Longitudinal measurement using a novel Western blot assay of glycosylation of alpha dystroglycan in patients with Limb-Girdle Muscular Dystrophy Type 2I/R9 FKRP-related: preliminary results from MLB-01-001

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Limb Girdle Muscle Dystrophy Type 2I (LGMD2I) Background

Limb Girdle Muscular Dystrophy Type 2I (LMGD2I) is an autosomal recessive disease of striated muscle caused by mutations in the fukutin-related protein gene, *FKRP,* which codes for the glycosyltransferase enzyme that is critical for the glycosylation of alpha-dystroglycan (αDG) (Figure 1). The heavily glycosylated αDG is a component of the dystrophin-glycoprotein complex that anchors the intracellular cytoskeleton of muscle cells to the extracellular matrix through interactions of the matriglycan on αDG (Figure 2).

In LGMD2I, αDG is hypo-glycosylated due to partial loss of function mutations in *FKRP* making muscle cells susceptible to contraction-induced injury that results in inflammation, fibrosis, and fatty infiltrate leading to muscle wasting and impaired function. Currently, there is no approved treatment available for LGMD2I, however, studies in LGMD2I mouse models have shown that ribitol increases αDG glycosylation (Cataldi et al, 2018).

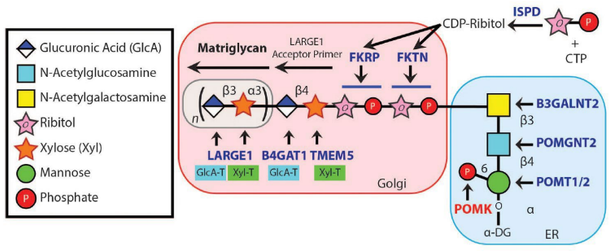


Figure 1: FKRP plays a critical role in priming αDG for additional glycosylation (Figure from Walimbe et al, 2020)

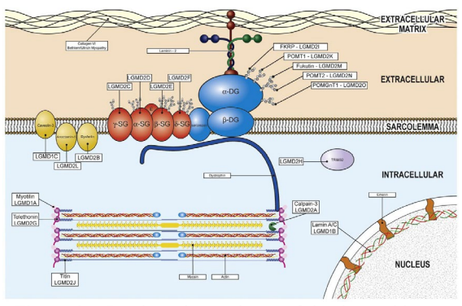


Figure 2: αDG is an integral part of the dystrophin-glycoprotein complex (Figure from Wicklund et al, 2014).

Study Hypothesis

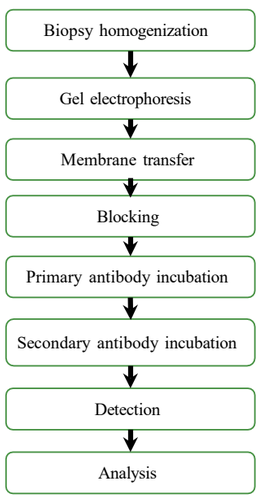
To support development of BBP-418 as a potential therapy for LGMDI2I, we sought to identify a biomarker that could reflect the glycosylation state of αDG, as hypo-glycosylation of αDG is the single causal pathway of disease. BBP-418 increases the intracellular concentration of CDP-Ribitol helping to drive residual activity of the mutant FKRP enzyme and increasing αDG glycosylation levels. Hence, we rationalized that measurement of glycosylated αDG levels would be a viable biomarker strategy in LGMD2I. ClinicalTrials.gov Identifier: NCT04202627

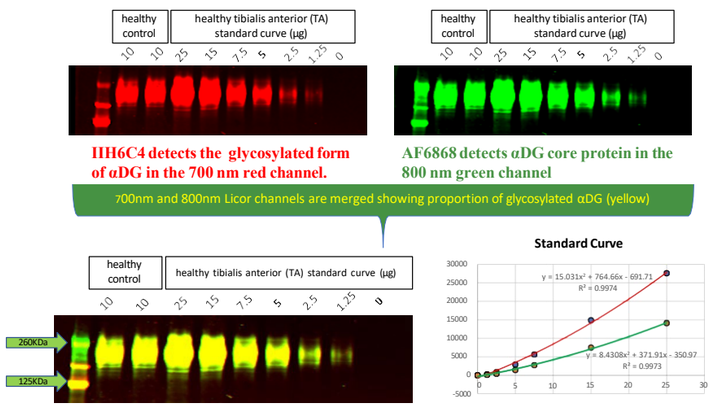
Methods

Tissue Lysis and sample preparation: Tissue samples were processed in lysis buffer supplemented with protease inhibitors. Total protein in lysate was determined by BCA (Thermo Fisher) for RIPA and protein 660 kit (Thermo Fisher) for SDS-Urea and tested at ≥40µg for L276I/L276I homozygous samples and ≥ 60µg for other FKRP genotype samples.

Western Blotting: For detection of target proteins αDG -Total and αDG-Glycan, membrane was probed simultaneously with primary antibodies AF6868 alpha Dystroglycan (R&D Systems) and IIH6C4 alpha Dystroglycan (Millipore), followed by fluorescent secondary antibodies IR800CW Mouse Anti-Sheep and IR680 Goat Anti-Mouse secondary antibodies (LiCor). The membrane was visualized in Odyssey CLx imager (LiCor) and intensities of the 700 (αDG-Glycan, red bands) and 800 (αDG-Total, green bands) nm channels were recorded. Merged channel resulted in yellow bands when glycosylated and total αDG signals overlap.

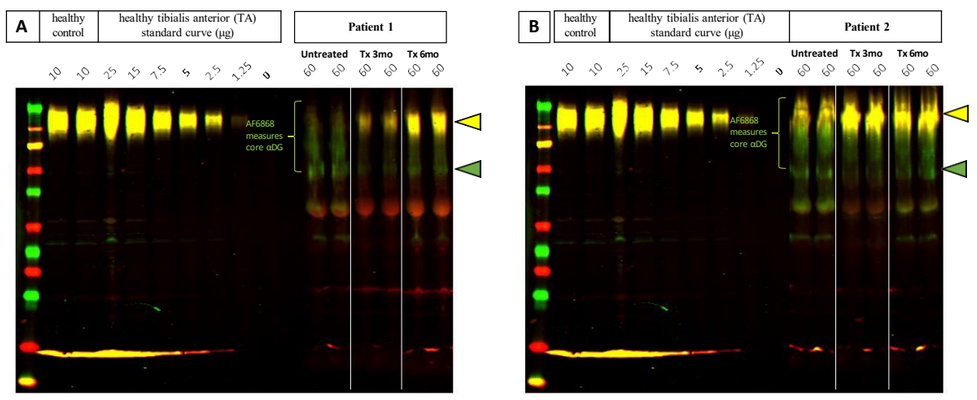
Data Analysis: Integration boxes were drawn using Image Studio software with specific dimensions over molecular weight range for standards, test articles and control samples. Data analysis was done in Excel (Microsoft). The 700nm and 800nm raw signals of test articles were interpolated to healthy control TA standard curve using regression to a quadratic equation, to give relative healthy TA amounts of glycosylated and total αDG. Healthy positive control (HPC) at 10µg was deployed to monitor assay performance.





Multiple sections of a single healthy donor TA biopsy sample are homogenized, and the resulting lysate is pooled together and stored in single use aliquots at –80° C. These aliquots are used to generate a standard curve which is used in every assay. In addition, a quality control of 10µg lysate is used to assess assay performance by measuring interpolatability to the standard curve.

Muscle biopsies from a Phase 2 trial of BBP-418 showed increases in αDG glycosylation

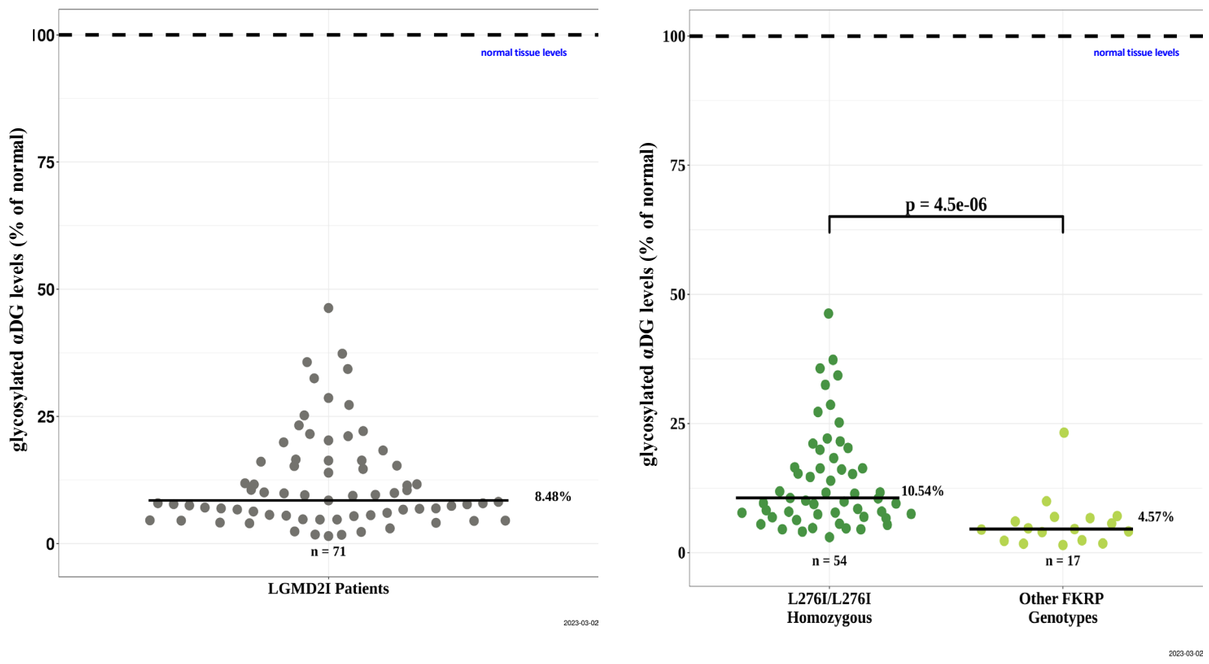


Note: blots have been reordered to allow for side-by-side comparison of treatment effect within a patient
◀ mature glycosylated species (~160kDa) ◀ lower non-glycosylated species (70kDa)

- Due to low levels of glycosylated αDG at baseline (compared to healthy individuals), LGMD2I patient samples require higher protein loading to detect glycosylated αDG levels.

LGMD2I patients have less functional, glycosylated αDG

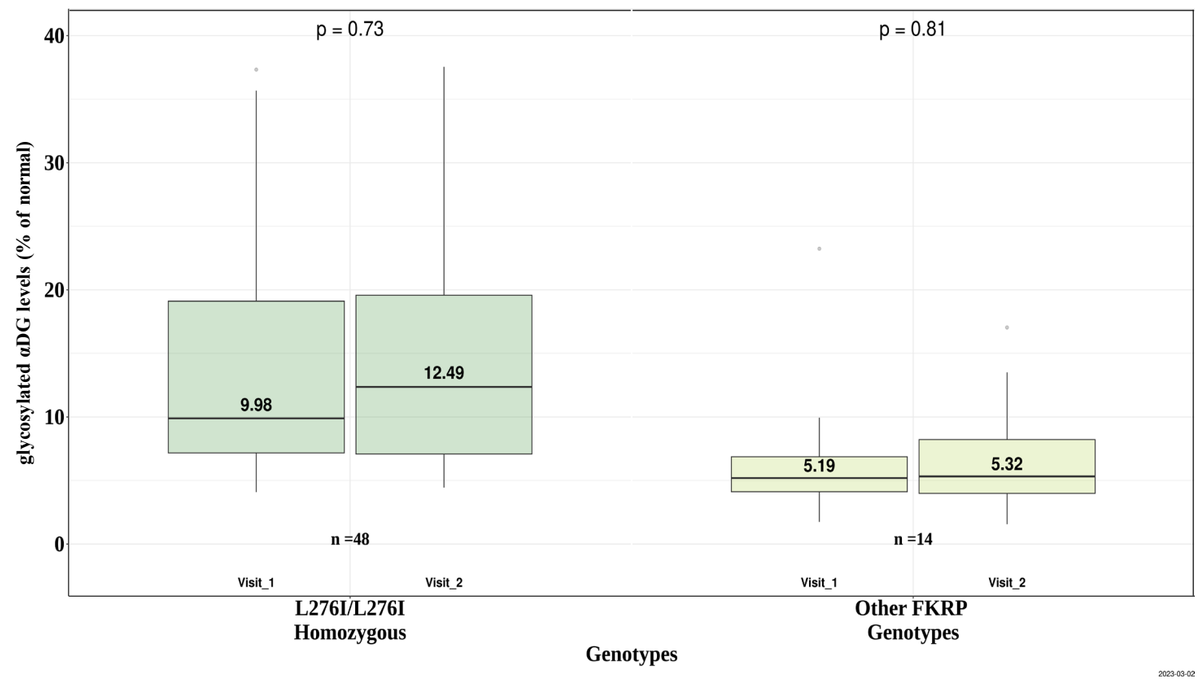
- Glycosylated αDG levels are reduced in LGMD2I patients relative to healthy tibialis anterior muscle.



Bars denote median values, boxes denote 25-75% percentile, whiskers show 1.5X IQR
Wilcoxon test used to determine significance

Glycosylated αDG levels remain stable over time

- Glycosylated αDG levels remain stable at 2nd visit (6, 9, or 12 months) compared to first visit in untreated LGMD2I patients.



Bars denote median values, boxes denote 25-75% percentile, whiskers show 1.5X IQR
Wilcoxon test used to determine significance

Conclusions

- Data from a natural history study of patients with LGMD2I suggest that a novel, validated Western blot assay can be used to evaluate glycosylation of αDG, a measure of the prognostic biomarker reflecting the root cause of LGMD2I.
- A clear difference in glycosylated αDG content in patients with LGMD2I relative to normal control tissue is observed.
- Additionally, glycosylated αDG content reflects the LGMD2I genotype: Other (non-L276I homozygous) patients showed a greater loss in glycosylation of αDG compared to L276I/L276I homozygous patients.
  - This observation aligns with known differences in disease onset and progression as these patients exhibit an earlier disease onset and more rapid progression than L276I homozygous patients.
- These natural history data suggest that longitudinal measurement of glycosylated αDG remains stable over 6—12 months.
- Measurement of αDG glycosylation may provide a relevant approach to assess the impact of potential therapies for LGMD2I.

References

1. Walimbe AS, et al. DOI: 10.7554/eLife.61388 "POMK regulates Dystroglycan function via LARGE1-mediated elongation of matriglycan".

2. Wicklund MP, et al. DOI: 10.1016/j.ncl.2014.04.005 "The limb-girdle muscular dystrophies".

3. Cataldi MP, et al. DOI: 10.1038/s41467-018-05990-z "Ribitol restores functionally glycosylated α-Dystroglycan and improves muscle function in dystrophic FKRP-mutant mice".

Disclosures

- BBP-418 is an investigational drug; BBP-418 has not yet been evaluated or approved to treat LGMD2I or any other disease or condition by any regulatory health authority.
- Presenters are employees of ML Bio Solutions, Inc. and BridgeBio Pharma, Inc.
- References made to a Phase 2 clinical trial refer to a trial that is currently ongoing and all results are preliminary and subject to change.