The effect of therapeutic hypothermia following HI on phosphorylated KCC2 expression.

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Background:
• Hypoxic ischemic encephalopathy (HIE) – a leading cause of disability and death in term-born infants.
• Therapeutic hypothermia (TH) – only clinical treatment available for HIE.
• Brain injury associated with HIE involves excitotoxic pathways.
• Excitotoxic injury is mediated by several neurotransmitters, receptors and transporters including the potassium-chloride cotransporter KCC2.
• Phosphorylation of KCC2 (pKCC2) is integral to its transporter function and may influence excitotoxic HI injury.
• pKCC2 function is reduced by cleavage of membrane-bound pKCC2 by the protease, m-calpain.

Methods:
• Newborn piglets (n=18) were anesthetised, and HI induced (30min) with 24h of TH (33.5 °C, HTH) or normothermia (38.5 °C, NTH), then recovered to 72h post-insult.
• Control animals (P4) did not undergo HI or TH.
• Western blotting (WB) – protein expression of pKCC2 & m-calpain.
• Immunofluorescence (IF) – localisation of KCC2 and m-calpain.

Discussion:
• pKCC2 expression was significantly reduced after HI (NTH) in parietal cortex and cerebellum with some recovery following TH.
• m-calpain expression was reduced in parietal cortex only after HI (NTH) with some recovery following HTH although non-significant.
• Although pKCC2 expression was reduced, there was no concomitant increase in m-calpain expression.
• IF co-localisation of KCC2 and m-calpain was seen in all groups with less labelling observed in HI (NTH) group.
• Earlier time points could be investigated throughout injury progression to identify changes in m-calpain activity.

Representative images - cerebellum at 40x magnification with Calpain (red), KCC2 (green), Neurofilament (magenta) and DAPI (blue).

Colocalisation of KCC2 and m-calpain evident (yellow, merged image)