

P010 Functional properties of the common and type 2 diabetes-associated R325W variant of the vesicular zinc transporter ZnT8

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Background: Zn^{2+} ions are important biological regulators. In the pancreatic β -cell, Zn^{2+} is thought to be of crucial importance for the maturation of the insulin crystal. A non-synonymous polymorphism in the *SLC30A8* gene, leading to a R325W mutation in the vesicular zinc transporter, ZnT8, has been shown in recent whole genome studies to be strongly associated with an increased risk of type 2 diabetes. Materials and Methods: cDNAs encoding *c-myc*-tagged forms of the common and at-risk R325W variants of ZnT8 were subcloned into plasmids pIRES2-eGFP or pIRES2-dsRed2 and expressed in INS-1 (832/13) β -cells. Cells were fixed using 3% paraformaldehyde for immunocytochemical analysis using Alexa-488, 568 or far-red-labelled secondary antibodies, and visualised on a Leica SP5 laser-scanning confocal microscope. Cytoplasmic $[Ca^{2+}]$ and $[Zn^{2+}]$ were measured using entrapped fura-redAM and Fluor-Zin3AM respectively. Results: Both wild-type and R325W ZnT8 were closely colocalised (>80%) with insulin-containing vesicles. No differences in cytoplasmic $[Ca^{2+}]$ increases in response to 50 mmol/l KCl or 17 vs 3 mmol/l glucose were apparent between the variant and common forms of the transporter. Over-expression of either construct enhanced KCl-induced increases in cytoplasmic free $[Zn^{2+}]$. Conclusion: The variant and common forms of ZnT8 display similar subcellular targeting to dense-core vesicle, but subtle differences in cellular Zn^{2+} handling