P-2-401

GRB10 Knockdown in Zebrafish is associated with decreased weight-to-length ratio without alterations in AKT and ERK activity: a model to study human growth regulation

Chiara De Leonibus[1], Jack Broadbent[2], Philip Murray[1], Joseph Whitehead[2], Adam Hurlstone[2], Holly Shiels[2], Peter Clayton[1], Adam Stevens[1]

[1]Institute of Human Development, Faculty of Medical and Human Sciences, University of Manchester and Manchester Academic Health Science Centre, Royal Manchester Children's Hospital, Central Manchester University Hospitals NHS Foundation Trust. [2]Faculty of Life Sciences, Manchester University of Manchester.

Background

- Regulatory single nucleotide polymorphisms within *GRB10* have been associated with response to growth hormone (GH) therapy in children with GH deficiency[1]
- In humans GRB10 negatively regulates IGF-1 and GH signaling predominantly via the phosphorylation of PI3K/mTOR/AKT and MEK/ERK pathways
- We have previously shown that *Grb10* knockdown in Zebrafish results in overgrowth with an increase in length and head size

Aim

To develop a model to study weight and weight-to-length ratio in *Zebrafish* and to examine the mechanisms through which *Grb10* knockdown mediates overgrowth

Material and Methods

- *Grb10* knockdown was obtained by injecting splice-blocking morpholino oligonucleotides (MO) into one-cell stage *Zebrafish* embryos. Comparisons were made to sham injected controls (CT) and wild type uninjected animals (WT)
- Weight-to-length ratio (mg/mm^2) was assessed at 54, 72, 96 and 120 hours post-fertilisation (hpf) (n=8). These developmental periods were chosen to model early through late childhood growth. Respirometry was performed to measure O_2 comsumption using a FireStingO2 fiber-optical oxygen meter (Pyroscience, Aachen, Germany)
- Chemical inhibition of the PI3K/mTOR/AKT (NVPBEZ235) and the MEK/ERK pathways (PD184532) was performed from 30 to 72hpf. Total and phosphorylated AKT and ERK were evaluated on Western-Blot to assess the level of phosphorylation of these molecules

Results

- There were significantly greater weights and heights for the MO compared to the CT groups at 54 and 120 hpf ($p<0.01$) (**Figure 1A & 1B**)
- After 72 hours weight-to-length ratio was significantly decreased in MO *vs* CT ($p<0.05$) (**Figure 1C**)
- Decrease in weight-to-length ratio corresponds to an increase O_2 consumption in the MO *Zebrafish Grb10* knockdown compared to controls (n=58), R^2 O_2 consumption MO *vs* CT *vs* WT = 0.80 v 0.64 v 0.27 non-normalised (**Figure 2A**) & 0.74 v 0.55 v 0.13 weight-to-length ratio normalised (**Figure 2B**, $p<0.05$)
- Comparing Western-Blot in MO *vs* CT samples, indicated that global AKT and ERK phosphorylation were not affected by *Grb10* knockdown (Data not shown)

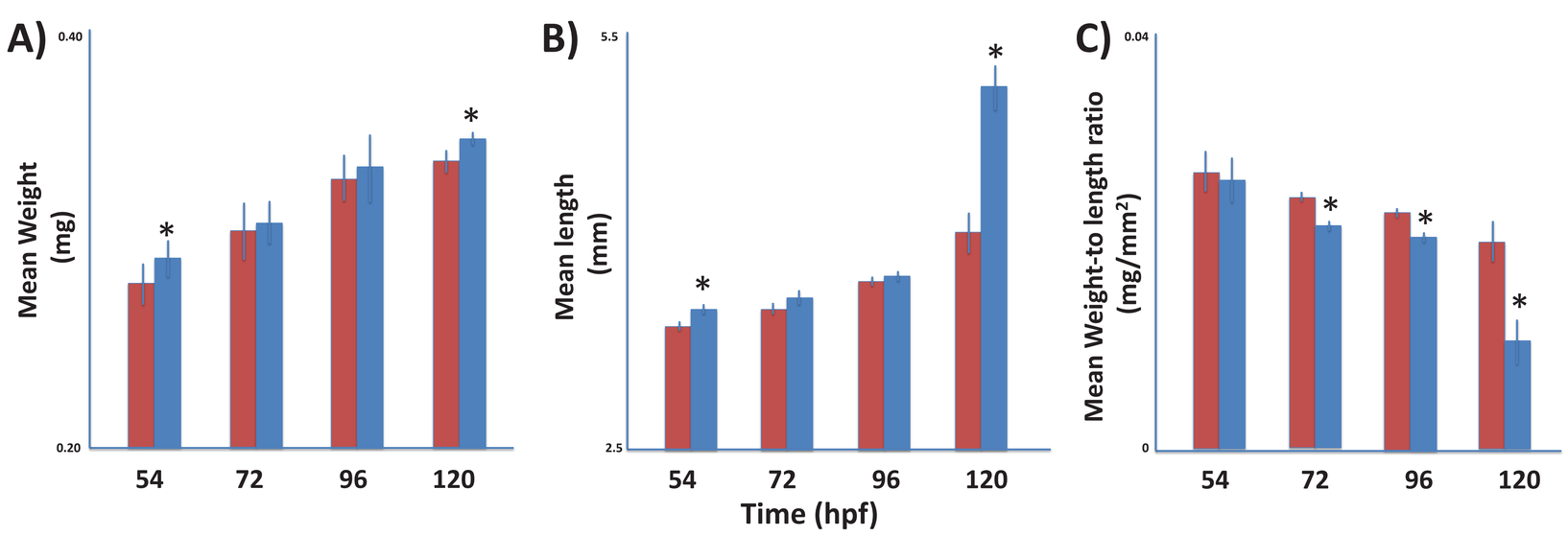

Figure 1. Comparison of height, weight and weight-to-length ratio in MO *vs* CT. * = $p<0.01$

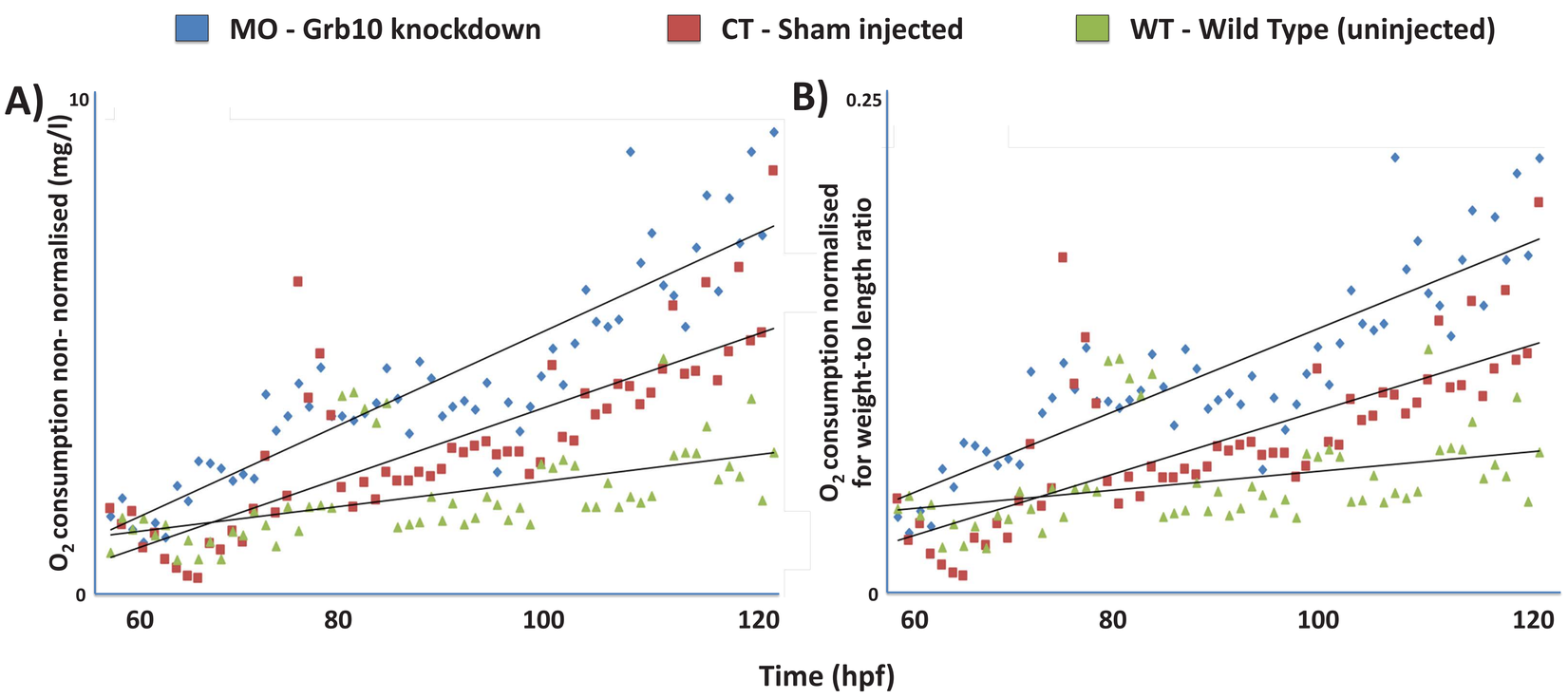

Figure 2. O_2 consumption in MO, CT and WT zebrafish.

Conclusions

- *Grb10* knockdown in the *Zebrafish* model increases length and weight while the ratio of weight-to-length decreases, associated with increased O_2 consumption
- Pathways other than PI3K/mTOR/AKT and MEK/ERK are responsible for the overgrowth of *Grb10* knockout *Zebrafish*
- *Grb10* knockdown in the *Zebrafish* generates a longer, leaner animal, a phenotype that is associated with increased O_2 consumption and provides a model to study the relationship between growth and metabolism.

[1]Clayton P *et al* 2013 Eur J Endocrinol 169 (3):277-289

ESPE 2015 401-P2
GH and IGF Physiology
Chiara De Leonibus
DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology
PosterSessionOnline.com