



$^{13}\text{C}/^{31}\text{P}$ Tissue Metabolic Biomarkers in a Mouse Model of Pompe Disease

Baligand, C. (Leiden University Medical Center, Netherlands); Todd, A.G. (Pharmacology and Therapeutics, UF); Lee-McMullen, B. (Stanford University, CA); Ravneet, S.V. (Physiology and Functional Genomics, UF); Byrne, B.J. (Pediatrics, UF); Falk, D.J. (Pediatrics, UF); Walter, G.A. (Physiology and Functional Genomics, UF)

Introduction - Pompe disease is a progressive degenerative neuromuscular disease characterized by a deficiency in alpha-glucosidase (GAA), the enzyme responsible for lysosomal glycogen breakdown. Although enzyme replacement therapy has been successful in improving survival rate, there is still no cure available. As an alternative, gene therapy has the potential to correct gene expression with a single administration of recombinant adeno-associated virus (rAAV). However, preclinical and clinical non-invasive biomarkers are currently lacking. In this study, we used a combination of high field (11.1T) $^{13}\text{C}/^{31}\text{P}$ MR spectroscopy (MRS) *in vivo* with tissue ^1H high-resolution magic-angle spinning NMR (^1H -HR-MAS) in the mouse model of the disease ($Gaa^{-/-}$) to 1) identify new metabolic biomarkers of the disease; 2) assess the effect of rAAV2/9-desmin-GAA treatment in skeletal muscle.

Experimental - $Gaa^{-/-}$ and wild-type mice were examined at 2 (n=6), 6 (n=4), 12 (n=7), and 18 months of age (n=5). Two-month-old $Gaa^{-/-}$ mice received unilateral rAAV injections in the leg ($Gaa^{-/-}$ +AAV, n=8) and were scanned after 28 days, as well as untreated (n=8) and controls (n=4). ***In vivo* ^{31}P and ^{13}C -MRS data were acquired on the 11.1T Agilent system available at the AMRIS facility at the University of Florida (Fig. 1).** ^{31}P and ^{13}C -MRS were acquired at the Ernst angle with TR=1s, and accumulated for 5 min and 1h, respectively, to obtain sufficient SNR. ^1H -WALTZ-16 decoupling was added for ^{13}C acquisitions. ^{13}C -glycogen signal-to-noise, and ^{31}P metabolites peak integrals (including Phosphomonoesters (PME)) were calculated in MestReNova 10.0. Tissue samples were scanned with **^1H -HR-MAS on the 600MHz Bruker system at the AMRIS facility** for glycogen, creatine and glucose-6-phosphate (G6P) detection using 1D-NOESY with presaturation. Metabolites were quantified using MestReNova Simple Mixture Analysis plugin. GAA activity and glycogen concentration were assessed biochemically.

Results - **1)** High C1-Glycogen signal could be detected *in vivo* in $Gaa^{-/-}$, while signal was within the noise level in control with physiological concentration (Fig.1A,B). **2)** ^{31}P PME/ATP_{total} was elevated in $Gaa^{-/-}$ mice at 2, 6, 12 and 18 mo compared to controls (Fig.1C,D). **3)** ^1H -HR-MAS revealed significantly higher G6P and glycogen-to-creatine levels in the gastrocnemius of $Gaa^{-/-}$ compared to controls (Fig.2). **4)** rAAV2/9-desmin-GAA injection in $Gaa^{-/-}$ +AAV successfully restored high levels of GAA activity. MRS showed that C1-glycogen level, *in vivo* PME levels, and intact tissue glycogen and G6P levels as measured by ^1H -HR-MAS were all corrected after treatment (Fig.2). GAA activity between 10-15 nmol/hr/mg was sufficient to significantly decrease all glycogen measures in $Gaa^{-/-}$ +AAV, and higher activity did not induce further glycogen clearance.

Conclusions - We identified PME as a new and sensitive ^{31}P -MRS biomarker of disease progression and response to gene therapy in $Gaa^{-/-}$ mice. rAAV treatment not only restored normal levels of GAA activity and induced glycogen clearance but also significantly decreased the PME signal. Elevated PME levels have previously been observed in patients with glycogen storage disorder type III.¹ Our HR-MAS results suggest that accumulation of G6P may greatly contribute to the higher *in vivo* PME signal. This is consistent with recent work in $Gaa^{-/-}$ mice reporting high G6P concentration and increased glycogen synthase activity.² Since ^{31}P -MRS has a higher sensitivity than ^{13}C , this approach has potential for clinical translation as a companion biomarker, in particular in the study of adjuvant therapy targeting glycogen metabolism. These results have been recently published in ref #3.

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References - [1] Wary, C., *et al.*, **Neuromuscul Disord.** 20(8):548-58 (2010). [2] Taylor, K.M. **PLoS One** 8: e56181 (2013). [3] Baligand, *et al.* **Molecular Therapy-Methods & Clinical Development** 7: 42-49 (2017).

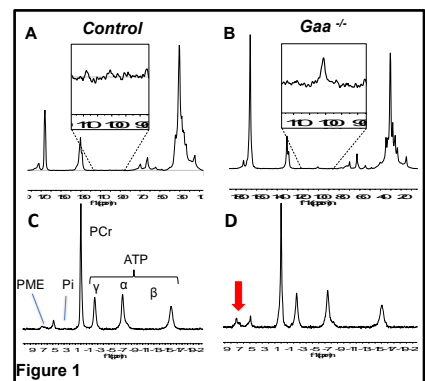


Figure 1

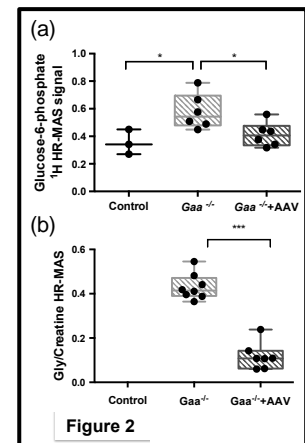


Figure 2