Islet Cell Proliferation is Inappropriately Maintained in the Pancreas of Children with Congenital Hyperinsulinism in Infancy

B Han¹, M Newbould², E Cheesman², G Batra³, RJ Craigie³, Z Mohamed¹,², L Rigby², R Padidela², M Skae², KE Cosgrove¹, MJ Dunne¹, I Banerjee²

¹Faculty of Life Sciences, University of Manchester ²Central Manchester University Hospitals NHS Foundation Trust (CMFT) ³Oxford Road, Manchester, M13 9PT, UK

Background
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 islet cell - diffuse CHI (CHI-D), or can be localised to a focal lesion, focal CHI (CHI-F). Increased rates of cell proliferation have been reported in the CHI-D and this may be linked to ABCC8 and KCNJ11 defects. Here, we examined the proliferative index (PI) of islet cells in CHI-D patients and compared this with focal CHI (CHI-F), which is caused by loss of cell cycle repression in β-cells specifically within the focal domain. We also examined islet PI in patient tissues with severe CHI unrelated to defects in ABCC8 and KCNJ11, atypical CHI (CHI-A).

Methods
Tissue was obtained following surgery from patients with CHI-D (n=10 patients with ABCC8 gene defects), CHI-F (n=6 patients with ABCC8 gene defects), and CHI-A (n=3 patients with unknown genetic causes of disease). Neonatal control (n=12, 2-days to 36-months of age), foetal tissues (n=5, 10 to 35 weeks post conception) and adult tissues (n=4) were included as control groups. Immunohistochemistry staining with Ki67 on histological sections (5 µm) and high-content analysis were used to calculate the proliferation index (PI) and changes in PI expressed as average fold-changes across the various groups. Data were analysed with One-way ANOVA followed by Tukey’s post hoc test.

Results 1

Graphical representations of proliferation index (PI) and fold changes in patients groups compared with age matched controls, foetal and adult control samples. Scatter plots of the differences in percentage of Ki67+ cells in CHI-D, CHI-A and age matched controls across whole pancreatic tissue (shown in A-i) and in islet specific regions (shown in B-i). Average fold changes in proliferation among CHI samples and age matched controls; data has been normalised to controls. P**<0.01.

Results 2

Graphical representations of PI and fold change in patients with various forms of CHI disease compared with age matched controls. Scatter plots of the differences in percentage of Ki67+ cells in CHI-D, CHI-F and age matched controls across whole pancreatic tissue (A) and in islet specific regions (B). Average fold changes in proliferation among CHI samples and age matched controls; data has been normalised to controls. P*<0.05; P**<0.01.

Conclusions
1. There is a negative correlation between age and cell proliferation in control pancreas. 2. All three forms of CHI are associated with increased rates of cell proliferation. 3. Although there is a negative correlation between age and cell proliferation in CHI tissue (islets and pancreas), it is quantitatively different and not directly associated with ABCC8 mutations.