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Altered cellular localisation and expression, together with unconventional protein trafficking, of prion protein, PrP^C, in type 1 diabetes

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Abstract

Aims/hypothesis—Normal cellular prion protein (PrP^C) is a conserved mammalian glycoprotein found on the outer plasma membrane leaflet through a glycophosphatidylinositol anchor. Although PrP^C is expressed by a wide range of tissues throughout the body, the complete repertoire of its functions has not been fully determined. The misfolded pathogenic isoform PrP^{Sc} (the scrapie form of PrP) is a causative agent of neurodegenerative prion diseases. The aim of this study is to evaluate PrP^C localisation, expression and trafficking in pancreases from organ donors with and without type 1 diabetes and to infer PrP^C function through studies on interacting protein partners.

Methods—In order to evaluate localisation and trafficking of PrP^C in the human pancreas, 12 non-diabetic, 12 type 1 diabetic and 12 autoantibody-positive organ donor tissue samples were analysed using immunofluorescence analysis. Furthermore, total RNA was isolated from 29 non-diabetic, 29 type 1 diabetic and 24 autoantibody-positive donors to estimate PrP^C expression in the human pancreas. Additionally, we performed PrP^C-specific immunoblot analysis on total pancreatic protein from non-diabetic and type 1 diabetic organ donors to test whether changes in PrP^C mRNA levels leads to a concomitant increase in PrP^C protein levels in human pancreases.

Results—In non-diabetic and type 1 diabetic pancreases (the latter displaying both insulin-positive [INS(+)] and -negative [INS(−)] islets), we found PrP^C in islets co-registering with beta

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Contribution statement The study was conceived and designed by HH, DEB, IK, MAA, HSN and CHW. HH, DEB, CY and ECJ provided design, data acquisition and interpretation of data. All authors contributed to the interpretation of the data and critical revision of the manuscript. HH, HSN and CHW drafted the manuscript. All authors read and approved the final manuscript. As guarantor of this work, CHW had full access to all of the data and takes full responsibility for the integrity of the data and accuracy of its analysis.

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cells in all INS(+) islets and, strikingly, unexpected activation of PrP^C in alpha cells within diabetic INS(−) islets. We found PrP^C localised to the plasma membrane and endoplasmic reticulum (ER) but not the Golgi, defining two cellular pools and an unconventional protein trafficking mechanism bypassing the Golgi. We demonstrate PrP^C co-registration with established protein partners, neural cell adhesion molecule 1 (NCAM1) and stress-inducible phosphoprotein 1 (STI1; encoded by *STIP1)* on the plasma membrane and ER, respectively, linking PrP^C function with cyto-protection, signalling, differentiation and morphogenesis. We demonstrate that both *PRNP* (encoding PrP^C) and *STIP1* gene expression are dramatically altered in type 1 diabetic and autoantibody-positive pancreases.

Conclusions/interpretation—As the first study to address PrP^C expression in non-diabetic and type 1 diabetic human pancreas, we provide new insights for PrP^C in the pathogenesis of type 1 diabetes. We evaluated the cell-type specific expression of PrP^C in the human pancreas and discovered possible connections with potential interacting proteins that we speculate might address mechanisms relevant to the role of PrP^C in the human pancreas.

Keywords

Cellular prion protein; Protein trafficking; Stress-induced phosphoprotein 1; Type 1 diabetes; Type 1 diabetes-dependent endocrine cell expression

Introduction

The cellular prion protein (PrP^C), encoded by the gene *PRNP*, is a glycophosphatidylinositol-anchored cell-surface glycoprotein expressed in numerous cell types [1–4]. PrP^C has been the subject of intense study since identification of the scrapie form of the prion protein, PrP^{Sc}, as an infectious agent [1, 5–7] transmissible through pathogenic conversion of the host-expressed PrP^C. As a pathogenic, alternatively folded aggregate, PrP^{Sc} is linked to a host of neurodegenerative disorders affecting animals and humans, including: scrapie; transmissible mink encephalopathy (TME); chronic wasting disease (CWD); bovine spongiform encephalopathy (BSE); feline spongiform encephalopathy (FSE); exotic ungulate encephalopathy (EUE); camel spongiform encephalopathy (CSE) and in humans disorders classified as Kuru, Creutzfeldt-Jakob disease (CJD), variant CJD (vCJD), Gerstmann–Sträussler–Scheinker syndrome (GSS) and fatal familial insomnia (FFI) [8–11]. Recently, Corbett et al [12] have demonstrated a PrP^c requirement in the toxicity of α-synuclein, amyloid-β and tau in induced pluripotent stem cell (iPSC)-derived neurons from patients with Alzheimer's, Lewy body and Pick's disease.

Although numerous studies have attempted to clarify PrP^C cellular function, including 3D protein structure and generation of several gene ablation models, a definitive biochemical function for PrP^C has not been elucidated [3, 8, 13]. Potential roles for PrP^C include modulation of signalling pathways, immune and hippocampal function, neuroprotection, T lymphocyte proliferation and anti-apoptotic activity, together with metal ion, mitochondrial and glucose homeostasis [3, 8].

Historically, Atouf et al [14] reported that *Prnp* gene expression was hormonally controlled in rodent pancreatic endocrine cell lines and primary islets. More recently, implementing

a PrP^C overexpressing mouse model, Ashok and Singh [15] demonstrated PrP^C expression in mouse pancreatic beta cells, promoting iron uptake through divalent metal transporters, thus modulating glucose homeostasis. Their results showed PrP^C silencing in 1.1B4 cells resulted in depletion of intracellular iron with GLUT2 and insulin upregulation. Using a rat model and mice lacking the prion protein, Strom et al [16, 17] reported that PrP^C is ubiquitously expressed in all four major endocrine cell types within rat islets and identified 'cytosolic inclusions' containing PrP^C exclusively in a subpopulation of insulin-producing beta cells; the study also implicated involvement of PrP^C in glucose homeostasis. Similarly, Amselgruber et al [18] demonstrated that PrP^C is strongly expressed in fetal and adult bovine pancreatic islets. PrP^C-null and -overexpressing mice fed a high-fat diet presented with insulin resistance including hyperglycaemia, hyperinsulinaemia and obesity [19, 20]. The majority of studies identify PrP^C expression in endocrine cells of rodents and ungulates, with some suggesting homeostatic functions for PrP^C. Furthermore, given the wealth of PrP^C data in the brain, and since neurons and pancreatic beta cells share numerous similarities [21], the question arises whether PrP^C has a role in the pathogenesis of type 1 diabetes. Therefore, given the resemblance between neurons and beta cells, we investigated the cell-type specific expression of PrP^C in human pancreas and tested possible connections with potential interacting proteins to address mechanisms relevant to the role of PrP^C in the human pancreas. To our knowledge, this study is the first attempt to evaluate such connections, expression and localisation of cellular prion protein in unaffected and type 1 diabetic human pancreases.

Methods

Human organ donors

Pancreas samples were obtained from nPOD (www.jdrfnpod.org). Procedures were approved by the University of Florida Institutional Review Board and the United Network for Organ Sharing (UNOS) according to federal guidelines, and informed consent obtained from each donor's legal representative. For each donor, a medical chart review was performed and C-peptide measured with type 1 diabetes diagnosis confirmed according to ADA guidelines. Demographics, length of hospitalisation, and organ transport duration information was obtained from hospital records or UNOS (ESM Tables 1 and 2). Cases were selected to represent type 1 diabetic donors with residual insulin-positive [INS(+)] islets, and type 2 diabetic, autoantibody-positive (AAb+) and non-diabetic control donors. Randomisation was not performed whereas blinding was only utilised in the immunoblot analysis. No data were excluded.

RNA extraction and qRT-PCR

Human pancreas tissue was cryo-preserved by flash freezing or in RNAlater (Qiagen, USA), ~16 h from cross clamp. Total RNA was isolated as described [22] including treatment with DNase 1 and analysed by quantitative RT-PCR (qRT-PCR). RNA concentrations were determined using a Nanodrop 2000C (Thermo Scientific, USA) and, when necessary, integrity verified by visualisation of ribosomal RNA by gel electrophoresis and ethidium bromide staining. Samples were confirmed to be free of DNA contamination in no-reverse-transcriptase controls with an exon/intron primer pair. cDNA was produced with Superscript

II (Invitrogen, USA) using oligo dT priming and subsequently utilised for qRT-PCR using Thermo Luminaris qPCR Master Mix (Thermo Scientific, USA). One microgram of total RNA was used for each 20 µl cDNA reaction, which was then diluted to 200 µl and 2 µl of diluted cDNA was employed for each 25 µl qRT-PCR reaction containing 600 nmol/l of each primer pair. Individual qRT-PCR reactions were carried out in duplicate in a Bio-Rad MyiQ system. Normalisation factors, based on the geometric mean of three reference genes, were applied to the expression of all genes in respective pancreases, with the fold difference reported as the ratio of the means (type 1 diabetic/non-diabetic). All qRT-PCR parameters were standardised in accordance with the Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines [23].

Immunohistochemistry and immunofluorescence

Formalin-fixed paraffin-embedded sections (4 µm) were evaluated by immunolocalisation, after deparaffinisation and rehydration, blocking, and incubation with primary antibodies to cellular prion (Sigma-Aldrich, USA, #HPA042754, 1:100), insulin (Agilent, USA, #IR002, undiluted), glucagon (Abcam, USA, #ab10988, 1:1600), somatostatin (Agilent, USA, #A0566, 1:1000), stress-inducible phosphoprotein 1 (STI1, Novus, USA, #H00010963-M11, 1:50) and neural cell adhesion molecule 1 (NCAM1, Bio-Rad, USA, #MCA2693GA, 1:50). Detection of primary antibody was performed by multiplex staining using polymer horseradish peroxidase (Akoya Biosciences, USA, HRP, #ARH1001EA) followed by tyramine amplification (TSA detection system, Akoya Biosciences, USA, NEL741001KT/744001KT/745001K). To assess amyloid-like plaques, slides were stained with Congo Red (Newcomer Supply Copper, USA, #9103) according to the manufacturer's protocol. Consecutive slides were stained for PrP^C primary antibody and then stained with 0.05% thioflavin T (in 50% ethanol) for 8 min at room temperature. To assess immunolocalisation, slides were deparaffinised, rehydrated and subjected to heat-induced antigen retrieval. Slides were blocked using 5% normal goat serum and incubated in primary antibodies to Golgin A2 (GM130; Abcam, USA, #ab169276, 1:50), Wolfram syndrome 1 (WFS1; LSBio, USA, #LS-B14378, 1:200) and CD99 (BioLegend, USA, #318002, 1:2000) in Dako diluent (Dako, USA, #S080983-2) overnight. Primary antibodies were visualised with H+L Alexa Fluor secondary antibodies (Thermo Scientific, USA), followed by nuclear staining with DAPI counterstain. Slides were analysed using a BZ-X710 Keyence florescence microscope, USA.

Tissue homogenisation

Human pancreases were homogenised in modified RIPA buffer (50 mmol/l Tris-HCl pH 7.4, 150 mmol/l NaCl, 5 mmol/l EDTA, 1 mmol/l EGTA, 1% NP-40, 0.5% sodium deoxycholate and 0.1% SDS) with protease and phosphatase inhibitor cocktails (Thermo Scientific, USA, #78430 and #78428, respectively) using a Tissue Tearor (BioSpec, USA), further disrupted with a Vibra-Cell sonicator (Sonics & Materials, USA) and placed on ice for 15 min after each sonication step. Tissue homogenates were centrifuged at 14,000 *g* for 20 min at 4°C and supernatants collected for protein concentration assay using a Pierce BCA assay kit (Thermo Scientific, USA, #23227).

Immunoblotting

Forty micrograms of protein lysates in Laemmli's buffer containing 2.5% β-mercaptoethanol was boiled for 5 min and loaded on 4–20% gradient Mini-PROTEAN TGX Stain-Free gels (Bio-Rad, USA, #456-8095). Gels were activated using a Gel DOC EZ imager (Bio-Rad, USA) and transferred onto nitrocellulose membranes (Li-Cor, USA, #926-31092). Membranes were scanned with the Gel DOC™ EZ imager and total protein staining visualised and quantified using Image Lab software version 5.2.1 (Bio-Rad, USA). Membranes were washed and blocked for 1 h at room temperature with Intercept Blocking Buffer (Li-Cor, USA, #927–60001) and incubated at 4°C overnight with mouse anti-PrP^C monoclonal antibody (BioLegend, USA, #SIG-39620), in Intercept Antibody Diluent (Li-Cor, USA, #927–60001). Membranes were washed with Tris-buffered saline containing 0.1% Tween 20 (TBST) and incubated with goat anti-mouse IRDye 800CW (Li-Cor, USA, #926–32210;) secondary antibody for 1 h at room temperature. Membranes were washed 3 times with TBST at 5 min intervals. Immunoreactive bands were densitometrically analysed using Odyssey infrared scanner software version 3.1 (Li-Cor, USA). PrP^c monoclonal antibody specificity was validated in human brain tissue lysate (Fig. 4b), consistent with published reports [24].

Quantification and statistical analysis

Statistical variables, including exact *n*, mean and SD are reported in the figures and figure legends. Data were judged statistically significant when $p < 0.05$ by a two-sample *t* test with Welch's correction for qRT-PCR and immunoblot analyses. Data were analysed and graphed using GraphPad Prism software version 8 (GraphPad, USA).

Results

Cellular localisation of PrP^C in human pancreases

To investigate the cellular localisation of PrP^C in human pancreas, PrP^C, insulin-producing beta cells, glucagon-producing alpha cells and somatostatin-producing delta cells were studied by immunofluorescence on paraffin-embedded tissue sections. We studied 12 non-diabetic and 12 type 1 diabetic pancreases (the latter all containing both residual INS-positive [INS(+)] and INS-negative [INS(−)] islets), together with 12 AAb+ human organ donor pancreases. Pancreases were obtained from the Network for Pancreatic Organ Donors with Diabetes (nPOD) biobank, with organ donor metadata and de-identified patient numbering (ESM Tables 1 and 2). We analysed PrP^C expression in islets of non-diabetic pancreases (Fig. 1a) compared with the expression in INS(+) (Fig. 1e) and INS(−) islets (Fig. 1i) from type 1 diabetic donors. High PrP^C immunoreactivity was observed in islets of non-diabetic and type 1 diabetic donors with a low level of expression in the exocrine pancreas (Fig. 1a, e, i). We deduced that in nondiabetic and INS(+) islets of type 1 diabetic donors, PrP^C was expressed mainly in beta cells, based on co-expression with insulin (Fig. 1c, g for non-diabetic and type 1 diabetic islets, respectively). We confirmed co-registration of PrP^C with insulin in beta cells by 3D image reconstruction analysis (Fig. 1d, h). Of particular note, even in the absence of insulin, PrP^C continues to be expressed in a large number of cells in type 1 diabetic INS(−) islets (Fig. 1i, k, l), implicating its co-expression

in an alternative endocrine cell type. These immunofluorescence results were consistently observed in all 12 non-diabetic and type 1 diabetic donor pancreases.

We found no co-registration of PrP^C with glucagon in both non-diabetic and type 1 diabetic INS(+) islets (Fig. 2a–j). However, by contrast, PrP^C was found expressed in alpha cells in type 1 diabetic INS(−) islets (Fig. 2k–o), suggesting that, in the absence of beta cells, PrP^C expression is activated in alpha cells. We also evaluated PrP^C expression in conjunction with somatostatin and found no evidence of PrP^C expression in somatostatin-positive delta cells in either non-diabetic or type 1 diabetic pancreases (ESM Fig. 1).

Intracellular localisation of PrP^C in the human pancreas

The majority of data on PrP^C trafficking is derived from results in the brain and spinal cord. In neurons, PrP^C is synthesised and processed following a pathway similar to other membrane or secreted proteins [8]. Synthesis originates on endoplasmic reticulum (ER)-bound ribosomes where a C-terminal, glycosyl-phosphatidylinositol (GPI) lipid anchor is added, followed by transit through the Golgi apparatus leading to GPI-dependent anchoring on the extracellular leaflet of lipid rafts [25]. To evaluate PrP^C intracellular localisation within human pancreatic endocrine cells, we performed co-staining of PrP^C with compartment-specific markers for ER (WFS1; [26]), Golgi (GM130; [27]) and plasma membrane (CD99; [28]). PrP^C was found to co-register with WFS1 (Fig. 3a, d, g) and CD99 (Fig. 3c, f, i) but not GM130 (Fig. 3b, e, h), based on 3D image reconstruction analysis, indicating that PrP^C is associated mainly with the ER and plasma membrane of beta cells in non-diabetic and type 1 diabetic INS(+) islets and in alpha cells in type 1 diabetic INS(−) islets. These results clearly demonstrate that PrP^C is not associated with the Golgi apparatus, as shown in Fig. 3b–h, together with additional patient data in ESM Fig. 2, suggesting that PrP^C in pancreatic endocrine cells is synthesised in the ER utilising an unconventional secretory pathway that bypasses the Golgi to reach the plasma membrane [29, 30].

PRNP gene and PrP^C protein expression in non-diabetic, type 1 diabetic and AAb+ organ donor human pancreases

To evaluate *PRNP* gene expression, mRNA expression levels were measured using qRT-PCR from total RNA isolated from freshly frozen pancreases from 29 non-diabetic and 29 type 1 diabetic organ donors (Fig. 4a). qRT-PCR was performed following strict MIQE guidelines by normalisation with the geometric mean of three reference genes [23]. The quantitative fold difference in gene expression between these two groups is reported as the ratio of the means. *PRNP* gene expression in type 1 diabetic donors revealed a highly significant increase of 10.85-fold; $p \leq 0.0001$ compared with non-diabetic donors (Fig. 4a). To test whether changes in *PRNP* mRNA levels leads to a concomitant increase in PrP^C protein levels, we performed PrP^C-specific immunoblot analysis on total pancreatic protein from non-diabetic and type 1 diabetic organ donors (Fig. 4b) with images for the intact blot and total protein stain included in ESM Fig. 3. The right panel illustrates the migration pattern for PrP^C in a human brain extract, for comparison (Fig. 4b, right). Consistent with the mRNA results (Fig. 4a), Fig. 4c provides a quantitative assessment of PrP^C protein levels from ten non-diabetic and ten type 1 diabetic donors, normalised to total protein, illustrating a significant increase of 2.16-fold between the type 1 diabetic and the non-diabetic groups.

We also evaluated *PRNP* gene expression in 23 AAb+ donor pancreases, from individuals at risk for type 1 diabetes (as the propensity for disease onset increases in the presence of multiple autoantibodies), and found a significantly decreased level of mRNA expression compared with the non-diabetic cohort (0.579-fold; $p \leq 0.012$; Fig. 4d). Given that PrP^C may function as an anti-apoptotic or cytoprotective protein [3], we also evaluated the expression of PrP^C in AAb+ donors by immunofluorescence and, as with the non-diabetic and type 1 diabetic donors, PrP^C was expressed primarily in islets and specifically in beta cells, based on co-registration with insulin and not glucagon (ESM Fig. 4). We also observed that in the majority of the 12 studied AAb + donors, PrP^C levels in pancreatic islets were consistently lower compared with non-diabetic islets, as observed for patient 6450 using identical imaging parameters (ESM Fig. 4).

PrP^C does not co-register with protein aggregates in type 1 diabetic pancreases

The hallmarks of prion-related diseases include protein aggregates and amyloid plaques, which in most cases positively stain for PrP^{Sc} [4, 8–11]. Similarly, islet amyloid has been most strongly associated with type 2 diabetes pathogenesis along with type 1 diabetes and cystic fibrosis-related diabetes (CFRD) [31–36]. To evaluate whether PrP^C co-registers with islet-associated protein aggregates previously documented in type 1 diabetic pancreases [31, 33], we identified three type 1 diabetic pancreases displaying protein aggregates from among our 12 type 1 diabetic donors, based on detection of amyloid using Congo Red stain [37] (Fig. 5a–c). A pancreatic section from a type 2 diabetic donor with known presence of amyloid was used as a positive control (Fig. 5d). To investigate whether these protein aggregates co-registered with PrP^C, we took consecutive sections from each of these donors and co-stained with a PrP^C antibody and thioflavin T (an additional amyloid marker) [38], with co-detection by fluorescence microscopy (Fig. 5e–h). Thioflavin T-positive regions of each islet correlated with the Congo Red staining; however, we found no colocalisation of PrP^C with the thioflavin T-positive regions in either the type 1 or type 2 diabetic pancreases.

STI1 and NCAM1 co-register with PrP^c in islets: implications for stress protection and cell morphogenesis

With no consensus on the physiological function of PrP^C [8], a powerful approach to infer function is by studying potential PrP^C binding proteins with known biological roles [13]. We utilised the Protein–Protein Interaction Networks Functional Enrichment Analysis software, STRING 11.0 (https://string-db.org), to generate a PrP^C-predicted human protein–protein interaction model (ESM Fig. 5). Based on this model and our own corroborating literature review, STI1 (encoded by *STIP1*) and NCAM1 (also known as CD56) were especially promising PrP^C binding partners [39, 40].

As a co-chaperone protein, STI1 coordinates the functions of heat-shock protein 70 (HSP70) and 90 (HSP90) in protein folding [41], whereas NCAM1 is a cell adhesion protein and member of the immunoglobulin superfamily directing cell–cell and cell–matrix interactions [42]. Zanata et al [43] demonstrated that interaction of PrP^C with STI1 activated anti-apoptotic pathways providing significant neuroprotection, as well as effects on neuritogenesis and survival of hippocampal neurons [44]. Mehrabian et al [45] have

demonstrated that, during epithelial-to-mesenchymal transitions (EMT), *PRNP* transcription is increased >10-fold and correlates with the polysialylation of NCAM1, controlled by PrP^C.

To investigate the expression of these prion interacting proteins in human pancreas, we stained pancreatic tissue sections from non-diabetic and type 1 diabetic donors for STI1, and found that STI1 is highly expressed in nondiabetic and type 1 diabetic INS(+) and INS(−) islets, together with lower levels in the exocrine pancreas (Fig. 6b, f, j). Moreover, we observed that STI1 highly co-registers with PrP^C primarily in the cytosol in islets, regardless of their insulin positivity, as shown in the 3D reconstruction images (Fig. 6d, h, l). As expected, co-registration of STI1 with insulin- and glucagon-positive cells was also observed in all three islet types (ESM Fig. 6). Interestingly, we determined that *STIP1* mRNA levels in type 1 diabetic donors were notably increased compared with the non-diabetic cohort (Fig. 6m: 13.42-fold; $p \leq 0.0001$). We also compared *STIP1* mRNA levels in non-diabetic and AAb+ donors, which showed a modest but significant increase (Fig. 6n: 2.09-fold; $p = 0.00641$. We similarly investigated NCAM1 and found that, in contrast to STI1, PrP^C co-registers with NCAM1 primarily at the cell membrane (Fig. 7a–l), thus establishing separate interacting partners for the two cellular pools of PrP^C in the human pancreas, with potentially independent cellular roles.

Discussion

The yin and yang prion proteins, PrP^C and PrP^{Sc}, have established themselves as singularly famous and infamous, in that the normal protein, after years of intense investigation lacks a consensus function, whilst the concept of an infectious protein particle remains a difficult concept to grasp. Previous studies implicated a unique expression of PrP^C in rodent and ungulate pancreatic islets, with a potential functional role for PrP^C in glucose homeostasis [15–18]. For example, with regard to normal or pathophysiological states, several studies in PrP^C knockout mice have demonstrated a role for PrP^C in gluco-regulation [17], together with a PrP^C-mediated modulation of glucose homeostasis through regulation of intracellular iron stores in pancreatic beta cells [15]. We have corroborated these findings by showing similar in situ expression of PrP^C in non-diabetic human islets in organ donor pancreases, specifically in beta cells, and extended these results to demonstrate that PrP^C is also expressed in type 1 diabetic islets that still harbour INS(+) beta cells. Strikingly, islets lacking beta cells [INS(−)] are still positive for PrP^C, but have switched expression of PrP^C to alpha cells. These results may implicate a beta cell regeneration mechanism relevant to the observation by Chakravarthy et al [46] that in type 1 diabetes patients, loss of DNA methyltransferase 1 (DNMT1) and aristaless related homeobox (ARX) in subsets of glucagon-expressing alpha cells leads to a population of cells expressing insulin and other beta cell-specific factors. The INS(−) islets may therefore be initiating a regeneration or survival mechanism involving PrP^C-positive alpha cells as beta cell progenitors. This is consistent with a purported role for PrP^C in pluripotency and stem cell differentiation [47, 48] and consistent with PrP^C interaction with NCAM1, discussed below.

As one might expect, PrP^C is expressed in beta cells in islets from AAb+ pancreases, identically to our non-diabetic cohort. However, close visual examination of numerous islets from each of our 12 AAb+ organ donor pancreases indicate that a vast majority, although

not quantified, appear to have diminished PrP^C expression compared with non-diabetic islets. This observation is in complete alignment with significantly diminished expression of *PRNP* mRNA in AAb+ pancreases observed from these donors. Of note, equally diminished expression is observed between AAb+ donors with single or multiple autoantibodies, with, in fact, ~75% of our cohort derived from single AAb+ donors. As a component of the cellular differentiation machinery, the reduction in PrP^C expression in AAb+ cohort could signal a prelude to a reduced capacity for beta cell regeneration, prior to extensive beta cell loss associated with insulin therapy accompanying type 1 diabetes diagnosis.

Another unique observation in the human pancreas from our studies indicates two cellular pools of PrP^C, localised to either the ER or plasma membrane, with the lack of expression in the Golgi apparatus in both beta and alpha cells. Conventional protein trafficking for secreted, integral membrane and GPI-anchored proteins involves synthesis in the ER, transit through the Golgi and vesicular transport of each of these protein classes to the plasma membrane [49, 50]. On the basis of our results, we propose that PrP^C in the human pancreas may use an unconventional protein trafficking mechanism that bypasses the Golgi, employing either a Golgi reassembly stacking protein 2 (GRASP55) or HSP70–DnaJ heat shock protein family (Hsp40) member C14 (DNAJC14) transport pathway [51].

In addition to the type 1 diabetes-dependent transition of PrP^C expression from beta to alpha cells in INS(−) islets, we have demonstrated a significant increase in *PRNP* transcription and protein synthesis in type 1 diabetic pancreases compared with our non-diabetic cohort. With no biological function for PrP^C to rationalise the increased levels in pancreases from the type 1 diabetic cohort, we studied the expression of PrP^C-interacting partners with known biological function to help provide a link to type 1 diabetes pathophysiology. Foremost, we found that PrP^C within its two distinct cellular pools co-registers with the co-chaperone STI1 in the ER/cytosol, whilst the PrP^C membrane fraction interacts with the cell adhesion protein, NCAM1. The existence of these two protein-protein complexes provides the opportunity to hypothesise on potential roles for PrP^C in type 1 diabetes pathogenesis. Based on its interaction with STI1 numerous studies have implicated the stress-protective properties of PrP^C [8, 43, 44, 52, 53]. In our results, not only did we observe type 1 diabetes-dependent increases in PrP^C expression, but we also demonstrate a parallel increase in gene expression for its binding partner *STIP1* in the type 1 diabetic pancreas. Therefore, increased levels of the PrP^C–STI1 complex may be an attempt by type 1 diabetic islets to induce cytoprotective measures circumventing further endocrine cell loss, potentially offering a therapeutic opportunity to stimulate alpha to beta cell replenishment. Furthermore, it has been shown that PrP^C can protect against Bax-mediated cell death in human primary neurons and cancer cells by inhibiting Bax activation through a pro-apoptotic conformational change and subsequent cytochrome C release [8, 54, 55]. This cytoprotective role may also be important in type 1 diabetes, based on recent evidence that loss of Bax and Bak protect against beta cell death under conditions of glucolipotoxicity and chronic ER stress [56].

Separately, co-registration of PrP^C at the membrane with NCAM1 may support a putative function in cell signalling, differentiation and morphogenesis in the human pancreas [39, 40, 45]. For example, Santuccione et al [57] have shown that PrP^C mediates the recruitment

of NCAM1 to lipid rafts leading to cellular signalling through activation of the proto-oncogene, tyrosine kinase, FYN. In relation to the comparative evolution of neurons and beta cells, PrP^C also regulates the neuronal polysialylation of NCAM1 thus controlling cell migration, axonal and dendritic projection, and synaptic targeting along with morphogenetic reprogramming specific to the epithelial-to-mesenchymal transition [58]. Linden [59] has, thus, consolidated results detailing a variety of potential PrP^C functions and proposed that PrP^C may propagate in lipid rafts a flexible cell-surface scaffold for the recruitment of a variety of signalling modules, thus providing a dynamic and adaptable platform for roles in physiology, behaviour and pathophysiology.

Our results on PrP^C expression and tissue and intracellular localisation, as well as PrP^C-interacting protein partners in non-diabetic, type 1 diabetic and AAb + pancreases provide potential new insights into the role PrP^C may play in the pathogenesis of type 1 diabetes. A schematic summary of our results is depicted in Fig. 8. We recognise that future efforts using alternative methods (e.g. isolated islets, surrogate beta cell lines, pancreatic slice cultures) may afford advances into an improved mechanistic understanding. It is, however, exciting to speculate that intervention through PrP^C protein–ligand interactions could be therapeutic in type 1 diabetes. However, without a universally accepted function, further mechanistic studies in models of type 1 diabetes remain highly speculative.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data availability

Application for datasets generated during and/or analysed during the current study may be considered by the corresponding author on reasonable request.

Abbreviations

AAb+	Autoantibody-positive
ER	Endoplasmic reticulum
GM130	Golgin A2

GPI	Glycosyl-phosphatidylinositol
HSP	Heat-shock protein
INS(+)	Insulin-positive
INS(−)	Insulin-negative
MIQE	Minimum Information for Publication of Quantitative Real-Time PCR Experiments
NCAM1	Neural cell adhesion molecule 1
PrP^C	Cellular prion protein
PrP^{Sc}	Scrapie form of prion protein (pathogenic, alternatively folded aggregate)
qRT-PCR	Quantitative RT-PCR
STI1	Stress-inducible phosphoprotein 1
UNOS	United Network for Organ Sharing
WFS1	Wolfram syndrome 1

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Research in context

What is already known about this subject?

- The normal cellular prion protein (PrP^C) is a conserved mammalian glycoprotein found on the outer plasma membrane and it is expressed by a wide range of tissues throughout the body
- The complete repertoire of its functions has not been fully determined, but a folded aggregate version (PrP^{Sc}) is linked to neurodegenerative disorders affecting both animals and humans
- PrP^C has been linked to modulation of signalling pathways, neuroprotection, anti-apoptotic activity and glucose homeostasis, among other things

What is the key question?

- Does PrP^C localise in human pancreases from organ donors with and without type 1 diabetes, and does it interact with partner proteins to affect cell function?

What are the new findings?

- PrP^C is highly expressed in the pancreatic islets of non-diabetic and autoantibody-positive donors and of donors with type 1 diabetes and with insulin-positive islets, and co-registers almost exclusively with beta cells
- In pancreatic endocrine cells PrP^C is synthesised in the endoplasmic reticulum, but utilises an unconventional secretory pathway that bypasses the Golgi to reach the plasma membrane
- Cellular prion protein co-registers with STI1 partner protein suggesting a PrP^C–STI1 complex that might induce cytoprotective measures to circumvent further endocrine cell loss

How might this impact on clinical practice in the foreseeable future?

- Our results provide potential new insights into the role that PrP^C may play in the pathogenesis of type 1 diabetes

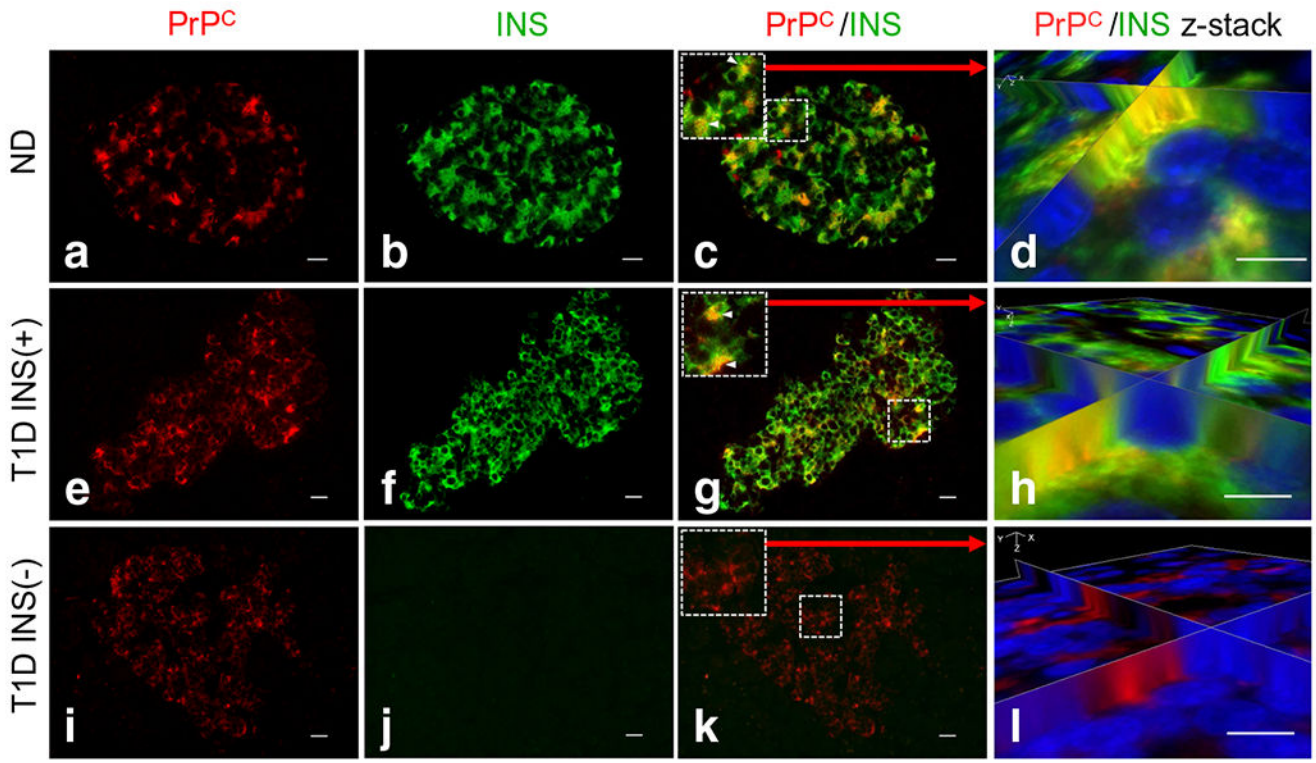

Fig. 1.

Cellular localisation of PrP^C in beta cells of human pancreases based on immunofluorescence analysis, **(a, e, i)** PrP^C was observed to be highly expressed in pancreatic islets of all three islet types [non-diabetic; type 1 diabetic INS(+); type 1 diabetic INS(−)] with low level expression in the exocrine pancreas, (**a**–**d**) PrP^C expression in non-diabetic donors localises to pancreatic islets and co-localises with beta cells (**c**). Co-registration of PrP^C/INS cells was confirmed using z-stack 3D image reconstruction analysis (**d**). (**e, i**) In our analysis of PrP^C in type 1 diabetic pancreas, we separated images from donors that contain both INS(+) (**e**) and INS(−) islets (**i**). (**e**)–**h**) As with non-diabetic donors, type 1 diabetic INS(+) islets show co-registration between PrP^C and INS cells (**g, h**). (**i**–**l**) In the absence of insulin [INS(−)], PrP^C continues to be expressed in a large number of cells (**i, k**). Scale bars, 20 αm. INS, insulin; ND, non-diabetic, T1D, type 1 diabetic

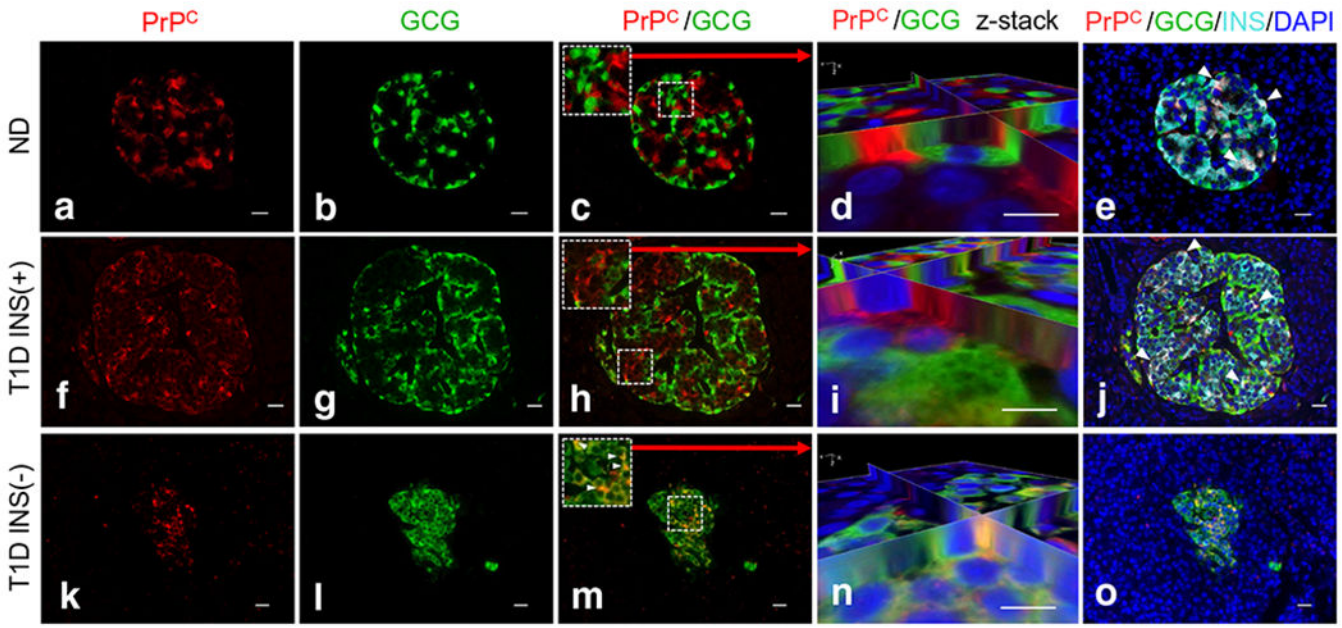

Fig. 2.

Cellular localisation of PrP^C in alpha cells of human pancreases based on immunofluorescence analysis. (**a**)–(**j**) We found no co-registration of PrP^C with glucagon in either non-diabetic or type 1 diabetic INS(+) islets (**k**)–(**o**) but did find co-localisation of PrP^C with insulin (white arrowheads **e** and **j**). PrP^C was found to be co-localised with alpha cells in type 1 diabetic INS(−) islets, suggesting that PrP^C expression is activated in alpha cells in the absence of beta cells in these islets. Co-registration was confirmed using z-stack 3D image reconstruction analysis shown in (**n**). Scale bars, 20 μm. GCG, glucagon; INS, insulin; ND, non-diabetic, T1D, type 1 diabetic

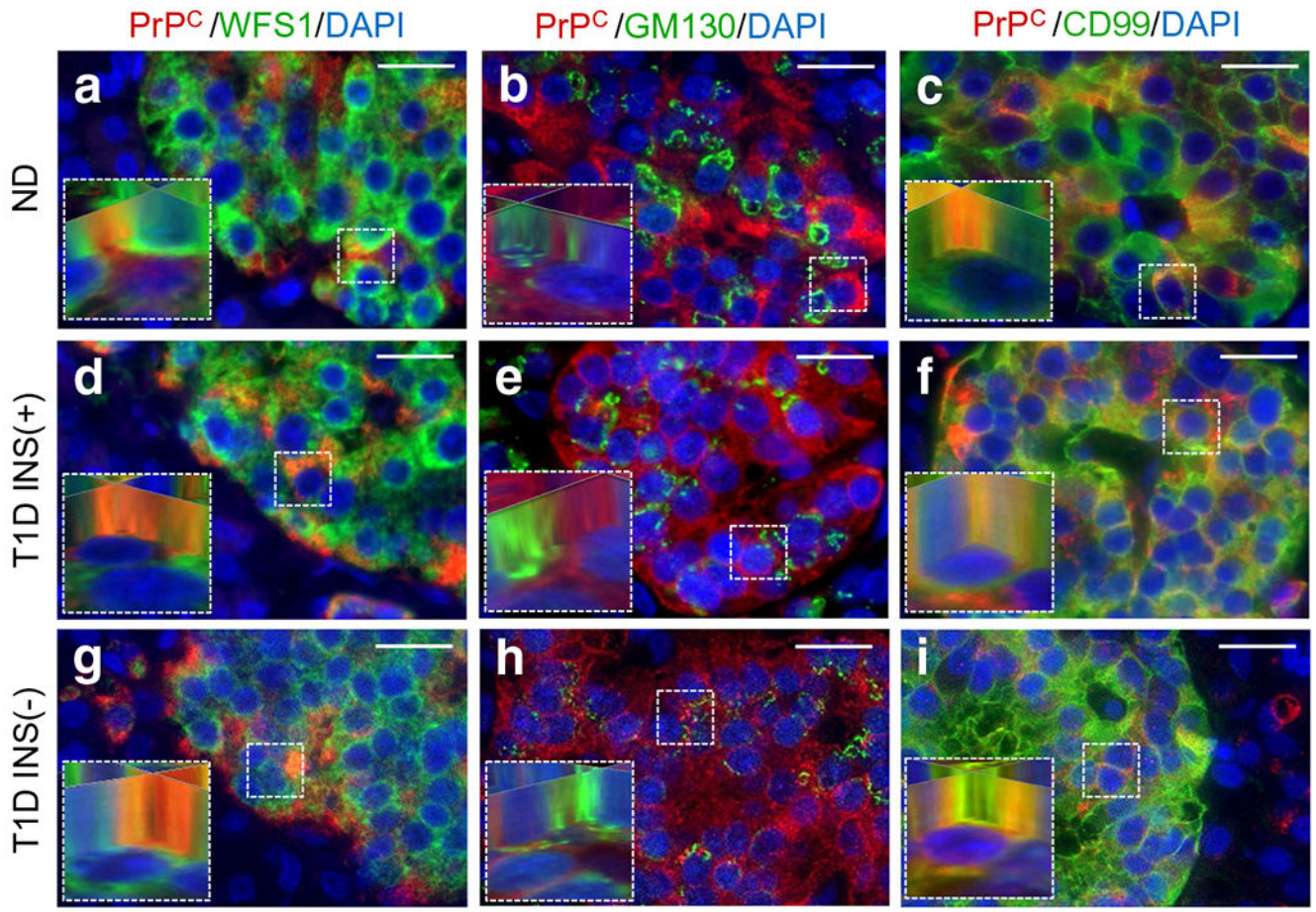

Fig. 3.

Intracellular localisation of PrP^C in the human pancreas. Specific co-staining of PrP^C and cellular markers for ER (WFS1), Golgi (GM130) and cell membrane (CD99) staining was implemented to assess PrP^C intracellular localisation within endocrine cells of the human pancreas. PrP^C was found to co-register with WFS1 (**a, d, g**) and CD99 (**c, f, i**) but not GM130 (**b, e, h**; ESM Fig. 2) based on 3D image reconstruction analysis (insets), indicating that PrP^C is mainly associated with the ER and plasma membrane of pancreatic endocrine cells. Scale bars, 20 μm. ND, non-diabetic, T1D, type 1 diabetic

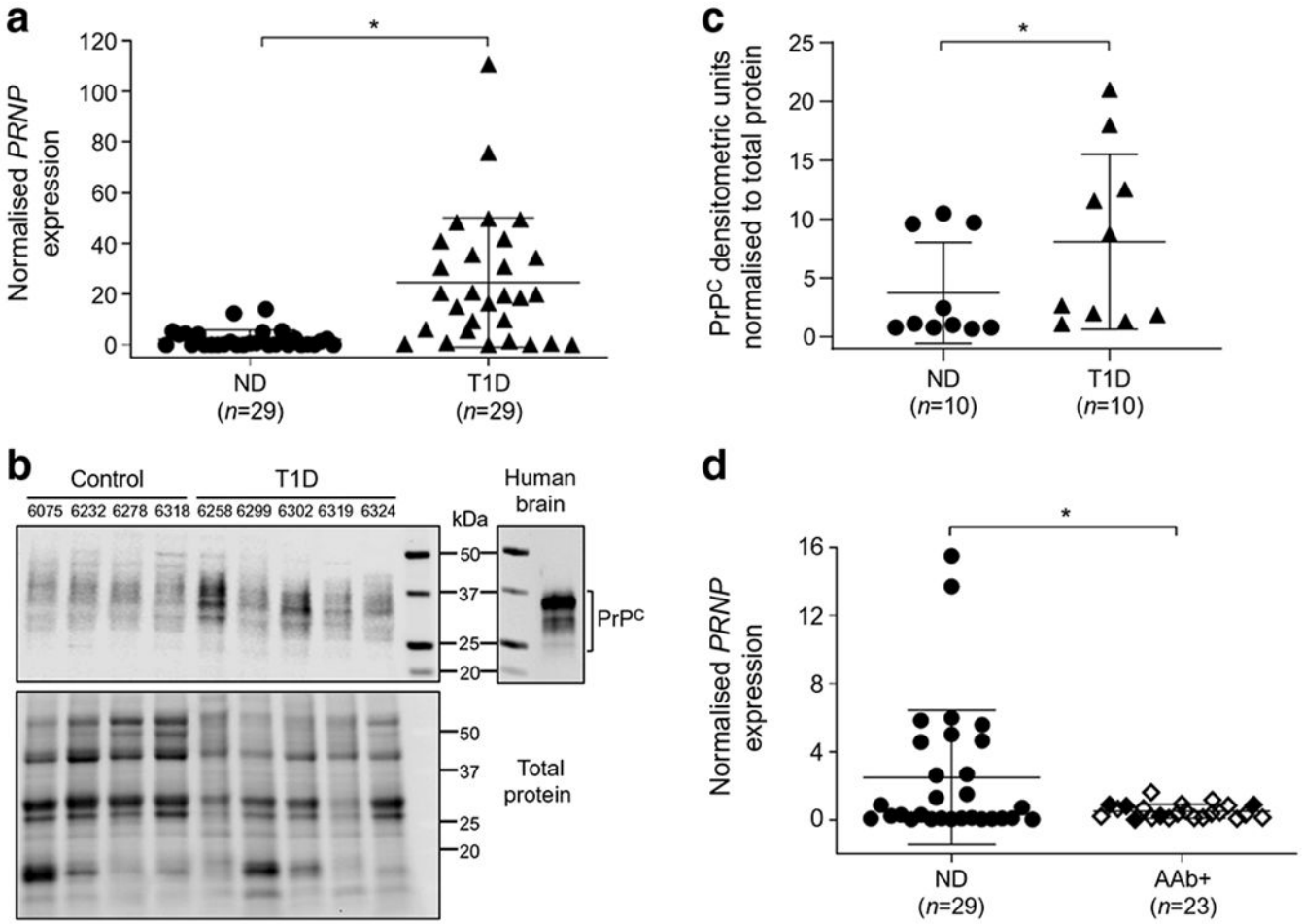

Fig. 4.

PRNP gene and PrP^C protein expression in non-diabetic, type 1 diabetic and AAb+ organ donor human pancreases. *PRNP* mRNA expression levels were measured by qRT-PCR from isolated total pancreatic RNA from 29 non-diabetic, 29 type 1 diabetic and 23 AAb+ donors. (**a**) *PRNP* gene expression in type 1 diabetes revealed a highly significant increase of 10.85-fold ($p \leq 0.0001$) compared with non-diabetic donors. (b) Representative PrP^C-specific immunoblot analysis on total pancreatic protein isolated from non-diabetic and type 1 diabetic organ donors was performed to test changes in PrP^C mRNA levels. Right panel: the migration pattern of PrP^C in human brain extracts; left panel: immunoblot analysis of purified human pancreas extracts of four representative non-diabetic and five representative type 1 diabetic donors. Full immunoblot for PrP^C and total protein can be found in ESM Fig. 3. (**c**) Densitometric analysis data from immunoblot shown in (**b**) combined with additional data (6 more non-diabetic; 5 more type 1 diabetic donors) normalised to total protein, illustrating a concomitant increase in PrP^C levels of 2.16-fold ($p = 0.0433$). (**d**) In contrast, AAb+ donors exhibited significantly lower *PRNP* mRNA levels when compared with non-diabetic donors (0.579-fold) ($p = 0.012$; white diamond, single AAb+; black diamond, multiple AAb+). *$p < 0.05$. ND, non-diabetic; T1D, type 1 diabetic

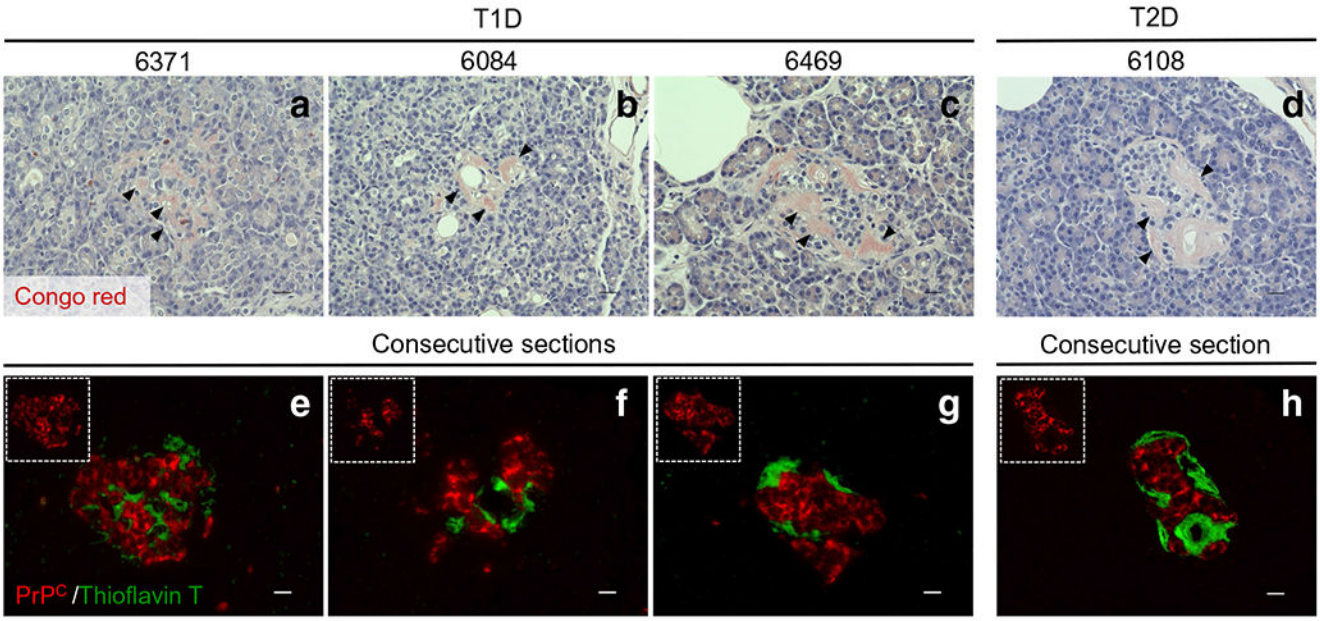

Fig. 5.

Assessment of possible interactions between PrP^C and protein aggregates in type 1 and type 2 diabetic donors. Among our 12 type 1 diabetic organ donors, three cases displayed amyloid-like plaques. In these three cases, we evaluated whether PrP^C co-localised with these islet-associated protein aggregates. (**a**)–**d**) The pathology stain, Congo Red, was used to identify amyloid/protein aggregates in the three type 1 diabetic donors as well as one type 2 diabetic donor (positive control). Black arrows show positivity for amyloid-like plaques (pink colour) in pancreatic islets. (**e**)–**h**) To visualise amyloid-like plaques and PrP^C expression together, consecutive slides were stained with thioflavin T (green) and PrP^C (red). We found no colocalisation of PrP^C-positive islet cells with the thioflavin T regions in either the type 1 or the type 2 diabetic pancreas samples. Scale bars, 20 μm. T1D, type 1 diabetic; T2D, type 2 diabetic

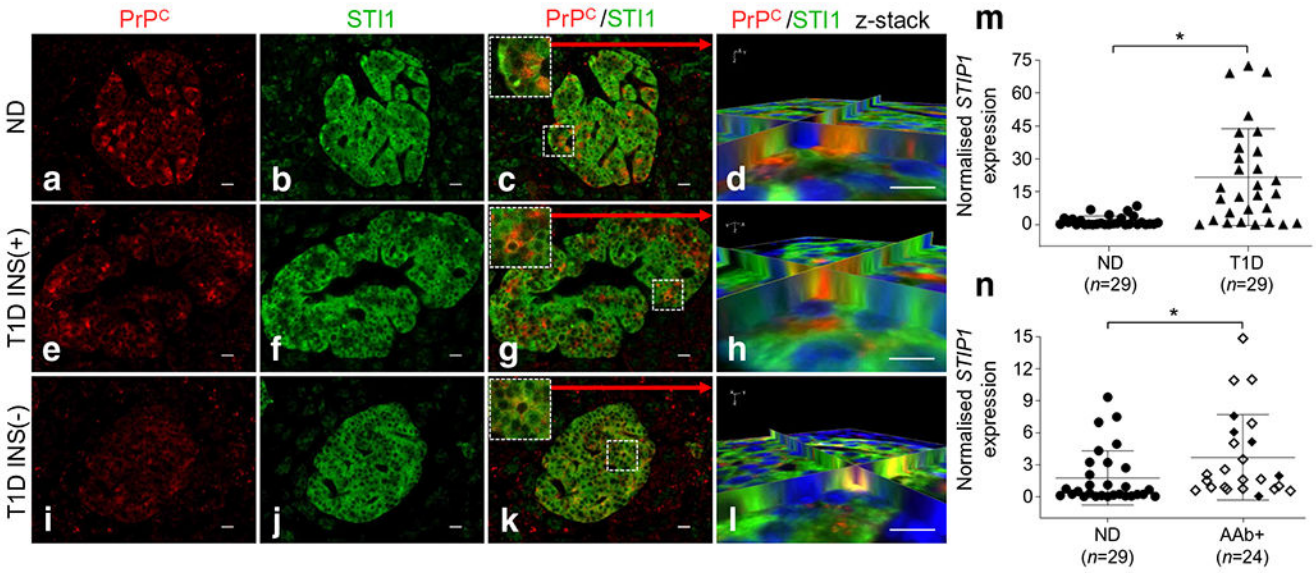

Fig. 6.

STI1 co-registers with PrP^C in islets. (**a, e, i**) PrP^C is expressed in non-diabetic, type 1 diabetic INS(+) and type 1 diabetic INS(−) islets. (**b, f, j**) STI1 is highly expressed in the pancreatic islets of non-diabetic, type 1 diabetic INS(+) and type 1 diabetic INS(−) islets. **(d, h, l)** We found that STI1 highly co-registers with PrP^C in all three groups, as shown in z-stack 3D reconstruction images. (**j, k, l**) Even with no insulin expression in INS(−) islets, STI1 expression is still evident, with co-registration with glucagon-positive cells (ESM Fig. 6). Scale bars, 20 μm. (**m**) *STIP1* total mRNA levels in type 1 diabetic donors is significantly increased compared with non-diabetic donors 13.42-fold ($p \leq 0.0001$). (**n**) We also compared mRNA levels in AAb+ donors to non-diabetic donors and observed increased *STIP1* expression of 2.09-fold ($p = 0.0064$) compared with non-diabetic donors (white diamond, single AAb+; black diamond, multiple AAb+). *$p < 0.05$. ND, non-diabetic; T1D, type 1 diabetic

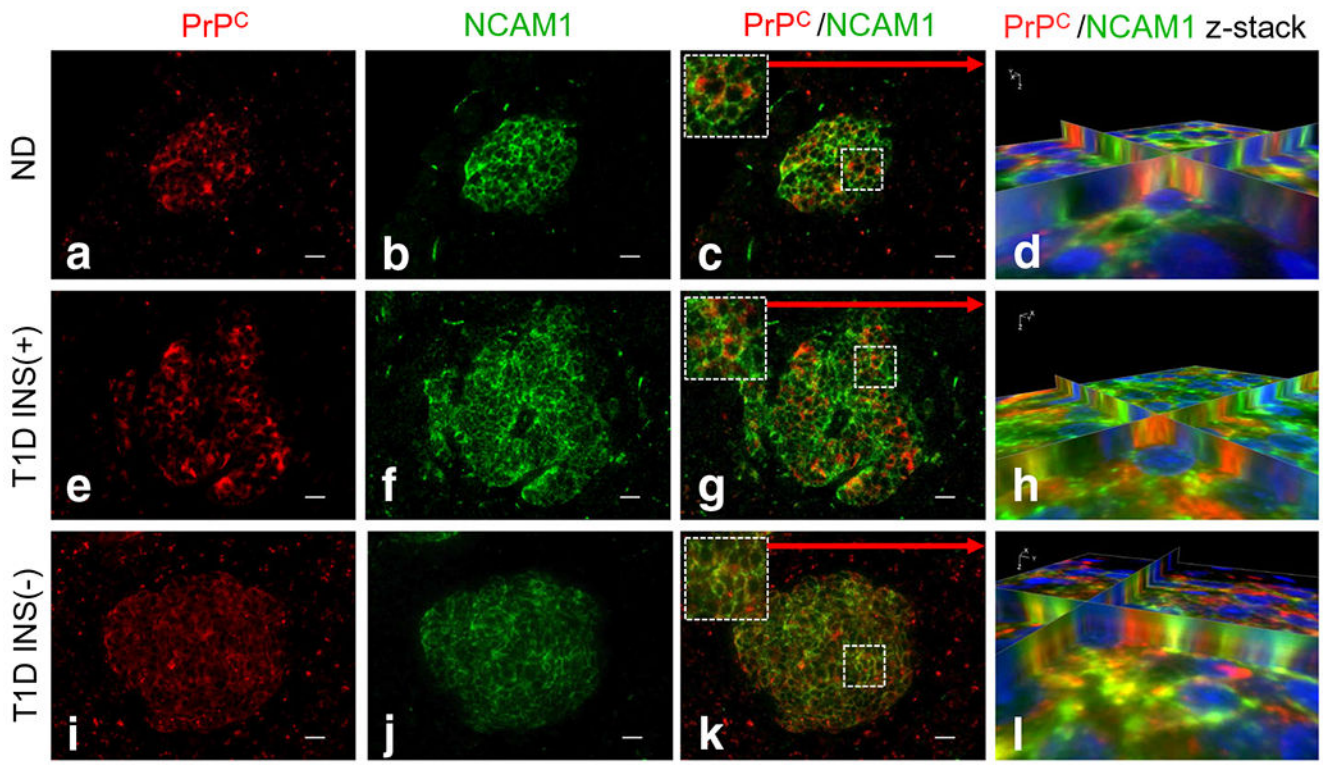

Fig. 7.

NCAM1 co-registers with PrP^C primarily in the cell membrane. (**a, e, i**) PrP^C is expressed in non-diabetic, type 1 diabetic INS(+) and type 1 diabetic INS(−) islets. (**b, f, j**) NCAM1 is highly expressed in the pancreatic islets of non-diabetic, type 1 diabetic INS(+) and type 1 diabetic INS(−) islets. (**c, g, k**) We found that NCAM1 highly co-registers with PrP^C in all three groups as shown in z-stack 3D reconstruction images **(d, h, l).** Scale bars, 20 μm. ND, non-diabetic; T1D, type 1 diabetic

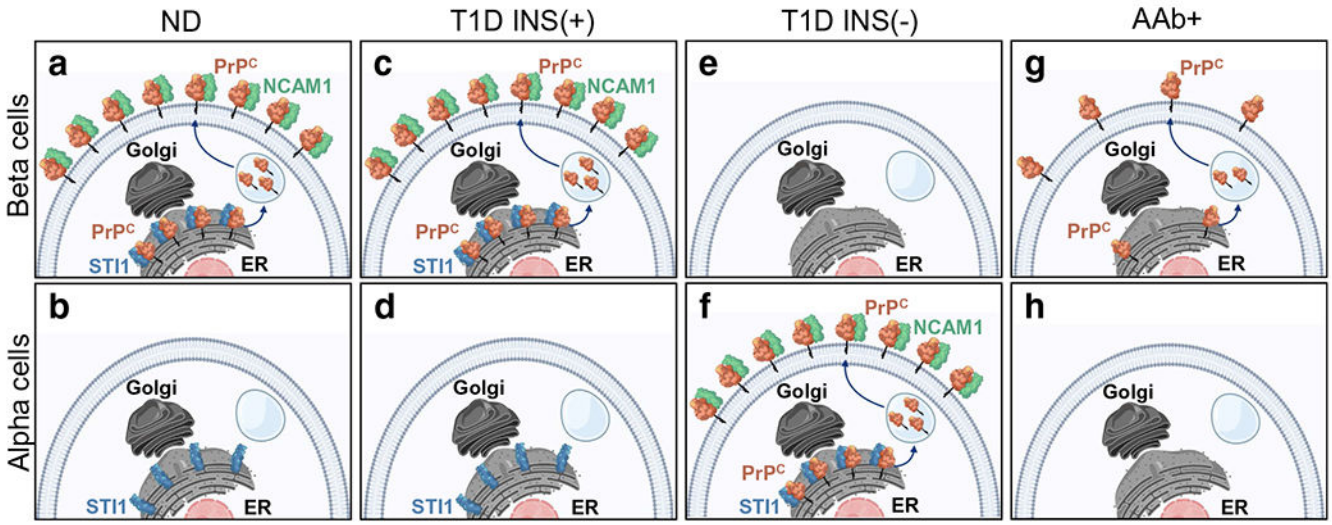

Fig. 8.

Schematic summary of human PrP^C cellular localisation, trafficking and interacting partners in beta and alpha cells in non-diabetic, type 1 diabetic and AAb+ human pancreas. (**a**) A summary of all the results in non-diabetic islets, including: PrP^C co-registration with beta cells; PrP^C existence in two cellular pools with interaction of PrP^C with NCAM1 on the plasma membrane and STI1 in the ER, along with the proposal that PrP^C must traffic from the ER to the plasma membrane bypassing transit through the Golgi. For illustration, the unconventional protein trafficking mechanism is depicted using vesicle-mediated transit from the ER to the plasma membrane. (**a, c, g**) PrP^C co-registers with beta cells in non-diabetic, type 1 diabetic INS(+) islets and AAb+ islets, with no co-registration with alpha cells (**b, d, h**). (**e, f**) PrP^C co-registers with alpha cells in type 1 diabetic INS(−) islets. (**g**) PrP^C is expressed at significantly lower levels in AAb+ beta cells compared with non-diabetic and INS(+) islets (**a, c**). Note: STI1 and NCAM1 were not measured in AAb+ (**g, h**). Graphic design created in biorender.com (https://biorender.com)