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Congenital Hyperinsulinism in Infancy: The Profiles of Insulin Secretory Granules are Markedly Different in Focal- and Diffuse β-Ccells

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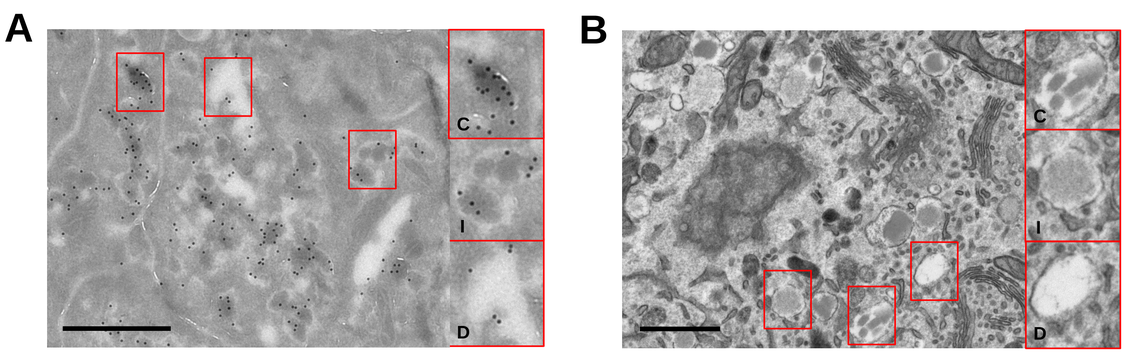
Background & Objectives

Congenital Hyperinsulinism of Infancy (CHI) is a potentially lethal condition of profound hypoglycaemia caused by unregulated insulin release in the neonatal period and early infancy. CHI mainly arises due to mutations in ATP-sensitive K^+-channel genes (*ABCC8* and *KCNJ11*) which can manifest in all islets cells – diffuse-CHI, or can be localised to a focal lesion, focal-CHI.

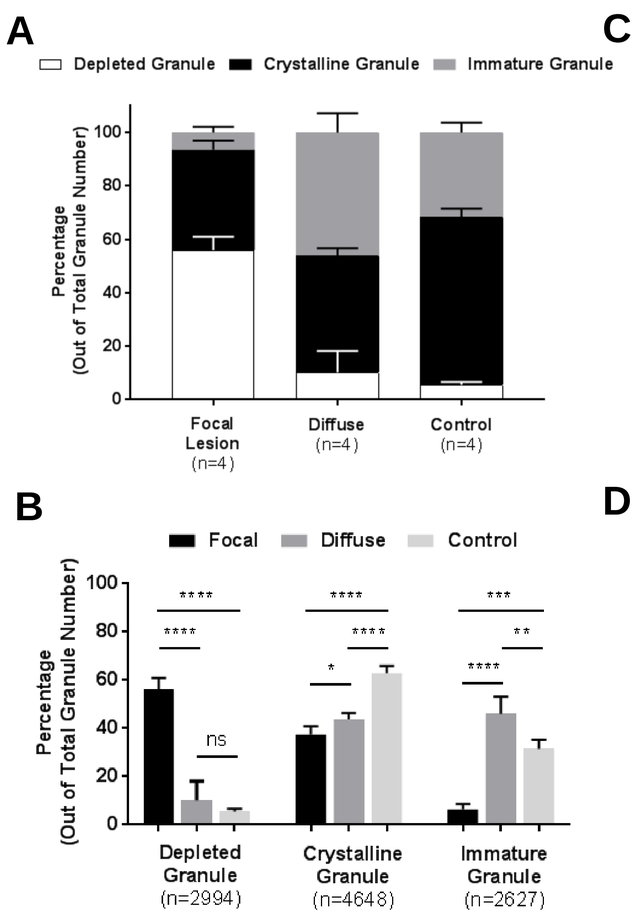
The mechanisms responsible for inappropriate insulin release have largely focused upon defects in K_{ATP} channels. As little is known about insulin biogenesis in CHI, our objectives were to assess the profiles of insulin-containing granules in β-cells from patients with diffuse- and focal disease.

1. Insulin-Containing Granules



β-cell and insulin secretory granules were identified using immuno-gold labeling (Fig. A) and routine TEM labeling (Fig. B).Three different stages of insulin granules were characterised: mature, dense-core / crystalline granules (labelled as C); immature secretory granules (labelled as I) and secretory granules that were depleted of insulin (labelled as D). Scale bars = 1µm.

2. The Profile of Insulin-Containing Granules

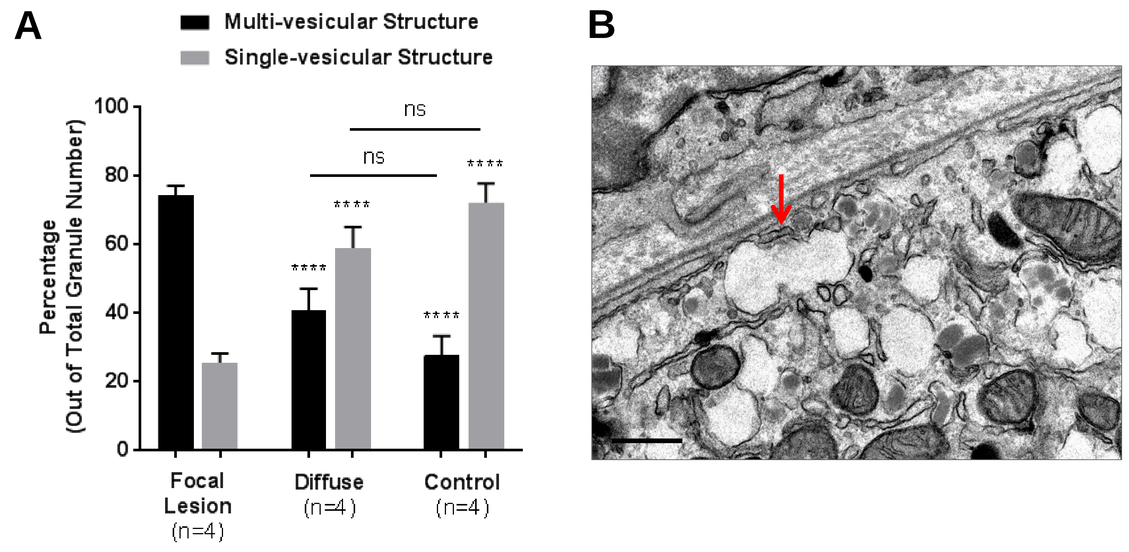


Panel A illustrates that there are marked differences in the profiles of insulin biogenesis in focal CHI compared to control and diffuse CHI tissue. Panel B indicates that the percentage of depleted granules is significantly higher in focal-CHI lesion while immature granules are significantly lower than control and diffuse CHI tissue. Mature insulin in the form of crystalline granules was similar between focal- and diffuse-CHI but did reach significance; $^{*}P=0.045$. $^{**}P<0.01$; $^{***}P<0.001$; $^{****}P<0.0001$. Panels C and D show representative TEM images for diffuse-CHI and focal-CH, respectively. Note that there are far fewer depleted granules in islets from diffuse CHI tissue. Scale bars = 0.5µm.

Methods

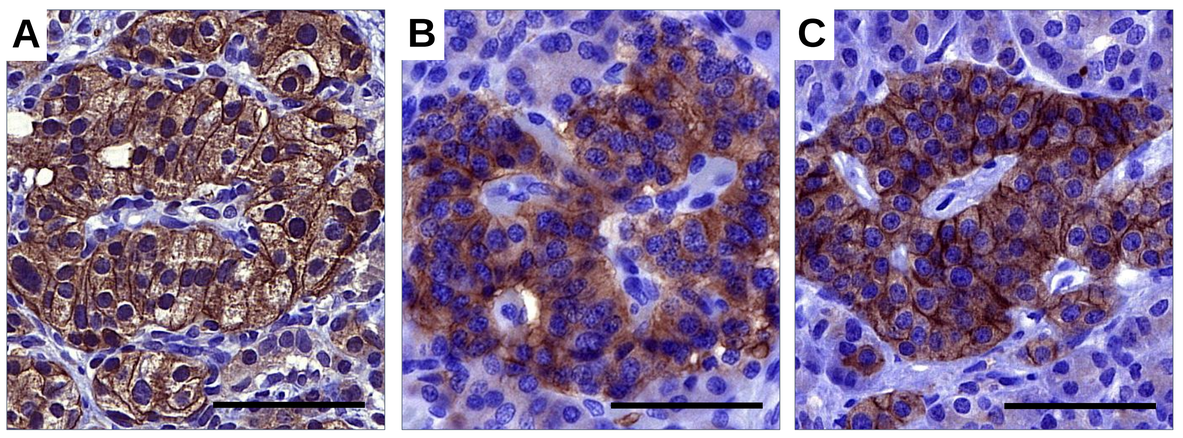
Tissue was obtained following surgery from patients with diffuse-CHI (n=4 patients with *ABCC8* gene defects), lesion from focal-CHI (n=4 patients with ABCC8 gene defects) and control samples (n=4 with no known genetic mutations related to CHI disease). Immunohistochemistry stains (IHC) with SNAP25 were performed on histological sections (5 µm). Ultrathin sections (70 nm) and cryo-sections (70nm) were cut for routine Transmission Electron Microscopy (TEM) and immuno-gold labelling, respectively. Insulin-containing granules were identified and quantified in image J and data were analysed for significance using One-way ANOVA followed by Tukey's post hoc test.

3. The Profile of Multi-vesicular Structures



Panel A shows the incidence of multi-vesicular insulin-containing granules in the different forms of CHI compared with controls. Note that insulin-containing secretory granules in focal-CHI are mainly found in multi-vesicular structures compared with diffuse and controls β-cells. No significant differences were detected between the diffuse-CHI and control. $^{****}P<0.0001$. Panel B shows a representative TEM image of the multi-vesicular structure (red arrow) in focal-CHI lesion. Scale bar = 0.5µm.

4. Expression of Crucial Exocytosis Related Protein



SNAP25 is a core component of exocytosis β-cells and amongst the top 0.2% of genes that are upregulated in focal-diffuse lesions ($P=2.1 \times 10^{-7}$, False Discovery Rate $=1.1 \times 10^{-4}$, *unpublished*). Using immunohistochemisty we found that the localisation of SNAP25 was markedly different in focal-CHI β-cells (A) compared to diffuse-CHI (B) and control tissue (C). Note how in focal CHI, SNAP25 has a far more marked association with the plasma membrane compared to diffuse disease. Scale bars = 50µm.

Conclusions

Our data imply that β-cells in focal-CHI have a greater secretory capacity (increased number multi-vesicular secretory granules, depleted granules, altered localisation of SNAP25) than in diffuse disease, despite the fact that both conditions associate with *ABCC8* gene defects.

55ESPE 549--P1

Perinatal Endocrinology

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DOI: 10.3252/pso.eu.55ESPE.2016

Poster presented at: ESPE 2016 PARIS

PosterSessionOnline.com