basal insulin secretion of PINK1<sup>-/-</sup> islets. Mitochondrial enlargement in PINK1<sup>-/-</sup>  $\beta$ -cells is present at 4 months (Figure 4B and 4G) and persists at 8 months (Figure 4D and 4F). These data show that loss of PINK1 leads to mitochondrial dysfunction in pancreatic  $\beta$ -cells.

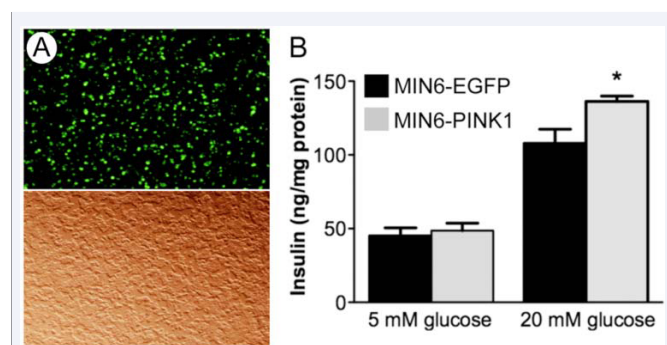
### PINK1-deficient pancreatic $\beta$ -cells display accumulation of autophagosomes and ubiquitin-positive inclusions

In addition to promoting mitophagy of depolarized mitochondria PINK1 interacts with Beclin-1 to stimulate macroautophagy (autophagy) [13]. In pancreatic  $\beta$ -cells autophagy is important for the degradation of ubiquitinated protein aggregates that form as a result of diabetes-induced oxidative stress [24]. To study if the loss of PINK1 alters  $\beta$ -cell autophagy we stained sections of the pancreas with an antibody against LC3, which when incorporated into autophagosomes (LC3II isoform) appears as punctae in cells. Compared to WT mice, pancreatic  $\beta$ -cells from PINK1-deficient mice contained significantly more punctae/autophagosomes (Figure 5A). Moreover, PINK1<sup>-/-</sup>  $\beta$ -cells accumulate ubiquitin-positive inclusions (Figure 5B). While increased numbers of autophagosomes seem inconsistent with the stimulatory role of PINK1 in autophagy induction [13], it should be noted that compensatory up-regulation of autophagy has been observed in PINK1-deficient neurons [29]. Thus, the higher abundance of ubiquitin-positive inclusions may be due to



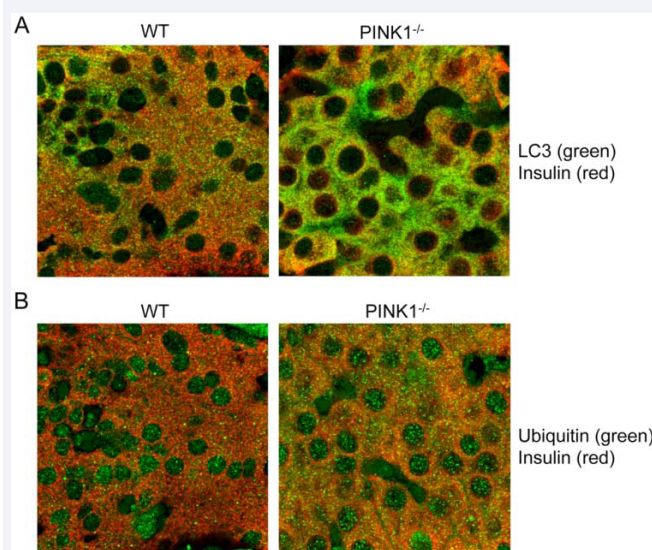
**Figure 4 Mitochondrial swelling in PINK1-deficient pancreatic  $\beta$ -cells:**

Pancreata were embedded, processed and cut for analysis by electron microscopy as described in Materials and Methods. (A-F) Electron microscopy images of mitochondria in pancreatic  $\beta$ -cells of 4-month and 8-month old WT (A,C,E) and PINK1<sup>-/-</sup> (B,D,F) mice (fold magnification indicated). (G) Statistical analysis of mitochondrial size. Thirty-seven to sixty-seven randomly chosen mitochondria in pancreatic  $\beta$ -cells were analyzed at 49,000 $\times$  magnification using NIH Image J software for n=3 mice per genotype and the means were compared by t-test. WT mice, black bars; PINK1<sup>-/-</sup> mice, grey bars; mean  $\pm$  SD; \*\*p<0.01. In panels B, D and F arrows point to enlarged/swollen mitochondria with cristae fragmentation. Asterisks in panels A-D indicate insulin granules. Scale bars, 0.5  $\mu$ m.



**Figure 3 PINK1 enhances glucose-stimulated insulin secretion in MIN6 cells:**

MIN6 cells were a gift from Dr. Sabire Ozcan (University of Kentucky). Cells were kept at passage number <30 and transfected when 75-80% confluent as described in Materials and Methods. (A) MIN6 cells transfected with EGFP expression plasmid, showing EGFP fluorescence (top) and bright field picture (bottom) of the same view field. (B) Basal and glucose-stimulated insulin secretion. GSIS assay was performed in MIN6 cells 48 hours after transfection as described in Materials and Methods (n=4-6 wells for each MIN6-EGFP and MIN6-PINK1). The amount of secreted insulin was normalized to total cellular protein. Mean  $\pm$  SEM, \*p<0.05. Experiments were repeated two different times with similar results.



**Figure 5 Increased numbers of autophagosomes and ubiquitin-positive inclusions in pancreatic  $\beta$ -cells of PINK1<sup>-/-</sup> mice:**

Paraffin sections of pancreata were stained with antibodies against (A) LC3 and insulin to detect autophagosomes and (B) ubiquitin and insulin to detect ubiquitinated inclusions in  $\beta$ -cells. Panels show overlays of green (Alexa488) and red (Alexa594) signals captured by confocal microscopy and are representative of three animals per genotype.

reduced clearance of autophagosomes (fusion with lysosomes) rather than decreased autophagy induction. However, further studies in the future are necessary to substantiate a direct role of PINK1 in the regulation of  $\beta$ -cell autophagy.

## DISCUSSION

Clinical studies suggest a link between T2D and PD, especially in younger patients [25,26]. Both PD and diabetes are characterized by mitochondrial dysfunction [21,30] and several genes linked to early-onset familial PD regulate mitochondrial function and quality control [30,31]. However, to date little is known about how mutations in familial PD genes affect the function and homeostasis of tissues implicated in the development of T2D. Here, we studied the effects of PINK1 deficiency on mitochondrial integrity, autophagy and insulin secretion in pancreatic  $\beta$ -cells. We show that PINK1 deficiency results in a significant decrease in GSIS from pancreatic islets without affecting intracellular insulin levels. The insulin secretion defect is associated with abnormally swollen mitochondria in PINK1-deficient  $\beta$ -cells *in vivo*, many of which develop cristae fragmentation. We recently showed that similarly enlarged mitochondria with disintegrated cristae are present in primary neurons of PINK1-deficient mice [17]. Results presented here demonstrate that mitochondrial quality control exerted by PINK1 is also important in  $\beta$ -cells. Moreover, we found that loss of PINK1 leads to an increased number of LC3II-positive autophagosomes and accumulation of ubiquitinated protein inclusions, suggesting altered autophagy in PINK1-/-  $\beta$ -cells. However, our experiments do not prove a direct involvement of PINK1 in the regulation of  $\beta$ -cell autophagy. It is possible that loss of PINK1 results in compensatory up-regulation of autophagy secondary to other defects (e.g., oxidative stress), as has been shown in cultured neuronal cells [29]. Future studies investigating whether PINK1 binds to Beclin-1 in mouse  $\beta$ -cells and/or if accumulation of autophagosomes is due to reduced autophagic flux are necessary to conclude that PINK1 directly controls autophagy in pancreatic  $\beta$ -cells. Nonetheless, it is interesting that several phenotypes of PINK1-/-  $\beta$ -cells are also observed in states of diabetes. For example, diabetes-induced oxidative stress in pancreatic  $\beta$ -cells leads to ubiquitination and storage of proteins into cytoplasmic aggregates, the number of which increases upon autophagy inhibition [24]. In addition,  $\beta$ -cell specific ablation of the autophagic machinery results in progressive accumulation of ubiquitinated proteins and swollen mitochondria as well as reduced glucose-stimulated insulin secretion [32,33]. Finally, long-term exposure to fatty acids and glucose blocks autophagic flux in  $\beta$ -cells, leading to the accumulation of autophagosomes, impaired autophagic turnover of long-lived proteins and reduced insulin secretion [34]. The autophagy inducer rapamycin ameliorated defects in autophagic flux and insulin release, showing that impaired autophagy contributes to insulin secretion defects [34]. Taken together, these results demonstrate the critical importance of mitochondria and autophagy for GSIS and suggest that mutations in PINK1 may be a risk factor for diabetes by causing abnormalities in mitochondrial integrity and autophagy in  $\beta$ -cells similar to those induced by high-calorie diet and obesity. It has recently been shown that DJ-1, which acts in parallel with PINK1 and Parkin to control mitochondrial function and autophagy [35], is a target for oxidation in MIN6 cells treated with high glucose

[36]. Moreover, DJ-1 over-expression protected MIN6 cells from oxidative stress-induced cell death and impairments in insulin secretion [36]. Like PINK1, over-expression of DJ-1 increased GSIS from MIN6 cells [36]. Finally, over-expression of  $\alpha$ -Synuclein ( $\alpha$ S), which is linked to sporadic and dominant familial PD, reduced insulin secretion from MIN6 cells and mouse islets, albeit only at low glucose concentrations [37]. In contrast, islets from  $\alpha$ S knockout mice showed elevated insulin secretion [37]. As a mechanism, it has been suggested that  $\alpha$ S binds to insulin granules and the  $K_{ATP}$  channel, acting as an inhibitor of insulin secretion [37]. However,  $\alpha$ S also inhibits mitochondrial complex I activity and autophagy [38,39], which if it occurred in  $\beta$ -cells may contribute to impaired insulin release. Finally, it has been shown that mice lacking insulin-degrading enzyme (*Ide* knockout mice) have impaired GSIS and autophagic flux [40]. *Ide* knockout mice showed accumulation of  $\alpha$ S in pancreatic  $\beta$ -cells and over-expression of  $\alpha$ S alone was sufficient to reduce GSIS [40].

## CONCLUSIONS

The results presented in this work and previous studies implicating DJ-1 and  $\alpha$ S in islet cell function and stress resistance support the notion that mutations in familial PD genes may be risk factors for T2D and provide a potential explanation for the known association between T2D and PD. Abnormal mitochondrial function and autophagy could be shared pathogenic mechanisms, because these processes are affected by all PD genes discussed above (PINK1, DJ-1,  $\alpha$ S) and are also compromised in sporadic PD [30,41,42]. Consequently, bestowing  $\beta$ -cells with an enhanced capacity for mitochondrial quality control and stress resistance through over-expression or activation of recessive PD genes [8,10,11,36] may lead to improved treatments for T2D in the future. The fact that over-expression of both PINK1 (shown here) and DJ-1 [36] increases GSIS in MIN6 cells lends further support to this idea. However, autophagy needs to be well balanced because both reduced and excessive autophagy can be detrimental to  $\beta$ -cell function. Defects other than those investigated here may exist and contribute to the phenotype of reduced GSIS in PINK1-deficient islets, most notably increased oxidative stress [8,29,43]. In addition, lack of PINK1 may affect glucose-dependent intracellular signaling through effects on Akt activity [9,19]. Finally, because all our studies have been performed with isolated islets future experiments with animals are necessary to determine if reduced insulin secretion leads to a diabetic status *in vivo*. This may depend on age, genetic background and how loss of PINK1 affects the physiology of other organs involved in glucose homeostasis, including skeletal muscle and liver.

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