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Abnormal Expression of an Insulin Synthesizing Enzyme in Islets of Adult Autoantibody Positive Donors

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Summary
The observation that the two active forms of proprotein convertase 1/3 (PC1/3) were differentially expressed in beta cells of normal islets raised the possibility that this heterogeneity is lost during type 1 diabetes (T1D) progression. To test this hypothesis, the expression of the convertase was evaluated by confocal microscopy in sections of human pancreas of autoantibody positive (AA+) and T1D donors and compared with that of control. Islets of T1D pancreas were comprised of beta cells expressing either low or high PC1/3 levels and all islets of a pancreatic section contained only one beta cell type. Pancreata of AA+ donors contained either of these two classes of islets intermixed with normal islets comprised of beta cells with heterogeneous PC1/3 expression. This alteration affected the expression of proinsulin and insulin, which in most AA+ and T1D donors were lower than in controls. The present results indicate that the heterogeneity of PC1/3 expression is lost in all beta cells in a subset islets of AA+ donors and in all islets of T1D donors. These findings suggest that the heterogeneity of PC1/3 expression is a biomarker of human beta cell health and that its loss coincides with the initial stages of T1D. (J Histochem Cytochem 70: 695–706, 2022)

Keywords
insulin synthesizing enzyme, PC1/3, proinsulin, proprotein convertase 1/3, type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an autoimmune disease where auto-aggressive CD8+ T-cells infiltrate the islets causing beta cell death.[1–4] The development of autoimmunity is marked by the appearance of autoantibodies in the circulation. These autoantibodies are not believed to be pathogenetic but serve as biomarkers that may predict destructive autoimmunity. It has been reported that patients positive for autoantibodies show an elevation of the proinsulin to insulin ratio in blood, which indicates the presence of alteration in proinsulin processing.[5,6] These observations suggest that beta cell deficiency precedes the diagnosis of hyperglycemia.[7–9] While the identity of the cellular signals responsible for the appearance of pathological traits in islet cells before T1D diagnosis is currently unknown, there is a current view that the triggering defect lies within the beta cell itself.[10]

To gain an insight into possible causes of beta cell dysfunction, the current study sought to ascertain whether the expression of three markers of beta cell activity become abnormal before T1D diagnosis in humans. Those three molecules are the hormone precursor proinsulin, the main enzyme involved in the conversion of proinsulin into insulin termed proprotein convertase 1/3 (PC1/3),[11–14] and the mature hormone. We previously reported that normal human beta cells differ in their level of expression of PC1/3.[15] Correlation of the expression of the convertase with that of proinsulin and insulin in individual beta cells revealed the presence of three cell types in human islets. One of these cell types expressed ProIN but lacked PC1/3 (ProIN+ PC1/3–), a second beta cell type had both ProIN and PC1/3 (ProIN+ PC1/3+) expression, with

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variable levels of the proconvertase, while a third cell type lacked ProIN but expressed the converting enzyme (ProIN– PC1/3+). All three cell types contained insulin, but the ProIN+ PC1/3– cells had low levels of hormone expression.[16] This analysis suggested that the three beta cell types reflected different stages of proinsulin processing and implied the presence of a critical relationship between the levels of the proconvertase and proinsulin to produce the mature hormone.

The present study sought to determine whether the heterogeneity of PC1/3 expression characteristic of normal human beta cells is abrogated in pancreatic islets during progression to T1D diagnosis and if so, whether those alterations are correlated with defects in the expression of insulin and proinsulin. The expression of proSAAS, a regulator of PC1/3 activity,[17–19] was also examined.

Methods

Paraffin sections of human pancreas were provided by the network of Pancreatic Organ Donors (nPOD). Immunohistochemical staining of sections was carried out essentially as previously described.[16] Briefly, monoclonal antibody to proinsulin was purchased from Novocastra (PROIN-1G4; Buffalo Grove, IL) and from the Developmental Biology Hybridoma Bank (GS-9A8; Nashville, TN). These two antibodies recognize the junction between B-chain and C-peptide and label both intact proinsulin and the low abundant proinsulin conversion intermediate des-64,65 proinsulin.[20,21] Rabbit antibody to PC1/3, a generous gift from Iris Lindberg (University of Maryland), was generated by D.F. Steiner (University of Chicago). This antibody recognizes the active 87 and 66 kDa forms of the enzyme.[22] Guinea pig antibodies to insulin (Linco, EMD Millipore, Burlington, MA) colocalized in beta cells with mouse anti-insulin (05-1108; EMD Millipore). The mouse MAb binds to mature insulin and/or the des-31.32 proinsulin intermediate that has been processed by both PC1/3 and CPE[21] suggesting similar properties for the Linco guinea pig antibody. Rabbit antibody to proSAAS was a generous gift from Dr. I. Lindberg (University of Maryland) who also determined its specificity to that molecule.[23,24] Secondary antibodies such as Antirabbit Alexa fluor 488 IgG (green) and goat-anti mouse Alexa fluor 594 (red) were purchased from Molecular Probes (Eugene, OR); donkey-anti guinea pig Cy5 IgG (blue) was purchased from Jackson ImmunoResearch (West Grove, PA).

Sections were heated in 10 mmol/l citrate buffer, pH 6, for 30 min in a water bath at 100C and incubated overnight at 4C with the three primary antibodies. The following day, sections were incubated with the corresponding secondary antibodies for 1 hr at room temperature and cover slipped.

Fluorescence Microscopy

Images were captured with a Leica SP5 confocal microscope and most islets from each stained section were captured. Confocal single plane images were acquired with an Argon (488 nm), HeNe (543 nm), and HeNe$^{(633)}$ laser source using an oil immersion 40 × lens of 1.25 NA. Images were scanned sequentially to prevent crosstalk between the three fluorophores. The same confocal microscope setting for laser power, photomultiplier voltage gain, offset, and pinhole value were used to collect the signals. Characteristics of the donors examined are presented in Table 1.

The insulin, PC1/3, and ProIN positive area of each islet was determined using Image J (National Institute of Health, NIH) and expressed as a percentage of the total islet area. To eliminate the unidentified green cells from the fluorescent intensity of PC1/3, images of islets that were double-labeled for PC1/3 (green) and insulin (blue) were first delineated using the free-hand selection of Image J. The selection included blue/green cells (insulin + PC1/3) cells but avoided green non-insulin cells. Then, using the color-balance tool, the blue component of the image was eliminated, and a measurement of the selected area was determined. At least 15 islets from autoantibody positive donors/tissue section and 5 to 10 islets from T1D donors were evaluated.

Statistical Analysis

Islets from two regions of each pancreas per donor were examined. In several T1D donors, the values of the fluorescence intensity of islets from both pancreatic regions were combined due to the small number of insulin positive islets present. Statistical significance was calculated using the Student's *t*-test.

Results

Expression of Beta Cell Functional Markers Is Altered Before T1D Diagnosis

To ascertain whether alterations in beta cell properties develop before hyperglycemia, the expression level of proinsulin, insulin, and PC1/3 was measured in islets of two regions of autoantibody positive and T1D pancreas. The results, shown in Table 1, indicate significant differences in the expression of the markers between islets of several autoantibody positive and controls pancreas. While in some donors, islets displayed a single abnormal marker, pancreatic islets of

Table 1. Donors Characteristics: Autoantibodies, Sex, age and marker expression levels of the Donors.

	GAD	IA2	mIAA	ZnT8	Age/sex	PR	ProIN	PC1/3	IN
Control							22.1 ± 1.3	17.1 ± 0.6	38.4 ± 2
6424	+	–	+	–	17.6 years, M	PB	11.8 ± 0.3*	11.7 ± 0.6*	27.8 ± 0.8**
						PT	9.6 ± 1.0**	8.5 ± 0.7**	26.0 ± 1.7**
6505	+	–	+	–	20.5 years, F	PB	30.2 ± 1.8*	9.5 ± 0.6**	34.4 ± 0.3
						PT	16.5 ± 1.2*	11.9 ± 1.3*	38.3 ± 2
6314	+	–	–	–	21 years, M	PB	17.8 ± 1.0	11.8 ± 0.8*	34.0 ± 1.3
						PT	18.1 ± 0.9	26.7 ± 0.9**	38.1 ± 1.8
6450	+	–	–	–	20.5 years, F	PH	14.6 ± 0.8**	5.54 ± 0.5**	36.6 ± 2.2
						PB	15.0 ± 1.0**	12.8 ± 1.2*	32.0 ± 2.0
6517	+	–	–	+	22.1 years, M	PB	21.3 ± 1.4	11.7 ± 0.6**	33.4 ± 1.2
						PT	19.3 ± 1.3	13.3 ± 1.0**	39.3 ± 2.0
6123	+	–	–	–	23.2 years, F	PB	23.8 ± 1.1	27.6 ± 1.0**	44.2 ± 1.4**
						PT	21.3 ± 0.3	29.2 ± 1.0	39.6 ± 1.4
6397	+	–	–	–	21.1 years, F	PB	13.3 ± 0.9**	8.9 ± 1.8**	29.2 ± 1.9**
						PT	16.5 ± 0.7**	14.3 ± 0.6**	29.5 ± 0.9**
6429	+	–	+	–	22.1 years, M	PB	15.1 ± 1.0**	18.1 ± 1.8	39.0 ± 1.7
						PT	14.9 ± 1.0**	20.1 ± 1.4	38.5 ± 2.0
6520	+	+	–	+	21.6 years, M	PB	12.6 ± 2.3**	7.9 ± 0.7**	43.8 ± 2.3
						PT.	3.5 ± 1.5**	28.9 ± 1.8**	44.2 ± 3.1
6362	+	–	–	–	24.9 years, M	PB	8.7 ± 1.0**.	29.6 ± 2.4**	45.2 ± 2.0
						PT	2.4 ± 0.8**.	15.8 ± 1.6*	43.0 ± 2.2
6325	+	+	+	–	20 years, F	PB+ PT	11.1 ± 0.72**	24.0 ± 1.0*	36.3 ± 3.8
5000	+	–	+	–	18.7 years, F	PB+ PT	12.2 ± 1.7**	9.8 ± 0.56**	25.3 ± 3.3

Fluorescence intensity/islet area of each functional marker. Two regions of the pancreas per donor and 10 to 25 islets per region of pancreas were evaluated. Italics: type 1 diabetic donors. In some of these donors, values from both pancreatic regions are combined. *X = $p<0.05$;**X = $p<0.005$
Abbreviations: IA2, tyrosine phosphatase-like insulinoma antigen; GAD65, glutamic acid decarboxylase-65; ZnT8, zinc transporter protein 8; micro mIAA, insulin; PC1/3, proprotein convertase 1/3; PR, pancreas region; ProIN, proinsulin; IN, insulin; PT, pancreas tail; PB, pancreas body; PH, pancreas head.

other donors showed alterations in all three traits. Interestingly, there was a lack of correlation between markers in the presence and/or type of change. Thus, islets of donor #6123 had normal proinsulin levels but an increased level of expression of PC1/3 (PC1/3^{high}) compared with control, while islets of donor #6429 had decreased ProIN and normal PC1/3 levels. These observations indicate that the expression of the markers varied independently of each other.

Comparison of the fluorescent intensity/islet area of each marker with the list of circulating autoantibodies determined in each donor (Table 1) indicates a lack of correlation between the two measurements. For instance, donors #6123 and #6397 have similar profiles of autoantibodies, but they differ in the expression of the three functional markers.

Gradual Loss of PC1/3 Heterogeneous Expression in Islets of Autoantibody Positive Donors

To ascertain whether the heterogeneity in PC1/3 expression found in beta cells of controls is abrogated in T1D, pancreatic islets from four T1D donors were examined (Table 1). A striking characteristic in all these tissues was the homogeneous expression of PC1/3. In pancreas body (pb) and pancreas tail (pt) of donor #5000, pt of donor #6362, and pb of donor #6520, all beta cells showed a dramatic decrease in PC1/3 expression (PC1/3^{low} phenotype). In contrast, PC1/3 levels in beta cells of islets of the pt region of donor #6520, the pb region of donor #6362, and all islets of donors #6325 pt, pb were homogeneously high (PC1/3^{high} phenotype). Representative images of a control islet and islets with the two types of abnormal PC1/3 phenotypes are illustrated in Fig. 1.

Analysis of pancreata of autoantibody positive donors indicated that PC1/3 heterogeneity in beta cells is lost before T1D diagnosis. Abnormal islets of autoantibody positive donors were concentrated in one pancreatic region (Fig. 2) or distributed in both pancreatic regions examined (Fig. 3), were either PC1/3^{high} or PC1/3^{low} but never a mixture of both abnormal islet types. This observation indicates that the change in the expression of the proconvertase followed the same pattern as in T1D donors. As

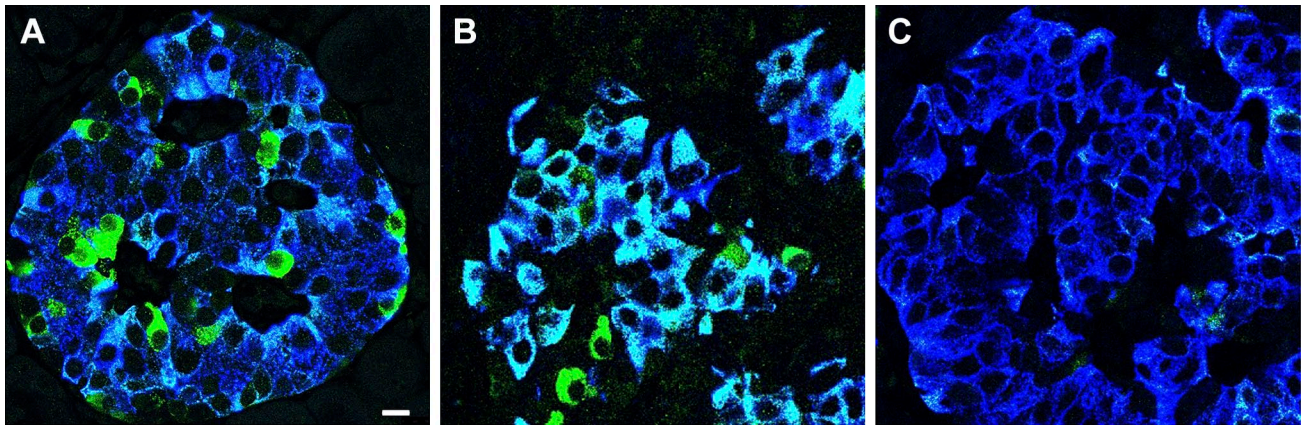


Figure 1. Abnormal PC1/3 phenotypes in beta cells of type 1 diabetes donors. Photomicrographs illustrate T1D islets costained for PC1/3 (green) and insulin (blue). Photomicrographs A to C are representative of all islets of each donor. While expression of PC1/3 in beta cells of islet of control (A) is heterogeneous, the level of the convertase in all beta cells of type 1 diabetes donors is homogeneous and either very high (B) or very low (C). Donor identification is indicated. Cells with green fluorescence remain to be identified. Bar: 12 um. Abbreviations: PC1/3, proprotein convertase 1/3; T1D, type 1 diabetes.

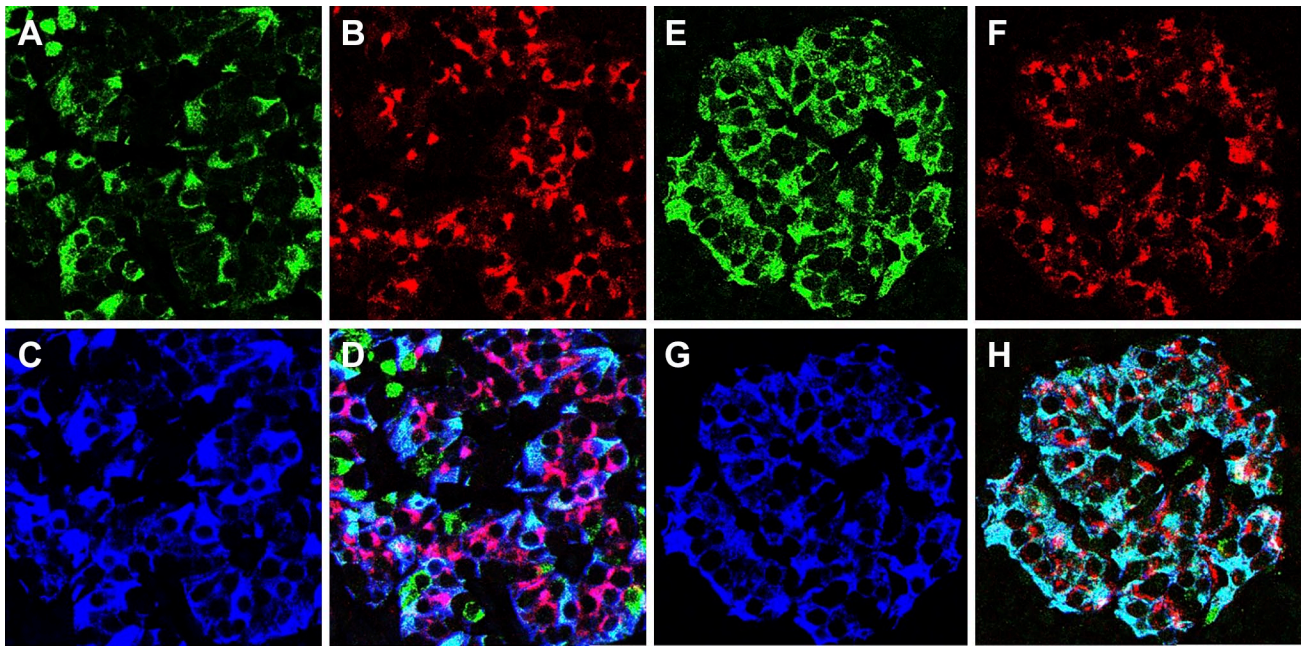


Figure 2. Different pancreatic regions contain either normal or abnormal islets. Islets were triple-labeled for PC1/3 (A, E), proinsulin (B, F), and insulin (C, G); D, H illustrate the overlap of the three labels. Photomicrographs of pancreas of donor # 6397 document the presence of normal islets in the tail region (A–D) and PC1/3^{high} islets in the body region (E–H) of the pancreas. Note that the expression of PC1/3 is heterogeneous in A and homogeneously high in E. Bar: 20 um. Abbreviation: PC1/3, proprotein convertase 1/3.

indicated in Table 1, islets in the pancreas of both autoantibody positive and T1D donors could be PC1/3^{high} in a pancreatic region but PC1/3^{low} in a consecutive area of the pancreas (Table 1), indicating a regional specificity in the defect. Pancreata from PC1/3^{high} donors with T1D also contained islets populated by cells expressing PC1/3 but lacking insulin and/or ProIN (Fig. 4D). Presumably, these represent some of the insulin negative islets described by others.[25–28]

While pancreata of autoantibody are populated by a mixture of abnormal islets and islets populated by normal beta cells with heterogeneous PC1/3 expression (Figs. 2 and 3), all islets in T1D tissues show abnormal expression of the convertase. Evaluation of the ratio of the fluorescent intensity of PC1/3/islet area in islets of

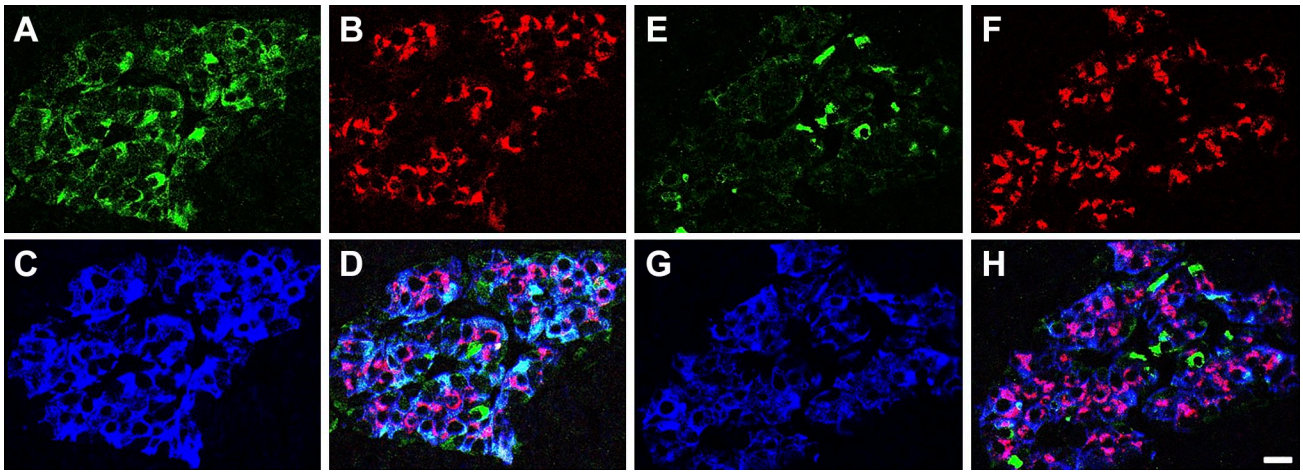


Figure 3. Coexistence of normal and abnormal islets in the same pancreatic region. Photomicrographs of pancreatic islets of donor #6540 illustrate islets triple-labeled for PC1/3 (A, E), proinsulin (B, F), insulin (C, G). D and H show the overlap of the three markers. Note that PC1/3 expression is heterogeneous in A and homogeneously low in E. Bar: 15 um. Abbreviation: PC1/3, proprotein convertase 1/3.

autoantibody positive over control and of T1D over control revealed the presence of two groups of islets (Table 2) that reflected the different contributions of islet monotypes (PC1/3^{low} or PC1/3^{high}) and normal islets with heterogeneous expression of the proconvertase. As expected, this ratio was similar for both regions of the pancreas in some donors but different in others. The presence of two groups of islets according to the PC1/3 expression is also illustrated in the histogram in Table 2.

The Level of Expression and Subcellular Localization of ProIN Differ Between Donors

ProIN expression decreased significantly relative to control in islets of all T1D donors examined (Table 1). This change was already present before diagnosis as the ratio of proinsulin: islet area in autoantibody positive over control was either similar or lower to controls in pancreatic islets from most donors (Table 3). The exception were islets in a pancreatic section of a donor (#6505) in which the ratio was significantly higher than normal values. In contrast to PC1/3, where islets in different pancreatic regions may express dissimilar phenotypes, in most donors, the fluorescent intensity of ProIN was similar in both regions of the pancreas examined (Table 3).

The subcellular localization of proinsulin, which varied between autoantibody positive donors, is not correlated with its fluorescent intensity, as revealed by the difference in the location of the prohormone in islets from different pancreas with similar fluorescent intensity (Fig. 4A, B). The difference in the allocation of the prohormone to distinct cytoplasmic compartment also lacks a correlation with the two abnormal PC1/3 traits as donors with either PC1/3^{low} or PC1/3^{high} phenotypes display similar proinsulin localization (Fig. 4C, G). In addition, the displacement of ProIN immunoreactivity from the perinuclear localization to the cytoplasm is not linked to the number of circulating autoantibodies. Thus, donors with single and double autoantibodies were found to display similar prohormone distributions (Fig. 4B, G). Pancreatic islets from some donors contained insulin cells with negligible levels of both ProIN and PC1/3 (Fig. 4E, F). Other tissues with islets displaying abundant cytoplasmic localization of the prohormone lacked PC1/3 and insulin (Fig. 4H, I). These observations suggest that these two latter beta cell types are inactive cells. As cells with cytoplasmic localization of proinsulin were described in T1D islets,[7] the present findings indicate that the presence of these abnormal beta cell types precedes diagnosis.

In common with islets of autoantibody positive donors, changes in the level of expression of PC1/3 in islets of T1D donors were independent from that of ProIN, further supporting the contention that the expression of each marker is regulated independently of the other markers examined.

The Level of Expression of Insulin in Islets Remains Constant During Disease Progression

In contrast with the variability in the expression and cytoplasmic localization of the prohormone and the convertase, the fluorescent intensity/islet area of

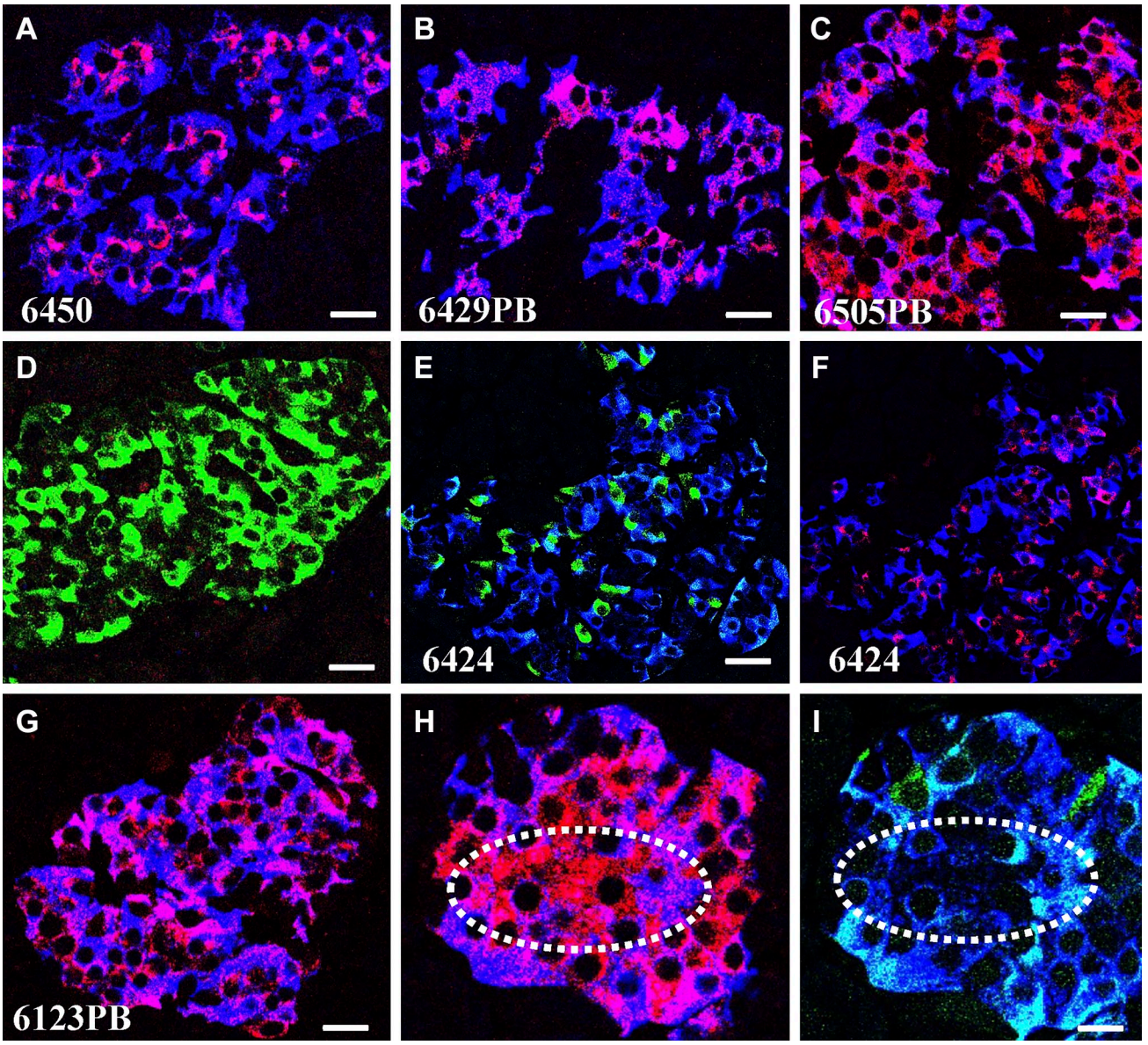


Figure 4. Fluorescent intensity and cellular allocation of proinsulin and insulin. Photomicrographs show sections of pancreas immunostained for ProIN (red), PC1/3 (green), and IN (blue). Figures illustrate beta cells with similar ProIN levels but different localization of the prohormone (A, donor #6450; B donor #6429); similar ProIN localization in donors with different number of autoantibodies (B, donor #6429; G, donor #6123); different PC1/3 phenotype but similar ProIN localization (C, donor #6505 and G, donor #6123); high ProIN immunostaining but very low IN and PC1/3 (H illustrates staining for ProIN and IN, I illustrates staining for PC1/3 and insulin, donor #6505); low ProIN and PC1/3 (E illustrates staining for PC1/3 and insulin and F shows immunostaining for ProIN and insulin, donor #6424). (D) Islet of T1D donor stained for insulin, proinsulin and PC1/3. Note that cells express only the proconvertase. Bar: 15 um. Abbreviation: PC1/3, proprotein convertase 1/3.

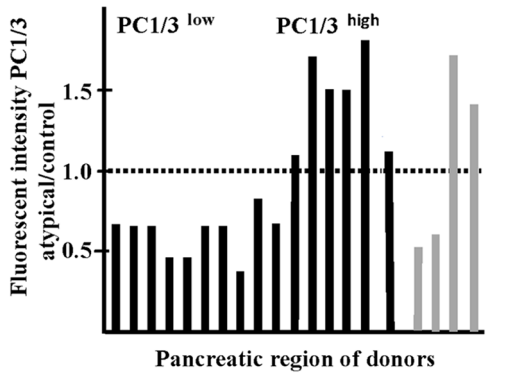
insulin in islets of autoantibody positive and T1D donors was similar in beta cells from both pancreatic regions examined and was similar or lower than that of controls (Table 3). In view of the observed variations in PC1/3 and proinsulin, the presence of a constant level of the hormone in beta cells is likely to be due to alterations in the regulation of hormone secretion (reviewed in Tsai et al.[29]).

T1D Also Affects the Expression of proSAAS, a Regulator of PC1/3 Activity

proSAAS is an endogenous binding protein that is a potent inhibitor of the PC1/3 in vitro.[17,19,23,24] To determine whether the expression of proSAAS in beta cells is affected by T1D, sections of pancreas from controls and T1D donors from the PC1/3^{high} and

Table 2. Classification of Donors According to Their PC1/3 Phenotype.

		R < 1	R > 1
6424	PB	0.68	
	PT	0.47	
6505	PB	0.5	
	PT	0.7	
6314	PB	0.7	
	PT		1.5
6450	PH	0.4	
	PB	0.7	
6517	PB	0.68	
	PT	0.76	
6123	PB		1.7
	PT		1.5
6397	PB		1.1
	PT	0.8	
6429	PB		1.8
	PT		1.1
6520	*PB + PT*	0.46	
6362	*PB + PT*		1.7
6325	*PB + PT*		1.4
5000	*PB + PT*	0.5	



The table illustrates the ratio (R) of the mean fluorescent intensity of PC1/3 per islet area in all islets of each donor over that of control. The same value for control islets was used for all calculations. Note that islets in different regions of the pancreas of autoantibody positive donors are populated by either PC1/3^{high} or PC1/3^{low} beta cells. Islets with PC1/3^{high} and PC1/3^{low} expression do not coexist in the same pancreatic region. At least 15 islets were evaluated for each pancreatic region per donor. Values close to that of control are due to the coexistence of normal and abnormal islets in the same section. Histogram illustrates the values of Table 2. Black lines: autoantibody positive donors; gray lines: type 1 diabetic donors. Note the presence of two abnormal groups according to their PC1/3 phenotype.
Abbreviations: PC1/3, proprotein convertase 1/3; PB, pancreas body; PT, pancreas tail; PH, pancreas head.

PC1/3^{low} groups, respectively, were immunostained for visualization of the granin and insulin. The results show that expression of proSAAS is localized to insulin cells (Fig. 5A), although it also labels unidentified cells that surround the islet. proSAAS expression persists in the PC1/3^{high} group (Fig. 5B) and decreases significantly in the PC1/3^{low} group (Fig. 5C), in agreement with the changes in the expression of the convertase.

Discussion

It is known that an increase in the proinsulin to insulin ratio in the circulation is a hallmark of the initial stages of type 2 diabetes[30] and is a predictor of abnormal glucose regulation in T1D.[9,31,32] While studies on NOD mice, a classical model of T1D, revealed the presence of markers of endoplasmic reticulum stress that are activated under conditions of inflammation[31,33,34] leading to abnormal proinsulin processing, the role of endoplasmic reticulum (ER) stress in defects of hormone production in T1D progression in humans remains unclear. However, the results of the present study strongly suggest that alterations in PC1/3, reflected by the loss of its heterogenic expression, are likely to result in the observed decrease in prohormone processing.

The hypothesis suggesting a relationship between proper beta cell function and heterogeneity in the expression of PC1/3 also raises a fundamental question regarding the mechanisms involved in the regulation of convertase expression. The PCSK1 gene encodes the 753-amino acid precursor preproPC1/3, from which the signal peptide is removed in the ER.[35–38] The resulting product is a proPC1/3 product of 94 kDa that undergoes propeptide cleavage in the Golgi, yielding an 87-kDa form.[37,39] The 87-kDA form is packaged into immature secretory granules,[40] where it generates the 77- and 66-kDa forms.[11,41–43]

The antibody to PC1/3 used in the studies reported here recognizes the 87 and 74/66 kDa active forms of the enzyme.[22] These forms differ in their enzymatic activity with the 74/66 kDa form more active but less stable than the high molecular mass form.[44–46] In vitro analysis indicated that the enzyme exists in multiple ionic forms due to oligomerization and aggregation.[42] It should also be noted that PC1/3 was found to have hysteretic properties, which indicates that it is present in forms with different kinetic properties.[47,48] Taken together, those reports suggest that the variation in

Table 3. Classification of Donors According to Their Proinsulin (ProIN) and Insulin (IN) Phenotypes.

		Ratio ProIN		
		<1	>1	Ratio IN
6424	PB	0.5		0.71
	PT	0.4		0.68
6505	PB		1.4	0.89
	PT	0.75		1.0
6314	PB	0.8		0.89
	PT	0.8		1.0
6450	PH	0.7		0.94
	PB	0.7		0.94
6517	PB	0.9		0.84
	PT	0.8		0.89
6123	PB	0.9		1.0
	PT	1.0		1.1
6397	PB	0.6		1.02
	PT	0.75		0.76
6429	PB	0.7		0.76
	PT	0.9		1.0
6520	*PB + PT*	0.5		1.0
6362	*PB + PT*	0.1		1.1
6325	*PB + PT*	0.1		0.9
5000	*PB + PT*	0.5		0.7

The table illustrates the ratio (R) of the mean fluorescent intensity per islet area of ProIN and IN in islets of each donor over that of control. The same value for control islets was used for all calculations. Note that different pancreatic regions of each donor have similar ProIN phenotype, except for donor #6505. Levels of proinsulin in pancreas of type 1 diabetes donors (italics) are similar or lower than that of autoantibody positive cohort.
Abbreviations: IN, insulin; ProIN, proinsulin; PB, pancreas body; PT, pancreas tail; PH, pancreas head.

the expression of the enzyme in normal beta cells may reflect changes not only in its affinity for the antibody but also in its molecular structure that results in differences in the rate of insulin synthesis. If these structural modifications are required for proper proinsulin processing, a perturbation of these changes may play a role in the increase in the proinsulin/insulin ratio in serum characteristic of the disease.[32]

The presence of both normal and abnormal islets in pancreas of autoantibody positive donors but not in tissues from T1D donors raises the hypothesis that there is a gradual change in islet type during progression of the disease from one containing the three beta cell types with heterogeneous PC1/3 expression to monotypes with either high (PC1/3^{high}) or low (PC1/3^{low}) levels of the proconvertase (Fig. 6). This analysis also indicates that alterations in PC1/3 expression is an early indicator of disease progression, and it could be considered as a biomarker of the development of beta cell abnormalities. These alterations in expression also affect proSAAS, a chaperone that functions as a regulator of PC1/3 activity in vitro. ProSAAS, encoded by the mouse gene Pcsk1n, is a 225-residue polypeptide[17,49] with broad neuronal distribution.[50–52] Notably, in islets of T1D donors, the expression of proSAAS changes in accordance with that of PC1/3, decreasing in PC1/3^{low} and increasing in PC1/3^{high} donors, respectively. This observation suggests that the expression of PC1/3 and proSAAS is coordinated.

Measurement of the level of expression of proinsulin and the proconvertase indicated the presence of significant differences in the values of each of the markers in islets from different autoantibody positive donors. This comparison also indicated that the expression of each marker is differentially affected by diabetogenic signals as islets may have abnormal PC1/3 expression but normal level of ProIN. Another important conclusion from this analysis is that variations in the expression of PC1/3 were specific for a pancreatic region as islets in one region could be PC1/3^{high} while those in other region expressed a PC1/3^{normal} or PC1/3^{low} phenotype. In contrast to the variability in PC1/3 levels reported here, mass spectrometry quantitation of PC1/3 in isolated T1D islets indicated a characteristic reduction in enzyme levels.[8,53] However, variation in proconvertase levels between donors and between different pancreatic regions of each donor, such as those reported in the present study, are likely to affect those quantitative results.

As changes in the value of the functional markers examined in this study may occur in response to specific signals, the presence of different values for PC1/3 expression in two regions of the same pancreas suggest that this is the result of two different signaling systems affecting the convertase. The lack of correlation between the changes in PC1/3 and ProIN expression supports the presence of an additional set of signals affecting prohormone levels. Taken together, the results of the present analysis indicate that the position of the islets in the pancreas determines how their properties are modified in disease, and that the incorporation of the positional information of the islets will aid in the identification of possible causes of the change.

While the alteration in PC1/3 expression is an early marker of a beta cell defect, there is recent evidence indicating that alpha cells also become functionally deficient in the prediabetic stage.[54] It is unclear if these defects include the convertase, as the presence of PC1/3 in alpha cells is controversial.[55,56] However, in view of the known interactions between alpha and

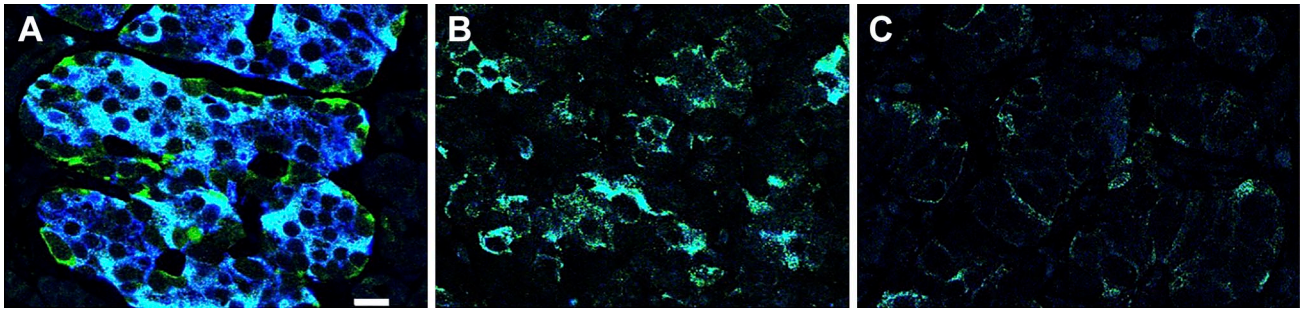


Figure 5. Type 1 diabetes affects proSAAS expression. Photomicrographs show: (A) the localization of proSAAS (green) in an islet of control. proSAAS localize to cells also stained for insulin (blue) and to an unidentified cell type that surrounds the islet. (B) proSAAS expression in an islet of PC1/3^{high} type 1 diabetic donor. (C) proSAAS expression in a PC1/3^{low} type 1 diabetic donor illustrates the near absence of both labels. Bar: 20 um. Abbreviation: PC1/3, proprotein convertase 1/3.

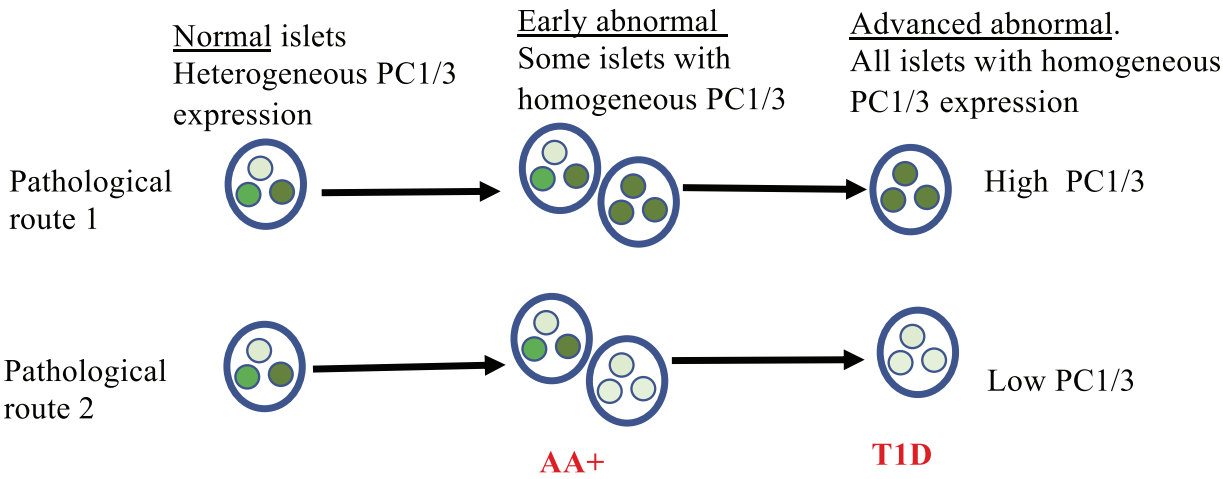


Figure 6. Postulated interpretation of results. Normal islets are populated by three beta cell types displaying variations in PC1/3 expression. Alterations in PC1/3 expression occur in a subset of islets of autoantibody positive donors; the expression of the convertase in beta cells of these abnormal islets is homogeneous, is either high or low, and is characteristic for a pancreatic region. It is hypothesized that there is a gradual change in islet type during progression to type 1 diabetes from one containing three beta cell types with heterogeneous PC1/3 expression to a monotype with either high or low levels of the proconvertase. It is also proposed that the alteration in the expression of the enzyme results in a reduction of its enzymatic activity. Large circle: islet; small circles, beta cells. Shades of green in small circles indicate level of PC1/3 expression. Abbreviation: PC1/3, proprotein convertase 1/3.

beta cells,[57–61] the elucidation of a possible correlation between the alterations in these two islet cell types may provide important insight into causative signals.

In summary, the present study provides evidence indicating that the conversion of PC1/3 expression from a heterogeneous to a homogeneous pattern occurs before T1D and that this conversion may indicate an early beta cell dysfunction during progression of the disease. It is likely that studies analyzing the mechanisms regulating PC1/3 activity will provide invaluable insight into functional beta cell defects that lead to T1D.

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Competing interest

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