A novel mechanism regulating insulin secretion involving *Herpud1* in mice

N. Wong · G. Morahan · M. Stathopoulos · J. Proietto · S. Andrikopoulos

Abstract

Aims/hypothesis Type 2 diabetes results from beta cell dysfunction after prolonged physiological stress, which causes oversecretion of insulin. We recently found that insulin hypersecretion is mediated by at least two genes. Among mouse models of type 2 diabetes, the DBA/2 mouse strain is more susceptible to diabetes than is the C57BL/6J (B6J) strain. One distinctive feature of the DBA/2 mouse is that it hypersecretes insulin, independent of changes in insulin sensitivity; we identified *Nnt* as a gene responsible for this trait.
Methods To identify the other gene(s) affecting insulin hypersecretion, we tested a panel of recombinant inbred BXD strains, which have different combinations of B6 and DBA/2 alleles.
Results We found that 25% of the BXD strains hypersecreted insulin in response to glucose. Microarray profiling of islets from high- and low-secretor strains showed that at least four genes were differentially expressed. One gene was consistently underexpressed in islets from both DBA/2 and the high-secretor BXD strains. This gene (*Herpud1* or *Herp*) encodes the 54 kDa endoplasmic reticulum stress-inducible protein (HERP) that resides in the integral endoplasmic reticulum membrane. To test directly whether *Herpud1* can interact with *Nnt*, *Herpud1* was either knocked down or overexpressed in

MIN6 cells. These results showed that when *Herpud1* was suppressed, *Nnt* expression was reduced, while overexpression of *Herpud1* led to increased *Nnt* expression. Furthermore, *Herpud1* suppression resulted in significantly decreased glucose-stimulated insulin secretion in the DBA/2 islets but not B6J islets.
Conclusions/interpretation We conclude that *Herpud1* regulates insulin secretion via control of *Nnt* expression.

Abbreviations

B6J	C57BL/6J
B6N	C57BL/6N
ER	Endoplasmic reticulum
ERAD	ER-associated degradation
GWAS	Genome-wide association studies
HERP	Endoplasmic reticulum stress-inducible protein
NNT	Nicotinamide nucleotide transhydrogenase
SiRNA	Small interfering RNA
UPR	Unfolded-protein response

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Introduction

Type 2 diabetes results from a complex interplay between genetic and environmental factors, which lead to two pathophysiological defects—insulin resistance and impaired insulin secretion. Hyperglycaemia caused by reduced insulin secretion results from beta cell dysfunction and/or loss. It is well established that a number of stressors, such as glucose toxicity, lipotoxicity and cytokine exposure, can contribute

to this beta cell dysfunction/death [1–3]. However, the underlying molecular cause of beta cell dysfunction has not yet been fully defined. There is strong evidence that type 2 diabetes is a polygenic disease [4]. Recent genome-wide association studies (GWAS) have identified a number of risk genes, including *TCF7L2*, *CDKAL1*, *HHEX*, *SLC30A8*, *CDKN2A/B*, *IGF2BP2*, *KCNJ11* and many more [5]. It is important to note that the cases used in some of these GWAS were chosen for their early age of onset and severity of disease so may not be representative of the typical individual with type 2 diabetes. Therefore, these genes may only account for a small proportion of the genetic susceptibility of type 2 diabetes, so further studies are warranted to identify more genes contributing to the disease process.

While the exact cause of beta cell dysfunction in type 2 diabetes is not known, it is evident that stimulation of the beta cell to secrete insulin under conditions when it is already maximally stressed (e.g. obesity and insulin resistance) can lead to deterioration in cellular function [6]. The DBA/2 mouse strain is a model of diabetes susceptibility, as compared with the C57BL/6J (B6J) strain which is resistant to diabetes. When DBA/2 mice were subjected to metabolic stress by the mutation of the leptin receptor (*db/db*), they developed overt diabetes with failure and death of the beta cells, whereas the same mutation in the B6J strain led to obesity, insulin resistance and islet hyperplasia [7]. We recently showed that a mechanism for beta cell failure following high glucose exposure of DBA/2 islets is oxidative stress [8]. We also reported that a distinctive feature of the DBA/2 islet is that it hypersecretes insulin in response to glucose both in vivo and in vitro, and this seems to be independent of changes in insulin sensitivity [9]. We conducted a genome-wide genetic scan and found that the glucose-induced insulin hypersecretion of the DBA/2 mouse is mediated by at least two genes, one of which was identified as the gene for nicotinamide nucleotide transhydrogenase located at the end of chromosome 13. *Nnt* is expressed in DBA/2 islets at levels more than fivefold higher than in islets from the B6J strain [10]. It is worth noting that B6J mice harbour a truncated *Nnt* allele, while DBA/2 mice have a full-length gene [10]. Identification of *Nnt* as a diabetes-susceptible gene in the DBA/2 strain led us to propose that beta cell stimulation could itself precipitate type 2 diabetes in susceptible individuals [6]. This was supported in a clinical setting: the ADOPT (A Diabetes Outcome Progressional Trial) study showed that in newly diagnosed individuals with type 2 diabetes those treated with insulin secretagogues (sulfonylureas) had worse glycaemic control and progressed to beta cell failure and monotherapy faster than those treated with insulin sensitisers [11].

However, from our previous studies it was not clear why *Nnt* was upregulated in the DBA/2 and not in the B6J strain [10, 12]. A possible explanation could be that other unidentified gene(s) were involved in driving *Nnt* upregulation in the DBA/2 mouse. Therefore, the aim of this study was to identify other gene(s) associated with insulin hypersecretion. To do so, we used the recombinant inbred BXD strain set. The genomes of BXD strains are derived from the DBA/2 and B6J strains and have been extensively genotyped across all chromosomes, facilitating gene mapping and systems genetic studies [13–15].

Methods

Animals B6J and DBA/2 mice were purchased from The Walter and Eliza Hall Institute for Medical Research (WEHI, Kew, VIC, Australia). The B6J mice were acquired by WEHI from The Jackson Laboratory and re-derived at the WEHI animal facility in 1989. C57BL/6-NTac (B6N) mice were purchased from Taconic Farm (Germantown, NY, USA) and maintained at the BioResources Facility Austin Health (Heidelberg, VIC, Australia). BXD lines were maintained at the Animal Resources Centre (Murdoch, WA, Australia). All mice were fed a standard rodent control chow diet (74% carbohydrate, 20% protein and 3% fat by weight; Ridley AgriProducts, Pakenham, VIC, Australia). Experimental procedures were performed using male mice at 8 weeks of age. All procedures were approved by the Austin Health Animal Ethics Committee (AEC No.: A2007/03055, A2005/02110 and A2010/04104).

Intravenous glucose tolerance test, plasma glucose and insulin assays The mice were fasted overnight (approximately 16 h) before the intravenous glucose tolerance test (IVGTT) as previously described [10]. Plasma glucose concentrations were measured using a GM7 Analyser (Analox Instruments, London, UK). Plasma insulin levels were determined by a rat specific insulin radioimmunoassay kit (Merck Millipore, Billerica, MA, USA).

Nicotinamide nucleotide transhydrogenase enzyme activity assay Pancreatic nicotinamide nucleotide transhydrogenase (NNT) enzyme activity was performed as previously described [10]. Islet NNT enzyme activity was determined using the Biovision NADP$^+$/NADPH quantification kit (Cat. No. K347-100; BioVision Research Products, Milpitas, CA, USA). Approximately 20 mg of pancreatic tissue was weighed out for each assay, washed with cold PBS and then homogenised with 400 μl of NADP/NADPH extraction buffer in a microcentrifuge tube. The samples were centrifuged at 18,500 g for 5 min and the extracted NADP/NADPH solution was transferred into new tubes. NADP cycling buffer and NADP cycling enzyme mix was added to each NADPH sample and incubated at room temperature for 5 min to convert NADP to NADPH. An NADPH developer was then added to each sample and

developed for 1–4 h before reading the absorbance at 450 nm. The final NADPH levels were calculated by a standard curve. All samples were performed in duplicate. For further details please refer to protocol provided by the supplier.

Gene knockdown and overexpression Herpud1 siRNAs (Cat. 4392421 ID: s82159 and s82160; please refer to electronic supplementary material [EMS] Table 1 for sequences) from Life Technologies (Melbourne, VIC, Australia) or pCMV.Sport6-Herpud1 vector (a pre-constructed plasmid expression vector containing *Herpud1* mouse cDNA) from Invitrogen (Cat. 4236803) and *Nnt* (PCI.Neo-Nnt) [12] was transfected into islets or MIN6 cells by following the manufacturer's specification for Lipofectamine 2000 (Invitrogen, Mount Waverley, VIC, Australia).

Insulin secretion assay The parental and transfected cells were preincubated in KRB with 2.8 mmol/l glucose for 90 min and then stimulated for 60 min with either 2.8 mmol/l or 20 mmol/l glucose as previously described [16].

Pancreatic insulin content, islet isolation and cDNA preparation These techniques were performed as previously described [10, 12].

Gene expression microarray and analysis Total RNA was extracted from isolated islets and sample quality was verified by the Agilent Bioanalyser (Mulgrave, VIC, Australia). Complimentary RNA (cRNA) was generated from mRNA by the Illumina(r) TotalPrep RNA Amplification Kit from Life Technologies (Cat. AMIL1797). The cRNA was used for Whole-Genome Gene Expression by direct hybridisation following the manufacturer's specification (Illumina, Part: 11322355). The raw gene expression data was analysed using the Illumina Genome studio software. The candidate regions were selected by the genotype of the low- and high-secretor BXD strains using the publicly available genotype dataset from the Gene Network [14]. More specifically, these are regions that were marked with B6J alleles in the low secretors and DBA/2 alleles in the high secretors. Genes that showed greater than a twofold difference from the microarray and located within these candidate regions were identified for further analysis.

Real-time PCR analysis Gene expression was determined using primers and probe (Taqman Gene Expression Assays) purchased from Applied Biosystems (Scoresby, VIC, Australia). The expression of 18S RNA was used as an endogenous control. Amplification and detection were performed with the ABI Prism 7700 Sequence Detector and Sequence Detection System software (version 1.9.1; Applied Biosystems). Each measurement was carried out in duplicate. Difference in gene expression was expressed as percentage of fold-change from control and was calculated using the $2^{-\Delta\Delta C_t}$ method using 18S as the internal control.

Western blot analysis The expression of endoplasmic reticulum stress-inducible protein (HERP) in isolated islets was determined using western blot analysis as previously described [8]. The following antibodies from Abcam (Redfern, NSW, Australia) were used: anti-Herpud1 antibody (ab73669) and anti-α-tubulin antibody (ab4074) as loading control.

Statistical analysis All data are expressed as mean ± SEM from repeated experiments. The statistical analyses were determined by a two-tailed, unpaired (two-sample assuming equal variances) Student's *t* test for parametric data. The level of statistical significance was taken as $p \leq 0.05$.

Results

Characterisation of BXD strains We searched for gene(s) associated with insulin hypersecretion by performing an IVGTT on available BXD strains. Low and high secretors were defined as described previously [10]. Upon administration of glucose, four BXD lines (BXD-9, -11, -24 and -32) out of 20 that were available to us for testing, hypersecreted insulin at the 2 min time point, which is the peak of first phase in the insulin secretion test (Fig. 1a). This insulin hypersecretion was clearly independent of fasting plasma insulin (Fig. 1b) and fasting plasma glucose levels (Fig. 1c) because no correlation was observed among these traits in the BXD strains. As expected from our earlier study, the BXD strains that hypersecreted insulin all had DBA/2-derived alleles of *Nnt* [14]. However, it is important to note that not all of the BXD strains that had DBA/2 *Nnt* alleles hypersecreted insulin, clearly demonstrating the involvement of other gene(s) in controlling this trait. For example, BXD lines 6, 22, 28, 31 and 62 all had DBA/2-derived alleles at 64.0 cM on chromosome 13 but all secreted insulin at levels similar to B6J. Insulin hypersecretion was predominantly associated with an increase in *Nnt* expression and NNT activity [10]. Consistent with these findings, the low-secretor strains BXD75 and 96 had lower NNT activity, similar to B6J, while the high secretors (BXD 9 and 11) had higher NNT activity, like DBA/2 controls (Fig. 2a), confirming that higher *Nnt* at least in part contributed to the insulin hypersecretion phenotype. Taken together, our data suggest that the BXD strains that failed to hypersecrete insulin despite having a DBA/2-derived *Nnt* allele lacked critical regulatory factor(s) for facilitating *Nnt* expression.

Differential gene expression between high- and low-secretor strains To identify genes associated with altered *Nnt* levels, we employed a systems genetics approach [17], integrating
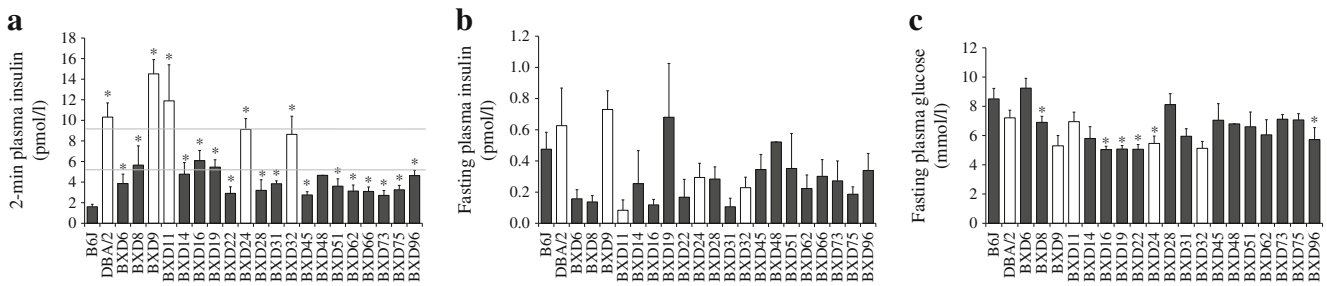
Fig. 1 BXD lines 9, 11, 24 and 32 hypersecreted insulin independent of changes in fasting plasma insulin and glucose level. Plasma insulin concentrations at 2 min (**a**), fasting plasma insulin concentrations (**b**), and fasting glucose levels (**c**) during an IVGTT (1 g/kg glucose bolus) in 8- to 10-week-old B6J, DBA/2 and BXD strains. Results are presented as means ± SEM (*n*=4–10). *$p<0.05$ vs B6J. White bars indicate high secretors

genetic and gene expression information. We reasoned that all high-secretor strains would share DBA/2 alleles of these genes while all low-secretor strains would have B6J-derived alleles. With the information that we were able to use from the relatively small number of BXD strains that we had access to, we found candidate regions on chromosomes 2, 3, 4, 5, 6, 8, 9, 12, 13, 14 and16 (Fig. 2b) for further analysis.

Next, a series of microarrays was performed and the differential gene expression profile in the pancreatic islets

Fig. 2 NNT enzyme activity is enhanced in the high-secretor BXD strains, which may be associated with gene(s) located in several chromosomal hotspot regions. (**a**) Pancreatic NNT activity level in 8- to 10-week-old B6J, DBA/2 and BXD strains 75, 96, 11 and 9. Results are presented as means ± SEM (*n*=4–10). *$p<0.05$ vs B6J. AcPy, 3-acetylpyridine analogue of NADH. (**b**) A schematic diagram of the hotspot locations on the chromosomes

determined. Genes that showed greater than a twofold difference in expression and positioned within the candidate regions were identified. These analyses showed eight candidate genes that were differentially expressed between the high- and low-secretor strains (Table 1). Four genes, namely, frizzled-related protein (*Frzb*; chromosome 2), nuclear factor, interleukin3 (*Nfil3*; chromosome 13), coagulation factor II (*F2r*; chromosome 13) and homeocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1 (*Herpud1*; chromosome 8), were selected for further investigation based on their known function. Real-time PCR was then performed to confirm the level of gene expression. Only one gene, *Herpud1* (also called *Herp*), was consistently underexpressed in both the high-secretor BXD strains and DBA/2 mouse islets (Fig. 3a–d). HERP protein levels were also lower in DBA/2 than in B6J islets (Fig. 3e). *Herpud1* encodes HERP, a 54 kDa protein that resides in the integral endoplasmic reticulum (ER) membrane [18]. However, the role of *Herpud1* in pancreatic beta cells has not been fully defined.
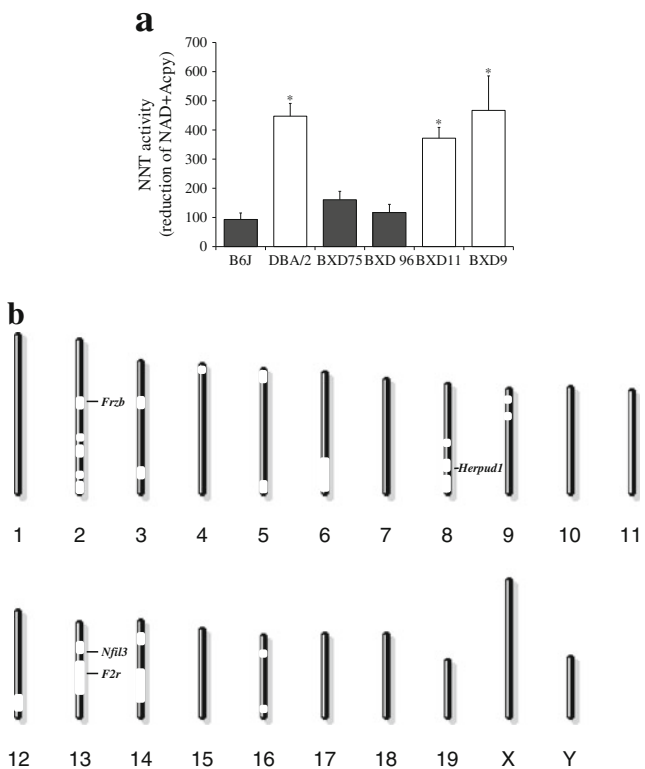
Characterisation of Herpud1 To determine whether *Herpud1* has a role in regulating *Nnt* expression, it was either knocked down or overexpressed in the pancreatic MIN6 cell line and

Table 1 Chromosomal location and microarray expression value of eight candidate genes associated with insulin hypersecretion

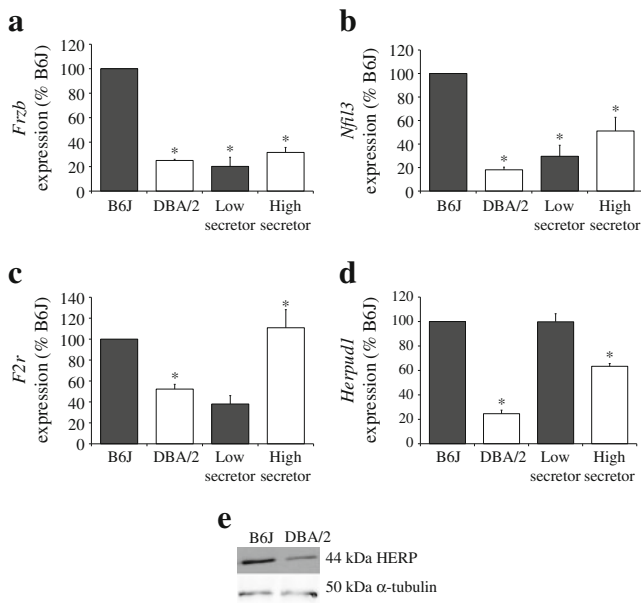| Chromosome	Location	Gene	Value
2	2 C3; 2	*Frzb*	5.06
5	5 G2; 5	*Actb*	3.75
6	6 E3; 6	*Depp*	2.88
8	8 B3.1; 8	*Sc4mol*	2.89
8	8 C5; 8	*Herpud1*	2.54
12	12 E; 12	*Ifi27l2a*	3.19
13	13 B1; 13	*Nfil3*	2.77
13	13 D1; 13	*F2r*	2.33

Value, change in gene expression in low vs high secretors

Fig. 3 *Herpud1* is consistently underexpressed in islets from high-secretor B6J and from DBA/2 mice. mRNA levels of *Frzb* (**a**), *Nfil3* (**b**), *F2r* (**c**) and *Herpud1* (**d**) in islets from B6J, DBA/2, BXD 22 (low secretor) and BXD 11 (high secretor). (**e**) Representative western blot for HERP in islets isolated from B6J and DBA/2 mice. Results are presented as means ± SEM ($n=5$). *$p<0.05$ vs B6J
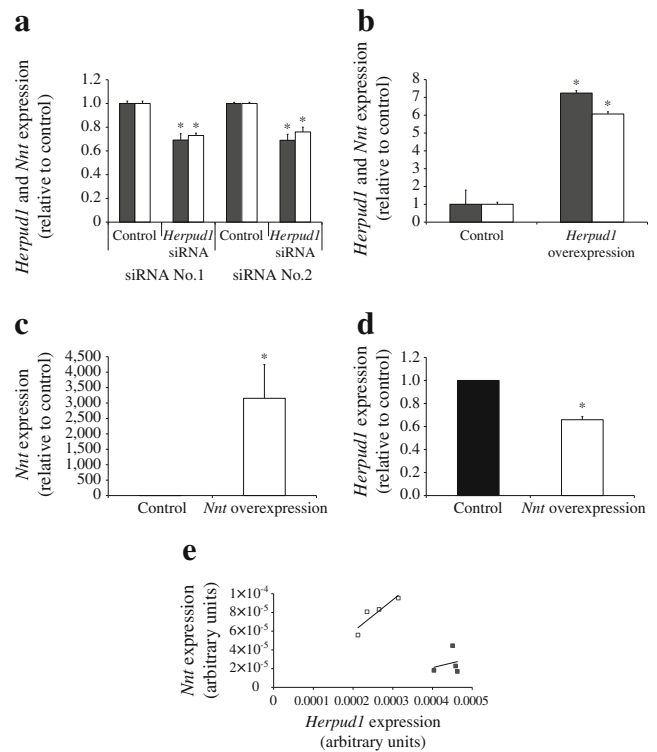

Fig. 4 Downregulation of *Herpud1* resulted in reduced *Nnt* mRNA levels while overexpression elevated *Nnt* in a one-directional manner suggesting that *Herpud1* is upstream of *Nnt*. (**a**) mRNA levels of *Herpud1* (black bars) and *Nnt* (white bars) in MIN6 cells 24 h post knockdown of *Herpud1* using siRNA. Results are presented as means ± SEM ($n=5$ or 6). *$p<0.05$ vs control. (**b**) mRNA levels of *Herpud1* (black bars) and *Nnt* (white bars) in MIN6 cells 24 h following overexpression of *Herpud1*. Results are presented as means ± SEM ($n=5$ or 6). *$p<0.05$ vs control. (**c**) mRNA levels of *Nnt* in MIN6 cells 24 h following overexpression of *Nnt*. Results are presented as means ± SEM ($n=5$ or 6). *$p<0.05$ vs control. (**d**) RNA levels of *Herpud1* in MIN6 cells 24 h following overexpression of *Nnt*. Results are presented as means ± SEM ($n=5$ or 6). *$p<0.05$ vs control. (**e**) Correlation of *Herpud1* and *Nnt* gene expression in islets from B6 mice (black squares), $R^2=0.0472$ and DBA/2 mice (white squares), $R^2=0.8101$. Data presented as absolute values ($n=4$)

real-time PCR was performed to determine gene expression levels. If *Herpud1* plays a role in regulating *Nnt* expression, altering its expression should also affect expression levels of *Nnt*. Using two sets of siRNA oligonucleotides, we found a reduction in the expression levels of both *Herpud1* and *Nnt* (Fig. 4a). Conversely, *Herpud1* overexpression in MIN6 cells led to increased *Nnt* expression (Fig. 4b). In addition, increasing *Nnt* by gene overexpression in MIN6 cells suppressed *Herpud1* expression (Fig. 4c,d), suggesting a reciprocal regulation between *Herpud1* and *Nnt*. Furthermore, a plot between *Herpud1* and *Nnt* revealed a positive correlation between these two genes in DBA/2, but not B6J, islets (Fig. 4e). An unexpected finding of this study was that *Herpud1* mRNA levels were higher in B6J than in DBA/2. This may have been due to the 5-exon deletion mutation in the B6J *Nnt* allele [10] with feedback causing higher *Heprud1* expression. Taken together, these data provide the first direct evidence for an interaction between these two genes, with *Herpud1* regulating *Nnt* expression.

We next knocked down *Herpud1* using the same set of siRNA oligonucleotides in isolated B6J and DBA/2 islets, and insulin secretion was determined. The results showed that there was no difference in insulin secretion between the siRNA-negative control and *Herpud1* siRNA-treated B6J islets (Fig. 5a). However, a significant decrease in insulin secretion was observed in the *Herpud1* siRNA-treated DBA/2 islets stimulated at 20 mmol/l glucose (Fig. 5a). Insulin content was also assessed in these islets to determine

whether the decrease in insulin secretion was due to a functional defect. The results, although not statistically significant, showed that the total insulin content was in fact trending towards an increase in the DBA/2 islets following the knockdown of *Herpud1* (Fig. 5b). We also attempted to measure HERP protein levels in islets treated with siRNA; however, the western blot technique may not have been sensitive enough to produce reliable results. Nonetheless, real-time PCR and NNT activity assay was also performed on these islets to confirm the gene knockdown of *Herpud1* and, again, the data showed that reducing *Herpud1* also reduced *Nnt* expression (Fig. 5c) and NNT activity (Fig. 5d).

We investigated whether the difference between the response of B6J and DBA/2 could be due to the *Nnt* exonic deletion in the latter strain. To do so, we compared the effect of upregulation of *Herpud1* on insulin secretion in B6J and
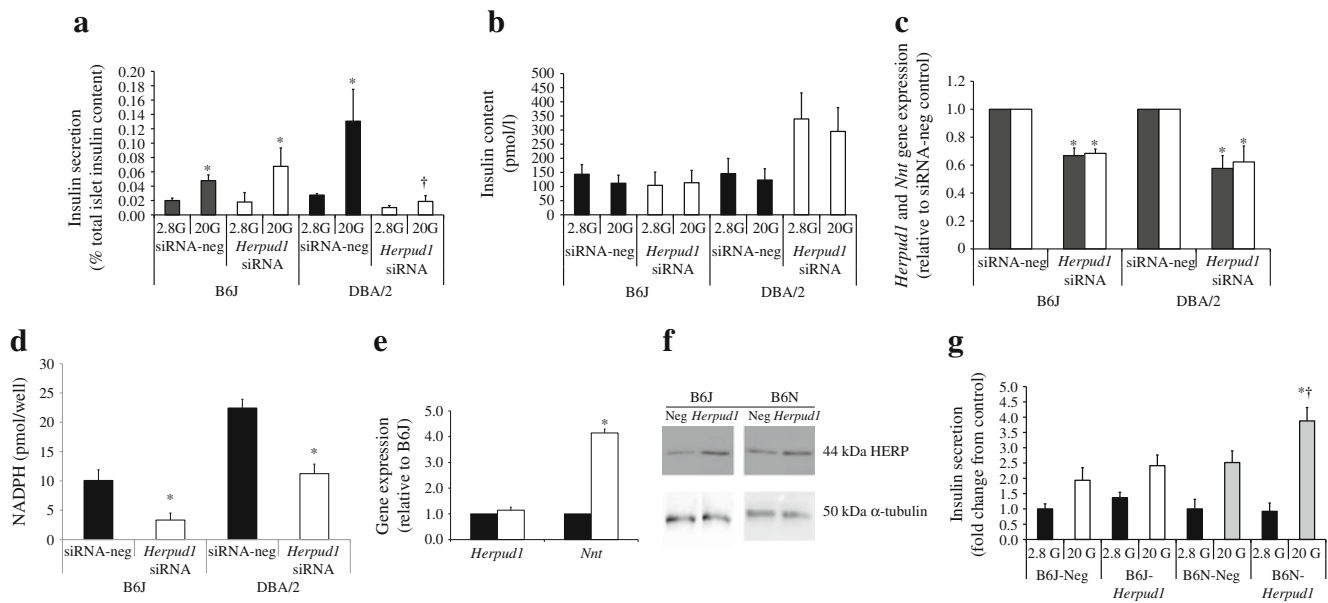
Fig. 5 HERP knockdown significantly impaired insulin secretion in islets expressing the full-length *Nnt* while overexpression of *Herpud1* led to enhanced insulin secretion. (**a**) Insulin secretion in B6J and DBA/2 islets under low (2.8 mmol/l; 2.8 G) and high (20 mmol/l; 20 G) glucose 48 h post treatment with scrambled control (siRNA-neg) or *Herpud1* siRNA. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs siRNA-neg. (**b**) Insulin content in B6J and DBA/2 islets 48 h post treatment with scrambled control (siRNA-neg) or *Herpud1* siRNA. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs siRNA-neg. (**c**) *Herpud1* (black bars) and *Nnt* (white bars) mRNA in B6J and DBA/2 islets following treatment with scrambled control (siRNA-neg) or *Herpud1* siRNA. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs siRNA-neg. (**d**) Islet NNT enzyme activity 48 h post *Herpud1* siRNA treatment in B6J and DBA/2 islets. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs siRNA-neg. (**e**) *Herpud1* and *Nnt* mRNA level 24 h post overexpression of *Herpud1* in B6J (black bars) and B6N (white bars) islets. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs siRNA-neg. (**f**) Representative western blots of HERP in islets from B6J and B6N overexpressing *Herpud1* vs control (Neg). (**g**) Insulin secretion in B6J and B6N islets under low (2.8 mmol/l) and high (20 mmol/l) glucose 24 h post overexpression of *Herpud1*. Results are presented as means ± SEM (n=5 or 6). *p<0.05 vs control, †p<0.05 vs B6J

the C57BL/6N (B6N) substrain, which possesses the full-length NNT gene [12]. The results showed that transient 24 h overexpression of *Herpud1* led to similar *Herpud1* expression levels in the B6J and B6N islets (Fig. 5e). However, *Nnt* upregulation was only detected in B6N islets and not B6J islets (Fig. 5e). We confirmed that overexpressing *Herpud1* in islets increased HERP protein levels using western blotting (Fig. 5f). Consistent with these results, *Herpud1* overexpression led to no change in glucose-stimulated insulin secretion in the B6J islets (Fig. 5g). However, the B6N islets showed a significant increase in insulin secretion, similar to that seen in the DBA/2 islets (Fig. 5e). Taken together, our data demonstrate that *Herpud1* not only regulated *Nnt* expression but also had a functional effect in the pancreatic beta cell.

Discussion

It is well established that beta cell dysfunction leads to hyperglycaemia in type 2 diabetes. Despite many years of effort, the exact cause of beta cell dysfunction is still not fully understood, although environmental factors in a permissive genetic environment are expected to be involved.

Since genetic susceptibility is predetermined, understanding type 2 diabetes genetics is vital in developing new therapeutic targets to treat and prevent this disease. There is accumulating evidence to suggest that overstimulating the beta cell may be an important risk factor in the development of its dysfunction: some individuals with obesity and a family history of type 2 diabetes, who are at increased risk of developing the condition, paradoxically hypersecrete insulin. In support of this, a variant in the sulfonylurea receptor gene (*SUR1*, also known as *ABCC8*) resulting in elevated fasting and glucose-induced plasma insulin concentrations has been described in non-diabetic Mexican Americans, an ethnic group with a high incidence of type 2 diabetes [19]. Moreover, it was shown in Pima Indians, who have the highest incidence of diabetes of any ethnic group in the world, that fasting hyperinsulinaemia was a significant predictor of the disease, independent of insulin resistance [20]. Primary defects causing insulin hypersecretion can also lead to beta cell dysfunction; for example, patients with a mutation in the *GCK* gene develop persistent hyperinsulinaemic hypoglycaemia of infancy and diabetes requiring insulin later in life [21].

We have previously reported that increased *Nnt* gene expression was associated with insulin hypersecretion in the DBA/2 mouse. However, the transcription regulation mechanism of *Nnt* was not yet known. Here we have identified *Herpud1*, which not only regulates the expression of *Nnt* but can also modulate insulin secretion in the pancreatic beta cell. *Herpud1* is a mammalian ubiquitin domain-containing protein, which is highly induced by the unfolded-protein response (UPR) in response to ER stress. However, its function in insulin secretion in the pancreatic beta cell has not previously been investigated. Recent studies on *Herpud1* revealed an important role for this gene in facilitating the ER-associated degradation (ERAD) system in the ER. For example, *Herpud1* was shown to interact with members of the ubiquitin family and functions as a shuttle factor to deliver ubiquitinated substrates to the proteasome for degradation [22]. Similarly, *Herpud1* was found to be involved in the regulation of Hrd1 ubiquitinylation, a ubiquitin–ligase complex responsible for rapid protein degradation in the ER [23]. Others showed that HERP was required for linking BiP substrates with the proteasome for target protein degradation [24]. Also, HERP can function as a substitute for Usa1p, a yeast protein that has a well established role in the ERAD system [25].

Although HERP was identified as a stress-responsive protein, evidence suggests that it can behave differently in different tissues. Interestingly, unlike other ER stress-induced molecules residing in the ER lumen, a major portion of the HERP molecule is present on the cytoplasmic side of the ER membrane [18]. Therefore, it has been suggested that HERP may have a role distinct from that of molecular chaperones. In fact, evidence suggests that the ubiquitin-like domain of HERP could have diverse roles. For example, using a human embryonic cell line, HERP was reported to be involved in the generation of amyloid beta proteins found in Alzheimer's disease [26]. Others showed that *Herpud1* was upregulated by Wnt-1 signalling, which regulates morphogenesis and mitogenesis in both murine and human colorectal cells [27]. Moreover, *Herpud1* was also shown to play a significant role in regulating Ca^{2+} homeostasis as well as mitochondrial function in neuronal cell cultures [28], while others suggested that it might be involved in the immune system [29]. Taken together, the above evidence suggests that *Herpud1* has a wide range of biological functions, although the role of this gene in the beta cell remains to be elucidated. A recent study reported that *Herpud1* null mice had impaired glucose tolerance, which appeared to be independent of insulin sensitivity [30]. Interestingly, these mice were produced on a C57BL/6CrSlc background, a substrain of C57BL/6 that carries the full-length *Nnt* [31]. It was unclear how *Herpud1* deficiency led to impaired glucose tolerance but since insulin sensitivity was not affected it is possible that there may be an insulin secretory defect. Although neither insulin secretion nor the expression of *Nnt* was assessed in these animals, this study highlights the importance of *Herpud1* in glucose homeostasis. More importantly, our study provides the first direct evidence to show a functional role for *Herpud1* in insulin secretion and that this could be mediated through the *Nnt* gene. Exactly how *Herpud1* regulates *Nnt* expression is not clear, although it appears that the full-length *Nnt* is required for this regulation to be effective. It is also possible that the effects of *Herpud1* on insulin secretion may be implicated by the indirect effects of this UPR target protein on the expression and function of other proteins involved in the secretory pathway. In addition, there are also strain differences between B6 and DBA/2, such as insulin secretion, islet mass and diabetes susceptibility, which may also contribute to the phenotype [8, 10, 32, 33]. However, it is worth noting that the effects of *Herpud1* on insulin secretion were observed in two different animal models (i.e. DBA/2 and B6N), therefore the effects of *Herpud1* on insulin secretion are likely to be independent of strain variations. Instead, it is possible that the ubiquitin-like domain sequence in *Herpud1*, which can have diverse functions, may act as a transcriptional factor to alter *Nnt* levels, with subsequent changes in insulin secretion. In addition, the function of *Herpud1* in Ca^{2+} homeostasis may also be a mechanism for the regulation of *Nnt*, leading to secretory changes. Moreover, significant quantitative trait loci on chromosome 8 for glucose intolerance and pancreatic beta cell function have also been reported in human and rodent studies [34, 35]. In conclusion, our data not only present a new role for *Herpud1* in beta cell function but are also the first to show that *Herpud1* has a functional effect in the regulation of insulin secretion in the pancreatic islet.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement NW contributed to the conception and design, acquisition of data, data interpretation and analysis, and drafting the article and revising it critically for important intellectual content. GM, MS and JP were responsible for acquisition of data, analysis and interpretation of data and revising the article. SA contributed to the design and conception of the study, data analysis and interpretation and assisted in drafting and critically revising the article. All authors approved the final version.

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