ACTN3 genotype influences androgen receptor signalling and skeletal muscle mass regulation in health and disease

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Muscle wasting due to ageing, disease or chronic illness is a debilitating condition associated with a reduction in quality of life and life expectancy. α-Actinin-3 (*ACTN3)* is a cytoskeletal protein integral to muscles contractile properties that interacts with a wide array of structural, metabolic and signalling proteins. Homozygosity for the null allele (577XX) results in *ACTN3* deficiency in 1 in 5 humans worldwide and is associated with reduced muscle mass and sprint/power performance in elite athletes and the general population. *ACTN3* deficiency is also a known risk factor for falling in the elderly and a genetic modifier of muscle disorders.

Using an *Actn3* knockout (KO) mouse that models *ACTN3* deficiency in humans we have demonstrated the traits of reduced muscle mass and strength. We are exploring the mechanisms resulting in the reduced mass. Muscle mass regulation involves a complex network of pathways including PI3K/Akt/mTOR and Androgen Receptor (AR) *via* androgen signalling. Androgens such as testosterone signal skeletal muscle hypertrophy through activation of the AR signalling and PI3K/Akt/mTOR pathways. Studies now show *ACTN3* genotype influences muscle mass through regulation of the PI3K/Akt/mTOR pathway. A study of elite Russian athletes (209) showed significantly higher testosterone in male and female athletes carrying the *ACTN3* R-allele with *ACTN3* genotype explaining >12.5% of variation in testosterone levels (Ahmetov *et al.*, 2014). The α-actinins are known primary co-activators and enhancers for AR activity (Huang *et al.*, 2004) and are known to interact with key players in the PI3K/Akt/mTOR signalling pathway; PI3K, PIP2 and mTOR (Lek and North 2010; Norman *et al.*, 2014). This link between *ACTN3* genotype and testosterone levels in elite athletes has focussed our studies on these pathways but how *ACTN3* deficiency influences these pathways has not been explored.

Microarray analyses have shown a significant reduction in AR levels at a transcript (~25%) in *Actn3* KO muscles, including transcript expression of androgen responsive genes *Odc1, Amd2, Smox and Itgb1bp3.* AR protein levels in both skeletal muscles and testes were also greatly reduced in the *Actn3* KO. Localisation of AR shown by IHC is also altered, while circulating testosterone levels were unchanged. Effects of androgen deprivation were also investigated by a castration model (N=6 per genotype/treatment) to determine how α-actinin-3 deficiency would influence muscle wasting. Mice were given pre-emptive analgesia (buprenorphine 0.1mg/kg), anaesthetized with isoflurane before receiving either sham or castration surgery. Mice were euthanised 12 weeks post-surgery. Our pilot castration studies show that androgen deprivation may be detrimental to α-actinin-3 deficient individuals with greater response to muscle atrophy.

We have also explored protein synthesis by surface sensing of translation (SUnSET) pathways including PI3K/Akt/mTOR by Western blotting analyses. A sub group of mice were given an intraperitoneal injection of either puromycin (0.04 μmol/g) [WT n=8, KO n=7] or vehicle only (PBS) [WT n=6, KO n=6] were sacrificed, 30 minutes post procedure. Intriguingly, male *Actn3* deficient mice also demonstrate increased levels of protein synthesis ($P < 0.01$)specifically in the PI3K/AKT/mTOR pathways. Preliminary findings suggest an up-regulation of TGFβ pathway members including SMAD2, 3 and 4.

These findings suggest *ACTN3* genotype influences muscle mass regulation through reduced AR availability and altered regulation of these pathways. Understanding how *ACTN3*, PI3K/Akt/mTOR and AR signalling interact, we will provide insights into muscle wasting conditions and their treatments.