Magnetic Resonance Studies in a New Rodent Model of Acid α-glucosidase Deficiency

Falk, D. (UF, Pediatrics), Díaz-Manera, J. (Hospital de la Santa Creu i Sant Pau, Spain), Zeng, H. (UF, AMRIS), Baligand, C. (CEA, France), Vaught, L. (UF, Pediatrics), Batra, A.(UF, Physiology), Walter G.(UF, Physiology)

Introduction - Pompe disease presents as a cardiac and neuromuscular disorder resulting from an absence or deficiency in the gene encoding acid α-glucosidase (GAA). Progressive lysosomal glycogen accumulation occurs in virtually all tissues, as GAA is necessary to degrade lysosomal glycogen. Glycogen accumulation creates a significant disturbance in cellular morphology, metabolism, and physiology. In this project, a genetically engineered rat model of GAA deficiency was created to examine changes in MR parameters (proton transverse relaxation time -T_2, magnetization transfer contrast -MTC and ^{31}P, ^{13}C, and ^{1}H MR visible metabolites and chemical exchange saturation transfer (CEST) spectra.

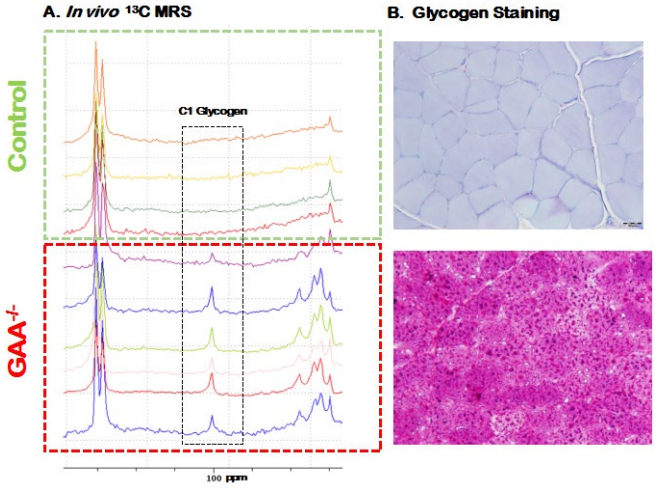

Fig.1 Glycogen accumulation in the posterior, hindlimb muscles of control and *Gaa*$^{-/-}$ rats was detected *in vivo* using ^{13}C NMR (**A**) and confirmed *ex vivo* by histology (**B**). Muscle tissue sections were stained with periodic acid schiff (PAS) reagent for glycogen (red).

Experimental - *Rodent model:* The *Gaa*$^{-/-}$ rat was generated with zinc finger nucleases targeting the ORF of *Gaa* resulting in complete absence of Gaa enzymatic activity and progressive lysosomal glycogen accumulation. This results in higher degree of mortality where male KO (*Gaa*$^{-/-}$) median lifespan is ~5 months of age. *MRI*: Quantitative T_2 images were acquired using a MSME sequence with the following parameters using a quadrature birdcage coil with 30mm i.d.: FOV=22x22mm, 3 slices with 0.7mm slice thickness, TR=5s, TE=3.1ms, ΔTE=3.1ms, echoes=48. *MRS*:^{1}H: Localized ^{1}H STEAM spectra from the gastrocnemius with the following parameters: voxel size 2x2x2mm^3, TR=2s, NA=128, SW = 6kHz, 4096 complex data points; ^{31}P: Surface coil (8x12mm) localized spectra were acquired from the posterior compartment (primarily gastrocnemius) with TR=2s, SW= 8kHz, NA=128x4, 2048 data points, 90 degree block pulse or adiabatic excite pulse; ^{13}C: Surface coil (9x12mm) localized spectra were acquired from the posterior compartment (primarily gastrocnemius) with TR= 0.5s, SW =25kHz, 512 data points, NA = 4096, 90 degree hard block pulse, and ^{1}H Waltz16 decoupling only during acquisition.

Results and Discussion- *Gaa*$^{-/-}$ rat muscles displayed definitive and significant glycogen accumulation based on biochemical (data not shown), *in vivo* detection of the C1 from glycogen (Fig1A), and histological staining (Fig 1B). Due to the large musculature of rats, high SNR and well resolved ^{31}P and ^{1}H *in vivo* spectra can be readily acquired. Moreover, both saturation transfer experiments of exchangeable ^{31}P and ^{1}H metabolites can also be measured. CEST spectra are being acquired using ^{1}H-STEAM to compare ^{1}H-MRS and CEST determined levels of creatine and glycogen in wild-type and *Gaa*$^{-/-}$ muscle.

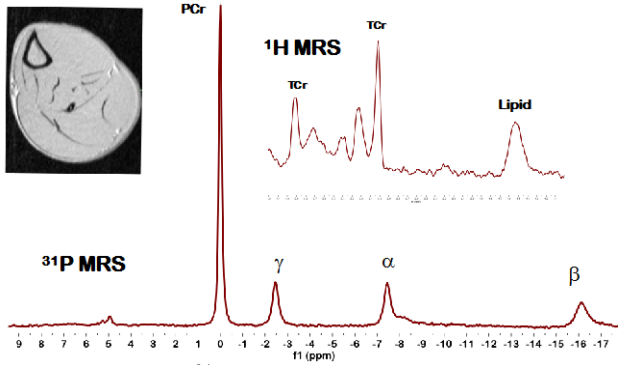

Fig.2 *In vivo* MRI, ^{31}P and 1H spectra from the gastrocnemius of a *Gaa*$^{-/-}$ rat. **Upper left**: Single echo image from multislice, multiecho spin echo sequence to map muscle T_2 values. **Bottom**: ^{31}P spectra acquired from the posterior compartment (α,β,γ ATP resonances are indicated). **Upper right**: localized 1H STEAM spectra from the gastrocnemius (resonances from creatine (TCr) and -CH_2- of the lipids are indicated).

Conclusions-NMR can be used to noninvasively detect the massive amount of glycogen deposition in the muscles of rats when Gaa has been genetically deleted. We have previously found that certain 31P metabolites can be used as robust biomarkers for Gaa expression in the mouse (1) and future studies are aimed at confirming these observations in this larger rodent following the viral-mediated gene replacement of Gaa.

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References- [1] Baligand, C., et al. *Molecular Therapy-Methods & Clinical Development* 7 (2017): 42-49.