The proinsulin, insulin and PC1/3 trichotomy: understanding beta cell function and dysfunction through image analysis.

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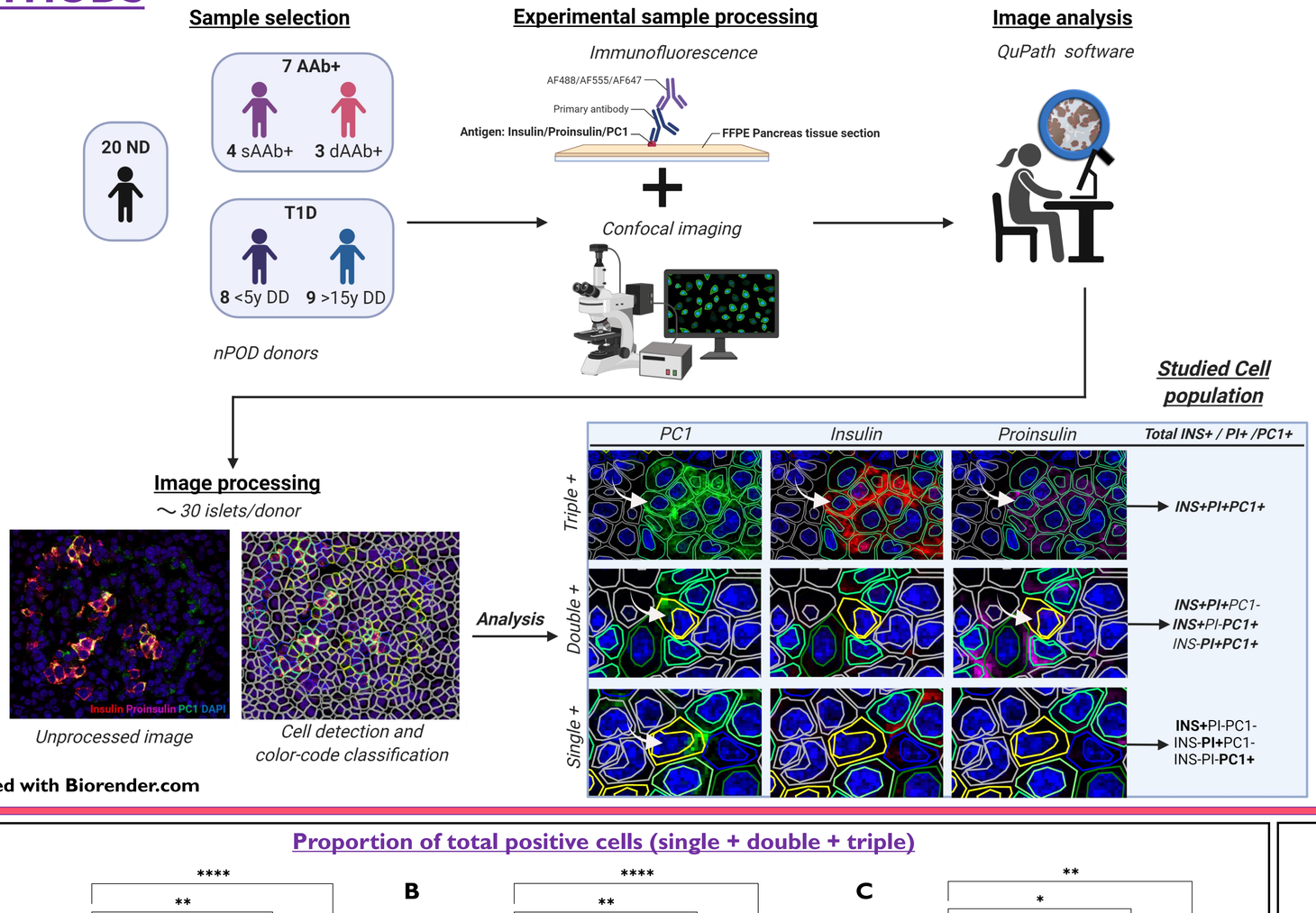
PURPOSE

Investigations in mice and/or human pancreas showed a general decay in beta cell mass, which may correlate with loss of beta cell function in type 1 diabetes (T1D). Detection of different biomarkers in blood such as proinsulin, insulin and/or C-peptide may help predict what happens to islet function and beta cell mass during disease progression. In this study, we aimed to provide an in-depth characterization of not only insulin and proinsulin expression but also of the prohormone processing enzyme PC1/3 (PC1) in non-diabetic (ND), single and double autoantibody-positive (sAAb+/dAAb+), and T1D donors (<5 years disease duration (DD) and >15 years DD) using high-resolution confocal imaging and state-of-the-art image analysis. In addition, we aimed to correlate islet beta cell phenotype and islet morphology to account for possible changes in cell size, number and islet area during disease progression.

METHODS



RESULTS

The proportion and density of **total positive cells (single + double + triple positive)** (figure 1) and **triple positive cells** (**INS+PI+PC1+)** (figure 2) was lower in 2 out of 3 double AAb+ donors, in most short-duration T1D donors, and in all long-duration T1D donors.

As observed in table 1A-B the number of PC1/3 negative beta cells (**INS+PI+**PC1-, 24.34 ± 17.44%) was higher in double AAb+ donors. Conversely, it was lower in long-duration T1D donors (0.01 ± 0.02%) compared to ND donors (8.46± 10.36%). A decrease in the proportion of PI negative beta cells (**INS+**PI-**PC1+)** was observed in double AAb+ (0.27 ± 0.12%) and long-duration T1D (0.26 ± 0.35%), compared to ND donors (0.79± 0.61%). In contrast, a higher proportion was observed in short-duration T1D donors (1.77 ± 1.94%). There were no differences in cells negative for insulin (INS-**PI+PC1+**) between the groups, with the exception of T1D donors with long disease duration (0.16 ± 0.16% vs 1.75± 1.32% in ND).

The density and proportion of cells positive for just PC1 (INS-PI-**PC1+**) was higher in T1D donors (both short and long disease duration) (figure 3). The proportion of cells staining positive for just PI (INS-**PI+**PC1-) or INS (**INS+**PI-PC1-) was very low in all the groups (table 2A-B).

When **islet cellularity and morphology** were analyzed, the mean number of cells per islet was comparable in all groups (152.2 ± 41.9 cells/islet). Interestingly, cell density (number of cells/islet area) was reduced in double AAb+ donors (suggesting possible cell hypertrophy), yet increased in all T1D donors (suggesting possible cell atrophy) compared to ND donors (figure 4-5).

Proportion of total positive cells (single + double + triple)

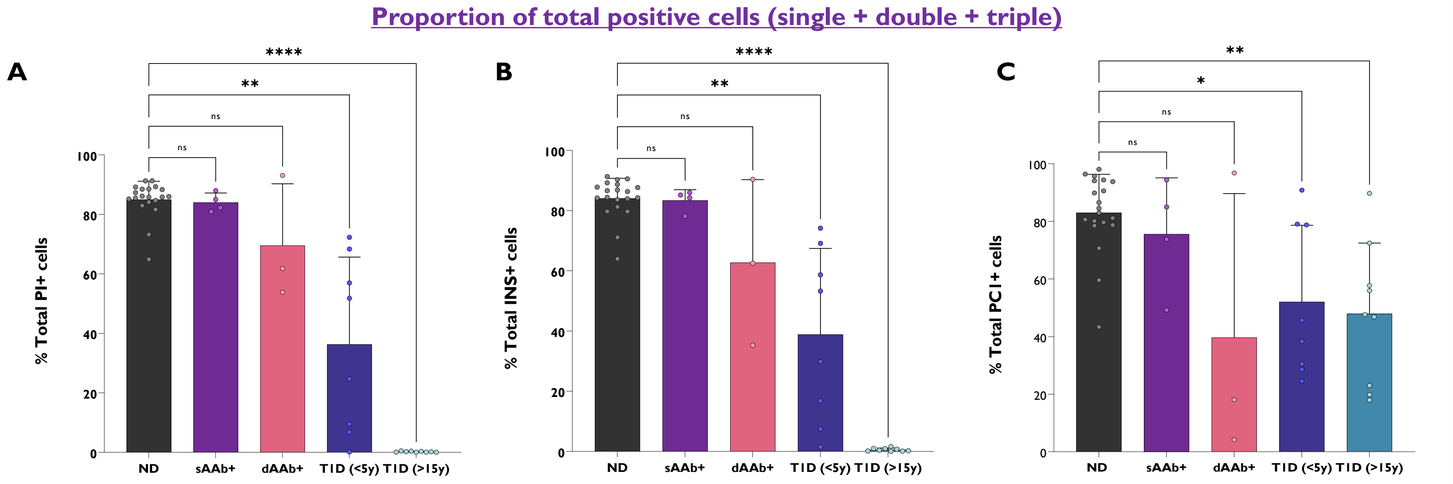


Figure 1. Analysis of total endocrine cells positive for A) proinsulin (PI+); B) insulin (INS+) and the prohormone processing enzyme PC1/3 (PC1+). Bar graphs represents the mean and SD of the proportion (%) of each protein per group. Each dot represents a donor. ns: not significant; * p< 0.05; ** p< 0.01 and **** p< 0.0001.

Triple positive cells (density and %)

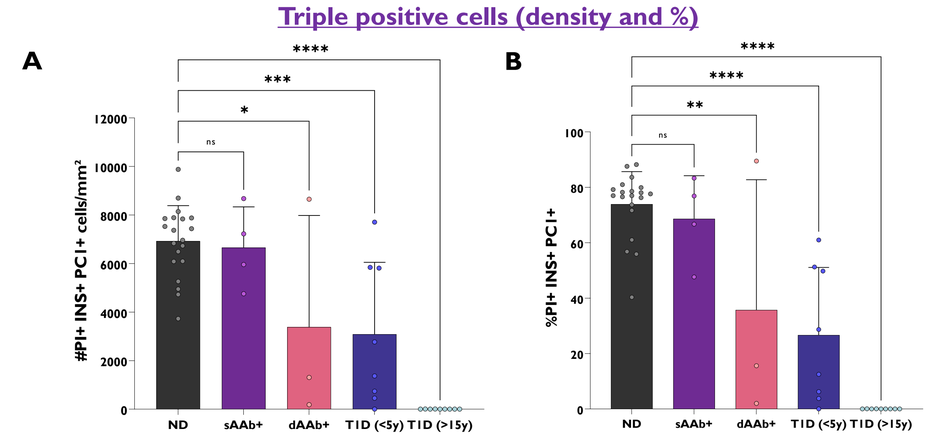


Figure 2. Analysis of endocrine cells positive for the three proteins (**PI+INS+PC1+).** A) Bar graph represents the mean and SD of the cell density expressed as number of positive cells/islet area (mm²) per group. B) Bar graph represents the mean and SD of the proportion of triple positive cells per group. Each dot represents a donor. ns: not significant; * p< 0.05; ** p< 0.01; *** p< 0.001 and **** p< 0.0001.

Double positive cells

Table 1. Analysis of the endocrine cells positive for two proteins (cell density). Highlighted in white the positive proteins. Values in bold purple indicate increased and light purple decreased values compared with the ND controls.

Group/Density	PI+INS+PC1-	PI-INS+PC1+	PI+INS-PC1+
ND	761.76 ± 962.15	74.1 ± 58.10	170.20 ± 134.14
sAAb+	1211.99 ± 1433.63	67.35 ± 60.08	187.53 ± 127.47
dAAb+	**2119.50 ± 1826.89**	23.92 ± 13.13	119.68 ± 138.85
TID (<5y)	844.47 ± 1235.92	**154.36 ± 175.08**	155.91 ± 269.79
TID (>15y)	0.73 ± 2.20	30.83 ± 42.15	18.81 ± 20.01

Table 2. Analysis of endocrine cells positive for two proteins (proportion). Highlighted in white the positive proteins. Values in bold purple indicate increased and light purple decreased values compared with the ND controls.

Group	% PI+INS+PC1-	% PI-INS+PC1+	% PI+INS-PC1+
ND	8.46 ± 13.63	0.79 ± 0.63	1.75 ± 1.35
sAAb+	12.47 ± 14.25	0.68 ± 0.56	1.93 ± 1.25
dAAb+	**24.34 ± 17.44**	0.27 ± 0.12	1.26 ± 1.15
TID (<5y)	8.08 ± 12.85	**1.77 ± 1.94**	1.30 ± 2.25
TID (>15y)	0.01 ± 0.02	0.26 ± 0.35	0.16 ± 0.17

Islet cellularity and morphology

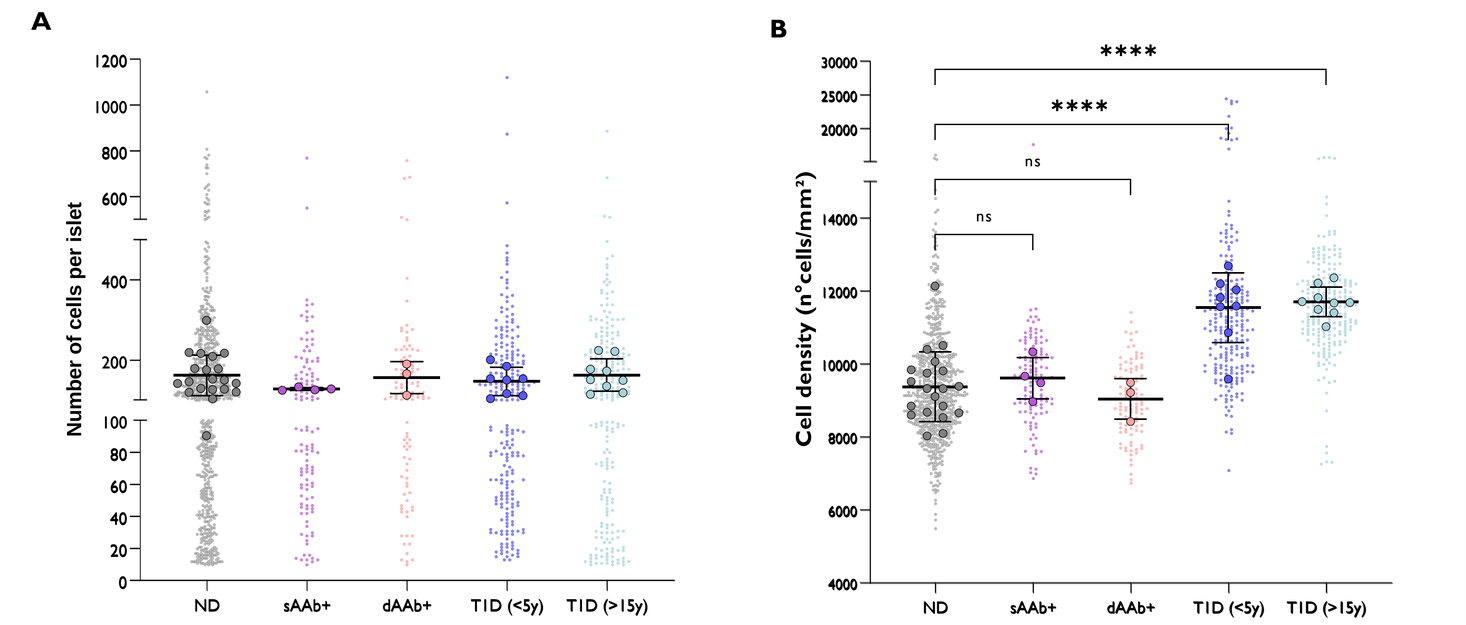


Figure 4. Islet cellularity and morphology **a**nalysis of islets per group. A) Superplot graph represents the mean and SD of the number of cells contained per islet. B) Superplot graph represents the mean and SD of the endocrine cell density per islet area (mm²) and per group. Each big dot represents the mean of a donor and the small dots represent individual islets. Islets with <10 cells were not included in the analysis. ns: not significant and **** p< 0.0001.

Single positive cells

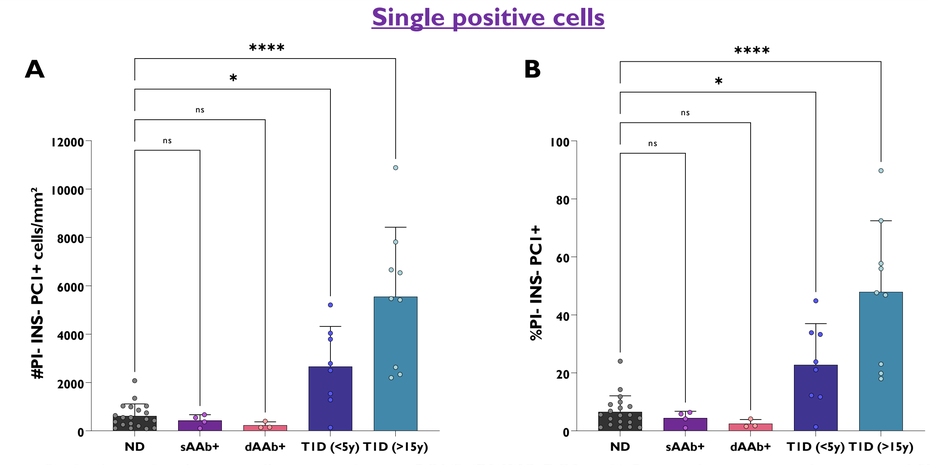


Figure 3. Analysis of endocrine cells positive for just PC1/3 (**PI-INS-PC1+**). A) Bar graph represents the mean and SD of the cell density expressed as number of positive cells/islet area (mm²) per group. B) Bar graph represents the mean and SD of the proportion of single positive cells per group. Each dot represents a donor. ns: not significant; * p< 0.05 and **** p< 0.0001.

Table 3. Analysis of endocrine cells positive for one protein (cell density). Highlighted in white the positive protein. Values in bold purple indicate increased and light purple decreased values compared with the ND controls.

Group/Density	PI+INS-PC1-	PI-INS+PC1-	PI-INS-PC1+
ND	77.66 ± 72.67	84.59 ± 64.66	613.46 ± 501.93
sAAb+	95.67 ± 52.30	154.69 ± 179.63	422.91 ± 243.96
dAAb+	**743.96 ± 988.55**	**208.31 ± 198.77**	229.11 ± 146.56
TID (<5y)	30.71 ± 45.36	**308.96 ± 281.40**	**2660.15 ± 1657.47**
TID (>15y)	1.86 ± 5.59	33.86 ± 37.76	**5549.48 ± 2871.38**

Table 4. Analysis of endocrine cells positive for one protein (proportion). Highlighted in white the positive protein. Values in bold purple indicate increased and light purple decreased values compared with the ND controls.

Group	% PI+INS-PC1-	% PI-INS+PC1-	% PI-INS-PC1+
ND	0.87 ± 0.84	0.91 ± 0.68	6.52 ± 5.55
sAAb+	1.01 ± 0.60	1.59 ± 1.78	4.36 ± 2.40
dAAb+	**8.25 ± 10.76**	**2.41 ± 2.38**	2.48 ± 1.43
TID (<5y)	0.28 ± 0.44	**2.80 ± 2.65**	**22.77 ± 14.22**
TID (>15y)	0.02 ± 0.05	0.29 ± 0.33	**47.91 ± 24.55**

CONCLUSIONS

During the progression to T1D, it is known that beta cells undergo functional and morphological changes. Our data showed a general decrease of cells expressing insulin, proinsulin and PC1/3 in double AAb+, and T1D donors with short and long disease duration. Changes in distinct double-positive cell subpopulations may represent different stages of beta cell dysfunction. The higher proportion and density of single PC1/3+ cells in T1D donors could be due to an increase in PC1/3+ alpha cells, or to the presence of remaining beta cells that are no longer able to produce proinsulin and insulin. Lastly, islet morphological changes (cell hypertrophy or atrophy) before and after onset of the disease might reflect possible alterations in cell function. These findings complement the current knowledge in beta cell pathophysiology, and contribute to our understanding of T1D pathogenesis.

HELMHOLTZ MUNICH

DZD Deutsches Zentrum für Diabetesforschung

nPOD Network for Pancreatic Organ Donors with Diabetes

JDRF IMPROVING LIVES. CURING TYPE 1 DIABETES.